

Compass Software User Guide

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ProteinSimple 3001 Orchard Parkway San Jose, CA 95134 Toll-free: (888) 607-9692 Tel: (408) 510-5500 Fax: (408) 510-5599 email: support@proteinsimple.com web: www.proteinsimple.com Compass Software User Guide for Wes, Sally Sue and Peggy Sue P/N 031-109 Revision 3, August 2015 For research use only. Not for use in diagnostic procedures

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Let's Get Started

Chapter Overview

- Launching Compass
- Compass Overview
- Software Menus
- Changing the Compass Main Window Layout
- Software Help
- Checking for and Installing New Versions of Compass
- Viewing Release Notes
- Viewing the Software Log
- Compass Version Information
- Directory and File Information

Launching Compass



To open Compass, double-click the icon on the computer desktop.

Compass Overview

Compass has three main screens:

- Assay You'll create and review your assay.
- Run Summary Check out the status of your run.
- Analysis Take a look at the data from your experiment.

Changing the Screen View

To move between the Assay, Run Summary and Analysis screens, just click the button in the screen tab located in the upper right corner of the main window.



Assay Screen

The Assay screen is used to create, view, and edit assays. You can assign well locations for assay plate reagents, modify assay protocol steps, enter assay notes and add annotations for individual wells on the assay plate.

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Ready Start																			Assay ᠿ Run Summary					alysis			
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													Seco	ndary An	tibody												ĺ
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Run Summary Screen

The Run Summary screen is used to monitor status of a run in progress, watch movies of the separation in the capillaries, and view current and voltage plots for each run.

2012-03-05	_11-51-19_He	alaControlER	Kassay - Cor	npass								- • •						
File Edit In	istrument \	Window He	elp															
											Assay Run Summ	ary 🏭 Analysis						
Run: 2012-0	03-05_11-51-3	19_HelaCont	rolERKassay		Separation	L IV Plot												
① Status																		
Ru Pat Assa Schedul Instrumer Starte Complete	Run 2012-03-05_11-51-19_HelaControlERKassay Path C\Users\ptung\Documents\ProteinSimple\2012-03-05_11-51-19_HelaControlERKassay Assay HelaControlERKassay Schedule Overlapping with hold nstrument PL0004 - PL0004 Started Mon 11:56 AM Mar 5, 2012 PST Ture 6:35 AM Mar 6, 2012 PST										Cycle 3							
Cycle	Sample	Sep	Hold	1*	2*	3°	Detect	Results										
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2	1:10 PM	+ 1:14 PM	() 2:24 PM	1° 🕑 7:27 PM	2° 🕑 7:53 PM	3°	11:10 PM	11:44 PM										
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	2:24 PM	2:28 PM	3:37 PM	8:46 PM	9:13 PM	11:22 PM	12:30 AM	1:04 AM										
4		•	\odot	1° 🕛	2° 🕒	3° 🕐		4										
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7	7:19 PM	• 7:23 PM	() 8:32 PM	1° 🕐 12:20 AM	2°(-) 12:46 AM	3° 🕑 2:55 AM	4:03 AM	4:37 AM										
8	8:32 PM	+ 8:37 PM	① 9:46 PM	1° 🕐 1:09 AM	2°() 1:36 AM	3° 🕑 3:44 AM	4:53 AM	5:26 AM	•									

Analysis Screen

The Analysis screen is used to view data from your assay, including the graph view (electropherograms), lane view (shown below), capillary images, and a table with your results. You can also analyze your data here after the run is finished.



Screen Panes

Assay, Run Summary and Analysis screens all have multiple screen panes that let you view the individual components of a run, assay or data file. Each pane has a labeled tab and a unique icon. We'll describe panes specific to each screen later in the individual screen sections.

The active pane in a screen is blue. To view a pane, click in the pane or on its tab. The example below shows panes in the Analysis screen, and the Lane pane is active:



Title Bar

In the title bar you will see the run file name and the icons that allow the main Compass window to be minimized, maximized or closed.

😻 Simple Western ERK Demo - Compass	X	

Main Menu

Access to various software, instrument and screen operations is available through the main menu. More details on menu commands can be found in "Software Menus" on page 7.

File Edit View Instrument Window Help

Instrument Status Bar

The instrument status bar is used to start runs and cleaning protocols, relay system status and show run progress. More details on instrument control and status can be found in Chapter 7, "Controlling Wes, Sally Sue and Peggy Sue".

Running Stop	
WS2003 Mon 3:19 PM	Mon 5:25 PI
Run: 2013-11-18_15-16-25_Wes_EZ_1200V_W2003C1087_A791_insulated	Separation 🛃 IV Plot

NOTE: You will only see the instrument status bar when Compass is connected to an instrument. There is no status bar on computer workstations that you're only using for data analysis.

Screen Tab

The screen tab lets you move between Assay, Run Summary or Analysis screens and is located in the upper right corner of the main window. Just click a button to view a screen.



View Bar

The view bar is only displayed in the Analysis screen as part of the main menu bar and allows you to switch between displaying sample chemiluminescent data, fluorescent standards or capillary registration information, data for a single capillary or all capillaries in the run, or grouped capillary data. View bar options are detailed in "Switching Between Sample, Standards and Registration Data Views" on page 160 for size assays or page 302 for charge assays, and "Using Groups" on page 189 for size assays or page 328 for charge assays.

🚊 Standards 🛛 🛧 Reg. 🚉 Samples 🛛 🔳 📰

Compass Status Bar

The status bar is located in the lower right corner of the main window. It displays active software processes and their progress.

Analyzing: Simple Western ERK Demo 🛛 🛁 📼

Software Menus

A brief description of the software menus in the main menu are described in this section. Not all menus are available in every screen, and menu commands change depending on what screen is active. The menus and commands available for each screen will be detailed in the individual screen sections.

File Menu

The File menu contains basic file commands.



Edit Menu

The Edit menu contains basic editing commands, analysis and preferences options. Specific details on preferences are described in Chapter 10, "Setting Your Preferences".

Edit	Instrument	Window	Help						
	Cut								
	Сору		Ctrl+C						
	Paste		Ctrl+V						
	Default Analysis								
	Analysis								
	Preferences								

View Menu

The View menu can only be seen in the Analysis screen, and allows you to change how your data is displayed.



page 8

Instrument Menu

The Instrument menu is only available when Compass is connected to directly to your instrument. Instrument control options are explained in Chapter 7, "Controlling Wes, Sally Sue and Peggy Sue".

Inst	rument Window Help
	New Run
	Open Trays
	Manual Clean
	Cleanup
	Self Test
	Leveling
	Runs
	Properties
	Disconnect

Window Menu

The Window menu allows you to switch between Assay, Run Summary or Analysis screens, and restore screens to the Compass default layout.

Win	dow Help
	Assay
	Run Summary
	Analysis
	Default Layout

- Assay Displays the Assay screen where you can create, view, and edit assays.
- Run Summary Displays the Run Summary screen which tells you the status of a run in progress.
- **Analysis** Displays the Analysis screen which is used to view sample electropherograms, lane data and results.
- **Default Layout** Restores the individual panes in the current screen back to their default size and location.

Help Menu

The Help menu provides access to Help, software updates, release notes and other software information.



- Help Contents Displays the Compass Help file.
- Check for Updates Automatically checks to see if a new version of Compass is available.
- **Release Notes** Displays Compass release notes for the current and prior versions.
- **Compass Log** Displays the Compass software log file.
- About Compass Displays the Compass software version and build information.

Changing the Compass Main Window Layout

You can easily resize the Compass main window and the individual panes in each screen. Screen panes can also be moved outside of the main window.

Resizing the Main Compass Window

To resize the main window, roll the mouse over a corner or border until the sizing arrow appears. Then just click and drag to resize.

Resizing the Screen Tab

The screen tab can be sized to show all or just some of the screen buttons. To resize, roll the mouse over the left edge of the tab until the sizing arrow appears, then click and drag to resize. If a screen button is hidden, a double arrow will display in the tab. Click to display and select the hidden screen.

💾 Assay	>>	
🕒 Run Summary		

Resizing Screen Panes

- **To resize a pane** Roll the mouse over the pane border until the sizing arrow appears. Then just click and drag to resize.
- **To maximize a pane** Click the maximize button in the upper right corner or double-click the tab. The other panes in the screen will automatically minimize to pane bars in the task area along the window border.



To restore all minimized panes - Click Restore on the minimized pane bar.



To restore only one minimized pane - Click the pane icon on the minimized pane bar.

•

•

To restore a maximized pane to its original size - Double-click the tab or right click the tab and click **Restore**.



• To restore all panes to their original sizes - Select Window in the main menu and click Default Layout.

Changing the Location of Screen Panes

Panes can be moved to different locations within a screen.

- **To move a pane** Click on its tab and drag it to the new location. As the pane is moved, area guides will display to assist you in choosing a drop location.
 - Area guides with a black arrow let you know that if the pane is dropped at that location, it will be resized and relocated as an individual pane in that area of the screen.



Area guides with a folder let you know that if the pane is dropped at that location, it will be added as a new tab in an area with one or more pane tabs.



•

File Edit View Instrument Window Help												
🚊 🗮 Standar	ds 🛧	Reg. 🚖 Sam	ples 🔳 📰			E	Assay	Run	Summary	Analysis]	
Exp	- 0	🛵 Graph	🕲 Image 🖽 Lane 🛛 🕺 🚺 🗮 📃 💳 🗖	III Peaks	Capillaries					-		
Sample	Prima		C1:9	Sample	Primary	Cap	Peak	Name	Position	MW (kDa)	-	
Biot. L	Antib	1 1000 1000		Biot. L	Antibo	C3:1	3	Ldr 66	756	66	-	
Sample	Prima	1		Biot. L	Antibo	C3:1	4	Ldr 116	813	116		
Sample	Prima	87.		Biot. L	Antibo	C3:1	5	Ldr 180	871	180		
Sample	Prima		12 40 68 H6 120 220 MVV(KDa)	Biot. L	Antibo	C3:1	6	Ldr 230	906	230		
Sample	Prima		C1:10	Sample	Primar	C3:7	1	PI3K	786	109		
Biot. L	Antib	2 -m		Sample	Primar	C3:8	1	PI3K	776	108		
🚯 Sample	Prima			Sample	Primar	C3:9	1	PI3K	792	108		
🚯 Sample	Prima	₿ .	, e 1 1	Sample	Primar	C3:10	1		724	61		
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3 Sample	Prima			Biot. L	Antibo	C4:1	1	Ldr 12	497	12		
Biot. L	Antib	g 200 (C3:1	Biot. L	Antibo	C4:1	2	Ldr 40	655	40		
Sample	Prima	E E	ura uro 17	Biot. L	Antibo	C4:1	3	Ldr 66	757	66	=	
Sample	Prima		$\land \land $	Biot. L	Antibo	C4:1	4	Ldr 116	815	116		
Sample	Prima			Biot. L	Antibo	C4:1	5	Ldr 180	874	180		
Sample	Prima		MW (kDa)	Biot. L	Antibo	C4:1	6	Ldr 230	908	230	_	
Biot. L	Antib		C3:7	Sample	Primar	C4:7	1	PBK	798	108		
3 Sample	Prima	8		Sample	Primar	C4:8	1	PI3K	795	107		
Sample	Prima	100		Sample	Primar	C4:9	1	PI3K	805	107		
Sample	Prima	87.		Sample	Primar	C4:10	1	DIDIK	726	60	- 11	
Bist I	Antik		12 40 58 +15 120 220 MW(kDa)	Diet 1	Primar	CE:1	1	PISK Label 2	795	105	-	
BIOT. L	Antib		C3:8	BIOT. L	Antibo	C5:1	1	Lar 12	500	12		
Sample	Drima	2 50		Biot. L	Antibo	C5:1	2	Ldr 66	757	40		
Sample	Drima			Biot I	Antibo	C5:1	4	Ldr 116	814	116		
Sample	Prima			Biot I	Antibo	C5:1	5	L dr 180	872	180		
Biot. I	Antib	- °		Biot. L	Antibo	C5:1	6	L dr 230	907	230		
Sample	Prima			Sample	Primar	C5:7	1	PI3K	795	108		
Sample	Prima	2 200	C3:9	Sample	Primar	C5:8	1	PI3K	794	108	- 1	
3 Sample	Prima		1	Sample	Primar	C5:9	1		745	61	- 1	
Sample	Prima		3	Sample	Primar	C5:9	2	PI3K	811	108	-	
<	F.			<							F I	
						:					_	

The following figure shows the Analysis screen after moving the Graph pane.

To detach a pane from the main window - Click on its tab and drag it outside the main Compass window or right click the tab and click **Detached**.

í en u el c	<u>`</u>	
	Detached	
, et	Restore	
Lado	Move	•
	Size	+
P €	Minimize	
33	Maximize	
	Close	

- To move a detached pane back inside the main window Right click the tab and deselect Detached.
- To restore all panes to their original locations Select Window in the main menu and click **Default Layout**.

•

Restoring the Main Window to the Default Layout

To restore screen pane sizes and locations to the original Compass layout, select **Window** from the main menu and click **Default Layout**.

Software Help

Select Help and click Help Contents to view the Compass Help file.

Checking for and Installing New Versions of Compass

Compass can automatically check to see if a newer version of software is available. To do this:

- 1. Make sure the computer being used has an active internet connection.
- 2. Select Help and click Check for Updates. If an update is found, the following screen will display:

😺 Update	
Available Updates A new update has been found for Compass.	
Compass 2.3.1	*
	*
	Finish Cancel

- 3. Click **Finish** to start the download and install the update.
- 4. Follow the on-screen instructions to complete the software installation.
- 5. Reboot the computer before using the new version of Compass.

Viewing Release Notes

Select **Help** and click **Release Notes** to view feature updates and bug fixes for new and past versions of Compass. We recommend you review these notes whenever a software update is installed.



Viewing the Software Log

Select Help and click Compass Log to view the software log file.

Compass Version Information

Select Help and click About Compass to view the software version and build number information.



Directory and File Information

The main Compass directory is located in the **Program Files** folder, and also contains PDF files of the Wes and Sally Sue/Peggy Sue User Guides.

😋 🔵 🗢 📕 🕨 Computer 🕨 Local Di	sk (C:) 🕨 Progran	n Files (x86) 🕨 Compass 🕨	_	-	
File Edit View Tools Help					
Organize 🔻 Include in library 👻	Share with 🔻	Burn Compatibility files New fo	older		
▷]] Intel	^	Name	Date modified	Туре	Size
PerfLogs		Configuration Examples	10/11/2012 12:06 PM 10/11/2012 12:06 PM	File folder File folder	
 Program Files Program Files (x86) Adobe 		🍑 features 🍑 jre	10/11/2012 12:06 PM 10/11/2012 12:06 PM	File folder File folder	
 Adobe Media Player Bonjour 		µ p2 µ plugins	10/11/2012 12:06 PM 10/11/2012 12:06 PM	File folder File folder File folder	
 Cisco Citrix 		 templates .eclipseproduct 	10/11/2012 12:06 PM 10/11/2012 12:06 PM 7/29/2010 10:36 AM	File folder ECLIPSEPRODUCT	1 KB
Common Files Compass Compass		 artifacts.xml Compass for NanoPro 1000.pdf 	10/8/2012 11:05 AM 10/8/2012 10:56 AM	Safari Document Adobe Acrobat D	36 KB 6,002 KB
 Image: Configuration Image: Configuration		Compass for Peggy.pdf Compass for Sally.pdf Compass for Sally.pdf Compase for Simon pdf	10/8/2012 10:56 AM 10/8/2012 10:56 AM	Adobe Acrobat D Adobe Acrobat D	7,184 KB 7,184 KB 5.191 KB
⊳ 퉲 jre ⊳ 퉲 p2	=	 Compass.exe Compass.ico 	10/8/2012 11:04 AM 10/8/2012 11:04 AM	Application Icon	52 KB 24 KB
 b logins i readme b logination 		 Compass.ini compass_data_file.ico 	10/8/2012 11:05 AM 10/8/2012 11:07 AM	Configuration sett Icon	1 KB 25 KB
e computes		eclipsec.exe eff epl-v10.html eff license.rtf	10/8/2012 11:04 AM 2/25/2005 6:53 PM 10/8/2012 10:56 AM	Application HTML Document Rich Text Format	24 KB 17 KB 139 KB
		NanoPro 1000 User Guide.pdf Anotice.html	10/8/2012 10:56 AM 2/4/2011 3:39 PM	Adobe Acrobat D HTML Document	6,002 KB 9 KB
		 Peggy User Guide.pdf Sally User Guide.pdf 	10/8/2012 10:56 AM 10/8/2012 10:56 AM	Adobe Acrobat D Adobe Acrobat D	3,741 KB 3,741 KB
		🔁 Simon User Guide.pdf	10/8/2012 10:56 AM	Adobe Acrobat D	1,082 KB



Compass assay and run files are located in the **Documents** folder in the User directory on your computer:

- Assays Folder Contains all assay files that you've saved.
- New Assays Folder Contains Simple Western assay template files.
- Runs Folder Contains all run files. Run data is automatically written to this folder.

NOTE: When a Compass software update is performed, the template assays in the New Assays folder are overwritten. If you have customized these assays, we recommend saving them in a unique subfolder prior to updating the software, then transferring them back to the New Assays folder after the update to avoid losing your assay customizations.

File Types

The following file types are used by Compass:

- Assay Files Use an *.assay file extension.
- Run Files Use a *.cbz file extension. The default file format for run files is Date_Time_AssayName. An example run file name would be 2012-09-28_18-50-53_Simple Western.cbz.
- **Protocol Files** Exported protocol files use a *.protocol file extension.
- Template Files Exported template files use a *.template file extension.
- Analysis Settings Files Exported analysis settings files use a *.settings file extension.

Chapter 2: Size Assays

Chapter Overview

- Assay Screen Overview
- Reagent Color Coding
- Opening an Assay
- Immunoassays: Creating a New Assay
- Total Protein Assays: Creating a New Assay
- Making Changes to an Existing Assay
- Switching Between Open Assays
- Creating a Template Assay
- Viewing and Changing the Detection Exposures
- Copying Protocols and Templates
- Printing Protocols and Templates
- Importing and Exporting Protocols and Templates

Assay Screen Overview

The Assay screen is used to create, view, and edit assays. To access this screen, click **Assay** in the screen tab:



Assay Screen Panes

The Assay screen has four panes:

- Layout Displays a map of the assay plate and shows where assay reagents will be located.
- **Protocol** Lists individual assay protocol steps and parameters that Wes, Sally Sue or Peggy Sue will execute for each of the capillaries.
- **Notes** Lets you enter specific assay information that is saved with the assay and can be used for future reference.
- **Template** Enter annotations for the individual well and row reagents in the assay plate.

la F	Sue Size - Compass													
1C D	dit Instrument V	Vindow Help												
											l.	Assay	Run Summary	Analysis
ssay	Sally Sue Size				Protocol 🔚	History 👖 Notes							-	
-		× 8 6											E E	Add 🔻 Rei
JLay							Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8
	12345	6 7 8 9 10 11 12 13 14 15 9	17 18 19 20 21 22 21 24		Separation Mat	ń.	cycler	cycle 2	cycles	cycle i	cycles	cycles	cycle /	cycleo
	A 123	4 5 6 7 8	A B		Stacking Matrix									
	C 1 2 3 D 1 2 3	4 5 6 7 8 4 5 6 7 8	SSSSSSS 8		Sample									
	F SSSS		888888888 F		Separation Tim	e (min)	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
	G COCCC		G H		Separation Volt	age (volts)	250	250	250	250	250	250	250	250
	1 2 3	4 5 6 7 8	555555555555555555555555555555555555555		Matrix Removal									
	E COCC		COCCOCCC E		Antibody Dilue	nt Time (min)	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0
	N COCO	12 - 230kDa Sep. M	atrix N		Primary Antibo	dy Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
	P 00000	Statking matrix	7770000000 P		Secondary Anti	body Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
					Detection									
TC.	inpiere													
_														
_	1	2	3	4	5	6	7	8		9	10	11		12
A	1 Biot. Ladder	2	3	4	5	6	7 Sample	8		9	10	11		12
AB	1 Biot. Ladder	2	3	4	5	6 Antibody	7 Sample 7 Diluent	8		9	10	11		12
A B C	1 Biot. Ladder Blocking	2	3	4	5	6 Antibody	7 Sample (Diluent Primary Antibo	8 ody		9	10	11		12
A B C D	1 Biot. Ladder Blocking Streptavidin	2	3	4	5	6 Antibody F	7 Sample 7 Diluent Primary Antibo econdary Antil	8 ody oody		9	10	11		12
A B C	1 Biot. Ladder Blocking Streptavidin	2	3	4	5	6 Antibody F	7 Sample Y Diluent Primary Antibo	8 ody oody		9	10	11		12

Software Menus Active in the Assay Screen

You can use the following software menus:

- File
- Edit
- Instrument (when Compass is connected to Wes, Sally Sue or Peggy Sue)
- Window
- Help

The File and Edit menu options specific to the Assay screen are described next.

File Menu

The following File menu options are active:



- New Assay Creates a new assay from a starter template.
- **Open Assay** Opens an existing assay.
- Save Saves the open assay.
- Save As Saves the open assay under a different file name.
- Import Protocol Imports a saved protocol file into an assay.
- Import Template Imports a saved template file into an assay.
- Export Protocol Exports the current protocol file for future use.
- Export Template Exports the current template file for future use.
- Print Prints the information in the Protocol or Template panes.
- Exit Closes Compass.

Edit Menu

The following Edit menu options are active:



- Copy Copies the information in the Protocol or Template panes into other documents.
- **Default Analysis** Displays the default settings that will be used to analyze the run data generated with an assay.
- Analysis Not active in this screen.
- **Preferences** Set and save your preferences for data export, plot colors in the graph and Twitter settings. See Chapter 10, "Setting Your Preferences" for more information.

Reagent Color Coding

Immunoassays

The Assay screen uses color coding to identify various assay reagents in all panes. The Layout pane is shown in the following example:

2	3	4	5 6	7	8 9	10	11 12	13 14 15 16 17 18 19 20 21 22 23 24
1	2	3	4	5	0	- (8	
1	2	3	4	5	6	7	8	
1	2	3	4	5	6	7	8	
	2	3	4	5	6	7	8	
								000000000000000000000000000000000000000
								000000000000000000000000000000000000000
								000000000000000000000000000000000000000
								000000000000000000000000000000000000000
								000000000000000000000000000000000000000
	2	3	4	5	6	7	8	000000000000000000000000000000000000000
х.	20	0	X	101	00	20	UC.	000000000000000000000000000000000000000
				1	2 -	23	DkD	a Sep. Matrix
					-	Star	ckin	Matrix 0000
				-				

- Orange Samples and Ladder
- Magenta Blocking reagent (Antibody Diluent)
- Light Teal Primary antibody
- Teal Secondary HRP conjugate
- Gold Luminol/Peroxide mix

- No color coding Separation Matrix (clearly designated)
- No color coding Stacking Matrix (clearly designated)
- Light Blue Water dispensed around Separation and Stacking Matrices

Total Protein Assays

The Assay screen uses color coding to identify various assay reagents in all panes. The Layout pane is shown in the following example:



- Orange Samples and Ladder
- Magenta Labeling reagent
- Light Teal Antibody Diluent
- Teal Total Protein Streptavidin-HRP
- **Gold** Luminol/Peroxide mix
- No color coding Separation Matrix (clearly designated)
- No color coding Stacking Matrix (clearly designated)
- Light Blue Water dispensed around Separation and Stacking Matrices

Opening an Assay

To open an existing assay:

1. Select File in the main menu and click Open Assay.



- 2. A list of the last five assays opened will display. Select one of these assays or click **Browse** to open the Assay folder and select a different assay.
- 3. To make changes to the assay, follow the instructions under "Immunoassays: Creating a New Assay" on page 25 for changing the assay parameters. Select **File** from the main menu and click **Save**.

Immunoassays: Creating a New Assay

To create a new assay, we recommend using one of the template assays and making any necessary modifications from there.

Step 1 - Open a Template Assay

1. Select File in the main menu and click New Assay:



2. Select the template assay for your instrument by selecting **Size** as your assay type, **Size Range** and **Cartridge Type** if running Wes, or choose **Open Assay** to select from the menu of saved assays.

New Sally Sue Assay	X	💿 New Wes Assay		×
Assay Type Size Total Protein Size	Size Range	Assay Type Size Total Protein Size	Size Range 12-230 kDa 66-440 kDa	Cartridge 25 13
ОК	Cancel		ОК	Cancel

If you're running a new size assay with split Running Buffer, the window has a Split Running Buffer check box to indicate the use of split buffer. This box is checked by default.

👀 New Sally Sue Assay	
Assay Type ◎ Size ● Total Protein Size	Size Range 12-230 kDa 66-440 kDa
📝 Split Running Buffer	
ОК	Cancel

NOTES:

Compass v2.7 or higher and the latest embedded are needed to run the split Running Buffer assays.

If you make changes to the assay, such as defining the wells in the plate, be sure to select **File** and **Save** before proceeding.

Split Running Buffer will be noted above the protocol steps and plate layout if that assay was selected for Sally Sue or Peggy Sue.

	Edit Instrument	Window Help)											
											Ass	ay 🕐 Run Su	ımmary 🏨 A	Analysis
ssay	: Sally Sue Size Split	Running Buffer	r	Protoco	ol 🔚 History 🚺	Notes								-
La	yout		- [5								I	🕂 📄 Add	▼ Rem
	×	S B 1		Split Runn	ning Buffer		Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycl
-				Separa	tion Matrix									
	12345678910	0 11 12 13 14 15 16 17 18 19	20 21 22 23 24	Stackir	ng Matrix									
	B 1 2 3 4 5 6 7		E B	Sample	e									
	D 1 2 3 4 5 6 7			Separa	tion Time (min)		40.0	40.0	40.0	40.0	40.0	40.0	40.0	
	F CONCERNING		88888 E	Separa	tion Voltage (volts)		250	250	250	250	250	250	250	
	1		88888 P	Matrix	Removal									
	J 1 2 3 4 5 6 7 K	8	Jessee K	Antibo	ody Diluent Time (m	nin)	23.0	23.0	23.0	23.0	23.0	23.0	23.0	
	L	000000000000000000000000000000000000000	See M	Primar	ry Antibody Time (r	nin)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	
	N 0 12 - 23 Sta	30kDa Sep. Matrix acking Matrix	88888 N	Second	dary Antibody Time	e (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	
	P 0000000000		X00000 P	Detect	ion									
Te	mplate													
Te	mplate													C
Te	mplate													c
Te	mplate	0							0	0	40		40	C
Te	mplate1	2	3	4	5	6	7		8	9	10	11	12	
Te	mplate 1 Biot. Ladder	2	3	4	5	6	7 Sampl	e	8	9	10	11	12	
Te	1 Biot. Ladder	2	3	4	5	6 Antil	7 Sampi body Diluent	le	8	9	10	11	12	3
Te	1 Biot. Ladder	2	3	4	5	6 Anti	7 Sampl	le	8	9	10	11	12	
Te	1 Biot. Ladder Blocking	2	3	4	5	6 Antil	7 Sampi body Diluent Primary An	e tibody	8	9	10	11	12	
Te	Biot. Ladder Biot. King Biocking Streptavidi	2	3	4	5	6 Antil	7 Sampl Dody Diluent Primary An econdary Ant	ie tibody ibody HRP	8	9	10	11	12	
Te A B C D	1 Biot. Ladder Blocking Streptavidi	2	3	4	5	6 Antii	7 Sampl Dody Diluent Primary An econdary Ant	le tibody ibody HRP	8	9	10	11	12	

Step 2 - Assign Assay Plate Reagents (Optional)

1. Click on the Layout tab. Displayed are the default row locations where each reagent should be placed on the assay plate.

NOTE: For Sally Sue and Peggy Sue, up to 96 different samples or conditions (lysates, proteins, antibodies or incubation times) can be assayed using the full eight cycles to accommodate experimental needs.
Wes plate layout:



- Row A Biotinylated Ladder (A1: 12-230 kDa or 66-440 kDa) and Sample (A2-25)
- Row B Blocking (Antibody Diluent)
- Row C Antibody Diluent (C1) and Primary antibody (C2-C25)
- Row D Streptavidin-HRP (D1) and Secondary HRP conjugate (D2-D25)
- **Row E** Luminol-S/Peroxide mix
- Row G Wash buffer
- Row H Wash buffer
- Row I Wash buffer
- Row M-P Pre-filled Separation Reagents

Sally Sue/Peggy Sue plate layout:



- Row A Biotinylated Ladder (A1: 12-230 kDa or 66-440 kDa) and Sample (A2-12)
- **Row B** Blocking (Antibody Diluent)
- Row C Antibody Diluent (C1) and Primary antibody (C2-C12)
- Row D Streptavidin-HRP (D1) and Secondary HRP conjugate (D2-D12)
- **Row J** Luminol-S/Peroxide mix

- **Row M** Water (M5-M20)
- Row N Water (N5-N6 and N19-N20) and Separation Matrix (N7-N18)
- Row O Water (05-06 and 019-020) and Stacking Matrix (07-018)
- **Row P** Water (P5-P20)

NOTE: For details on sample, reagent and assay plate preparation, please refer to the product insert provided with the Simple Western kits.

- 2. If needed, well assignments can be modified as described below. Any row assignments changed in the **Layout** pane are updated in the Protocol pane automatically.
 - To move a reagent row to another location Click the row in the Layout pane, then drag and drop it on the new location. The row from which it was moved will be reassigned as empty.



To insert a sample row - Click an empty row or the row below where the new sample row should be inserted, then click the S icon (Insert a sample row) in the Layout pane toolbar. A new sample row will be added in the empty row or inserted above the selected row.

Layout	× SB 1 2 3 4 5 D -
	Insert a Sample Row
	1 2 4 5 6 7 8 8 1 1 2 3 4 5 6 7 8
	1 2 3 4 5 6 7 8 K L M N 866 - 440kDa Sep. Matrix N Stacking Matrix P

To insert a detection row - Click an empty row or the row below where the new detection row should be inserted, then click the D icon (Insert a detection row) in the Layout pane toolbar. A new detection row will be added in the empty row or inserted above the selected row.

🖻 Layout	× S B 1 2 3 4 5 D	
	Insert a De	etection Row
	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24	Separation
	A 1 2 3 4 5 6 7 8	Stacking I
	C 1 2 3 4 5 6 7 8	Sample
		Separatio
		Separatio
		Matrix Rei
	K	Antibody
		Primary A
	O Stacking Matrix	Secondary
	P 000000000000000000000000000000	Quaterna
		Detection

 To insert a fourth or fifth incubation reagent - Click an empty row or the row below where the new incubation reagent should be inserted, then click the 4 icon (Insert a quaternary row) in the Layout pane toolbar. A new incubation reagent row will be added in the empty row or inserted above the selected row.



A fifth incubation reagent can now be added by repeating the above and clicking the **5** icon (Insert a Quinary row) instead.

NOTES:

When using the fourth and fifth incubation steps, only five cycles can typically be performed per run as more water is needed per cycle for the additional wash steps.

Row J is the last row assignment that can be used on the assay plate. New, sample, incubation or luminol rows cannot be inserted if row J already has an assigned reagent. To insert a row in this case, you must first move the contents of row J to another area on the plate.

• **To delete a row** - Click the row to be deleted, then click the red **X** icon (Delete a row) in the toolbar. Only empty rows and fourth incubation rows can be deleted. Rows for required assay reagents cannot be deleted.



NOTES:

For Sally Sue and Peggy Sue, you can put samples, antibodies and blocking buffer in Rows A-J and in columns 1-12 or 13-24.

We recommend keeping two rows empty between the substrate and the closest dispensed HRP conjugate to avoid any interaction with the substrate.

Rows K and L are purposely left empty, don't put anything into these wells.

Separation and Stacking matrices can only be pipetted into the designated wells.

Water is used in the wells to reduce the potential of evaporation of the matrices.

Step 3 - Modifying the Assay Protocol (Optional for All Instruments)

1. Click on the Protocol tab. This pane shows the individual steps of the assay protocol and allows you to change parameters. When creating a new assay, the default protocol automatically assigns all reagent locations for the assay. The Sally Sue assay is shown as an example:

Protocol 🔚 History 👖 Notes									- 0
								Ŧ	📄 Add 🔻 Remove
	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8	
Separation Matrix									
Stacking Matrix									
Sample									
Separation Time (min)	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	
Separation Voltage (volts)	250	250	250	250	250	250	250	250	
Matrix Removal									
Antibody Diluent Time (min)	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0	
Primary Antibody Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	
Secondary Antibody Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	
Quaternary Antibody Time (min)	120.0	120.0	120.0	120.0	120.0	120.0	120.0	120.0	
Detection									

Each row contains an assay protocol step. Each step can contain a reagent assay plate row assignment and one or more parameter settings. To view the details for a step, click on the white arrow next to the step name. We recommend using the default protocol settings for the assays. An expanded list of the default protocol step parameters is shown here for a protocol with split Running Buffer:

Split Running Buffer	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8
Separation Matrix								
Well Row	N7							
Load Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Stacking Matrix								
Well Row	07	07	07	07	07	07	07	07
Load Time (sec)	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
⊿ Sample								
Well Row	A1							
Load Time (sec)	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
Separation Time (min)	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
 Separation Voltage (volts) 	250	250	250	250	250	250	250	250
Standards Exposure (sec)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
EE Immobilization Time (sec)	250.0	250.0	250.0	250.0	250.0	250.0	250.0	250.0
Matrix Removal								
Matrix Removal Time (sec)	230.0	230.0	230.0	230.0	230.0	230.0	230.0	230.0
Matrix Washes	3	3	3	3	3	3	3	3
Matrix Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
 Antibody Diluent Time (min) 	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0
Well Row	B1							
Washes	2	2	2	2	2	2	2	2
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
 Primary Antibody Time (min) 	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Well Row	C1							
Washes	2	2	2	2	2	2	2	2
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
 Secondary Antibody Time (min) 	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Well Row	D1							
Washes	2	2	2	2	2	2	2	2
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
A Detection								
Well Row	J1							
Detection Profile	7 Exposures							

2. You can change the primary or secondary antibody incubation time. Click the cell in the value column next to Primary Ab Time (min) or Secondary Ab Time (min) and enter a new value in minutes:

								I.
Split Running Buffer	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8
Separation Matrix								
Stacking Matrix								
Sample								
Separation Time (min)	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Separation Voltage (volts)	250	250	250	250	250	250	250	250
Matrix Removal								
Antibody Diluent Time (min)	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0
Well Row	B1							
Washes	2	2	2	2	2	2	2	2
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Primary Antibody Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Well Row	C1	C						
Washes	2	2	2	2	2	2	2	
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Secondary Antibody Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Well Row	D1							
Washes	2	2	2	2	2	2	2	2
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Detection								

3. You can change the primary and secondary antibody incubation reagent row location. Click the cell in the value column next to Well Row and select a different row on the assay plate:

Protocol 🛛 🔚 History 👖 Notes	i)							
								Ŧ
Split Running Buffer	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8
Separation Matrix								
Stacking Matrix								
Sample								
Separation Time (min)	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Separation Voltage (volts)	250	250	250	250	250	250	250	250
Matrix Removal								
Antibody Diluent Time (min)	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0
Well Row	B1	B1	B1	B1	B1	B1	B1	B1
Washes	2	2	2	2	2	2	2	2
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Primary Antibody Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Well Row	<u>c1</u> –	C1						
Washes	2	2	2	2	2	2	2	2
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Secondary Antibody Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Well Row	D1	D1	D1	D1	D1	D1	D1	D1
Washes	2	2	2	2	2	2	2	2
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Detection								

NOTE: Only rows you've designated as primary antibody in the Layout tab can be selected in the Well Row drop-down menu.

4. Additional exposures can be collected in the assay if desired. To do this, click the white arrow next to Detection to expand the row. Click the cell in the exposure column next to Detection Profile to open the Detection Profile window. Additional times can be added to the protocol by clicking the **Add** button, entering the values and selecting **OK**.

B1	B1	B	1 B1	B1	B1	B1
2				2	2	2
150.0	S Detection	n Profile		150.0	150.0	150.0
30.0				30.0	30.0	30.0
C1	Add	Remove		C1	C1	C1
2			2	2	2	2
150.0	Exposure	e (sec)		150.0	150.0	150.0
30.0	5.0			30.0	30.0	30.0
D1	15.0)		D1	D1	D1
2	30.0		2	2	2	2
150.0	60.0			150.0	150.0	150.0
	120	0				
J1	120.	0		J1	J1	J1
7 Exposure:	240.	0	1	7 Exposures	7 Exposures	7 Exposures
	400.	0				
	ОК		Cancel			
	B1 2 150.0 30.0 C1 2 150.0 D1 2 150.0 7 Exposure:	B1 B1 2 150.0 30.0 C1 2 150.0 30.0 Exposure 30.0 D1 150.0 15.0 2 30.0 150.0 15.0 7 Exposure: M 7 Exposure: M	B1 B1 B 2 150.0 Solution Profile 30.0 Add Remove 2 150.0 Solution Solution 30.0 D1 Solution Solution 30.0 D1 Solution Solution 30.0 Solution Solution Solution 30.0 Solution Solution Solution 150.0 Solution Solution Solution 7 Exposure: Add Remove 0K OK Solution Solution	B1 B1 B1 B1 B1 2 150.0 30.0 C1 150.0 30.0 D1 2 150.0 30.0 D1 2 150.0 30.0 D1 2 150.0 30.0 60.0 120.0 240.0 480.0	B1 B1 B1 B1 B1 2 150.0 2 30.0 Add Remove 2 150.0 30.0 C1 2 150.0 30.0 5.0 150.0 30.0 150.0 5.0 150.0 30.0 150.0 5.0 150.0 15.0 30.0 15.0 150.0 120.0 120.0 120.0 240.0 480.0	B1 B1 B1 B1 B1 B1 B1 2 2 2 2 150.0 30.0 30.0 30.0 C1 C1 C1 2 2 2 150.0 5.0 150.0 30.0 5.0 15.0 30.0 30.0 30.0 30.0 5.0 15.0 30.0 15.0 30.0 30.0 15.0 150.0 30.0 15.0 150.0 10 11 11 7 Exposure(m) OK Cancel

5. You can modify any other protocol parameters as needed.

NOTES:

For more information on changing protocol step parameters other than incubation times, contact ProteinSimple Technical Support or your local Field Application Specialist.

When a protocol is being edited, an asterisk will appear in the Protocol tab to indicate changes have been made and should be saved.

Step 4 - Add Assay Notes (Optional)

- 1. Click on the Notes tab.
- 2. Click in the pane and type any assay or protocol notes. This information will be stored with the assay and run data.

Trotocol T *Notes	- 8
This is our default assay for STAT5 analysis for samples run in triplicate.	

NOTE: When notes are being edited, an asterisk will appear in the Notes tab to indicate changes have been made and should be saved.

Step 5 - Select a Schedule (Optional for Sally Sue and Peggy Sue)

Sally Sue and Peggy Sue can execute cycles serially or in parallel. To choose a schedule option, select **Edit** and click **Schedule**.

😵 Schedule 🛛 💌
Serial
Overlapping
Overlapping with hold
OK Cancel

- Serial Each cycle is executed in sequential order, and each cycle completes before the next one starts. Selecting this option result in the longest run times.
- **Overlapping** Cycle steps are executed in parallel if timing permits shared use of the separation tray and robot. Each cycle will run through to completion once started. This option offers a reduction in overall run time without affecting the individual run time for each cycle.
- **Overlapping with Hold (default)** Each cycle executes through the separate step in sequence and then enters a variable length hold step where capillaries remain in the incubation tray. More sample stability is provided with this option as proteins are 'locked' to the capillary wall and therefore more stable than samples on the assay plate. This option also results in the most significant reduction in overall run time.

NOTE: We recommend using the **Overlapping with hold** option. For more information on when to use other schedule options, please contact ProteinSimple Technical Support or your local Field Application Specialist.

Step 6 - Add Assay Plate Annotations (Optional)

Custom reagent names, notes and annotations can be entered for individual rows and wells on the assay plate. This information will be stored with assay and run data. During post-run analysis, this information will be used to apply custom analysis settings to specific sample names, blocking reagent names, primary antibody names or sample and primary antibody attributes. This will be explained in more detail in "Compass Analysis Settings Overview" on page 232.

NOTE: Template pane information can also be added or updated after a run is complete.

To enter annotations:

1. Click on the Template tab. The default annotations for reagent rows and individual wells on the assay plate will display:

目 Ter	mplate												- 8
													Edit
	1	2	3	4	5	6	7	8	9	10	11	12	
Α	Biot. Ladder						Sample						
в						Antibod	y Diluent						
с	Blocking						Primary Antibody						
D	Streptavidin					s	econdary Antiboo	ly					
J						Dete	ection						

- 2. Change or add row and well annotations as needed. To do this:
 - a. To enter annotations for a specific well Right click the well and select Edit or click Edit in the



upper right corner of the pane or double click the selected well. The following box will display:

Enter the reagent name, notes or specific attributes (for example, sample concentration or dilution ratio):

🛞 Well Cont	ient 💽
Name:	HeLa
Attribute:	1 mg/mL
	OK Cancel

Click **OK**. The new information will display in the selected well:

9 10 11 12
9 10 11 12

b. To enter annotations for multiple wells or a row - To select individual wells, press and hold the Control key and then select individual wells. To select a sequential set of wells or a full row, press and

hold the Shift key, then select the first well and last well. Next, right click and select **Edit** or click **Edit** in the upper right corner of the pane. The following box will display:

E	Te	emplate												- 8
														Edit
		1	2	3	4	5	6	7	8	9	10	11	12	
	A	Biot. Ladder						Saniple	e					
	в					🛞 Well C	Content	×						
	с	Blocking				Attribu	te: Sample							
	D	Streptavidin					ОК	Cancel						
	J						Dete	ection	J					
														_

Enter the reagent name, notes and specific attributes (for example, sample concentration or dilution ratio):

🕲 Well Cont	tent 💌
Name:	HeLa
Attribute:	1 mg/mL
	OK Cancel

Click **OK**. The new information will display in the selected wells:

1	2											
1	2											Edit
	~	3	4	5	6	7	8	9	10	11	12	
liot. Ladder	1 mg/mL	1 mg/mL	1 mg/mL	1 mg/mL	1 mg/mL	HeLa 1 mg/mL	1 mg/mL	1 mg/mL	1 mg/mL	1 mg/mL	1 mg/mL	
					Antibody	y Diluent						
Blocking					1	Primary Antibody						
reptavidin					s	econdary Antiboo	ly					
					Dete	ction						
ſ	3locking	Slocking sptavidin	1 mg/mil. 1 mg/mil. 3locking 1 sptavidin	1 mg/mL 1 mg/mL 1 mg/mL 3locking	Impirit. Impirit. Impirit. Impirit. Impirit. Impirit. Slocking Impirit. Impirit. sptavidin Impirit. Impirit.	Impirit. Impirit. Impirit. Impirit. Impirit. Impirit. Impirit. Impirit. Slocking Impirit. Impirit. Impirit. Impirit. Impirit. Impirit. Impirit. Impirit. Impirit. Impirit. Impirit. Impirit. Impirit. Impirit. Impirit. Impirit. Impirit. Impirit. Impirit.	Implifie Implifie	Ingited Ingited <thingited< th=""> <thingited< th=""> <thi< td=""><td>I mgint. 1 mgint.</td><td>Name 1 mg/mL <th1 mg="" ml<="" th=""> <th1 mg="" ml<="" th=""> <th1 mg<="" td=""><td>Ingint Ingint Ingint<</td><td>And the sector of the secto</td></th1></th1></th1></td></thi<></thingited<></thingited<>	I mgint. 1 mgint.	Name 1 mg/mL 1 mg/mL <th1 mg="" ml<="" th=""> <th1 mg="" ml<="" th=""> <th1 mg<="" td=""><td>Ingint Ingint Ingint<</td><td>And the sector of the secto</td></th1></th1></th1>	Ingint Ingint<	And the sector of the secto

NOTE: When annotations are being edited, an asterisk will appear in the **Template** tab to indicate changes have been made and should be saved. Only Name and Attribute will be used by Compass to annotate the data.

Step 7 - Save the Assay

1. Select File from the main menu and click Save As. Enter the assay name and click Save.

Save As			×
Organize Vew folde	r	Search Assay:	s ⊂ ≣ ▼ @
☆ Favorites ■ Desktop	Documents library Assays	Arrang	je by: Folder 🔻
Downloads Except Places Desktop Comparis Documents My Documents Compass Assays	 Name SW long incubation.assay Simple Western 2.assay Test assay.assay Simple Western.assay Erk Assay.assay Erk Assay.assay Simon Decontamination Procedure.assay Generic Simple Western-9-9-2011.assay Simole Western Demo Plate assay 	Date modified 9/29/2011 3:27 PM 9/29/2011 3:24 PM 9/29/2011 3:21 PM 9/29/2011 3:18 PM 9/12/2011 1:11 PM 9/12/2011 1:11 PM 9/9/2011 7:18 PM	Type Compass Assay File Compass Assay File Compass Assay File Compass Assay File Compass Assay File Compass Assay File
New Assays Runs Eile name: My New Assays	Simple Western Demo Plate.assay	979/2011 7:18 PM	Compass Assay File
Save as type: Assay	w Assay] File (*.assay)		•
Hide Folders		Save	Cancel

NOTE: New assays are saved in the Compass Assays directory.

Step 8 - Modify Default Analysis Parameters (Optional)

You can preset the parameters used to analyze run data generated with the assay.

🛞 Default Analysis			
type filter text	Advanced		⇔ • ⇔ • •
Advanced Images	Analysis Settings	Analysis Settings: Advanced	
Peak Fit Peak Names	Advanced	Standards	
Standards		Peak Width Allowable Drift	15
		Sample	100
	Add	Peak Fit Starting Width Ratio	0.5
	Default Advance	Image d Median Filter Threshold Ratio	0.5
	Override	Median Filter Threshold Limit	100
	Apply To Settings		
	Add Remo	ve	
			Restore Original
Import Export		ок	Cancel

1. Select Edit from the main menu and click Default Analysis. The following screen will display:

2. We recommend using the default parameters for Simple Western assays. However, you can modify any you want, then click **OK**. For detailed information on analysis parameters, please refer to "Compass Analysis Settings Overview" on page 232.

Total Protein Assays: Creating a New Assay

To create a new assay, we recommend using one of the template assays and making any necessary modifications from there.

Step 1 - Open a Template Assay

1. Select File in the main menu and click New Assay:



 Select the template assay for your instrument by selecting Total Protein Size as your assay type, Size Range and Cartridge Type if running Wes, or choose Open Assay to select from the menu of saved assays.

🛞 New Sally Sue Assay	X	۲	New Wes Assay		X
Assay Type Size Total Protein Size	Size Range ⊚ 12-230 kDa ⊚ 66-440 kDa		Assay Type Size Total Protein Size	Size Range	Cartridge ② 25 ③ 13
ОК	Cancel			ОК	Cancel

If you're running a new Total Protein assay with split Running Buffer on Sally Sue or Peggy Sue, the window has a Split Running Buffer check box to indicate the use of split buffer. This box is checked by default.

New Sally Sue Assay	X
Assay Type	Size Range
Size	12-230 kDa
Total Protein Size	🔘 66-440 kDa
Split Running Buffer	
ОК	Cancel

NOTES:

Compass v2.7 or higher and the latest embedded are needed to run the split Running Buffer assays.

If you make changes to the assay, such as defining the wells in the plate, be sure to select **File** and **Save** before proceeding.

Split Running Buffer will be noted above the protocol steps and plate layout if that assay was selected for Sally Sue or Peggy Sue.

								Assay 🕑 R	un Summary	🏨 Analys
say: Sally Sue Total Protein High MW Split Running Buffer	💷 Protocol 🛛 📙 History 🎵 N	otes								
Layout X S B 1 2 3 4 5 D 9									E E	Add 👻 R
	Split Running Buffer	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8	
	Separation Matrix									
1234567899912114555599272233	Stacking Matrix Sample									
A 1 2 3 4 5 6 7 8 A B	Separation Time (min)	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	
C 1 2 3 4 5 6 7 8 D 1 2 3 4 5 6 7 8 D 2 1 2 3 4 5 6 7 8	Separation Voltage (volts)	275	275	275	275	275	275	275	275	
F	Biotin Labeling Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	
12345678	Antibody Diluent Time (min)	26.0	26.0	26.0	26.0	26.0	26.0	26.0	26.0	
Ř.	Total Protein HRP Time (min	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	
M M 66 - 440kDa Sep. Mabrix N Stacking Matrix O	Detection									
P 00000000000000000000000000000000000										
Femplate										
emplate										
emplate										
1 2 3	1 5	6	7	8	9		10	11		12
1 2 3	4 5	6	7	8	9		10	11		12
Template	\$	6 San	7 nple	8	9		10	11		12
I 2 3 Biot. Ladder	i 5	6 Sam otal Protein Biotin	7 nple Re	8 eagent	9		10	11		12
1 2 3 Biot. Ladder	1 5 1	6 San otal Protein Biotin	7 nple n Labeling Re	8 eagent	9		10	11		12
Template	\$ 5	6 San otal Protein Biotin Antibody Diluent	7 nple n Labeling Re	8 eagent	9		10	11		12
1 2 3 Biot. Ladder	1 5 T T Total 1	6 otal Protein Biotin Antibody Diluent Protein Streptavid	7 nple n Labeling Re	8 Bagent	9		10	11		12
1 2 3 Biot. Ladder	\$ 5 7 7 7 7 7 7 7 7	6 otal Protein Biotin Antibody Diluent Protein Streptavid	7 nple Re n Labeling Re	8 eagent	9		10	11		12
1 2 3 Biot. Ladder	1 5 7 7 7 7 7 7 7 7 7 7	6 San otal Protein Biotin Antibody Diluent Protein Streptavid Detection	7 nple Re n Labeling Re in HRP	8 eagent	9		10	11		12
1 2 3 Biol. Ledder	а 5 Тота I	6 San otal Protein Biotis Antibody Diluent Protein Streptavid Detection	7 nple 2 n Labeling Re in HRP 2	8 Pagent	9		10	11		12

Step 2 - Assign Assay Plate Reagents (Optional)

1. Click on the Layout tab. Displayed are the default row locations where each reagent should be placed on the assay plate.

NOTE: For Sally Sue and Peggy Sue, up to 96 different samples or conditions (lysates, proteins, antibodies or incubation times) can be assayed using the full eight cycles to accommodate experimental needs.

Wes plate layout:



- Row A Biotinylated Ladder (A1: 12-230 kDa or 66-440 kDa) and Sample (A2-A25)
- Row B Antibody Diluent (B1) and Labeling Reagent (B2-25)
- Row C Antibody Diluent
- Row D Total Protein Streptavidin-HRP
- **Row E** Luminol-S/Peroxide mix
- Row G Wash buffer
- Row H Wash buffer
- Row I Wash buffer
- Row M-P Pre-filled Separation Reagents

Sally Sue/Peggy Sue plate layout:



- Row A Biotinylated Ladder (A1: 12-230 kDa or 66-440 kDa) and Sample (A2-A12)
- Row B Antibody Diluent (B1) and Total Protein Labeling Reagent (B2-12)
- Row C Antibody Diluent
- Row D Total Protein Streptavidin-HRP
- **Row J** Luminol-S/Peroxide mix
- Row M Water (M5-M20)
- Row N Water (N5-N6 and N19-N20) and Separation Matrix (N7-N18)
- Row O Water (05-06 and 019-020) and Stacking Matrix (07-018)
- Row P Water (P5-P20)

NOTE: For details on sample, reagent and assay plate preparation, please refer to the product insert provided with the Simple Western kits.

- 2. If needed, well assignments can be modified. Any row assignments changed in the **Layout** pane are updated in the Protocol pane automatically.
 - To move a reagent row to another location Click the row in the Layout pane, then drag and drop it on the new location. The row from which it was moved will be reassigned as empty.

12345678910112134155678192021222124
B 1 2 3 4 5 6 7 8 B B C 1 2 3 4 5 6 7 8 C C 1 2 3 4 5 6 7 8 C
D 1 2 3 4 5 6 7 8 D E
б 1 4 3 4 3 6 7 6 н
J J 1 2 3 4 5 6 7 8
L COCCOCCOCCOCCOCCOCCOCCOCC
N 66 - 440kDa Sep. Matrix N Stacking Matrix O

To insert a sample row - Click an empty row or the row below where the new sample row should be inserted, then click the S icon (Insert a sample row) in the Layout pane toolbar. A new sample row will be added in the empty row or inserted above the selected row.

🗐 Layout	× SB 1 2 3 4 5 D - D
	Insert a Sample Row

To insert a detection row - Click an empty row or the row below where the new detection row should be inserted, then click the D icon (Insert a detection row) in the Layout pane toolbar. A new detection row will be added in the empty row or inserted above the selected row.

🔳 Layout	× S B 1 2 3 4 5 D ⁻ -	
	I 2 3 4 5 6 7 8 9 10 10 10 10 10 10 10 10 10 10 10 10 10	Detection Row Separation
	B 1 2 3 4 5 6 7 8 C C 1 2 3 4 5 6 7 8 C D 1 2 3 4 5 6 7 8 C E 1 2 3 4 5 6 7 8 C	Sample
	G H J J 1 2 3 4 5 6 7 8	Separation Matrix Rer
	K L L M 66 - 440kDa Sep. Matrix N O Stacking Matrix O	Antibody Primary A Secondan
	P	Quaternar Detection

• To insert a fourth or fifth incubation reagent - Click an empty row or the row below where the new incubation reagent should be inserted, then click the 4 icon (Insert a quaternary row) in the **Layout** pane toolbar. A new incubation reagent row will be added in the empty row or inserted above the selected row.



A fifth incubation reagent can now be added by repeating the above and clicking the **5** icon (Insert a Quinary row) instead.

NOTES:

When using the fourth and fifth incubation steps, only five cycles can typically be performed per run as more water is needed per cycle for the additional wash steps.

Row J is the last row assignment that can be used on the assay plate. New, sample, incubation or luminol rows cannot be inserted if row J already has an assigned reagent. To insert a row in this case, you must first move the contents of row J to another area on the plate.

• **To delete a row** - Click the row to be deleted, then click the red **X** icon (Delete a row) in the toolbar. Only empty rows and fourth incubation rows can be deleted. Rows for required assay reagents cannot be deleted.

🖹 Layout	🗙 S B 1 2 3 4 5 D 🖓 🗖
	Delete a row
	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
	A 1 2 3 4 5 6 7 8 A B 1 2 3 4 5 6 7 8 B C 1 2 3 4 5 6 7 8 C D 1 2 3 4 5 6 7 8 C D 1 2 3 4 5 6 7 8 C D 2 1 2 3 4 5 6 7 8 C D 2 1 2 3 4 5 6 7 8 C
	E F G H G H
	J <u>1 2 3 4 5 6 7 8</u> K
	M 66 - 440kDa Sep. Matrix N O Stacking Matrix O P P

NOTES:

We recommend keeping two rows empty between the substrate and the closest dispensed HRP conjugate to avoid any interaction with the substrate.

Rows K and L are purposely left empty, don't put anything into these wells.

Separation and Stacking matrices can only be pipetted into the designated wells.

Water is used in the wells to reduce the potential of evaporation of the matrices.

Step 3 - Modifying the Assay Protocol (Optional for All Instruments)

1. Click on the Protocol tab. This pane shows the individual steps of the assay protocol and allows you to change parameters. When creating a new assay, the default protocol automatically assigns all reagent locations for the assay. The Sally Sue assay is shown as an example:

👕 Protocol 🛛 🔚 History 🏋 Notes									
								Ŧ	🖻 Ado
Split Running Buffer	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8	
Separation Matrix									
Stacking Matrix									
Sample									
Separation Time (min)	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	
Separation Voltage (volts)	250	250	250	250	250	250	250	250	
Matrix Removal									
Biotin Labeling Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	
Antibody Diluent Time (min)	26.0	26.0	26.0	26.0	26.0	26.0	26.0	26.0	
Total Protein HRP Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	
Detection									

Each row contains an assay protocol step. Each step can contain a reagent assay plate row assignment and one or more parameter settings. To view the details for a step, click on the white arrow next to the step name. We recommend using the default protocol settings for the assays. An expanded list of the default protocol step parameters is shown:

Split Running Buffer	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8
Stacking Matrix								
Well Row	07	07	07	07	07	07	07	07
Load Time (sec)	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
⊿ Sample								
Well Row	A1							
Load Time (sec)	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
Separation Time (min)	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
 Separation Voltage (volts) 	250	250	250	250	250	250	250	250
Standards Exposure (sec)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
EE Immobilization Time (sec)	250.0	250.0	250.0	250.0	250.0	250.0	250.0	250.0
Matrix Removal								
Matrix Removal Time (sec)	230.0	230.0	230.0	230.0	230.0	230.0	230.0	230.0
Matrix Washes	3	3	3	3	3	3	3	3
Matrix Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
 Biotin Labeling Time (min) 	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Well Row	B1							
Washes	0	0	0	0	0	0	0	0
Wash Soak Time (sec)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
 Antibody Diluent Time (min) 	26.0	26.0	26.0	26.0	26.0	26.0	26.0	26.0
Well Row	C1	CI						
Washes	2	2	2	2	2	2	2	2
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Total Protein HRP Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Well Row	D1							
Washes	2	2	2	2	2	2	2	2
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Detection								

2. You can change the biotin labeling and Total Protein streptavidin-HRP incubation time. Click the cell in the value column next to Biotin Labeling Time (min) or Total Protein HRP Time (min) and enter a new value in minutes:

Protocol 🔚 History 🏋 Notes								
								(+
Split Running Buffer	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8
Separation Matrix								
Well Row	N7							
Load Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Stacking Matrix								
Sample								
Separation Time (min)	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Separation Voltage (volts)	250	250	250	250	250	250	250	250
Matrix Removal								
Biotin Labeling Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Well Row	B1							
Washes	0	0	0	0	0	0	0	0
Wash Soak Time (sec)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Antibody Diluent Time (min)	26.0	26.0	26.0	26.0	26.0	26.0	26.0	26.0
> Total Protein HRP Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Detection								

3. You can also change the biotin labeling and Total Protein streptavidin-HRP row location. Click the cell in the value column next to Well Row and select a different row on the assay plate:

								-
Split Running Buffer	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8
Separation Matrix								
Well Row	N7							
Load Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Stacking Matrix								
Sample								
Separation Time (min)	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Separation Voltage (volts)	250	250	250	250	250	250	250	250
Matrix Removal								
Biotin Labeling Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Well Row	B1 👻	B1						
Washes	0	0	0	0	0	0	0	C
Wash Soak Time (sec)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Antibody Diluent Time (min)	26.0	26.0	26.0	26.0	26.0	26.0	26.0	26.0
> Total Protein HRP Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Detection								

NOTE: Only rows you've designated as labeling reagent in the Layout tab can be selected in the Well Row drop-down menu.

4. Additional exposures can be collected in the assay if desired. To do this, click the white arrow next to Detection to expand the row. Click the cell in the exposure column next to Detection Profile to open the Detection Profile window. Additional times can be added to the protocol by clicking the **Add** button, entering the values and selecting **OK**.

Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
N7	N7	N7	N7	N7	N
150.0	150.0	150.0	150.0	150.0	150.0
(• • • · · ·		x		
	Detection i	rofile			
40.0				40.0	40.0
250	Add	Remove		250	250
30.0	Exposure (ec))	30.0	30.
26.0	5.0			26.0	26.
30.0	15.0			30.0	30.0
	30.0				
J1	60.0			J1	J
7 Exposure:	120.0		5	7 Exposures	7 Exposure
	240.0				
	490.0				
	400.0				
	OK				
	OK	Can	:el		
	N7 150.0 40.0 250 30.0 26.0 30.0 26.0 30.0 7 Exposure:	N7 N7 150.0 150.0 (*) Detection F 40.0 250 30.0 26.0 30.0 7 Exposure: 7 Exposure: 10 0 0 0 0 0 0 0 0 0 0 0 0 0	N7 N7 N7 150.0 150.0 150.0 (***) Detection Profile 40.0 250 30.0 26.0 30.0 26.0 30.0 15.0 30.0 15.0 30.0 15.0 30.0 15.0 30.0 15.0 30.0 15.0 30.0 0 (***) 240.0 480.0 0 (***) 0 (***) 0 (***) 0 (***) 0 (***) 0 (***) 0 (***) 0 (***) 0 (***) 0 (***) 0 (***) 0 (***) 0 (***) 0 (***) 0 0 0 0 0 0 0 0 0 0 0 0 0	N7 N7 N7 N7 150.0 150.0 150.0 40.0 250 30.0 26.0 30.0 15.0 30.0 15.0 30.0 15.0 30.0 15.0 30.0 15.0 30.0 15.0 30.0 15.0 30.0 15.0 16.0 16.0 16.0 17.0 10.0	N7 N7 N7 N7 N7 150.0 150.0 150.0 150.0 40.0 250 30.0 26.0 30.0 26.0 30.0 15.0 15.0 30.0 5.0 15.0 30.0 15.0 30.0 15.0 15.0 30.0 15.0 10.0 15.0 10.

5. You can modify any other protocol parameters as needed.

NOTES:

For more information on changing protocol step parameters other than incubation times, contact ProteinSimple Technical Support or your local Field Application Specialist.

When a protocol is being edited, an asterisk will appear in the Protocol tab to indicate changes have been made and should be saved.

Steps 4 - 8

Steps 4 through 8 for creating a Total Protein Assay are the same as when you're creating an Immunoassay. Please go to *"Step 4 - Add Assay Notes (Optional),"* starting on page 34 to continue.

Making Changes to an Existing Assay

1. Select File in the main menu and click Open Assay.



- 2. A list of the last assays opened will display. Select one of these assays or click **Browse** to open the assay folder and select a different assay.
- 3. Follow the steps in "Immunoassays: Creating a New Assay" on page 25 to make changes and save the assay.

Switching Between Open Assays

If more than one run file is open, you can switch between viewing the assays for each run. To do this:

1. Click the down arrow in the run box.



2. Select the run for the assay you want to view from the drop down list.

Creating a Template Assay

You can create and save template assays that can be used as a starting point for creating new assays. To do this:

- 1. Select **File** in the main menu. Click **Open Assay** to open an existing assay or click **New Assay** to open an existing template assay.
- 2. Follow the steps in "Immunoassays: Creating a New Assay" on page 25 to make changes to the assay.
- 3. When changes are complete, select **File** in the main menu and click **Save As**. Select the New Assays folder:

🔕 Save As			×
😋 🕞 🗢 📙 « My Documents I	Compass 🕨 New Assays	✓ ✓ Search New As.	says 🔎
Organize 🔻 New folder			•== • • • • • • • • • • • • • • • • • •
▲ 🔆 Favorites 💻 Desktop	Documents library New Assays	Arrange	by: Folder 🔻
🐌 Downloads 🗐 Recent Places	Name Simple Western.assay	Date modified 9/26/2011 3:25 PM	Type Compass Assay File
Desktop Desktop Decuments My Documents My Documents Assays New Assays Runs	• • • [
File name: Protein Test			•
Save as type: Assay File (*.a:	ssay)		•
Hide Folders		Save	Cancel

- 4. Type the name for the new template assay and click **Save**.
- 5. Select **File** in the main menu and click **New Assay**. The new template assay will now be available in the drop down list:

File	Edit Instrument	Wind	low Help
	New Assay	×	Protein Test
	Open Assay	•	Simple Western
	Save Save As		

Viewing and Changing the Detection Exposures

To view the current detection profile, roll the cursor over the exposures cell in Protocol pane:

Total Protein HRP Time (min)	30.0	30.0	30.0	30.0
Well Row	D1	D1	D1	D1
Washes	2	2	2	2
Wash Soak Time (sec)	150.0	150.0	150.0	150.0
⊿ Detection				
Well Row	J1	Л	J1	J1
Detection Profile	7 Expos	res	7 Exposures	7 Exposures
	sign	al, 5.0 sec		
	sign	al, 15.0 sec		
	sign	al, 30.0 sec		
	sign	al. 120.0 sec		
	sign	al, 240.0 sec		
	sign	al, 480.0 sec		

While we recommend using the default assay detection profile, you can change the profile, exposure times and exposure sequences if needed. To do this:

1. Select the exposures cell in the Protocol pane and click the button, or double-click in the cell. The following screen will display:

🛞 Detection Pr	ofile	×
Add	lemove	
Exposure (se	ec)	
5.0		
15.0		
30.0		
60.0		
120.0		
240.0		
480.0		
ОК		Cancel
		curreer

Each row represents an individual exposure that will be taken during the run.

a. To change an existing exposure time - Click in the exposure cell and enter a new time in seconds:

Detection Profile	×
Add	DVe
Exposure (sec)	*
15.0	
30.0	
60.0	
120.0	=
240.0	
480.0	
500.0	
	*
ОК	Cancel

- b. To delete an existing exposure Select a type or exposure cell and click Remove.
- c. **To add a new exposure** Select **Add**. A new exposure will be added to the end of the list. Click in the exposure cell and enter an exposure time in seconds.
- 2. Click **OK** to save and exit.

Copying Protocols and Templates

The steps and parameters in the Protocol pane can be copied and d into other documents, as can the graphic image of the annotations in the Template pane.

Copying an Assay Protocol

- 1. Click on the Protocol tab.
- 2. Select Edit in the main menu and click Copy.
- 3. Open a document (Microsoft[®] Word[®], Excel[®], etc.). Right click in the document and select **Paste**. All assay protocol steps and parameters will be copied into the document as a list in the same format shown in the Protocol pane.

Copying an Assay Template

- 1. Click on the Template tab.
- 2. Select **Edit** in the main menu and click **Copy**.
- 3. Open a document (Microsoft[®] Word[®], Excel[®], etc.). Right click in the document and select **Paste**. The assay template will be copied into the document as an image, exactly as it is shown in the Template pane.

Printing Protocols and Templates

The information in the Protocol pane can be printed, as can a graphic image of the information in the Template pane.

Printing an Assay Protocol

- 1. Click on the Protocol tab.
- 2. Select File in the main menu, click Print, and then click Print Protocol.

File	Edit Instrument	Windo	w	Help)			
	New Assay	•						
	Open Assay	•	⊢					Pro
	Save							
	Save As		F					
	Import Protocol		2	3	4	5	L	
	Import Template		F	-	-			Se
	Export Protocol							In
	Export Template							Pr
_						_		Se
	Print	•		Prir	nt Pr	otoc	ol	
	Exit			Prir	nt Te	mpl	ate	

All assay protocol steps and parameters will be printed as a list in the same format shown in the Protocol pane.

Printing an Assay Template

- 1. Click on the Template tab.
- 2. Select File in the main menu, click Print, and then click Print Template.

File	Edit Instrument	Windo	w	Help)			
	New Assay	•	1					
	Open Assay	+	⊢					Pro
	Save							
	Save As		F				. 🗆	
	Import Protocol		2	3	4	5	L	5.
	Import Template		F		_	-		Se
	Export Protocol							In
	Export Template							Pr Se
	Print	•		Prin	nt Pr	otoc	ol	
	Exit			Prin	nt Te	mpl	ate	

The assay template will print as an image, exactly as it is shown in the Template pane.

Importing and Exporting Protocols and Templates

The assay protocol in an open assay or run file can be exported as a separate file, as can the assay template annotation information. This allows the same assay protocol and template information to be imported into another assay at a later time, rather than having to re-enter assay protocol and template information manually.

Importing an Assay Protocol

NOTE: Importing an assay protocol imports information into the Protocol pane only.

- 1. Open the assay you want to import the assay protocol in to.
- 2. Select File in the main menu and click Import Protocol.
- 3. Select a protocol file (*.protocol) and click **OK**. The imported information will display in the Protocol pane.

Exporting an Assay Protocol

NOTE: Exporting an assay protocol exports information in the Protocol pane only.

- 1. Open the assay you want to export the assay protocol from.
- 2. Select File in the main menu and click Export Protocol. The following window displays:

S Export Protocol				×
OO V 📕 « Do	cuments 🕨 My D	ocuments 🕨 Compass 🕨 Assays	✓ ⁴ → Search Assays	٩
Organize 🔻 New	w folder			i - 🕡
🔆 Favorites 📃 Desktop	<u> </u>	Documents library Assays	Arrang	e by: Folder 🔻
Downloads	E	Name	Date modified	Туре
Recent Places		Standard sample protocol.protocol	10/4/2011 9:57 PM	PROTOCOL File
Compass Compass Compass Assays Runs	nents s ssays	< III		
File name:	New protcol	<u></u>		
Save as type:	Protocol File (*.pr	otocol)		
Aide Folders			Save	Cancel

- 3. The default directory is Compass/Assays. Change the directory if needed.
- 4. Enter a protocol name and click **Save**. The protocol will be saved as a *.protocol file.

Template Export and Import

The Template information including the sample and reagent names can be exported to a file. As an example, a default Wes Template is displayed in the Assay view.

Tem	plate	_																								- 1
																										Edi
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
Α	Bio												San	nple												
в												Antil	oody Dil	uent												
с	Blo											Р	rimary .	Antibod	у											
D	Str											Seco	ndary A	ntibody	HRP											
Е												Lumi	nol/Per	oxide												

There are three file format options:

- A comma separated CSV file that is best opened in a spreadsheet.
- An XML template file that has full control over the sample and reagent names and layout.
- A tab delimited TXT file that can be opened in a spreadsheet even when the decimal separator is set to a comma.

Exporting the Template to a CSV file

Follow the steps below to easily import a template into Compass, populating it with reagent names (sample, antibody, etc.) and their corresponding attributes (concentration, dilution factor, etc.). This example uses a Wes Template, but the same steps can be followed for a Sally Sue or Peggy Sue plate template.

1. Select File in the main menu and click Export Template. The following window displays:

🍪 Template File			X
🚱 🗢 🖡 « Documents 🕨	Compass 🕨 👻 🍕	Search Compass	٩
Organize 🔻 New folder			?
★ Favorites ▲ Desktop ★ Recent Places	Documents library Compass	Arrange by: Folder	•
 Dropbox (ProteinSimp OneDrive Downloads 	Assays New Assays Runs		
□ Libraries □ Documents □ Music □ Pictures □ Videos			
Tile server Was 25 Siz	< <u> </u>		4
Save as type: Text File, co	omma delimited (*.csv)		•
🔿 Hide Folders		Save Cance	I

Make sure the Save as type is set to CSV.

File name:	Wes-25 Size.csv	¥
Save as type:	Text File, comma delimited (*.csv)	~
	Text File, comma delimited (*.csv)	
Browse Folders	Template File (*.template) Text File, tab delimited (*.bxt)	

- 2. Enter a protocol name and click **Save**. The protocol will be saved as a *.csv file.
- 3. Open the CSV file in a spreadsheet program like Microsoft® Excel®.

	Α	В	С	D	E	F
1	Biot. Ladder	Sample	Sample	Sample	Sample	Sample
2						
3	Antibody Diluent	Antibody Diluent	Antibody Diluent	Antibody Diluent	Antibody Diluent	Antibody Diluent
4						
5	Blocking	Primary Antibody	Primary Antibody	Primary Antibody	Primary Antibody	Primary Antibody
6						
7	Streptavidin HRP	Secondary Antibody HR				
8						
9	Luminol/Peroxide	Luminol/Peroxide	Luminol/Peroxide	Luminol/Peroxide	Luminol/Peroxide	Luminol/Peroxide

The names in the spreadsheet are arranged in the same order as the Compass Template, and the rows alternate between names and attributes.

NOTE: The default assay has no attributes so these rows will be empty.

4. Edit the names and add attributes, then save the spreadsheet as a CSV file.

NOTE: Make sure not to edit the first column of the spreadsheet, this corresponds to the ladder wells.

5. To import the edited CSV file into Compass, select **File** in the main menu, click **Import Template** and then browse to the .cvs file you just saved. Once imported, the edited CSV file displays the edited Sample names and Primary Antibody with attributes in the Template.

Tem	plate	_																								
																										Ed
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
Α	Bio	F	Patient	A	F	Patient	В	1	Patient	C	F	Patient	D	F	atient	E I	F	atient	F	P	atient	G	1	Patient	н	
в												Anti	body Di	luent												
С	Blo	1 ma/ml	10ma/	40ma/	1ma/ml	10ma/	40mg/	1ma/ml	10ma/	40ma/	1mg/ml	F	Primary	Antiboo	y 10ma/	40ma/	1ma/ml	10ma/	40ma/	1ma/ml	10mg/	40ma/	1ma/ml	10ma/	40mg/	
D	Str	Ting/inc	rongr	40mg/	Tingrine	Tomg	worngr	mgnic	rongr	40mg/	mgmic	Seco	ondary A	ntibody	HRP	-tonigr	Tingrine	rongr	40mgr	Tingrine	rongr	Homg	ring/inc	rong	wong	
Е												Lumi	inol/Per	oxide												

Template Cut and Paste

The names and attributes in the Template can be copied and pasted within Compass, between two copies of Compass, and between Compass and a spreadsheet like Microsoft[®] Excel[®].

All you need to do is enter names in a spreadsheet row, then copy to the clipboard. Next, select a well in the Template and paste from the clipboard. The new names will be pasted into the row at the selected column and columns to the right. Here's a quick example of how to do it:

1. Copy these three rows from a spreadsheet:



2. Select a well in the Template, A2 is selected for this example:



3. Paste the names from the clipboard.

Note: Make sure not to select any of the ladder wells (A1, B1, C1, etc.)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Α	Bio	S1	S2	S3										S	ample										
в												Antib	ody Dil	uent											
С	Blo											Pi	rimary /	Antibod	у										
D	Str											Secor	ndary A	ntibody	HRP										
Е												Lumir	nol/Per	oxide											

4. To include attributes, add them in the next row:



5. Pasting into the Template will update the names and attributes.



	Α	В	C
1	S1	S2	S3
2	100	200	300
3	AD1	AD2	AD3
4	101	201	301
5	AB1	AB2	AB3
6	102	202	302
7	HRP1	HRP2	HRP3
8	103	203	303
9	LUM1	LUM2	LUM3
10	104	204	304

Multiple rows can also be copied and pasted with names and attributes on alternate rows:

Only wells in the same row can be selected, but multiple rows will be pasted into the rows below.

Note: Make sure not to select any of the ladder wells (A1, B1, C1, etc.)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
А	Bio	S1	S2	S3										S	ample										
		100	200	300																					
_		AD1	AD2	AD3																					
в	Ant													Antib	ody Dilu	ient									
		101	201	301							_	_	_	_						_	_	_	_	_	
C	Blo	AB1	ABZ	AB3										Drima	n Antik	who									
0	510	102	202	302										1 11110	iy Andi	Joury									
		HRP1	HRP2	HRP3																		_			
D	Str												Se	condar	y Antibo	ody HR	P								
		103	203	303																					
		LUM1	LUM2	LUM3																					
E	Lum													Lumin	ol/Pero	xide									
1		104	204	304																					
Chapter 3: Running a Size Assay

Chapter Overview

- Starting a Run
- Stopping a Run

Starting a Run

Step 1 - Get Ready

- 1. Create or open the an assay file in Compass.
- 2. Prepare the assay plate using the information provided in the product insert.

IMPORTANT

To prevent well evaporation and ensure best results, keep a lid on the assay plate until ready to use.

3. Prepare the instrument following the procedure described in the User Guide.

IMPORTANT

Capillaries are light sensitive. Keep them covered until you are ready to transfer the capillary cartridge or box to the instrument.

4. Place assay plate into the sample tray of the instrument.

Step 2 - Start the Run

You can start a run in one of two ways depending on whether you want to run an assay using existing parameters or set up a new assay.

- 1. New run of an existing assay:
 - a. Select File in the main menu and click Open Assay.



- b. A list of the last five assays opened will display. Select one of these assays or click **Browse** to open the Assay folder and select a different assay.
- 2. Alternatively, choose **New Assay** and select one of the size assays to get the default assay conditions for using Wes, Sally Sue or Peggy Sue.
 - a. The **Start** button will display. This indicates that an assay has been loaded.



- a. Go to the Assay screen and verify this is the assay you want to use. If not, select **File** in the main menu, click **Open Assay**, and select another assay.
- 3. Click **Start** to begin the run.
 - a. For Wes: Instrument status will change to Running, the Start button will change to Stop and the Run Progress Bar will be displayed:



b. For Sally Sue/Peggy Sue: This will launch the Start Run Wizard (continue on to the next step).

NOTE: If you didn't clean the manifold in Sally Sue or Peggy Sue prior to starting the run, a message will appear. If you see this message, click **Yes** to cancel the run and perform the manifold cleaning.

4. Check Water and Waste. The fluid levels in the accessory module bottles will be checked by the software. If the levels in both bottles will allow Sally Sue an Peggy Sue to complete the run, the wizard screen will display Water Level OK and Waste Level OK messages. Click Next to proceed.

Start Run	X
Check water and waste Make sure there is enough water and room in the waste bottle	
Waste Water The run will be terminated if the water bottle empties or the waste bottle fills during the run.	
Water level OK	
Waste level OK	
< Back Next > Start Ca	ncel

NOTE: If the waste level is too high or the water level is too low to complete the run, messages to indicate one or both will be presented in this screen. If this occurs, fill or empty each bottle as indicated using the procedures outlined in the Sally Sue/Peggy Sue User Guide. When this is complete, the error status will be automatically updated and allow the **Start Run Wizard** to proceed.



5. **Replace Sponge.** You should use a new sponge each time a new experimental run is started. Discard the old sponge and put a new sponge in the manifold wash station. Click **Next** to proceed.

6. **Start.** The resource tray will automatically open. Fill the Wash Buffer, Running Buffer, and Matrix Removal Buffer cups and insert the capillary box in the primary capillary box position. Add a secondary capillary box if more capillaries are required. Click **Next** when finished. The resource tray will close.

NOTE: Make sure enough capillaries are loaded to complete the run. A capillary box should be inserted in the primary position first and if additional capillaries are needed, use the secondary position. When the primary box is depleted, Sally Sue or Peggy Sue will automatically move to the secondary box. Discard left-over Running Buffer, Wash Buffer and Matrix Removal Buffer prior to refilling bottles for new run.



NOTE: You an also refer to the labels on the resource tray for proper insertion of reagents.



7. Load Sample Plate. The sample tray will automatically open. Place the lidded 384-well plate on the cooling block in the tray, ensuring that the A1 well position is aligned with the upper left corner of the cold block. When this is complete, click **Next**. The sample tray will close.

oad Sample	Plate
Remove the o	d sample plate and load the new plate
	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
	A O O O O O O O O O O O O O O O A B B C O O O O O O O O O O O O O O O O O O
	D D D E F
	H J
	K K L
	N



NOTES:

Plate lids be used on sample plates for Sally Sue and Peggy Sue. If a lid is not detected, a message will be displayed in the Start Run Wizard. Compass software will reopen the sample tray to allow you to insert a lid.

When inserting the sample plate, ensure that the plate is firmly seated and level on the cold block. Plates that are not level can interfere with the movement of the sample tray.

8. **Begin the Automated Run.** The data file name will automatically default to the assay name appended with the current date and time. To change the file name, begin typing in the text box. To change the directory where the data file will be stored, click **Browse**:

Start Run
Begin the Automated Run The following protocol will be run. You can change the results location and prefix
Assay : Multi Western_Hela-HUT78
Cycles : 8
Schedule : Overlapping with hold
Run name:
2012-05-31_09-29-06_Multi Western_Hela-HUT78
Location: C:\Users\pfung\Documents\Compass\Runs
< Back Next > Start Cancel

Click **Start** to begin the run. Instrument status will change to **Running**, the **Start** button will change to **Stop** and the **Run Progress bar** will display:



The run will continue until complete (~14-19 hours depending on the assay).

Step 3 - Post-Run Procedures

- 1. Remove the capillary cartridge (Wes) or empty the capillary discard tray (Sally Sue/Peggy Sue).
- 2. Remove the assay plate.
- 3. Dispose of the assay plate and capillaries. Disposal will depend on the samples that have been assayed. If sample origins are unknown, we recommend that used capillaries cartridges, capillaries and plates be disposed of in biohazard waste.

!WARNING! SHARPS HAZARD

The capillaries may present a potential sharps hazard. Dispose of used capillaries in biomedical waste sharps containers.



WARNING! BIOHAZARD

Samples and waste bottle contents should be handled by procedures recommended in the CDC/NIH manual: Biosafety in Microbiological and Biomedical Laboratories (BMBL). The manual is available from the U.S. Government Printing Office or online at http://www.cdc.gov/biosafety/publications/bmbl5/.

Depending on the samples used, waste bottle contents may constitute a biohazard. Use precaution when emptying the waste bottle. Dispose of waste bottle contents in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations. Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste vial before you store, handle, or dispose of chemical waste.

Stopping a Run

- 1. To stop a run, click **Stop**.
 - a. **Wes:** When the run stops, Wes's status will go to **Not Ready** and he'll start a process that plugs the capillaries in the cartridge. Once that's done, you can then remove the capillary cartridge and plate and discard them.
 - b. Sally Sue/Peggy Sue: When the run stops, Sally Sue's or Peggy Sue's status will go to Not Ready and a Cleanup button displays:



NOTE: If a run is stopped prior to completion, Sally Sue and Peggy Sue must perform a cleaning protocol to remove used capillaries from system trays. This is required to prepare the system for the next run.

2. Click Cleanup.

~ .				Cleaning	
. 2	Cleaning	Stop]
PL0005			Wed 3:48 PM	Wed 3:56 PM	

Allow Sally Sue and Peggy Sue to complete the cleaning protocol which takes about ten minutes. When complete, instrument status will change to **Ready** and a new run can be started.

Chapter 4: Charge Assays

Chapter Overview

- Assay Screen Overview
- Reagent Color Coding
- Opening an Assay
- Creating a New Assay
- Making Changes to an Existing Assay
- Switching Between Open Assays
- Creating a Template Assay
- Viewing and Changing the Detection Exposures
- Copying Protocols and Templates
- Printing Protocols and Templates
- Importing and Exporting Protocols and Templates

Assay Screen Overview

The Assay screen is used to create, view, and edit assays. To access this screen, click **Assay** in the screen tab:



Assay Screen Panes

The Assay screen has four panes:

- Layout Displays a map of the assay plate and shows where assay reagents will be located.
- **Protocol** Lists individual assay protocol steps and parameters that Peggy Sue will execute for each of the 12 capillaries simultaneously.
- **Notes** Lets you enter specific assay information that is saved with the assay and can be used for future reference.
- **Template** Enter annotations for the individual well and row reagents in the assay plate.

😺 Peggy S	oue Charge - Comp	ass													J .
File Edit	Instrument Win	dow Help													
												Assay	🕒 Run Summ	ary 🅼 Ana	lysis
Assay: Peg	ggy Sue Charge				Protocol 🔚 Hi	story 📧 Notes									- 0
E Layout		X O S B	1234										E	🖹 Add 🔻	Remove
							Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8	
	A 1 2 3 4 5 6	7 8 9 10 11 12 13 14 15 16 17	18 19 20 21 22 23 24		Sample										
	B 1 2 3 4	5 6 7 8	B		Separation										
	P		0000000 P		Immobilization 1	me (sec)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	
	F CONTRACTOR		G G		Primary Antibody	Time (min)	120.0	120.0	120.0	120.0	120.0	120.0	120.0	120.0	
	H SSSSSS		8888888 H		Detection	dy Time (min)	00.0	00.0	60.0	00.0	60.0	00.0	60.0	00.0	
	K 1 2 3 4	5 6 7 8	× ×		Detection										
	M		N N												
	0 P														
Templa	ite														- 0
															Edit
	1	2	3	4	5	6	7		8	9	10		11	12	_
A						Sa	ample								
в						Primar	y Antibody								
с						Seconda	ary Antibody								
J						Lumino	ol/Peroxide								

Software Menus Active in the Assay Screen

The following software menus are available:

- File
- Edit
- Instrument (when Compass is connected to Peggy Sue)
- Window
- Help

The File and Edit menu options specific to the Assay screen are described next.

File Menu

The following File menu options are active:



- New Assay Creates a new assay from a starter template.
- **Open Assay** Opens an existing assay.
- Save Saves the open assay.
- Save As Saves the open assay under a different file name.
- Import Protocol Imports a saved protocol file into an assay.
- Import Template Imports a saved template file into an assay.
- **Export Protocol** Exports the current protocol file for future use.
- Export Template Exports the current template file for future use.
- Print Prints the information in the Protocol or Template panes.
- Exit Closes Compass.

Edit Menu

The following Edit menu options are active:



- Copy Copies the information in the Protocol or Template panes into other documents.
- **Default Analysis** Displays the default settings that will be used to analyze the run data generated with an assay.
- Analysis Not active in this screen.
- **Preferences** Set and save custom preferences for data export, plot colors in the graph and Peggy Sue's Twitter settings. See Chapter 10, *"Setting Your Preferences"* for more information.

Reagent Color Coding

The Assay screen uses color coding to identify various assay reagents in all panes. The Layout pane is shown in the following example:



- Orange Samples
- Light Blue Primary antibody
- Teal Tertiary antibody
- Yellow Secondary HRP conjugate
- **Gold** Luminol/Peroxide mix

Opening an Assay

To open an existing assay:

1. Select File in the main menu and click Open Assay.



2. A list of the last five assays opened will display. Select one of these assays or click **Browse** to open the Assay folder and select a different assay.

Creating a New Assay

To create a new assay, we recommend using the Peggy Sue template assay and modifying from there as needed.

Step 1 - Open a Template Assay

1. Select File in the main menu and click New Assay:



2. A list of template assays that can be used as a starting point for new assays will display. Click **Peggy Sue Charge**. The Peggy Sue template assay and default settings will display in the Assay screen:

😵 Peggy	Sue Charge - Con	ipass													J ×
File Edit	Instrument W	indow Help													
												Assay	🕒 Run Summ	ary 🅼 Anal	lysis
Assay: Pe	eggy Sue Charge				Protocol 📙 Hi	story 👖 Notes									- 0
E Layout	t	X O S 🖪	1234	5 D - D	<u> </u>								E	🖹 Add 👻	Remove
							Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8	
	12341	6 7 8 9 10 11 12 13 14 15 16 12	1 10 10 20 21 22 23 24		Sample										
	A 1 2 3 B 1 2 3		A B		Separation										
	P				Immobilization Ti	ime (sec)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	
	F COSC		F G		Primary Antibody	Time (min)	120.0	120.0	120.0	120.0	120.0	120.0	120.0	120.0	
	H COSC				Detection	ay time (min)	00.0	00.0	00.0	00.0	00.0	00.0	00.0	00.0	
	K COSC		K L												
	N COSC		N N												
	P 88888		500000000 P												
E Templ	late														
															Edit
	1	2	3	4	5	6	7		8	9	10		11	12	
A						Si	ample								
в						Primar	y Antibody								
						Carand	Anallanda								
						Second	ary Antibody								
J						Lumino	ol/Peroxide								

Step 2 - Assign Assay Plate Reagents (Optional)

1. Click on the Layout tab. This pane displays the default row locations of where each reagent should be placed on the assay plate.

NOTE: Up to 96 different samples or conditions (lysates, proteins, antibodies or incubation times) can be assayed using the full eight cycles to accommodate experimental needs.

• Row A - Samples



- **Row B** Primary Antibody
- Row C- Secondary Antibody
- Row D Tertiary Antibody (optional)
- **Row E** Luminol-S/Peroxide mix

NOTE: For details on sample, reagent and assay plate preparation, please refer to the product insert provided with the Simple Western kits.

- 2. If needed, well assignments can be modified as described below. Any row assignments changed in the **Layout** pane are updated in the Protocol pane automatically.
 - To move a reagent row to another location Click the row in the Layout pane, then drag and drop it on the new location. The row from which it was moved will be reassigned as empty.



To insert a new row - Click the row below where the new one should be inserted, then click
 Insert an empty row (circle icon) in the Layout pane toolbar. A new row will be inserted above
 the selected row.



• To insert a sample row - Click an empty row or the row below where the new sample row should be inserted, then click Insert a sample row (S icon) in the Layout pane toolbar. A new sample row will be added in the empty row or inserted above the selected row.

Insert a sample row
A 1 2 3 4 5 6 7 8 9 10 1 20 10 10 10 10 10 10 10 10 10 10 10 10 10
B 1 2 3 4 5 6 7 8 C 1 2 3 4 5 6 7 8 C 1 2 3 4 5 6 7 8
P 1 2 3 4 5 6 7 8
F CONCERNING CONCERNING F
H CONCERNMENT CONCERNMENT
J 1 2 3 4 5 6 7 8
K L
M N N N
9 COCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
FULL CONTRACTOR F

• To insert a detection row - Click an empty row or the row below where the new detection row should be inserted, then click Insert a detection row (D icon) in the Layout pane toolbar. A new detection row will be added in the empty row or inserted above the selected row.

	Insert a Detection Row
	Separatic Immobil Primary J Seconda Tertiary J
L L H N O P	Detection

 To insert a fourth or fifth incubation reagent - Click an empty row or the row below where the new incubation reagent should be inserted, then click Insert a Quaternary Row (4 icon) in the Layout pane toolbar. A new incubation reagent row will be added in the empty row or inserted above the selected row.



A fifth incubation reagent can now be added by repeating the above and clicking **Insert a Quinary Row** (5 icon) instead.

NOTES:

When using the fourth and fifth incubation steps, only five cycles can typically be performed per run as more water is needed per cycle for the additional wash steps.

Row J is the last row assignment that can be used on the assay plate. New, sample, incubation or luminol rows cannot be inserted if row J already has an assigned reagent. To insert a row in this case, you must first move the contents of row J to another area on the plate.

• **To delete a row** - Click the row to be deleted, then click **Delete** (red x icon) in the toolbar. Only empty rows and fourth incubation rows can be deleted. Rows for required assay reagents cannot be deleted.



NOTE: Samples, antibodies and blocking buffer can be dispensed in Rows A-J and in columns 1-12 or 13-24. Rows K-P cannot be used for assay reagents.

Step 3 - Modifying the Assay Protocol (Optional)

1. Click on the Protocol tab. This pane displays the individual steps of the assay protocol and allows you to change parameters as needed. When creating a new assay, a default protocol will display which automatically assigns all reagent locations for Cycle 1:

*Protocol 👖 Notes			- 0
		Add 🗨	• Remove
	Cycle 1		
Sample			
Separation			
Immobilization Time (sec)	100.0		
Primary Ab Time (min)	120.0		
Secondary Ab Time (min)	60.0		
> Tertiary Ab Time (min)	10.0		
Detection			

Each row contains an assay protocol step. Each step can contain a reagent assay plate row assignment and one or more parameter settings. To view the details for a step, click on the white arrow next to the step name. We recommend using the default protocol settings for Peggy Sue assays. An expanded list of the default protocol step parameters is shown:

		Add
	Cycle 1	
Sample		
Well Row	A1	
Load Time (sec)	25.0	
Separation		
Separation Profile	Power 1 Step	
Standards Exposure (sec)	3.0	
Immobilization Time (sec)	100.0	
Washes	2	
Wash Load Time (sec)	20.0	
Wash Soak Time (sec)	150.0	
Primary Ab Time (min)	120.0	
Well Row	B1	
Load Time (sec)	2.0	
Washes	2	
Wash Load Time (sec)	20.0	
Wash Soak Time (sec)	150.0	
Secondary Ab Time (min)	60.0	
Well Row	C1	
Load Time (sec)	2.0	
Washes	2	
Wash Load Time (sec)	20.0	
Wash Soak Time (sec)	150.0	
Tertiary Ab Time (min)	10.0	
Well Row	D1	
Load Time (sec)	2.0	
Washes	2	
Wash Load Time (sec)	20.0	
Wash Soak Time (sec)	150.0	
Detection		
Well Row	F1	
Wash Load Time (sec)	2.0	
Detection Profile	5 Exposures	

- 2. Five incubation steps are allowed per protocol. You can select the type of incubation for each step. The available incubation types and their default Simple Western use is as follows:
 - **First incubation** Primary antibody
 - Second incubation Secondary antibody
 - Third incubation User defined (tertiary antibody)
 - Fourth incubation User defined (quaternary antibody)
 - Fifth incubation User defined (custom)

To change the type, click the incubation step name and select an option from the drop down list.



3. If needed, change the primary incubation time. To do this, click the cell in the value column next to Primary Ab Time (min) and enter a new value in minutes:

*Protocol 👖 Notes			- 0
		Add	🔹 Remove
	Cycle 1		
Sample			
b Separation			
Immobilization Time (sec)	100.0		
Primary Ab Time (min)	120.0		
Well Row	B1		
Load Time (sec)	2.0		
Washes	2		
Wash Load Time (sec)	20.0		
Wash Soak Time (sec)	150.0		
Secondary Ab Time (min)	60.0		
> Tertiary Ab Time (min)	10.0		
Detection			

4. If needed, change the primary incubation reagent row location. To do this, click the cell in the value column next to Well Row and select a different row on the assay plate:

Protocol 👖 Notes		
		Add
	Cycle 1	
Sample		
Separation		
Immobilization Time (sec)	100.0	
Primary Ab Time (min)	120.0	
Well Row	B1 👻	
Load Time (sec)	B1	
Washes	Cl	
Wash Load Time (sec)	20.0	
Wash Soak Time (sec)	150.0	
Secondary Ab Time (min)	60.0	
Tertiary Ab Time (min)	10.0	
Detection		

NOTE: Only rows designated as primary antibody in the Layout tab can be selected in the Well Row dropdown menu.

5. If needed, change the secondary incubation time. To do this, click the cell in the value column next to Secondary Ab Time (min) and enter a new value in minutes:

*Protocol 👖 Notes		
		Add 🔻 Remove
	Cycle 1	
> Sample		
Separation		
Immobilization Time (sec)	100.0	
Primary Ab Time (min)	120.0	
 Secondary Ab Time (min) 	60.0	
Well Row	C1	
Load Time (sec)	2.0	
Washes	2	
Wash Load Time (sec)	20.0	
Wash Soak Time (sec)	150.0	
> Tertiary Ab Time (min)	10.0	
Detection		

6. If needed, change the secondary incubation reagent row location. To do this, click the cell in the value column next to Well Row and select a different row on the assay plate:

*Protocol 👖 Notes					6
				Add 🔻 R	Rer
	Cycl	e 1			
Sample					
Separation					
Immobilization Time (sec)	10	0.00			
Primary Ab Time (min)	12	20.0			
Secondary Ab Time (min)	(50.0			
Well Row	C1	-			
Load Time (sec)	C1				
Washes	D1				
Wash Load Time (sec)	1	20.0			
Wash Soak Time (sec)	1	50.0			
Tertiary Ab Time (min)	1	10.0			
Detection					

NOTE: Only rows designated as secondary antibody in the Layout tab can be selected in the Well Row drop-down menu.

7. Additional exposures can be collected in the assay if desired. To do this, click the white arrow next to Detection to expand the row. Click the cell in the exposure column next to Detection Profile to open the Detection Profile window. Additional times can be added to the protocol by clicking the **Add** button, entering the values and selecting **OK**.

Protocol 🛛 🔚 History 🎵	Notes	i)		
		Cycle 1	Cycle 2	
Sample				
Separation				
Immobilization Time (sec)		100.0	100.0	
Primary Antibody Time (m	iin)	120.0	120.0	
Secondary Antibody Time	(min)	60.0	60.0	
Tertiary Antibody Time (m	in)	120.0	120.0	
Quaternary Antibody Time	e (min)	120.0	120.0	
Detection				
Well Row		Л	Л	
Wash Load Time (sec)		2.0	2.0	
Detection Profile		6 Exposure:	6 Exposures	(
6	Dete	ction Profile	X	
				F
	Ad	d Remove		F
	- 44	u nemove		
		_		
	Expo	osure (sec)]	┝
5	Expo	osure (sec) 30.0		
5	Ехро	30.0 60.0		8
5	Expo	30.0 60.0 120.0		8
5	Expo	30.0 60.0 120.0 240.0		8
5	Expo	30.0 60.0 120.0 240.0 480.0		8
5	Expo	asure (sec) 30.0 60.0 120.0 240.0 480.0 960.0		8
5	Expo	osure (sec) 30.0 60.0 120.0 240.0 480.0 960.0		8
5	Expo	sure (sec) 30.0 60.0 120.0 240.0 480.0 960.0		8
5	Expo	sure (sec) 30.0 60.0 120.0 240.0 480.0 960.0		8
5	Expo	osure (sec) 30.0 60.0 120.0 240.0 480.0 960.0 OK	Cancel	8

- 8. Modify any other protocol parameters as needed.
- 9. To add additional cycles to the assay protocol, click in any cell with a value in a cycle column.
 - To add one cycle, either click Add or click the down arrow next to Add and select 1 Cycle
 - Select **4 Cycles** from the drop down menu to add four additional cycles
 - Select **All Cycles** from the drop down menu to add the number of cycles needed to reach the maximum of eight

Protocol 👖 Notes		
		Add 🔻 Remove
	Cycle 1	1 Cycle
Sample		4 Cycles
Separation		
Immobilization Time (sec)	100.0	All Cycles
Primary Ab Time (min)	120.0	
Well Row	B1	
Load Time (sec)	2.0	
Washes	2	
Wash Load Time (sec)	20.0	
Wash Soak Time (sec)	150.0	

*Protocol 🌃 Notes								- 8	
	Add 🔻 Remove								
	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8	
> Sample									
Separation									
Immobilization Time (sec)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	
Primary Ab Time (min)	120.0	120.0	120.0	120.0	120.0	120.0	120.0	120.0	
Secondary Ab Time (min)	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	
> Tertiary Ab Time (min)	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	
Detection									

New cycle columns will display using the same parameters used for cycle 1.

Repeat steps 1-8 to change parameters for the added cycles. As cycles are added and reagent locations are selected, cycle number assignments will update in the Layout pane assay plate map:



NOTES:

For more information on changing protocol step parameters other than incubation times, contact ProteinSimple Technical Support.

When a protocol is being edited, an asterisk will appear in the Protocol tab to indicate changes have been made and should be saved.

Step 4 - Add Assay Notes (Optional)

- 1. Click on the Notes tab.
- 2. Click in the pane and type any assay or protocol notes. This information will be stored with the assay and run data.



NOTE: When notes are being edited, an asterisk will appear in the Notes tab to indicate changes have been made and should be saved.

Step 5 - Select a Schedule (Optional)

Peggy Sue can execute cycles serially or in parallel. To choose an option, select Edit and click Schedule.

😵 Schedule 🛛 💌
🔘 Serial
Overlapping
Overlapping with hold
OK Cancel

- **Serial** Each cycle is executed in sequential order, and each cycle completes before the next one starts. Selecting this option result in the longest run times.
- **Overlapping** Cycle steps are executed in parallel if timing permits shared use of the separation tray and robot. Each cycle will run through to completion once started. This option offers a reduction in overall run time without affecting the individual run time for each cycle.
- **Overlapping with Hold (default)** Each cycle executes through the separate step in sequence and then enters a variable length hold step where capillaries remain in the incubation tray. More sample stability is provided with this option as proteins are 'locked' to the capillary wall and therefore more stable than samples on the assay plate. This option also results in the most significant reduction in overall run time.

NOTE: We recommend using the overlapping with hold option. For more information on when to use other schedule options, please contact ProteinSimple Technical Support.

Step 6 - Add Assay Plate Annotations (Optional)

Custom reagent names, notes and annotations can be entered for individual rows and wells on the assay plate. This information will be stored with assay and run data. During post-run analysis, this information will be used to apply custom analysis settings to specific sample names, primary antibody names or sample and primary antibody attributes. This will be explained in more detail in "Compass Analysis Settings Overview" on page 232.

NOTE: Template pane information can also be added or updated after a run is complete.

To enter annotations:

1. Click on the Template tab. The default annotations for reagent rows and individual wells on the assay plate will display:

Peagy	Sue Charge - Com	pass													a E
File Edit	Instrument Wi	ndow Help													
												Assay	😷 Run Summ	ary 🏭 Ana	lysis
Assay: P	eggy Sue Charge				Protocol 📙 H	istory 👖 Notes									- [
E Layou	ıt	X O S 🖪	1234	5 D - D									Œ	Add 🔻	Remov
							Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8	
	A 1 2 3 4 5	67891011121314151612	6020122234		Sample										
	B 1 2 3 4 C 1 2 3 4	5678	B C		Separation	ime (cas)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	
	P COSSO				Primary Antibody	(Time (sec)	120.0	120.0	120.0	120.0	120.0	120.0	120.0	120.0	
	G		G G		Secondary Antibo	ody Time (min)	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	
	1 2 3 4	1 5 6 7 8			Detection										
	L COSCO														
	N COSCO		800000 N												
🖶 Temp	late														- 1
															Edi
	1	2	3	4	5	6	7		8	9	10		11	12	
Α						S	ample								
в						Prima	ry Antibody								
c						Second	ary Antibody								
J						Lumin	ol/Peroxide								
															-

- 2. Change or add row and well annotations as needed. To do this:
 - a. To enter annotations for a specific well Right click the well and select Edit or click Edit in the upper right corner of the pane or double click the selected well. The following box will display:



Enter the reagent name, notes or specific attributes (for example, sample concentration or dilution ratio):

😢 Well Cont	tent 💌
Name:	HeLa
Attribute:	1 mg/mL
	OK Cancel

Click **OK**. The new information will display in the selected well:



b. To enter annotations for multiple wells or a row - To select individual wells, press and hold the Control key and then select individual wells. To select a sequential set of wells or a full row, press and hold the Shift key, then select the first well and last well. Next, right click and select Edit or click Edit in the upper right corner of the pane. The following box will display:



E Template

A

в

с

D

Biot. Ladder

Blocking

Streptavidin

Well Content

Name: HeL:

Attribute: 1 mg/mL

OK Cancel

Enter the reagent name, notes and specific attributes (for example, sample concentration or dilution ratio):

Click **OK**. The new information will display in the selected wells:

4

5

NOTE: When annotations are being edited, an asterisk will appear in the **Template** tab to indicate changes have been made and should be saved. Only Name and Attribute will be used by Compass to

6

Antibody Diluent

Primary Antibody

Secondary Antibody

8

9

10

11

annotate the data.

2

- 8

12

Step 7 - Save the Assay

1. Select File from the main menu and click Save As. Enter the assay name and click Save.



NOTE: New assays are saved in the Compass Assays directory.

Step 8 - Modify Default Analysis Parameters (Optional)

You can preset the parameters used to analyze run data generated with the assay.

1. Select **Edit** from the main menu and click **Default Analysis**. The following screen will display:

🛞 Default Analysis			
type filter text	Advanced		(
Advanced Images	Analysis Settings	Analysis Settings: Advanced	
Peak Fit Peak Names	Advanced	Standards	
Standards		Peak Width	15
		Allowable Drift	100
		Sample	
	Add Remove	Peak Fit Starting Width Ratio	0.5
	Default Advanced 🗸	Image	
	Ouerride	Median Filter Threshold Katio	100
	Apply To Settings		
	Add Remove		
			Restore Original
Import Export		ОК	Cancel

2. We recommend using the default parameters for Simple Western assays. However, you can modify any parameters as needed, then click **OK**. For detailed information on analysis parameters, please refer to "Compass Analysis Settings Overview" on page 232.

Making Changes to an Existing Assay

1. Select File in the main menu and click Open Assay.



- 2. A list of the last assays opened will display. Select one of these assays or click **Browse** to open the assay folder and select a different assay.
- 3. Follow the steps in "Creating a New Assay" on page 79 to make changes and save the assay.

Switching Between Open Assays

If more than one run file is open, you can switch between viewing the assays for each run. To do this:

1. Click the down arrow in the run box.



2. Select the run for the assay you want to view from the drop down list.

Creating a Template Assay

You can create and save template assays that can be used as a starting point for creating new assays. To do this:

- 1. Select **File** in the main menu. Click **Open Assay** to open an existing assay or click **New Assay** to open an existing template assay.
- 2. Follow the steps in "Creating a New Assay" on page 79 to make changes to the assay.
- 3. When changes are complete, select **File** in the main menu and click **Save As**. Select the New Assays folder:

Save As			×
🚱 🕞 🗢 📙 « My Documents 🕨	Compass 🕨 New Assays 👻	Search New Assays	م
Organize 🔻 New folder			· •
▲ 🔆 Favorites	Documents library New Assays	Arrange by:	Folder 🔻
Downloads	Name Simple Western.assay	Date modified Type 9/26/2011 3:25 PM Com	pass Assay File
Desktop D			
File name: Protein Test			-
Save as type: Assay File (*.ass	ay)		•
) Hide Folders		Save	Cancel

- 4. Type the name for the new template assay and click **Save**.
- 5. Select **File** in the main menu and click **New Assay**. The new template assay will now be available in the drop down list:

File	Edit Instrument	Wind	ow Help
	New Assay	×	Protein Test
	Open Assay	×	Simple Western
	Save Save As		

Viewing and Changing the Detection Exposures

To view the current detection profile, roll the cursor over the exposures cell in Protocol pane:

	Cycle 1	Cycle
Sample		
Separation		
Immobilization Time (sec)	100.0	100
Primary Antibody Time (min)	120.0	120
Secondary Antibody Time (min)	60.0	60
Tertiary Antibody Time (min)	120.0	120
Detection		
Well Row	E1	E
Wash Load Time (sec)	2.0	2
Detection Profile	6 Expos	6 Eunacury
	sigi sigi sigi sigi sigi sigi sigi sigi	nal, 30.0 sec nal, 60.0 sec nal, 120.0 sec nal, 240.0 sec nal, 480.0 sec nal, 960.0 sec

While we recommend using the default assay detection profile, you can change the profile, exposure times and exposure sequences if needed. To do this:

1. Select the exposures cell in the Protocol pane and click the button, or double-click in the cell. The following screen will display:

😵 Detection	Profile	X
Add	Remove	
Exposure	sec)	
30.0		
60.0		
120.0		
240.0		
480.0		
960.0		
ОК		ancel
		ancer
Each row represents an individual exposure that will be taken during the run.

a. To change an existing exposure time - Click in the exposure cell and enter a new time in seconds:

1
-
Cancel

- b. To delete an existing exposure Select a type or exposure cell and click Remove.
- c. **To add a new exposure** Select **Add**. A new exposure will be added to the end of the list. Click in the exposure cell and enter an exposure time in seconds.
- 2. Click **OK** to save and exit.

Copying Protocols and Templates

The steps and parameters in the Protocol pane can be copied and d into other documents, as can the graphic image of the annotations in the Template pane.

Copying an Assay Protocol

- 1. Click on the Protocol tab.
- 2. Select **Edit** in the main menu and click **Copy**.
- 3. Open a document (Microsoft[®] Word[®], Excel[®], etc.). Right click in the document and select. All assay protocol steps and parameters will be copied into the document as a list in the same format shown in the Protocol pane.

Copying an Assay Template

- 1. Click on the Template tab.
- 2. Select Edit in the main menu and click Copy.
- 3. Open a document (Microsoft[®] Word[®], Excel[®], etc.). Right click in the document and select. The assay template will be copied into the document as an image, exactly as it is shown in the Template pane.

Printing Protocols and Templates

The information in the Protocol pane can be printed, as can a graphic image of the information in the Template pane.

Printing an Assay Protocol

- 1. Click on the Protocol tab.
- 2. Select File in the main menu, click Print, and then click Print Protocol.

File	Edit Instrument	Windo	w	Help)			
	New Assay	•	1					
	Open Assay	•	⊢				_	Pro
	Save							
	Save As		F				- 1	
	Import Protocol		2	3	4	5	L	
	Import Template			-	-			Se
	Export Protocol							In
	Export Template							Pr
			_			_		Se
	Print	•		Prir	nt Pr	otoc	ol	
	Exit		Prir	nt Te	mpl	ate		

All assay protocol steps and parameters will be printed as a list in the same format shown in the Protocol pane.

Printing an Assay Template

- 1. Click on the Template tab.
- 2. Select File in the main menu, click Print, and then click Print Template.

File	Edit Instrument	Windo	w	Help)			
	New Assay	•	1					
	Open Assay	•	⊢					Dro
	Save							FIG
	Save As		-					
	Import Protocol		2	3	4	5	1	6
	Import Template		F	-	-		_	Se
	Export Protocol							Im
	Export Template							Pr
	Print	•		Prir	nt Pr	otoc	:ol	Se
	Exit			Prir	nt Te	mpl	ate	

The assay template will print as an image, exactly as it is shown in the Template pane.

Importing and Exporting Protocols and Templates

The assay protocol in an open assay or run file can be exported as a separate file, as can the assay template annotation information. This allows the same assay protocol and template information to be imported into another assay at a later time, rather than having to re-enter assay protocol and template information manually.

Importing an Assay Protocol

NOTE: Importing an assay protocol imports information into the Protocol pane only.

- 1. Open the assay you want to import the assay protocol in to.
- 2. Select File in the main menu and click Import Protocol.
- 3. Select a protocol file (*.protocol) and click **OK**. The imported information will display in the Protocol pane.

Exporting an Assay Protocol

NOTE: Exporting an assay protocol exports information in the Protocol pane only.

- 1. Open the assay you want to export the assay protocol from.
- 2. Select File in the main menu and click Export Protocol. The following window displays:

			×		
Export Protocol					
🕞 🕞 🗸 📕 🖉 Documents 🕨 My D	Jocuments ► Compass ► Assays -	Search Assays	Q		
Organize 🔻 New folder			:= - 🕡		
★ Favorites	Documents library Assays	Arrange by: Folder 🔻			
Downloads 🛛 🗉	Name	Date modified	Туре		
🔠 Recent Places	Standard sample protocol.protocol	10/4/2011 9:57 PM	PROTOCOL File		
 Desktop Libraries Documents My Documents Compass Assays New Assays Runs 					
•	•		•		
File name: New protcol			-		
Save as type: Protocol File (*.pr	otocol)		-		
) Hide Folders		Save	Cancel		

- 3. The default directory is Compass/Assays. Change the directory if needed.
- 4. Enter a protocol name and click Save. The protocol will be saved as a *.protocol file.

Importing an Assay Template

NOTE: Importing an assay template imports information into the Template pane only.

- 1. Open the assay you want to import the assay template in to.
- 2. Select **File** in the main menu and click **Import Template**.
- 3. Select a template file (*.template) and click **OK**. The imported information will display in the Template pane.

Exporting an Assay Template

NOTE: Exporting an assay template exports information in the Template pane only.

- 1. Open the assay you want to export the assay template from.
- 2. Select File in the main menu and click Export Template. The following window displays:



- 3. The default directory will be Compass/Assays. Change the directory if needed.
- 4. Enter a template name and click Save. The template will be saved as a *.template file.

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Chapter 5: Running a Charge Assay on Peggy Sue

Chapter Overview

- Starting a Run
- Stopping a Run

Starting a Run

Step 1 - Get Ready

- 1. Open Compass software.
- 2. Prepare instrument: empty waste, refill water and add a new manifold sponge.
- 3. Create or open desired assay file.
- 4. Prepare assay plate following the procedure described in the product insert.

IMPORTANT

To prevent well evaporation and ensure best results, keep a lid on the assay plate until ready to use.

5. While plate is spinning, add Wash Buffer, Anolyte and Catholyte to resource tray cups. Place capillary box in the designated resource tray position.

IMPORTANT

Capillaries are light sensitive. Keep the cover on the box until you are ready to transfer the capillary box to the resource tray.

6. Place assay plate into the sample tray of the instrument and press Start.

Step 2 - Start the Run

1. New run of an existing assay:

a.Select File in the main menu and click Open Assay.



- b. A list of the last five assays opened will display. Select one of these assays or click **Browse** to open the Assay folder and select a different assay.
- 2. Alternatively, choose **New Assay** and select **Peggy Sue Charge** to get the default Peggy Sue assay conditions.
 - c. The Start button will display. This indicates than an assay has been loaded.



- a. Go to the Assay screen and verify this is the assay you want to use. If not, select **File** in the main menu, click **Open Assay**, and select another assay.
- 3. Click Start. This will launch the Start Run Wizard.

NOTE: If the manifold was not cleaned prior to starting the run, a message indicating this will display. If this occurs, click **Yes** to cancel the run and perform the manifold cleaning.

4. **Check Water and Waste.** The fluid levels in the accessory module bottles will be checked by the software. If the levels in both bottles will allow Peggy Sue to complete the run, the wizard screen will display **Water Level OK** and **Waste Level OK** messages. Click **Next** to proceed.

🔞 Start Run	X
Check water and waste Make sure there is enough water and room in the waste bottle	
The run will be terminated if the water bottle empties or the waste bottle fills during the run.	
Water level OK	
Waste level OK	
< Back Next > Start Can	cel

NOTE: If the waste level is too high or the water level is too low to complete the run, messages to indicate one or both will be presented in this screen. If this occurs, fill or empty each bottle as indicated using the procedures outlined in the Peggy Sue User Guide. When this is complete, the error status will be automatically updated and allow the **Start Run Wizard** to proceed.



5. **Replace Sponge.** A new sponge should be used each time a new experimental run is started. Discard the old sponge and put a new sponge in the manifold wash station.

6. **Start.** The resource tray will automatically open. Fill the Wash Buffer, Anolyte and Catholyte cups and insert the capillary box in the primary capillary box position. Add a secondary capillary box if more capillaries are required. Click **Next** when finished. The resource tray will close.

NOTE: Make sure enough capillaries are loaded to complete the run. A capillary box should be inserted in the primary position first and if additional capillaries are needed, use the secondary position. When the primary box is depleted, Peggy Sue will automatically move to the secondary box. Discard leftover Running Buffer, Wash Buffer and Matrix Removal Buffer prior to refilling bottles for new run.



NOTE: You an also refer to the labels on the resource tray for proper insertion of reagents.



7. Load Sample Plate. The sample tray will automatically open. Place the lidded 384-well plate on the cooling block in the tray, ensuring that the A1 well position is aligned with the upper left corner of the cold block. When this is complete, click **Next**. The sample tray will close.

oad Sample	Plate
Remove the	old sample plate and load the new plate
	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
	AA B
	F
	К
	P COCCOCCOCCCCCCCCCCCCCCCCCCCC



NOTES:

Peggy Sue requires that plate lids be used on sample plates. If a lid is not detected, a message will be displayed in the Start Run Wizard. Compass software will reopen the sample tray to allow you to insert a lid.

When inserting the sample plate, ensure that the plate is firmly seated and level on the cold block. Plates that are not level can interfere with the movement of the sample tray.

8. **Begin the Automated Run.** The data file name will automatically default to the assay name appended with the current date and time. To change the file name, begin typing in the text box. To change the directory where the data file will be stored, click **Browse**:

🔞 Start Run	23
Begin the Automated Run The following protocol will be run. You can change the results location and pref	ix 📳
Access Destein Test	-
Cycles: 8	
Schedule : Overlapping with hold	
Run name:	
2012-10-16_09-29-06_Protein Test	Browse
Location: C:\Users\pfung\Documents\Compass\Runs	
< Back Next > Start	Cancel
	concer

Click **Start** to begin the run. Instrument status will change to running, and the stop button and progress bar will display:



The run will continue until complete (~14-19 hours depending on the assay).

Step 3 - Post-Run Procedures

- 1. Empty the capillary discard tray.
- 2. Remove the assay plate.
- 3. Dispose of the assay plate and capillaries. Disposal will depend on the samples that have been assayed. If sample origins are unknown, we recommend that used capillaries and plates be disposed of in bio-hazard waste.

!WARNING! SHARPS HAZARD

The capillaries may present a potential sharps hazard. Dispose of used capillaries in biomedical waste sharps containers.



WARNING! BIOHAZARD

Samples and waste bottle contents should be handled by procedures recommended in the CDC/NIH manual: Biosafety in Microbiological and Biomedical Laboratories (BMBL). The manual is available from the U.S. Government Printing Office or online at http://www.cdc.gov/biosafety/publications/bmbl5/.

Depending on the samples used, waste bottle contents may constitute a biohazard. Use precaution when emptying the waste bottle. Dispose of waste bottle contents in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations. Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste vial before you store, handle, or dispose of chemical waste.

Stopping a Run

1. To stop a run, click **Stop**. When the run stops, instrument status will go to Not Ready and a Cleanup button displays:



NOTE: If a run is stopped prior to completion, Peggy Sue must perform a cleaning protocol to remove used capillaries from system trays. This is required to prepare the system for the next run.

2. Click Cleanup.



Allow Peggy Sue to complete the cleaning protocol which takes about ten minutes. When complete, instrument status will change to Ready and a new run can be started.

^{Chapter 6:} Run Status

Chapter Overview

- Run Summary Screen Overview
- Opening Run Files
- Viewing File and Run Status Information
- Watching Standards Separation Movies
- Viewing Current and Voltage Plots
- Switching Between Open Run Files
- Closing Run Files

Run Summary Screen Overview

The Run Summary screen is used to monitor run progress, watch movies of the fluorescent standards separation, and view current and voltage plots for a run. To access this screen, click Run Summary in the screen tab:

📑 Assay 💽 Run Summary 🏥 Analysis

Run Summary Screen Panes

The Run Summary screen has three panes:

- Status Displays run file information and current status of a run in progress.
- Separation Lets you view a movie of the fluorescent standards separation for each cycle of the experimental run.
- IV Plot Lets you view plots of the total current and voltage measured during separation for all capillaries for each cycle of the experimental run.

2015-07-17_14-03-48_Peggy Sue Size Split Running Buffer 8-cycle - Compass								
ne cat instrument window neip	Assay 🕀 Run Summary 🚑 Analysis							
Run: 2015-07-17_14-03-48_Peggy Sue Size Split Running Buffer 8-cycle	Separation 2 IV Plot							
🕑 Status 📕 History								
Path <u>C\Users\ppiatti\Desktop</u> Assay Peggy Sue Size Split Running Buffer Killufa Beguler, 12-231 kDa Split Bunning Buffer	Cycle 1							
Kit Into Regular: 12-230 KDa, Split Running Buffer Schedule Overlapping with hold Instrument Peggy-Sue : Peggy Sue SW0105 - SW0105								
Started Fri 2:09 PM Jul 17, 2015 PDT Completed Sat 5:57 AM Jul 18, 2015 PDT								
Cycle Sample Sep Hold B 1° Detect Results	-							
2:09 PM 2:17 PM 3:33 PM 1:22 AM 1:44 AM 2:23 AM 2:42 AM								
2 3:33 PM 3:41 PM 4:57 PM 1:49 AM 2:11 AM 2:49 AM 3:08 AM								
3 4:57 PM 5:05 PM 6:21 PM 2:15 AM 2:37 AM 3:16 AM 3:34 AM	▶ <1 IÞ							

Software Menus Active in the Run Summary Screen

The following software menus are available:

- File
- Edit
- Instrument (when Compass is connected to an instrument)
- Window
- Help

The File and Edit menu options specific to the Run Summary screen are described next.

File Menu

The following **File** menu options are active:



- Open Run Opens a run file.
- Add Run Open and view other run files in addition to the one that is already open.
- **Close** Closes the run file currently being viewed.
- Close All Closes all open run files.
- **Exit** Closes Compass.

Edit Menu

The following Edit menu options are active:

Edit	Instrument	Window	He
	Cut		
	Сору	Ctrl+C	
	Paste	Ctrl+V	
	Preferences		

• **Preferences** - Set and save custom preferences for data export, plot colors in the graph and Twitter settings. See Chapter 10, *"Setting Your Preferences"* for more information.

Opening Run Files

You can open one run file or multiple run files at a time to compare information between runs.

Opening One Run File

1. Select **File** in the main menu and click **Open Run**.

File) Edit Instr	ument	Win	dow Help		
	Open Run	•		Simple Western ERK Demo	1	
	Add Run	•		2011-08-31_16-38-23_test		
Close Close All				3 ab run DemoData		- 8
	Exit			Browse		
	Path	<u>C:\Us</u>	0.010		RK Demo.cbz	
	Assay Simple Western Demo Assay					

2. A list of the last 10 runs opened will display. Select one of these runs or click **Browse** to open the Runs folder and select a different file.

Opening Multiple Run Files

1. To open the first run file, select **File** in the main menu and click **Open Run**.

File	Edit Instr	ument	Win	dow Help	
	Open Run	×		Simple Western ERK Demo	1
	Add Run	•		2011-08-31_16-38-23_test	
	Close Close All			3 ab run DemoData	
	Exit			Browse	
	Path Assay	C:\Us Simpl	e We	estern Demo Assay	<u>RK Demo.cbz</u>

- 2. A list of the last 10 runs opened will display. Select one of these runs or click **Browse** to open the Runs folder and select a different file.
- 3. To open another run file, select **File** in the main menu and click **Add Run**.

File	Edit Instrument	Windo	w Help
	Open Run	- + I	
	Add Run	•	2012-03-05_11-51-19_HelaControlERKassay
	Close		Browse
	Close All	[
	Exit	ū	ng\Documents\ProteinSimple\2012-03-05_11-51-19

- 4. A list of the last 10 runs opened will display. Select one of these runs or click **Browse** to open the Runs folder and select a different file.
- 5. Repeat the last two steps to open additional runs.

Viewing File and Run Status Information

Information specific to each run file is shown in the Status pane:

6	015-07-17	_14-03-48_P	eggy Sue Siz	e Split Runn	ing Buffer 8-	cycle - Com	pass	
	File Edit In	strument \	Vindow He	lp				
	Run: 2015-0	07-17_14-03-4	18_Peggy Sue	e Size Split R	unning Buffe	r 8-cycle		
ſ	🕑 Status 🔪	🔡 History						
	R	un c6081	3_sw0105_	2015-07-17	_14-03-48_F	RB2revC-S	ep Buff_EZ	_8 cyc_1pt1
	Pa	ath <u>C:\Us</u>	ers\ppiatti\E	Desktop				
	Ass	ay Peggy	/ Sue Size S	Split Runnin	g Buffer			
	Kit Ir	nfo Regul	ar: 12-230 k	Da, Split Ru	inning Buffe	r		
	Schedu	ile Overla	apping with I	hold				
	Instrume	ent Peggy	/-Sue : Pegi	gy Sue SW	0105 - SW0	105		
	Start	ed Fri 2:0	9 PM Jul 17,	2015 PDT				
	Complet	ed Sat 5:5	57 AM Jul 18	3, 2015 PDT	Γ			
	Cycle	Sample	Sep	Hold	В	1*	Detect	Results
	1	JL.		\odot	в 🕛	1° 🕕	尺	
	· ·	2:00 PM	2:17 DM	2-22 DM	1.22 AM	1-44 AM	2.23 AM	2.42 AM
		2.03 FIVI	2.17 FIVI	5.55 PW	1.22 AW	1.44 AW	2.25 AW	2.42 AM
				Ð	B	1°())	- <u>R</u> -	4
	2	ш	*	9			\rightarrow	
		3:33 PM	3:41 PM	4:57 PM	1:49 AM	2:11 AM	2:49 AM	3:08 AM
				(The second seco	B	1°D		J:
	3		+	0		0	Δ	Contra de
		4:57 PM	5:05 PM	6:21 PM	2:15 AM	2:37 AM	3:16 AM	3:34 AM
	4	Ш	• -	\odot	в 🕒	1° 🕕	尺	

The run file name, path (directory location) and assay used is displayed along with instrument serial number and the run start/complete date and time.

- To go to the run file directory location Double click the path hyperlink, or right-click and select **Open Directory**.
- **To copy the path** Right-click on the path hyperlink and click **Copy**. The path can then be copied into documents. The path can also be copied into the Windows Explorer address bar to launch Compass and open the run file automatically.
- **Kit info** Compass v2.7 and higher displays the type of kit used to run the assay (regular for immunoassays, total protein or charge), the molecular range and whether or not the split Running Buffer was used.
- Plate S/N For Wes, the plate serial number (S/N) information is captured and displayed for all assays.



Assay Steps: Size-based Assays

Each assay step that is executed during the run is represented by an icon. The time the individual step is scheduled to start is shown under each icon. Details on what happens during each assay step is as follows:

Step	Description
Sample	Sample Loading Step - Capillaries are moved to the assay plate in the sample tray where Separation Matrix, Stacking Matrix, biotinylated ladder and samples are aspirated. Capillaries are then transferred to the separation tray.

Step	Description
Sep +	Separation Step - Samples and fluorescent standards are separated in the capillaries. Cap- illaries are then exposed to UV light, which immobilizes the separated proteins to the walls of each capillary. During the separation, voltage and current are monitored and displayed in the IV Plot. Separation of the fluorescent standards is recorded and a movie of the sepa- ration is compiled for viewing when separation is complete in the Separation pane. After separation, Matrix Removal Buffer is aspirated.
Block B 2:00 PM	Blocking Step - Capillaries are moved to the assay plate in the sample tray and blocking reagent (Antibody Diluent) is aspirated. Capillaries are then transferred to an incubator tray. When incubation is complete, Wash Buffer is aspirated.
1° 1° 2:16 PM	Primary Antibody or Total Protein Labeling Reagent (1°) Step - Capillaries are moved to the assay plate in the sample tray and primary antibody or labeling reagent is aspirated. Capillaries are then transferred to an incubator tray. When incubation is complete, Wash Buffer is aspirated.
2° 4:22 PM	Secondary Antibody or Total Protein Streptavidin-HRP (2°) Step - Capillaries are moved to the assay plate in the sample tray and secondary HRP conjugate and Streptavi- din-HRP (Immunoassays), or Total Protein Streptavidin-HRP (Total Protein Assays) is aspi- rated. Capillaries are then transferred to an incubator tray. When incubation is complete, Wash Buffer is aspirated.
Detect 5:28 PM	Detect Step - Capillaries are moved to the assay plate in the sample tray and Luminol-Per- oxide solution is aspirated. Capillaries are then transferred to the separation tray where the emitted chemiluminescent light is detected with the CCD camera.
Results	Results Step - Results are available in the Analysis screen.
Hold ① 1:10 PM	Hold Step - This step is used for overlapping or overlapping with hold schedule options. A cycle that has completed a specific step will go into a hold step while the remaining cycles execute the same step in parallel. For example, once cycle1 completes the separate step, it may go into a hold step while cycle 2 executes the separate step. This execute and hold pattern will continue until all cycles have completed the separate step.

When a run is in progress, icons for steps that have not executed will be grey (inactive). In the following example, the Secondary Antibody (2°) step is executing and the Detect and Results steps have not started:

Sample	Sep	Block	1°	2*	Detect	Results
	•	в	1° 🕛	2°()	<u> </u>	
12:54 PM	12:56 PM	2:00 PM	2:16 PM	4:22 PM	5:28 PM	6:00 PM

Assay Steps: Charge-based Assays

Each assay step that is executed during the run is represented by an icon. The time the individual step is scheduled to start is shown under each icon. Details on what happens during each assay step is as follows:

Step	Description
Sample	Sample Loading Step - Capillaries are moved to the assay plate in the sample tray and samples are aspirated. Capillaries are then transferred to the separation tray.
Sep • 12:56 PM	Separation Step - Samples, ampholyte mix and fluorescent pl standards are separated in the capillaries. Capillaries are then exposed to UV light, which immobilizes the separated proteins to the walls of each capillary. During the separation, voltage and current are monitored and displayed in the IV Plot. Separation of the fluorescent standards is recorded and a movie of the separation is compiled for viewing in the Separation pane after separation is complete.
1° 1° 2:16 PM	Primary Antibody (1°) Step - Capillaries are moved to the assay plate in the sample tray and primary antibody is aspirated. Capillaries are then transferred to an incubator tray. When incubation is complete, Wash Buffer is aspirated.
2° 2° 4:22 PM	Secondary Antibody (2°) Step - Capillaries are moved to the assay plate in the sample tray and secondary HRP-conjugated antibody is aspirated. Capillaries are then transferred to an incubator tray. When incubation is complete, Wash Buffer is aspirated.
Detect	Detect Step - Capillaries are moved to the assay plate in the sample tray and Luminol-Per- oxide solution is aspirated. Capillaries are then transferred to the separation tray where the emitted chemiluminescent light is detected with the CCD camera.
Results	Results Step - Results are available in the Analysis screen.

Step	Description
Hold ① 1:10 PM	Hold Step - This step is used for overlapping or overlapping with hold schedule options. A cycle that has completed a specific step will go into a hold step while the remaining cycles execute the same step in parallel. For example, once cycle1 completes the separate step, it may go into a hold step while cycle 2 executes the separate step. This execute and hold pattern will continue until all cycles have completed the separate step.

When a run is in progress, icons for steps that have not executed will be grey (inactive). In the following example, the Secondary Antibody (2°) step is executing and the Detect and Results steps have not started:



Watching Standards Separation Movies

You can view a movie of the fluorescent standards separation in all 12 capillaries. To do this:

1. Click the **Separation** tab.



2. The player control panel has play/pause, rewind and fast forward buttons, and a slider bar that allows you to scroll through the movie manually:



Click **Play** (button on far left) to view the movie. In the examples below, standards for a size assay are on the left, and standards for a charge assay are on the right:



NOTE: Complete separation movies of the fluorescent standards are not available until the separation step has finished executing. If the movie is played while the separation step is executing, the movie will only show separation progress up to the current point in time.

Viewing Current and Voltage Plots

You can view plots of the total current and voltage measured during separation for all 12 capillaries. To do this, click the **IV Plot** tab. In the examples below, the IV plot for a size assay is on the left, and the IV plot for a charge assay is on the right:



The blue Y-axis and plot shows the run voltage in volts (V), and the red Y-axis and plot shows the run current in micro amps (μ A). The X-axis displays time in seconds.

- To zoom in on an area of the plot Hold the mouse button down and draw a box around the area with the mouse.
- To zoom out Click Zoom Out in the upper right corner of the pane.

NOTE: The IV plot for a run in progress will not be available until the separation step starts executing. The plot is then displayed in real-time.

Switching Between Open Run Files

If more than one run file is open, you can switch between viewing the run information in each. To do this:

1. Click the down arrow in the run box.



2. Select the run you want to view from the drop down list.

Closing Run Files

If more than one run file is open, you can close just one file or all the open files at the same time.

- To close the run file being viewed Select File from the main menu and click Close.
- To close all open run files Select File from the main menu and click Close All.

Chapter 7: Controlling Wes, Sally Sue and Peggy Sue

Chapter Overview

- Instrument Control
- Self Test
- Viewing and Changing System Properties
- Viewing Log Files
- Status Modes

Instrument Control

The Instrument menu allows you to control Wes, Sally Sue and Peggy Sue.



NOTE: Instrument menu options are active only when a computer with Compass software is connected directly to Wes, Sally Sue or Peggy Sue.

Starting a New Run

To start a new run, select **Instrument** in the main menu and click **New Run**. Then follow the steps described in "Step 2 - Start the Run" on page 64 for size assays or "Step 2 - Start the Run" on page 106 for charge assays.

Opening Trays (Sally Sue and Peggy Sue)

To open any of the five trays, select **Instrument** and click **Open Trays**. The tray control window will appear:

🛞 Open Trays	23
Main Trays	Other Trays
Resources	Separation
	Upper Incubator
Samples	Lower Incubator

Open a tray by clicking on its button. The button will become highlighted indicating the tray is open.

NOTE: Only one tray can be open at a time.



To close a tray, click the corresponding tray button again.

NOTE: If the tray control window is closed when a tray is open, the tray will close automatically.

Cleaning

Two cleaning options are available for Sally Sue and Peggy Sue.

Manual Clean

This option is used for general manual cleaning and cleaning the manifold head. To do a manual cleaning, select **Instrument** and click **Manual Clean**. The manifold head will move to a safe position for easy access and the vacuum will turn on.

NOTE: Please contact Protein Simple Technical Support if you have any questions regarding the manifold cleaning procedure.

Cleanup

This option is a fully automated cleaning step. The manifold head is flushed, the separation tray troughs are aspirated and washed, and any capillaries left in the trays or gripper are picked up and discarded. This option should be selected when the instrument has not been used for more than a week or if a run error occurs. Cleaning takes about eight minutes to complete.

To start the protocol, select Instrument and click Cleanup. A window will appear with instructions:



Sally Sue's or Peggy Sue's status will change to cleaning, and the stop button and the cleaning progress bar display. The **Assay** screen provides cleaning status details:



When cleaning is complete, instrument status will change to Ready.



Cleaning After a Run Error

Additional cleaning steps are required if an error occurs that stops the run. When this happens, the red Error status light on Sally Sue's or Peggy Sue's front panel will come on.

Click on the **Reset** button displayed in Compass software. The following instructions will appear:



If the troughs in the separation tray are empty, click on **OK** and proceed with "Cleanup" on page 131.

If Running Buffer is present in the separation tray, click on **Cancel** and manually remove the buffer. Evaporation of the Running Buffer will result in a highly viscous residue which the automatic cleaning feature cannot remove.

To remove the Running Buffer:

1. Select Instrument and click Open Trays.

🔞 Open Trays	X
Main Trays	Other Trays
Resources	Separation
Samples	Upper Incubator Lower Incubator

- 2. Click **Separation** to open the separation tray.
- 3. Add 800 µL of deionized water to the troughs in the separation tray and soak for 20 minutes.
- 4. Remove the water by either aspirating with a pipette or with the vacuum wand located on the inside of Sally Sue's or Peggy Sue's left door.
- 5. Repeat the steps above until the Running Buffer or residues are completely removed.
- 6. To complete the cleaning process, select **Instrument** and click **Cleanup**.

Self Test

Wes, Sally Sue and Peggy Sue can perform a series of self tests to check for proper instrument performance. To start the test, select **Instrument** and click **Self Test**. The test takes approximately two minutes.

	Stop	Self	Test	Assay 🕑 Run Summary	Summary 👫 Analysis
PL0006	ising Stop	Sat 4:53 PM	Sat 4:55 PM		
Run: Self Test	-			Separation 🛃 IV Plot	- 8
Status			- 0		
Run	Self Test		Â		
Assay	Self Test				
nstrument Started	Peggy : Peggy S Sat 4:53 PM Jun	W1001-SW1001 9, 2012 PDT			

NOTE: We recommend performing the self test prior to starting a run.

To view the test log at completion of the test, select **Instrument**, click **Properties** and click **View Test Log**. See "Self Test Logs" on page 138 for more information.

Viewing and Changing System Properties

Select Instrument and click Properties to display system properties which include:

- Name
- Location
- Type
- Serial number
- Instrument software version (firmware)
- Network name and address
- Date and time of the instrument clock
| 🔞 Peggy SW10 | 02 Properti | es | | × |
|---------------|--------------|--------------------------|-----------------------------|--------|
| | | | | |
| Name: | Peggy SW1 | 1002 | | |
| Location: | | | | |
| | | | | |
| | Type: | Peggy | Network Name: sw1002.local. | |
| Seri | al Number: | SW1002 | Network Address: 10.1.2.158 | |
| Instrumer | nt Software: | 2.0.16919 | | |
| | | | | |
| Instrum | ent Date and | d Time | | |
| | 201 | 2 10 10 12 45 20 07 00 | | |
| | 201 | 12-10-18 12:45:29 -07:00 | Set to PC time | |
| | | | | - |
| | | | | |
| View Error Lo | g View | l est Log | OK | Cancel |
| | | | | |

- To change system name or location click in the name or location boxes and enter the new information.
- To sync the instrument clock with the computer click Set to PC time.

Viewing Log Files

Error Logs

- 1. Select Instrument in the main menu and click Properties to display system properties.
- 2. Click **View Error Log**. A list of system logs will display:

page	136
puge	150

Name	Date	Size	
embedded.log	2012-10-30 13:59	4729499	
temperature.log	2012-10-30 13:58	24198	
temperature.log.2012-10-29	2012-10-30 09:39	134780	
lamp.log	2012-10-30 03:28	153166	
error.log	2012-10-29 17:06	873831	
temperature.log.2012-10-28	2012-10-29 09:39	134811	
temperature.log.2012-10-27	2012-10-28 09:38	134807	
temperature.log.2012-10-26	2012-10-27 09:37	134725	
temperature.log.2012-10-25	2012-10-26 09:37	532087	
stdout.log	2012-10-24 06:54	244772	
temperature.log.2012-10-21	2012-10-22 10:44	134793	
temperature.log.2012-10-20	2012-10-21 10:43	134782	
embedded.log.1	2012-10-02 21:50	8388603	
embedded.log.2	2012-07-23 19:26	8388540	
embedded.log.3	2012-05-24 23:12	8388565	
embedded.log.4	2012-03-31 07:00	8388547	

3. Select a log file and click **View**. The log details will display:

2011-09-25 10:	19:30,793 temperatu	re: INFO SetPoint(C),23.0,Chamber(C),24.99,Ambient(0	C),24.99,LookupF	• WM(%)8
2011-09-25 10:	19:35,860 temperatu	re: INFO SetPoint(C),23.0,Chamber(C),24.99,Ambient((),24.99,LookupF	WM(%)8
2011-09-25 10:	19:40,878 temperatu	re: INFO SetPoint(C),23.0,Chamber(C),24.99,Ambient((),24.99,LookupF	WM(%)8
2011-09-25 10:	19:45,896 temperatu	re: INFO SetPoint(C),23.0,Chamber(C),24.99,Ambient(0),24.99,LookupF	PWM(%)8
2011-09-25 10:	19:50,911 temperatu	re: INFO SetPoint(C),23.0,Chamber(C),24.99,Ambient(0),24.99,LookupF	PWM(%)8
2011-09-25 10:	19:55,925 temperatu	re: INFO SetPoint(C),23.0,Chamber(C),24.99,Ambient(0),24.99,LookupF	PWM(%)8
2011-09-25 10:	20:00,941 temperatu	re: INFO SetPoint(C),23.0,Chamber(C),24.99,Ambient((),24.99,LookupF	WM(%)8
2011-09-25 10:	20:05,957 temperatu	re: INFO SetPoint(C),23.0,Chamber(C),24.99,Ambient(0),24.99,LookupF	PWM(%)8
2011-09-25 10:	20:10,971 temperatu	re: INFO SetPoint(C),23.0,Chamber(C),24.99,Ambient(0),24.99,LookupF	PWM(%)8
2011-09-25 10:	20:15,987 temperatu	are: INFO SetPoint(C),23.0,Chamber(C),24.99,Ambient(0),24.99,LookupF	PWM(%)8
2011-09-25 10:	20:21,003 temperatu	re: INFO SetPoint(C),23.0,Chamber(C),24.99,Ambient(0),24.99,LookupF	PWM(%)8
2011-09-25 10:	20:26,019 temperatu	are: INFO SetPoint(C),23.0,Chamber(C),24.99,Ambient(0),24.99,LookupF	PWM(%)8
2011-09-25 10:	20:31,035 temperatu	are: INFO SetPoint(C),23.0,Chamber(C),24.99,Ambient(0),24.99,LookupF	PWM(%)8
2011-09-25 10:	20:36,049 temperatu	are: INFO SetPoint(C),23.0,Chamber(C),24.99,Ambient(0),24.99,LookupF	PWM(%)8
2011-09-25 10:	20:41,062 temperatu	ure: INFO SetPoint(C),23.0,Chamber(C),24.99,Ambient((C),24.99,LookupF	2WM(%)8
2011-09-25 10:	20:46,077 temperatu	ure: INFO SetPoint(C),23.0,Chamber(C),24.99,Ambient((C),24.99,LookupF	2WM(%)8
2011-09-25 10:	20:51,093 temperatu	re: INFO SetPoint(C),23.0,Chamber(C),24.99,Ambient(0	C),24.99,LookupF	2WM(%)8
2011-09-25 10:	20:56,110 temperatu	re: INFO SetPoint(C),23.0,Chamber(C),24.99,Ambient(0	C),24.99,LookupF	2WM(%)8
2011-09-25 10:	21:01,128 temperatu	ure: INFO SetPoint(C),23.0,Chamber(C),24.99,Ambient(0	C),24.99,LookupF	2WM(%)8
2011-09-25 10:	21:06,146 temperatu	ure: INFO SetPoint(C),23.0,Chamber(C),24.99,Ambient(0	C),24.99,LookupF	2WM(%)8
2011-09-25 10:	21:11,163 temperatu	ure: INFO SetPoint(C),23.0,Chamber(C),24.99,Ambient(0	C),24.99,LookupF	2WM(%)8
2011-09-25 10:	21:16,180 temperatu	ure: INFO SetPoint(C),23.0,Chamber(C),24.99,Ambient(0	C),24.99,LookupF	200 WM
2011-09-25 10:	21:21,197 temperatu	re: INFO SetPoint(C),23.0,Chamber(C),24.99,Ambient(0),24.99,LookupF	20/WW
2011-09-25 10:	21:26,215 temperatu	re: INFO SetPoint(C),23.0,Chamber(C),24.99,Ambient(0	.),24.99,LookupF	2WM(%)8
2011-09-25 10:	21:31,232 temperatu	re: INFO SetPoint(C),23.0,Chamber(C),24.99,Ambient(0	.),24.99,LookupF	2WM(%)8
2011-09-25 10:	21:36,250 temperatu	ire: INFO SetPoint(C),23.0,Chamber(C),24.99,Ambient(0	.),24.99,LookupH	2007/WM
2011-09-25 10:	21:41,267 temperatu	ire: INFO SetPoint(C),23.0,Chamber(C),24.99,Ambient(0	.),24.99,LookupH	2007/WM
2011-09-25 10:	21:46,282 temperatu	ire: INFO SetPoint(C),23.0,Chamber(C),24.99,Ambient(0	.),24.99,LookupF	2001/00/08
2011-09-25 10:	21:51,298 temperatu	Ire: INFO SetPoint(C),23.0,Chamber(C),24.99,Ambient(0	.),24.99,LookupH	/WIVI(%)8
2011-09-25 10:	21:50,313 temperatu	Ire: INFO SetPoint(C),23.0,Chamber(C),24.99,Ambient(0	.),24.99,LookupH	/WIVI(%)8
2011-09-25 10:	22:01,329 temperatu	Ire: INFO SetPoint(C),23.0,Chamber(C),24.99,Amblent(0	2),24.99,LOOKUPF	VVIVI(76)8
2011-09-25 10:	22:00,344 temperatu	Ire: INFO SetPoint(C),23.0,Chamber(C),24.99,Amblent(0	.),24.99,LOOKUPH	VVIVI(76)8
2011-09-25 10:	22:11,500 temperatu 22:16 277 temperatu	ITE: INFO SetPoint(C) 22.0, Chamber(C),24.99,Amblent((2),24.99,LOOKupP	VVIVI(/6/0
2011-09-25 10:	22:10,577 temperatu 22:21,204 temperatu	ITE: INFO SetPoint(C),25.0,Chamber(C),24.99,Amblent((2),24.99,LOOKupP	VVIVI(/6/0
2011-09-25 10:	22:21,594 temperatu 22:26 412 temperatu	ITE: INFO SetPoint(C),25.0,Chamber(C),24.99,Amblent((2),24.99,LOOKupP	VVIVI(/6/0
2011-09-25 10:	22:20,412 temperatu 22:21,420 temperatu	ITE: INFO SetPoint(C),25.0,Chamber(C),24.99,Amblent((2),24.99,LOOKupP	VVIVI(/6/0
2011-09-25 10:	22:51,429 temperatu 22:26 446 temperatu	Ine: INFO SetPoint(C),25.0,Chamber(C),24.99,Ambient(() 24.99,Ambient((2),24.99,LOOKupF	20/0/0/ (20/0/ 20/
2011-09-25 10:	22:50,440 temperatu 22:41 462 temperatu	Ine: INFO SetPoint(C),25.0,Chamber(C),24.99,Ambient(() 24.99,Ambient((2),24.99,LOOKupF	20/0/0/ (20/0/ 20/
2011-09-25 10:	22:41,405 temperatu 22:46,490 temperatu	re: INFO SetPoint(C),25.0,Chamber(C),24.99,Ambient(() 24.00 Ambient((-),24.99,LOOKupF -) 24.99 LookupF	2)/////(///////////////////////////////
2011-03-25 10.	22.40,400 temperatu	ile. IN O SetFollit	c),25.0,Chamber(C	,24.35,Amblenu(.),24.55,200Kupr	
•						•

4. Click **Save File As** to save a copy of the log file.

Self Test Logs

- 1. Select Instrument in the main menu and click Properties to display system properties.
- 2. Click **View Test Log**. A list of self test logs will display:

😵 PL0003 S	elf Tests		23
Test Date		Status	
2012-10-23	_14.33.20	PASSED	
2012-10-11	_13.11.50	PASSED	
2012-10-10	_16.22.29	FAILED	
2012-10-10	_16.15.12	FAILED	
2012-10-10	_14.25.48	STOPPED	
2012-10-10	_14.24.41	FAILED	
2012-10-05	_14.05.02	PASSED	
2012-10-05	_14.02.32	PASSED	
2012-10-05	_13.59.20	PASSED	
2012-06-19	_16.05.22	PASSED	
		View	Done

3. Select a log file and click **View**. The individual test details will display:

() Self test: 2012-06-15_12.00.22_PASS	ED.txt				- ×
Name	Start	Duration (sec)	Result	Failure Reason	
Dark Masters	12:00:21	0.024	PASSED		
Camera	12:00:10	11.720	PASSED		
Fan Temp Sensors	12:00:08	0.056	PASSED		
Level	12:00:08	0.161	PASSED		E
Filter Not In	12:00:07	0.143	PASSED		
Filter Not Out	12:00:06	0.163	PASSED		
Filter Out	12:00:04	0.593	PASSED		
Filter In	12:00:03	0.665	PASSED		
LED Low Not On	12:00:02	0.025	PASSED		
LED Low On	12:00:00	0.017	PASSED		
LED Not On	11:59:59	0.016	PASSED		
LED On	11:59:58	0.016	PASSED		
Voltage On	11:59:48	8.965	PASSED		
Lid Remover Not Down	11:59:47	0.174	PASSED		
Lid Remover Not Up	11:59:46	0.147	PASSED		
Lid Remover Up	11:59:44	0.468	PASSED		
Lid Remover Down	11:59:43	0.309	PASSED		
Pipet Not Down	11:59:41	0.052	PASSED		
Pipet Not Up	11:59:40	0.053	PASSED		
Pipet Up	11:59:39	0.248	PASSED		
Pipet Down	11:59:38	0.321	PASSED		-
				Save File As	Cancel

4. Click **Save File As** to save a copy of the log file.

Status Modes

The instrument status bar displays status, buttons and progress bars depending on what Wes, Sally Sue or Peggy Sue is doing.

- **Ready/Start button** The instrument is ready and an assay is loaded. Click **Start** to begin a run.
- Not Ready/Clean button The instrument is not ready and must perform system cleaning. Click Clean to start the cleaning protocol.
- Not Ready/Reset button The instrument is not ready and must reinitialize. Click Reset to start the initialization protocol.
- **Running/Stop button** The instrument is running an assay. The run name, time the run started and when it will complete display in the run progress bar. Click **Stop** to stop the run.
- **Cleaning/button not active** The instrument is running a cleaning protocol. The time the cleaning protocol started and when it will complete display in the run progress bar.
- Error/Reset button An error has occurred. Go to the Status window in the Run Summary screen to view details. When the source of the error is corrected, click Reset.

Chapter 8: Size Assay Data Analysis

Chapter Overview

- Analysis Screen Overview
- Opening Run Files
- How Run Data is Displayed in the Analysis Screen
- Viewing Run Data
- Compass Run Data Notifications and Warnings
- Checking Your Results
- Group Statistics
- Copying Data Views and Results Tables
- Exporting Run Files
- Changing Sample Protein Identification
- Changing the Virtual Blot View
- Changing the Electropherogram View
- Closing Run Files
- Compass Analysis Settings Overview
- Advanced Analysis Settings
- Images Analysis Settings
- Peak Fit Analysis Settings
- Peak Names Settings
- Standard Curve Settings
- System or Loading Control Settings

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- Standard Curve Settings
- Importing and Exporting Analysis Settings

Analysis Screen Overview

The Analysis screen is used to view run data including electropherograms, capillary images, lane view data and tabulated results. Any post-run analysis is also done in this screen. To access this screen, click **Analysis** in the screen tab:

📑 Assay 🔮 Run Summary 🚛 Analysis

Analysis Screen Panes

The Analysis screen has six panes, each displays the following data for up to 96 capillaries per experimental run:

- Experiment Lists the assay protocol steps and assay template information.
- **Graph** Displays electropherogram data for sample proteins, fluorescent standards or capillary registrations.
- **Image** Displays a 12-capillary image of the separated sample proteins, fluorescent standards or capillary registrations.
- Lane Displays data for sample proteins, fluorescent standards or capillary registrations as bands in individual lanes. This virtual blot-like image is similar to traditional blot results.
- **Peaks** Lists the tabulated results for sample proteins and information for identified fluorescent standards and capillary registrations.
- **Capillaries** Displays a list of the sample proteins Compass named automatically using the userdefined peak name analysis parameters.

6	2013-11-2	7_15-05-4	8_Wes_recAKT	1_1200V_A9	27_C1234_	34308 -	Compass								- 0	x
F	ile Edit \	View Inst	rument Wine	dow Help												
	🗮 Standar	rds 🚖 Sa	mples									📑 Assay	🕒 Run Sum	imary 🚺	Analysi	is
	Experi	- 0	🖳 Graph 🔒	🛙 Image 🔳	Lane L	Std C	urve						۲	H2 8F	۵ 🖌	
Ir	Sample	Primary		*												
	✓ Biotin	None		Ladde												
	Jurkat	Anti-A.		Naleo.												
	Jurkat	Anti-A	ni ^{di}	res when when	t wheat wh	or where	what what	where where	where where	wheat wheat w	that what wh	at what what				
	Jurkat	Anti-A	kDa 💎	30 30	20 20	20	20 20	20 20	3° 3°	3° 3° 3'	- 30 30	20 20				
	Jurkat	Anti-A.	230													
	Jurkat	Anti-A	180-													
	Jurkat	Anti-A														
	🗸 Jurkat	Anti-A	116													
	Jurkat	Anti-A					_			_	_					
	Jurkat	Anti-A	66													
	Jurkat	Anti-A														
	🗸 Jurkat	Anti-A														
	Jurkat	Anti-A	40-													
	Jurkat	Anti-A														
	Jurkat	Anti-A														
	Jurkat	Anti-A														
	Jurkat	Anti-A														
	Jurkat	Anti-A	12-													
	Jurkat	Anti-A														
					~											
			Peaks	Capillari	es											
			Sample	Primary	Cap	Peak	Name	Position	MW (kDa)	Height	Conc	Area	% Area	Width	S/N	В ^
			Biotinylat	None	C1:1	5	Ldr 180	607	180	2000.6		23016		10.8	363.8	
			Biotinylat	None	C1:1	6	Ldr 230	636	230	1538.2		36941		22.6	206.1	
			Jurkat	Anti-A	C1:4	1	AKT1	495	64	4912.5	20.9	57238	24.3	10.9	237.8	
			Jurkat	Anti-A	C1:4	2	GST-A	533	95	16088.3	63.8	178251	75.7	10.4	795.3	
			Jurkat	Anti-A	C1:5	1	AKT1	495	63	4347.2	18.8	51364	39.4	11.1	236.3	
			Jurkat	Anti-A	C1:5	2	GST-A	533	94	7288.2	28.6	79051	60.6	10.2	405.5	-
	< III	F.	•						m							F.
											1					
											1					

NOTE: The reported molecular weight for sample proteins in Compass may vary slightly from predicted molecular weights based on sample and assay conditions.

Software Menus Active in the Analysis Screen

The following software menus are available:

- File
- Edit
- View
- Instrument (when Compass is connected to Wes, Sally Sue or Peggy Sue)
- Window
- Help

The File, Edit and View menu options specific to the Analysis screen are described next.

File Menu

The following File menu options are active:



- **Open Run** Opens a run file.
- Add Run Opens and views other run files in addition to those that are already open.
- Close Closes the run file currently being viewed.
- Close All Closes all open run files.
- Save Saves changes to the open run file.
- Save As Saves changes to the open run file under a different file name.
- Export Tables Exports the results for all capillaries in the run in .txt format.
- Export Spectra Exports the raw data traces for each capillary in the run in .txt or .cdf format.
- Exit Closes Compass.

Edit Menu

The following Edit menu options are active:

Edit) View Instru	ment	Windo
	Cut		
	Сору	Cti	rl+C
	Paste	Ct	rl+V
	Analysis Preferences		

- **Copy** Lets you copy data shown in the graph, lane, peaks or capillaries panes. See "Copying Data Views and Results Tables" on page 194 for more information.
- **Analysis** Displays the analysis settings used to analyze the run data and lets you change them as needed. See "Compass Analysis Settings Overview" on page 232 for more information.
- **Preferences** Lets you set and save custom preferences for data export, plot colors in the graph and Twitter settings. See Chapter 10, "Setting Your Preferences" for more information.

View Menu

The following View menu options are active:



- Single View Displays data in a per capillary (single) view format.
- Multiple View Displays data in a per 12- or 25-capillary (multiple) view format.
- Standards Lets you change the data view to show only the fluorescent standards.
- **Registration** Lets you change the data view to show only the capillary registrations (Sally Sue and Peggy Sue only).
- Samples Lets you change the data view to show sample proteins.
- Filter Lets you display data only for specific capillaries or named proteins.
- View Region Lets you change the molecular weight (x-axis) range of the data displayed.

Show Hidden- Shows capillaries that are hidden from the data view.

Opening Run Files

You can open one or multiple run files at a time to compare data between runs.

Opening One Run File

NOTE: If you need to open a run file when another run is executing, launch another instance of Compass first, then open the file.

1. Select **File** in the main menu and click **Open Run**.

Open Run	•	2013-11-18_13-30-23_Wes_Hela_1200V_A730_C998_35268
Add Run	+	DemoData_Charge_HeLa_ERK12
Close		Demo data_Hi-Low ERK (old)
Close All		2013-11-25_PI3Kdose-HeLa_lookup AC_33849_SW0018
Close All		Antibody screen 2013-10-17_14-12-31_eg10-14-13-1
Save		2013-11-27_15-05-48_Wes_recAKT1_1200V_A927_C1234_34308
Save As		2013-11-27_15-05-48_Wes_recAKT1_1200V_A927_C1234_34308
Export Tables		2013-12-26_Wes2008_Hela_ERK1-DIG_c1251
Export Spectra		2013-11-21_12-32-43_Wes_Hela_1200V_A914_C1148_34123
Exit		4691 and 4060 vs both lysates 2013-10-21_14-38-13_eg10-14-13-1
Hela DI3 Kin	1	Browse

2. A list of the last 10 runs opened will display. Select one of these runs or click **Browse** to open the Runs folder and select a different file.

Opening Multiple Run Files

NOTE: If you need to open a run file when another run is executing, launch another instance of Compass first, then open the file.

1. To open the first run file, select **File** in the main menu and click **Open Run**.



- 2. A list of the last 10 runs opened will display. Select one of these runs or click **Browse** to open the Runs folder and select a different file.
- 3. To open another run file, select File in the main menu and click Add Run.

File	Edit View Inst	rumen	Window Help
	Open Run	+	
	Add Run	+	2013-11-18_13-30-23_Wes_Hela_1200V_A730_C998_35268
	Close		Antibody screen 2013-10-17_14-12-31_eg10-14-13-1
	Close All		2013-11-27_15-05-48_Wes_recAKT1_1200V_A927_C1234_34308
	crose rui		2013-12-26_Wes2008_Hela_ERK1-DIG_c1251
	Save		2013-11-25_PI3Kdose-HeLa_lookup AC_33849_SW0018
	Save As		2013-11-21_12-32-43_Wes_Hela_1200V_A914_C1148_34123
	Export Tables		4691 and 4060 vs both lysates 2013-10-21_14-38-13_eg10-14-13-1
	Export Spectra	•	Antibody dilution2013-10-21_10-53-43_eg10-14-13-1
	Exit		Browse

4. A list of the last 10 runs opened will display. Select one of these runs or click **Browse** to open the Runs folder and select a different file. When a run is added, its data will append to the open run file, and will display as a second set of up to 25 capillaries (Wes) or 96 capillaries (Sally Sue/Peggy Sue) in all screen panes. The second run file name will also appear in the Compass title bar:



5. Repeat the last two steps to add additional runs.

How Run Data is Displayed in the Analysis Screen

Data in the run file is compartmentalized for easy review in one of six panes in the Analysis screen.

Experiment Pane: Assay and Capillary Information

The experiment pane displays assay and capillary information for each of the 25 capillaries (Wes) or 96 capillaries (Sally Sue/Peggy Sue) in the run. A maximized view of the experiment pane is shown below.

Experiment								
Sample	Primary	Cycle	Сар	s	1	2	3	
 Biotinylated Ladder 	Blocking	1	1	Cl	D1	E1	F1	
✓ K562	anti-ERK1/2	1	2	C2	D2	E2	F2	
K562	anti-ERK1/2	1	3	C3	D3	E3	F3	
✓ K562	anti-ERK1/2	1	4	C4	D4	E4	F4	
✓ K562	anti-ERK1/2	1	5	C5	D5	E5	F5	
✓ K562	anti-ERK1/2	1	6	C6	D6	E6	F6	
K562	anti-ERK1/2	1	7	C7	D7	E7	F7	
K562	anti-ERK1/2	1	8	C8	D8	E8	F8	
K562	anti-ERK1/2	1	9	C9	D9	E9	F9	
K562	anti-ERK1/2	1	10	C10	D10	E10	F10	
K562	anti-ERK1/2	1	11	C11	D11	E11	F11	
RTU K562	anti-ERK1/2	1	12	C12	D12	E12	F12	

- To view all columns Click the Experiment tab, then use the scroll bar or click Maximize in the upper right corner.
- To resize columns Click the Experiment tab. Roll the mouse over a column border until the sizing arrow appears, then click and drag to resize.

Experiment pane column descriptions for the default assay are as follows:

• **Sample** - Sample name. If sample names were entered in the assay template (Assay screen), those names will display here. Otherwise, Sample (default name) will display.

NOTE: Data notification icons will display in the sample column if Compass detected a potential analysis issue or data was manually modified by the user. For more information see "Compass Run Data Notifications and Warnings" on page 176.

- **Primary** Primary antibody name. If primary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Primary (default name) will display.
- **Cycle** Run cycle number. There are There are 25 capillaries in one cycle for Wes and 12 capillaries in one cycle for Sally Sue and Peggy Sue.

NOTE: Sally Sue and Peggy Sue can run up to eight cycles (12 capillaries per cycle) in an experiment. Each cycle is designated in the Experiment summary.

- **Cap** Capillary number.
- **S** Well on the assay plate used for sample.
- 1 Well on the assay plate used for primary antibody or Total Protein labeling reagent.
- 2 Well on the assay plate used for secondary HRP-conjugate or Total Protein Streptavidin-HRP.

Graph Pane: Electropherogram Data

Click the **Graph** tab to view data for sample proteins, fluorescent standards or capillary registrations. Data for samples is shown in the following example, and proteins are displayed as peaks:



More Graph view options will be described in more detail in "Changing the Electropherogram View" on page 209.

Image Pane: Capillary Separation Image Data

Click the **Image** tab to view final separation images of sample proteins, fluorescent standards or capillary registrations for up to 96 capillaries per experimental run. Image data for samples is shown in the following example:

raph 😢 Image 🔠 Lane	
Cycle 1, Samples, Exp. 30.0	

Lane Pane: Virtual Blot-Like Image Data

Click the **Lane** tab to view data for sample proteins, fluorescent standards or capillary registrations as bands in individual lanes. This virtual blot-like image is similar to traditional blot results. Data for samples in the lane view for an Immunoassay (immunodetected proteins) and from a Total Protein Assay are displayed as bands in the examples below.



To view information for a band, roll the mouse over a band until the info box appears.

NOTE: The reported molecular weight for sample proteins detected by an Immunoassay or a Total Protein Assay in Compass may vary slightly from predicted molecular weights based on sample and assay conditions.

Lane data displayed in the virtual blot is automatically aligned by Compass. To view raw, unaligned lane data and learn more about virtual blot viewing options, see "Changing the Virtual Blot View" on page 203.

Std Curve Pane: Standard Curve Fit Data

Click the **Std Curve** tab to view a linear or 4-parameter curve fit of your standard curve proteins. Data for the samples in the std curve view is shown in the following example where proteins are displayed as dots.



Concentration of the sample proteins is automatically determined by Compass based on defined values of the standard curve.

Peaks Pane: Calculated Results

Click the **Peaks** tab to view tabulated results for sample proteins, fluorescent standards or capillary registrations. Each row in the table shows the individual results for each peak detected in each capillary. Data for samples in the peaks table for both an Immunoassay and a Total Protein Assay are shown in the following examples:

🗰 Peaks 🛛 🛄	Capillaries											
Sample	Primary	Cap	Peak	Name	Position	MW (kDa)	Height	Area	% Area	Width	S/N	•
Biotinylat	Blocking	C1:1	5	Ldr 116	761	116	3175.2	59215		17.5	207.0	
Biotinylat	Blocking	C1:1	6	Ldr 180	865	180	3775.6	78654		19.6	229.9	Ξ
K562	anti-E	C1:2	1	ERK2	522	43	1380.3	21641	79.6	14.7	289.7	
K562	anti-E	C1:2	2	ERK1	549	47	353.2	5552	20.4	14.8	35.9	
K562	anti-E	C1:3	1	ERK2	528	43	1400.1	21546	78.8	14.5	205.6	
K562	anti-E	C1:3	2	ERK1	555	47	342.7	5792	21.2	15.9	25.7	
K562	anti-E	C1:4	1	ERK2	518	43	1373.1	21542	79.1	14.7	304.9	
K562	anti-F	C1:4	2	FRK1	545	47	359.6	5700	20.9	14.9	42.0	Ŧ

Peaks	📙 Peaks 🛛 🛄 Capillaries 🔤 🗖														
Sample	Primary	Cap	Peak	Name	Position	MW (kDa)	Height	Area	% Area	% Total	Width	S/N	Baseline 🔺		
Biot. Lad	Antibo	C1:1	5	Ldr 180	632	180	3547.2	66440		16.56	29.0	148.9	1034.6		
Biot. Lad	Antibo	C1:1	6	Ldr 230	657	230	3272.5	60980		15.20	35.0	138.2	1034.5		
HeLa	Antibo	C1:2	8	DNAK	536	73	1621.6	41485	100.0	14.11	31.0	18.8	1264.3		
HeLa + 3	Antibo	C1:3	7	DNAK	536	74	7017.3	131139	100.0	33.17	41.0	370.6	1408.6		
HeLa + 1	Antibo	C1:4	10	DNAK	536	75	5663.3	122330	100.0	23.36	41.0	219.0	1088.8		
HeLa + 7	Antibo	C1:5	8	DNAK	536	74	3467.4	77588	100.0	21.14	37.0	115.8	1310.0		
HeLa + 3	Antibo	C1:6	10	DNAK	536	74	2452.4	58625	100.0	17.03	35.0	71.7	1313.9		
HeLa	Antibo	C1:7	7	DNAK	533	72	1379.5	36572	100.0	14.21	32.0	17.3	1356.2 _		
HeLa + 3	Antibo	C1:8	8	DNAK	537	75	6249.9	113059	100.0	29.93	39.0	306.9	1322.4		
HeLa + 1	Antibo	C1:9	9	DNAK	537	75	5472.6	104042	100.0	21.65	36.0	162.8	1134.9		

NOTES:

Peaks that Compass names automatically using the user-defined peak name analysis parameters are color-coded.

The reported molecular weight for sample proteins detected using an Immunoassay or a Total Protein Assay in Compass may vary slightly from predicted molecular weights based on sample and assay conditions.

- To view all rows Click the Peaks tab, then use the scroll bar or click Maximize in the upper right corner.
- To resize columns Click the Peaks tab. Roll the mouse over a column border until the sizing arrow appears, then click and drag to resize.

Peak table column descriptions are as follows:

- **Sample** Sample name. If sample names were entered in the assay template (Assay screen), those names will display here. Otherwise, Sample (default name) will display.
- **Primary** Primary antibody name. For Immunoassays, if primary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Primary (default name) will display. For Total Protein Assays, Antibody Diluent will display as the default name.
- **Cap** Cycle and capillary number. For example, C1:3 indicates cycle 1, capillary 3.

NOTE: Sally Sue and Peggy Sue run up to eight cycles in an experiment, 12 capillaries at a time. Capillary and cycle numbers are displayed in the Experiment tab.

- Peak Peak number. Peaks are numbered in order of detection.
- **Name** Peak name. Displays peaks that Compass named automatically using the user-defined peak name analysis parameters. Cells will be blank if Compass was not able to name the peak or if naming parameters were not entered.
- **Position** Displays the pixel position of a peak in the image.
- **MW (kDa)** Displays the calculated molecular weight in kDa for the peak (shown for sample data only).
- Height Displays the calculated peak height.
- Area Displays the calculated peak area (shown for sample data only).
- % Area Reported when area is calculated using the Gaussian method (default for Immunoassays, see "Peak Find Settings" on page 245 for more information). Displays the calculated percent area for the named peak compared to all named peaks. This value results from dividing the individual peak area by the sum of all named peak areas for the capillary and multiplying by 100 (shown for named peak sample data only).
- **% Total** Reported when area is calculated using the Dropped Line method (default for Total Protein Assays, see "Peak Find Settings" on page 245 for more information). Displays the calculated percent area for the named peak compared to the total area measured in the capillary. This value results from dividing the individual peak area by the entire area under the curve for the capillary and multiplying by 100.
- **Conc (Concentration)** Displays the calculated concentration of protein for the named peak. This column will not appear unless a standard curve is defined.
- **Corr. Area** Displays the calculated area for the named peak compared to the area for the loading control. This column will not appear unless a loading control is defined.
- Width Displays the calculated peak width (shown for sample data only).
- S/N Displays the calculated signal to noise ratio for the peak (shown for sample data only).

Capillaries Pane: User-Specified Peak Names

Click the **Capillaries** tab to view tabulated results for sample proteins associated with specific primary antibodies or total protein detection in the run data. Compass labels these sample peaks automatically using user-defined peak name settings. Each row in the table shows the individual results for the named peaks detected in each capillary. Data for samples in the capillaries table is shown in the following example for an Immunoassay with Gaussian fit.

Peaks Capillaries												
					Area Area % Corr. Area Cor							
Sample	Primary	Capillary	System Co	ERK1								
HeLa	ERK1+	C1:2	44704	55899								
HeLa	ERK1+	C1:3	42886	63846								
HeLa	ERK1+	C1:4	50291	65303								
HeLa	ERK1+	C1:5	45954	57780								
HeLa	ERK1+	C1:6	45887	54196								
HeLa	ERK1+	C1:7	48500	68243								
HeLa	ERK1+	C1:8	47257	56120								
HeLa	ERK1+	C1:9	49193	59797								
HeLa	ERK1+	C1:10	47426	57923								
HeLa	ERK1+	C1:11	42898	45543								
HeLa	ERK1+	C1:12	43542	49701								

For Total Protein Assays where area calculation is handled via Dropped Lines by default, the Capillaries tab is shown below. Compass labels the sample peaks automatically using user-defined peak name settings. Each row in the table shows the individual results for the named peaks detected in each capillary.

Peaks 🗍	Capillaries				
					% Total Area % Ar
Sample	Primary	Capillary	Total Area	DNAK	
HeLa	Antibo	C1:2	294092	14.11	
HeLa + 3	Antibo	C1:3	395361	33.17	
HeLa + 1	Antibo	C1:4	523599	23.36	
HeLa + 7	Antibo	C1:5	367106	21.14	
HeLa + 3	Antibo	C1:6	344206	17.03	
HeLa	Antibo	C1:7	257414	14.21	
HeLa + 3	Antibo	C1:8	377685	29.93	
HeLa + 1	Antibo	C1:9	480506	21.65	
HeLa + 7	Antibo	C1:10	483433	20.36	
	a	~ ~ ~	acarar	10.11	

NOTES:

Peaks that Compass names automatically with user-defined peak name settings are color-coded.

Information displayed for fluorescent standards and capillary registration data will be for identified standards or registration peaks.

- To view all rows Click the Capillaries tab, then use the scroll bar or click Maximize in the upper right corner.
- To resize columns Click the Capillaries tab. Roll the mouse over a column border until the sizing arrow appears, then click and drag to resize.

Capillaries table column descriptions are as follows:

- **Sample** Sample name. If sample names were entered in the assay template (Assay screen), those names will display here. Otherwise, Sample (default name) will display.
- **Primary** Primary antibody name. If primary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Primary (default name) will display.
- Capillary Cycle and capillary number. For example, C1:3 indicates cycle 1, capillary 3.

NOTE: Sally Sue and Peggy Sue run12 capillaries at a time in a cycle and are able to run up to eight cycles in an experiment. The information on cycle and capillary number is displayed in the Experiment tab.

- **Peak Name Columns** An individual column per peak name will display for every peak identified by name in the run data. Cells for capillaries in these columns will be blank if Compass did not find peaks automatically using the user-defined peak name analysis parameters (or none were entered).
 - To view % area in the peak name columns the Gaussian Fit area calculation setting must first be selected. To do this, select **Analysis** from the **Edit** menu, then select **Peak Fit** page. In the Peak Find box, select **Gaussian Fit** for the Area Calculation setting (default for Immunoassays). Next, select **Area** % in the upper right corner of the Capillaries pane. This displays the calculated percent area for the named peak compared to all named peaks. This value results from dividing the individual peak area by the sum of all named peak areas for the capillary and multiplying by 100.

Peaks	Capillaries				- D
					Area 🛛 Krea % Corr. Area Conc 🌣
Sample	Primary	Capillary	System Co	ERK1	A
HeLa	ERK1+	C1:2	44704	55899	
HeLa	ERK1+	C1:3	42886	63846	_
HeLa	ERK1+	C1:4	50291	65303	=
HeLa	ERK1+	C1:5	45954	57780	
HeLa	ERK1+	C1:6	45887	54196	
HeLa	ERK1+	C1:7	48500	68243	
HeLa	ERK1+	C1:8	47257	56120	
HeLa	ERK1+	C1:9	49193	59797	
HeLa	ERK1+	C1:10	47426	57923	
HeLa	ERK1+	C1:11	42898	45543	
HeLa	ERK1+	C1:12	43542	49701	

- To view peak area in the peak name columns (default) Select Area in the upper right corner of the pane. This displays calculated peak area for the individual peak only.
- To view corrected peak area in the peak name columns Select Corr. Area in the upper right corner of the pane. This displays the corrected peak area for the named peak compared with the loading control peak.
- To view concentration in the peak name columns Select Conc. in the upper right corner of the pane. This displays the calculated concentration for the named peak compared with the standard curve.
- To view % total in the peak name columns The Dropped Lines area calculation setting must first be selected. To do this, select **Analysis** from the **Edit** menu, then select **Peak Fit** page. In the Peak Find box, select **Dropped Lines** for the Area Calculation setting (default for Total Protein Assays). Next, select % **Total** in the upper right corner of the Capillaries pane. This displays the calculated percent total for the named peak compared to the total area measured in the capillary. This value results from dividing the individual peak area by the entire area under the curve for the capillary and multiplying by 100.

Peaks 📗	Capillaries				
					% Total Area % Area
Sample	Primary	Capillary	Total Area	DNAK	
HeLa	Antibo	C1:2	294092	14.11	
HeLa + 3	Antibo	C1:3	395361	33.17	
HeLa + 1	Antibo	C1:4	523599	23.36	
HeLa + 7	Antibo	C1:5	367106	21.14	
HeLa + 3	Antibo	C1:6	344206	17.03	
HeLa	Antibo	C1:7	257414	14.21	
HeLa + 3	Antibo	C1:8	377685	29.93	
HeLa + 1	Antibo	C1:9	480506	21.65	
HeLa + 7	Antibo	C1:10	483433	20.36	
	A 144	~ ~ ~ ~	acarar		

Viewing Run Data

Each run file contains the following data for up to 96 capillaries:

- Sample data For the proteins in the sample.
- Standards data For the fluorescent standards run with each sample.
- **Registration data (Sally Sue and Peggy Sue only)** For tracking capillaries as they are moved for various assay steps.

Data can be viewed for one, all, or individually selected capillaries.

Switching Between Sample, Standards and Registration Data Views

You can switch between viewing sample, standards and registration data in a run file using the View bar or View menu:



Data buttons in the View bar:



Show Standards



Show Registrations



Show Samples



To view sample data - Click Show Samples in the View bar or select View in the main menu and click Samples:

- Data in this view is for sample proteins only.
- Graph view data displays electropherograms in chemiluminescence units (y-axis) and molecular weight in kDa (x-axis).
- Lane view data displays sample proteins only.
- Image view data displays sample proteins only.
- Results for each protein are shown in the peaks and capillaries tables.

NOTE: The reported molecular weight for sample proteins detected by an Immunoassay or a Total Protein Assay in Compass may vary slightly from predicted molecular weights based on sample and assay conditions.

For information on checking and identifying sample peaks, see "Step 4 – Checking the Ladder" on page 183 or "Step 5 – Checking Samples" on page 185.

To view standards data - Click Show Standards in the View bar or select View in the main menu and click Standards:



- Data in this view is for the fluorescent standards only. These standards are premixed with each sample prior to analysis.
- Graph view data displays electropherograms in fluorescence (y-axis) and position (x-axis).
- Lane view data displays fluorescent standards only.
- Image view data displays fluorescent standards only.
- Standards are highlighted in both the peaks and capillaries tables and marked with an S.

For information on checking and identifying standards peaks, see "Step 2 – Checking Fluorescent Sizing Standards" on page 178.

To view registration data (Sally Sue and Peggy Sue only) - Click Show Registrations in the View bar or select View in the main menu and click **Registration**:



- Data in this view shows capillary registration only.
- Graph view data displays electropherograms in fluorescence (y-axis) and position (x-axis).
- Lane view data displays capillary registration only.
- Image view data displays capillary registration only.
- Registrations are highlighted in both the peaks and capillaries tables and marked with an R.

Because capillaries in Sally Sue and Peggy Sue must be moved multiple times during the run, their positions are tracked to account for potential changes in imaging position as they are moved to and from the separation block. To do this, one of the standards is used as a capillary registration. An image of the standard used for registration is taken at the end of the separation step prior to chemiluminescent imaging. The position of this peak in the registration image is then compared to the position of the same standard peak in the standards image. The shift value listed in the peaks table is the change in pixel position between the two images. Compass data is then aligned to account for any changes in capillary position.

For information on checking and identifying registration peaks, see "Step 3 – Checking Capillary Registrations (Sally Sue and Peggy Sue Only)" on page 182.

Selecting and Displaying Capillary Data

You can choose to view data from one, multiple, or all capillaries at once.

• To look at data for one capillary - Click a row in the experiment pane. Data for just the row selected will display in all data views and tables. The following example shows sample data displayed in the lane and peaks panes when one sample is selected:



To look at data for multiple non-sequential capillaries - Hold the **Ctrl** key and select the rows you want to view in the experiment pane. Data for only the rows selected will display in all data views and results tables. The following example shows sample data displayed in the graph (in multiple view) and capillaries panes when multiple rows are selected:



To look at data for multiple sequential capillaries - Hold the **Shift** key and select the first and last rows you want to view in the experiment pane. Data for only the selected rows will display in all data views and results tables. The following example shows standards data displayed in the image and peaks panes when multiple sequential rows are selected:



To look at data for all capillaries - Hold the **Shift** key and select the first and last rows in the experiment pane. Data for all rows will display in all data views and results tables. The following example shows standards data displayed in the lane and peaks panes when all rows are selected. The pane scroll bars can be used to view all 8 cycles:



Switching Between Single and Multiple Views of the Capillaries

You can switch between displaying run data in the graph, image and lane panes in a per capillary (single) format or up to 96 capillary format. This view is selected in the View bar or the View menu.

∰ St	andar	rds 🛧 Reg. 🚖 Sample	s 🔳 🎛
	View	Instrument Window Single View Multiple View Standards Samples	
		Grouping Filter View Region Show Hidden	

Capillary view buttons in the View bar:



H

Single View

Multiple View

• To view data in a per capillary format - Click Single View in the View bar or select View in the main menu and click Single View:

🕲 2013-	11-27_15-0	-48_W	/es_recA	KT1_1200V_A9	27_C1234_34	1308 - Com	pass				-	-			• ×
File Ec	lit View I	nstrum	nent W	indow Help											
🗄 Sta	indards 📄	Samp	les]::								Assay	🕒 Run Sum	nmary 🚛	Analysis
Expe	riment		- 0	🛴 Graph	🕲 Image	🖽 Lane	Z Std C	urve					Ţ	:∎ ≡[
Sample	Primary	Cy	cle Ca						C1:4,	C1:12, C	1:17				
🗸 EZ	None	1	1	10 000											
Jurka	t Anti-A.	. 1	4	17 000							A	KT1 GST-	AKT		
Jurka	t Anti-A.	. 1	5	16.000								A A			
Jurka	t Anti-A.	. 1	6	15,000											
Jurka	t Anti-A.	. 1	7	14,000											
Jurka	t Anti-A.	. 1	8	13,000											
Jurka	t Anti-A.	. 1	9	පී 12,000	-										
🗸 Jurka	t Anti-A.	. 1	12	g 11,000											
Jurka	t Anti-A.	. 1	13	<u></u> 10,000											
Jurka	t Anti-A.	. 1	14	E 9,000	1										
Jurka	t Anti-A.	. 1	15	E 8,000	1										
🗸 Jurka	t Anti-A.	. 1	16	9 /,000]										
Jurka	t Anti-A.	. 1	17	5 000											
Jurka	t Anti-A.	. 1	20	4.000											
Jurka	t Anti-A.	. 1	21	3.000								Λ \square			
Jurka	t Anti-A.	. 1	22	2,000											
Jurka	t Anti-A.	. 1	23	1,000											
Jurka	t Anti-A.	. 1	24	0			~					~			
Jurka	t Anti-A.	. 1	25		L	12				40		66	116	180	230
										MW (ki	Da)				200
				Peaks	Capillari	es									- 6
				Sample	Primary	Cap	Peak	Name	Position	MW (kDa)	Height	Conc	Area	% Area	Width
				Jurkat	Anti-A	C1:4	1	AKT1	495	64	4912.5	20.9	57238	24.3	10.9
				Jurkat	Anti-A	C1:4	2	GST-A	533	95	16088.3	63.8	178251	75.7	10.4
				Jurkat	Anti-A	C1:12	1	AKT1	497	64	3906.6	16.1	43822	24.3	10.5
				Jurkat	Anti-A	C1:12	2	GST-A	535	94	13209.5	49.0	136548	75.7	9.7
				Jurkat	Anti-A	C1:17	1	AKT1	500	63	4132.0	17.4	47473	99.1	10.8
				Jurkat	Anti-A	C1:17	2	GST-A	547	103	52.7	0.7	411	0.9	7.3
•	III		+	•											Þ
			_				_								

Data for the row(s) selected in the experiment pane will display as follows:

- Electropherograms in the graph pane display overlaid or stacked (depending on the graph option chosen).
- Results for only the selected row(s) will display in the peaks and capillaries tables.
- The selected row(s) are highlighted in the image pane:
| 🔣 Graph 🎦 Image 🛛 🖽 Lane 🗠 Std Curve | - 8 |
|--------------------------------------|----------|
| | <u>^</u> |
| Cycle 1, Samples, Exp. 60.0 | |
| | |
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• Lanes for only the selected row(s) are displayed in the lane pane:



•

- C - X 8 2013-11-25_PI3Kdose-HeLa_lookup AC_33849_SW0018 - Compass File Edit View Instrument Window Help 🗄 Standards 🛧 Reg. 🚉 Samples 🔳 📰 Assay 🕒 Run Summary 🚛 Analysis 🖳 🗖 🔣 Graph 🛛 Image 🖽 Lane ~ - -Experiment Chemitminescence B B B B B B Sample Primary Cycle Biot. L... Blocking 1 HeLa PIBK CI... 1 E PI3K Cl... 1 HeLa MVV (KDa) HeLa PI3K CI... 1 C1:9 PBK CI... 1 HeLa 88888 **Atmitminscence** Biot. L... Blocking 3 HeLa PI3K CI... 3 🚯 HeLa PI3K CI... 3 🚯 HeLa PI3K CI... 3 🚯 HeLa PI3K CI... 3 C1:10 Biot. L... Blocking 4 Hel a PI3K CL., 4 HeLa PI3K CI... 4 HeLa PI3K CI... 4 HeLa PBK CI... Δ C3:1 Biot. L... Blocking 5 Chemiteminescence B B B B B B B HeLa PI3K CI... 5 PI3K Cl... 5 Hel a HeLa PI3K CI... 5 🚯 HeLa PBK CI... 5 🚯 Biot. L... Blocking 7 HeLa PBK CI... 7 Peaks Capillaries PIBK CI... 7 HeLa . Sample Primary Peak Name Position MW (kDa) Height % Area Width S/N Cap Area B HeLa PI3K Cl... 7 РВК 17443 100.0 162.2 HeLa PI3K CI.. C1:7 1 800 108 1381.4 11.9 HeLa PI3K CI... 7 PI3K CI... C1:8 РВК 794 108 4739.0 49578 100.0 9.8 562.4 HeLa Biot. L ... Blocking 8 PI3K CI... 8 HeLa PBK CI.. C1:9 1 PBK 796 107 11756.3 133763 100.0 10.7 1472.6 HeLa Hel a PI3K CL. C1:10 1 727 61 448.1 4338 9.1 43.6 HeLa PI3K CI... 8 🚯 HeLa HeLa PI3K Cl... C1:10 2 PBK 795 106 18007.9 232314 100.0 12.1 1512.1 PI3K CI... 8 Biot. L... Blocking C3:1 1 Ldr 12 497 12 1238.4 27711 21.0 120.1 PI3K CI... 8 🚯 HeLa • 111 ъ

To view data in a multiple capillary format - Click Multiple View in the View bar or select View in the main menu and click Multiple View:

Data for the row(s) selected in the experiment pane will display as follows:

- Electropherograms corresponding to the selected row(s) are highlighted and displayed in the graph pane.
- Results for selected capillaries display in the peaks and capillaries tables and are highlighted.
- The selected row(s) are highlighted in the image pane:



• All selected lanes display in the lane pane, and lanes corresponding to the selected row(s) are highlighted.



Hiding Capillary Data

Capillary data can be hidden from view if needed. To do this:

1. Click the **Experiment** tab.

2. Select the rows you want to hide, then right click on one of the selected rows and click **Hide**:

Data for selected rows will be hidden in all data views and results tables (except for the image pane).

- To view hidden rows Select View in the main menu and click Show Hidden. Hidden rows will become visible again in all panes, and hidden rows will be marked with an X in the experiment pane:
 - 8 🛄 Experiment Sample Primary Cycle ^ × Sample Primary 1 × Sample Primary 1 Sample Primary 1
- To unhide rows Select the hidden row(s). Right click on one of the selected rows and click Unhide. •

Setting Run Data Display Filters

The filter lets you auto-hide specific capillaries in the run file data, or only show data for peaks that Compass identified automatically using the user-defined peak name analysis parameters.

NOTE: When more than one run file is open, filter settings will be applied to all files.

To filter data to show specific capillaries only - Select View in the main menu and click Filter. • Uncheck the boxes for the capillaries you do not want shown, then click **OK**.

Experimer	nt		
Sample	Primary	Cycle	*
Sample	Primary	1	
Sample	Priman	1	
Sample	_P × Hi	de	
Sample	P CI	ear All	

🛞 Filter										
 ✓ Cycles ✓ 1 ✓ 2 ✓ 2 	Capillaries									
 ✓ 4 ✓ 5 ✓ 6 ✓ 7 	 ✓ 4 ✓ 5 ✓ 6 ✓ 7 									
▼ 8	 ✓ 8 ✓ 9 ✓ 10 ✓ 11 ✓ 12 									
Show named peaks or	l2 l2									
OK Cancel										

Only data for the checked capillaries will display in data views and results tables, unchecked capillaries will be hidden.

• To filter data to show named peaks only - Select View in the main menu and click Filter. Select Show named peaks only then click OK. Only data for peaks that Compass identified automatically using the user-defined peak name analysis parameters will display in data views and results tables, all other peak data will be hidden.

Compass Run Data Notifications and Warnings

If Compass detects a potential data issue, a notification or warning will display next to the row in the experiment pane. A list of warnings, notifications and corrective actions are as follows:



• **Manual correction of sample data notification** - Indicates the user modified sample data manually, such as adding or removing a sample peak. Rolling the mouse over the icon displays the type of data modification made.

Sample	Primary	Cycle	¢							
✓ High	ERK1/2	1	1							
Low p	ERK1/2	1								
High	ERK1/2 Baseline Manual									

Standards warning - Indicates that one of the standards may not be identified properly. This can be resolved by manually identifying the standards. Please refer to "Step 2 – Checking Fluorescent Sizing Standards" on page 178 for details. Rolling the mouse over the icon displays warning details.



- **Manual correction of standards data notification** Indicates the user modified the standards data manually. Rolling the mouse over the icon displays the type of data modification made.
- Registrations warning Indicates that a capillary registration was not identified properly. This can be resolved by manually identifying the registration peak. Please refer to "Step 3 Checking Capillary Registrations (Sally Sue and Peggy Sue Only)" on page 182 for details. Rolling the mouse over the icon displays warning details.



- Manual correction of registrations notification Indicates the user modified the capillary registration data manually. Rolling the mouse over the icon displays the type of data modification made.
- Peak fit warning Indicates that a peak cannot be fit properly. This is typically caused either by a very small peak in the electropherogram or a peak that is very close to the end of the molecular weight range. This can be resolved by removing the peak. Please refer to "Step 4 Checking the Ladder" on page 183 or "Step 5 Checking Samples" on page 185 for details. Rolling the mouse over the icon displays warning details.

🛆 Kit low-pho	anti-H	2	4
Kit low-pho	anti-E	2	5
Peak Fit Warning:	Too many	iteration	s 5

Checking Your Results

Compass detects proteins, fluorescent standards and capillary registrations and reports results automatically with no user-interaction. However, we recommend you review the data as outlined in this section as good general practice to ensure accurate results. If a data warning is presented in the experiment pane, the following steps will also help you identify and correct any issues.

Step 1 – Review the Fluorescent Sizing Standards Movie

All capillaries should have three fluorescent sizing standards. To verify the standards separated in the capillary correctly:

- 1. When the run has completed, click the **Run Summary** screen tab.
- 2. Click the **Separation** tab and play the movie (this will be the fluorescent standards separation).

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3. For each capillary, verify that the standards visibly separated. Each fluorescent point in the capillary represents one of the three sizing standards.

Step 2 – Checking Fluorescent Sizing Standards

To verify the standards are identified correctly:

- 1. Click the **Analysis** screen tab.
- 2. Click **Show Standards** in the View bar. Verification that the standards have been correctly identified can be done in either the graph or lane panes.

- a. Click **Single View** in the View bar.
- b. Click on the first row in the experiment pane, then click the **Graph** tab. Check that the electropherogram has three standard peaks labeled Std 1, Std 29 and Std 230 (for 12-230 kDa size assays) or Std 57 and Std 280 (for 66-440 kDa size assays). They will also be identified with a green **S** in the peaks table.





If standards are not identified correctly, they can be manually corrected as follows:

- If an incorrect peak is identified as a standard Right click the peak in the electropherogram or peaks table and select Not a Standard. Compass should correctly reassign the remaining peaks as standards, and update the peaks table.
- To set an unidentified peak as a standard Right click the peak in the electropherogram or peaks table and select Force Standard. Compass will assign the peak as a standard, and correctly reassign the remaining standard peaks. A lock icon indicating the standard was set manually will display next to the peak in the peaks table.

NOTE: To remove standards peak assignments that were made manually, right click on the peak in the electropherogram or peaks table and click **Clear**.

c. Repeat the previous steps for the remaining rows in the experiment pane to make sure all standards are identified correctly.

Lane Pane:

- a. Click Multiple View in the View bar.
- b. Click on the first row in the experiment pane, then click the **Lane** tab. Standards will be bands and identified with a green outline. Check that all standard bands are labeled: Std 1, Std 12 and Std 230

(for 12-230 kDa size assays) or Std 57 and Std 280 (for 66-440 kDa size assays). They will also be identified with a green **S** in the peaks table. To view band labels, roll the mouse over the individual bands.

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HeLa	PI3K CI 8	Biot. L	Blocking	CI:1	4	130	2.0							
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If standards are not identified correctly, they can be manually corrected as follows:

- If an incorrect band is identified as a standard Right click the band in the lane or peaks table and select Not a Standard. Compass should correctly reassign the remaining bands as standards.
- To set an unidentified band as a standard Right click the band in the lane or peaks table and select Force Standard. Compass will assign the band as a standard, and correctly reassign the remaining standard bands.
- c. Repeat the previous steps for the remaining rows in the experiment pane to make sure all standards are identified correctly.

Step 3 - Checking Capillary Registrations (Sally Sue and Peggy Sue Only)

All capillaries should have a single capillary registration peak. To verify the registrations are identified correctly:

- 1. Click the **Analysis** screen tab.
- 2. Click Show Registrations and Single View in the View bar.
- 3. Click the **Graph** tab.
- 4. Click on the first row in the experiment pane. The registration peak will be the first and largest peak in the electropherogram. Check that the registration peak is identified and labeled Reg 1 in the electropherogram. It will also be identified with a purple **R** in the peaks table.



5. If the registration peak is not identified correctly, right click the correct registration peak in the electropherogram or peaks table and select **Registration Force**. Compass will assign the new peak as the capillary registration. A lock icon indicating the registration was set manually will display next to the peak in the peaks table. NOTE: To remove registration peak assignments that were made manually, right click on the peak in the electropherogram or peaks table and click **Registration Clear**.

6. Repeat the previous steps for the remaining rows in the experiment pane to make sure all registrations are identified correctly.

Step 4 - Checking the Ladder

Depending on the size assay you are running, the biotinylated ladder should have the following sizing standards: 12, 40, 66, 116, 180 and 230 kDa (for 12-230 kDa size assays), or 66, 116, 200, 280 and 440 kDa (for 66-440 kDa assays). To verify the ladder standards are identified correctly:

- 1. Click the Analysis screen tab.
- 2. Click **Show Samples** in the View bar. Verification that the ladder standards have been correctly identified can be done in either the graph or lane panes, but manual corrections must be done in the graph pane.

Graph Pane:

- a. Click Single View in the View bar.
- b. Click on the row in the experiment pane that contains the ladder (typically row 1), then click the **Graph** tab. Check that the electropherogram has either six ladder peaks (for 12-230 kDa size assays) or five ladder peaks (for 66-440 kDa size assays). In the example below, the electropherogram has six peaks labeled Ldr 12, Ldr 40, Ldr 66, Ldr 116, Ldr 180 and Ldr 230.



If ladder peaks are not identified correctly, they can be manually corrected as follows:

- If an incorrect peak is identified as a ladder peak Right click the peak in the electropherogram or peaks table and select **Remove peak**. Compass should correctly reassign the remaining peaks as ladder standards.
- To set an unidentified peak as a ladder peak Right click the peak in the electropherogram or peaks table and select Add Peak. Compass will assign the peak as a ladder standard, and correctly reassign the remaining ladder standards peaks.

NOTE: To remove ladder peak assignments that were made manually and go back to the original view of the data, right click in the electropherogram and click **Clear All**.

Lane Pane:

a. Click either Single View or Multiple View in the View bar.

b. Click on the row in the experiment pane that contains the ladder (typically row 1), then click the Lane tab. Check that the lane has either six ladder bands (for 12-230 kDa size assays) or five ladder bands (for 66-440 kDa size assays). In the example below, the lane has six peaks labeled Ldr 12, Ldr 40, Ldr 66, Ldr 116, Ldr 180 and Ldr 230. To view band labels, roll the cursor over the individual bands. If ladder bands are not identified correctly, they must be corrected in the graph pane as described in the previous section.



Step 5 – Checking Samples

All sample proteins in the graph and lane panes will be labeled automatically with the calculated protein size.

NOTE: The reported molecular weight for immunodetected and total protein sample proteins in Compass may vary slightly from predicted molecular weights based on sample and assay conditions.

To verify that sample proteins are identified correctly:

- 1. Click the **Analysis** screen tab.
- 2. Click **Show Samples** in the View bar. Verification that sample proteins have been correctly identified can be done in either the graph or lane panes, but manual corrections must be done in the graph pane.

Graph Pane:

- a. Click **Single View** in the View bar.
- b. Click on the row in the experiment pane that contains the sample you wish to check, then click the **Graph** tab.



If sample peaks are not identified correctly, they can be manually corrected as follows:

• If an incorrect peak is identified as a sample peak - Right click the peak in the electropherogram or peaks table and select **Remove peak**. Compass will no longer identify it as a sample peak in the electropherogram and the peak data will be removed in the results tables. • To set an unidentified peak as a sample peak - Right click the peak in the electropherogram or peaks table and select Add Peak. Compass will calculate and display the results for the peak in the results tables and identify the peak in the electropherogram.

NOTE: To remove sample peak assignments that were made manually and go back to the original view of the data, right click in the electropherogram and click **Clear All**.

c. Repeat the previous steps for the remaining sample rows in the experiment pane to make sure all sample proteins are identified correctly.

Lane Pane:

- a. Click either **Single View** or **Multiple View** in the View bar.
- b. Click on the row in the experiment pane that contains the sample you wish to check, then click the **Lane** tab. To view sample protein band labels, roll the cursor over the individual bands. If sample bands are not identified correctly, they must be corrected in the graph pane as described in the previous section.

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Step 6 – Assigning Peak Names (Optional)

Compass can identify and automatically name sample proteins associated with specific primary antibodies in the run data using user-specified peak name settings. For more information on how to do this see "Peak Names Settings" on page 252.

Group Statistics

The Grouping View is used to analyze replicates by calculating the mean, standard deviation and CV of named proteins (see "Peak Names Settings" on page 252 for detailed information on entering named proteins). Statistics for each protein are also plotted for easy comparison between samples, antibodies, and proteins.

Using Groups

- 1. A group is automatically created for capillaries with the same sample and primary antibody name, so to use this feature, this information must first be entered into the Assay template. To do this:
 - a. Click the **Assay** tab and go to the Template pane.
 - b. Enter sample names and primary antibody names as described in "Step 6 Add Assay Plate Annotations (Optional)" on page 36. Be sure to enter the same sample and/or primary antibody names for the groups of samples you want to calculate statistics for.

In this example there are two sample types, **Sample A** and **Sample B** which were run with two different antibodies, **Primary 1** and **Primary 2**.

	1	2	3	4	5	6	7	8	9	10	11	12
Α	Biot. La	SampleA	SampleB	SampleA	SampleB	SampleA	SampleB	SampleA	SampleB		Control	
в						Antibody	/ Diluent					
С	Antibody	Primary 1	Primary 2	Primary 1	Primary 2	Primary 1	Primary 2	Primary 1	Primary 2	Primary 1	Primary 2	Primary 1
D	Streptav					Sec	ondary Antik	oody				
J						Dete	ction					

Each of the two samples were run with each of the two antibodies twice in every cycle. This creates four groups for the combination of two samples and two antibodies.

2. Select the **Analysis** tab. Group your results and view the associated statistics by selecting **View** and clicking on **Grouping**. In the box, click **Enable Grouping**.



3. Select a grouping option by clicking the box next to the option. These options allow you to group capillaries in multiple ways:



- **Group across runs** Groups capillaries from multiple runs. This option creates fewer groups and generates statistics across multiple runs.
- Group across cycles Groups capillaries run in different cycles.
- **No option selected** When only one run is open, groups will only contain capillaries from the same cycle. When more than one run is open, groups are created for each run.

Viewing Statistics

Peak and Capillary Groups

The Peak Groups pane reports statistics for each named protein in every group. Each group shows the statistics for named proteins which includes average area, standard deviation and %CV. The number in parenthesis after the sample name indicates the number of capillaries in the group.

Peak Groups	Capillary Groups 📊 Group F	Plot					Ŧ	
Sample	Primary	Cap	Name	Corr Area	Std.Dev.	% CV	SEM	
b HeLa (24)	ERK1+System Control		ERK1	127918	12886	10.1	2630	
HeLa (24)	ERK1+System Control		System Control	100000	0.0000	0.0	0.0000	

Clicking the arrow next to a group lists the individual capillaries in the group and reported data for each capillary:

Peak Groups	Capillary Groups 📊 Group P	lot					Ŧ	E - D
Sample	Primary	Cap	Name	Corr Area	Std.Dev.	% CV	SEM	*
⊿ HeLa (24)	ERK1+System Control		ERK1	127918	12886	10.1	2630	=
HeLa	ERK1+System Control	C1:2	ERK1	125041				
HeLa	ERK1+System Control	C1:3	ERK1	148874				
HeLa	ERK1+System Control	C1:4	ERK1	129850				
HeLa	ERK1+System Control	C1:5	ERK1	125733				
HeLa	ERK1+System Control	C1:6	ERK1	118107				
HeLa	ERK1+System Control	C1:7	ERK1	140706				
HeLa	ERK1+System Control	C1:8	ERK1	118756				-

The **Capillary Groups** pane pivots the **Peak Groups** results to show statistics for named protein peaks in individual columns.

🔛 Peak Groups 🔛 Capillary Groups 📊 Group Plot											
Sample	Primary	Capillary	Syste	Std.Dev.	%CV	SEM	ERK1	Std.Dev.	%CV	SEM	
HeLa (24)	ERK1+System Control		100000	0.0000	0.0	0.0000	127918	12886	10.1	2630	

Group Plots

The mean values for named peaks in each group are plotted in bar graphs with error bars showing the standard deviation. The plots compare different antibodies for the same sample and different samples for the same antibody to allow a choice of presentation. The y-axis on the plot will be concentration if a standard curve is defined.



Hiding or Removing Capillaries in Group Analysis

Hidden capillaries are not included in groups. However, hiding capillaries provides an easy way to reject individual capillaries from the statistical analysis. See "Hiding Capillary Data" on page 173 for details on how to do this.

Larger groups of capillaries can also be excluded from analysis. To do this, select View and click Filter.

🛞 Filter 📃 🔀		
 ♥ Filter ♥ Cycles ♥ 1 ♥ 2 ♥ 3 ♥ 4 ♥ 5 ♥ 6 ♥ 7 	Capillaries 1 2 3 4 5 6 7 7	
V / V 8	 7 8 9 10 11 12 	
Show named peaks only OK Cancel		

Uncheck the box next to the cycles or capillaries you wish to remove and click **OK**. This data will now be removed from the grouped view statistics.

Copying Data Views and Results Tables

You can copy and paste data and results tables into other documents, or save a data view as a graphic file.

Copying Data Views

- 1. Click in the graph or lane pane.
- 2. Select Edit in the main menu and click Copy, or right click and select Copy.
- 3. If you selected copy from the graph pane the following window will display, click **Copy**. If you selected copy from the lane pane skip to the next step.

Copy Graph		
Graph title:	Samples	
Metafile (EMF)		
🔘 Bitmap (PNG)		
Portable Document Format (PDF)		
Save	Copy Cancel	

4. Open a document (Microsoft[®] Word[®], Excel[®], PowerPoint[®], etc.). Right click in the document and select **Paste**. A graphic of the copied data view will be pasted into the document.

Copying Results Tables

- 1. Click in the peaks or capillaries pane.
- 2. Select one or multiple rows.
- 3. Select Edit in the main menu and click Copy, or right click and select Copy.
- 4. Open a document (Microsoft[®] Word[®], Excel[®], PowerPoint[®], etc.). Right click in the document and select **Paste**. Data for the rows selected will be pasted into the document.

Saving the Graph View as an Image File

- 1. Click in the graph pane.
- 2. Select Edit in the main menu and click Copy, or right click and select Copy.
- 3. Select an image option (EMF, PNG or PDF) in the pop-up window, then click Save.

Copy Graph			
Graph title:	Samples		
Metafile (EMF)			
 Bitmap (PNG) 			
Portable Document Format (PDF)			
Save Copy Cancel			

4. Select a directory to save the file to and enter a file name, then click **OK**.

Exporting Run Files

Results tables and raw plot data can be exported for use in other applications.

Exporting Results Tables

To export the information in the peaks and capillaries tables:

- 1. Click File in the main menu and click Export Tables.
- 2. Select a directory to save the files to and click **OK**. Data will be exported in .txt format.

NOTE: To exclude export of standards data or export results table data in .csv format, see "Setting Data Export Options" on page 413.

Exporting Raw Sample Electropherogram Data

To export raw sample plot data:

1. Click File in the main menu and click Export Spectra.

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	Save As	1	5000
	Export Tables		4500
	Export Spectra •		Text Format
	Exit		Andi Format
			S 2500

- To export data in .txt format Select Text Format. Plots will be exported in one file for all capillaries.
- To export data in .cdf format Select Andi Format. Plots will be exported in one file per capillary.
- 2. Select a directory to save the files to and click **OK**. Data will be exported in the selected format.

Changing Sample Protein Identification

Compass allows you to customize what sample proteins are reported in the results tables by making manual adjustments in the electropherogram or peaks table.

Adding or Removing Sample Data

- 1. Click **Show Samples** in the View bar.
- 2. Click **Single View** in the View bar.
- 3. Click on the row in the experiment pane that contains the sample you wish to correct, then click the **Graph** tab.
 - To remove a peak from the data Right click the peak in the electropherogram or peaks table and select **Remove peak**. Compass will no longer identify it as a sample peak in the electropherogram and the peak data will be removed in the results tables.



• To add an unidentified peak to the data - Right click the peak in the electropherogram or peaks table and select Add Peak. Compass will calculate and display the results for the peak in the results tables and identify the peak in the electropherogram.

NOTES:

To remove sample peak assignments that were made manually and go back to the original view of the data, right click in the electropherogram and click **Clear All**.

Virtual blot data in the lane pane will also update to reflect changes made in the graph pane.

Hiding Sample Data

You can hide the results for a sample protein in the results tables without completely removing it from the reported results. To to this:

- 1. Click **Show Samples** in the View bar.
- 2. Click Single View in the View bar.
- 3. Click on the row in the experiment pane that contains the sample you wish to correct, then click the **Graph** tab.
- 4. Right click the peak in the electropherogram or peaks table and select **Hide peak**. Compass will hide the peak data in the results tables.



- ~ 8 J. Graph 📲 Image 🖽 Lane Samples 1400 ERK2 1300 1200 1100 K562 1000 C1:5 Chemiluminescence 900 800 700 600 500 78 ERK1 400 300 200 100 0 -100 12 40 66 90 116 180 MW (kDa) - -Peaks Capillaries Sample Primary Cap Peak Position MW (kDa) Height Area % Area Width S/N Name K562 C1:5 ERK2 521 43 1264.2 20893 79.6 15.5 208.4 anti-E... 1 K562 ERK1 47 324.9 5364 15.5 anti-E.. C1:5 549 20.4 26.2 2 × K562 anti-E... C1:5 3 676 78 394.6 1155 2.8 31.2
- 5. To view hidden peak data, click **View** in the main menu and click **Show Hidden**. Hidden peak data will display in the results table and be marked with an **X**.

6. To unhide a peak, right click on the peak in the electropherogram or peaks table and select **Unhide Peak**.

Changing Peak Names for Sample Data

If Compass did not automatically name a sample protein associated with a specific primary antibody, this can be adjusted manually. To do this:

- 1. Click **Show Samples** in the View bar.
- 2. Click **Single View** in the View bar.
- 3. Click on the row in the experiment pane that contains the sample you wish to correct, then click the **Graph** tab.

4. Right click the peak in the electropherogram or peaks table and click **Name**, then click a name in the list. Compass will change the peak name in the electropherogram and results tables, and adjust peak names for other sample proteins accordingly.



NOTES:

For details on how to specify peak name settings, see "Peak Names Settings" on page 252.

Virtual blot data in the lane pane will also update to reflect changes made in the graph pane.

Displaying Sample Data for Named Peaks Only

You can adjust the sample data to display results only for user-specified named peaks. To do this:

- 1. Click **Show Samples** in the View bar.
- 2. Click **View** in the main menu and click **Filter**.
- 3. Check the Show Named Peaks only box and click OK.

😵 Filter	x			
 ✓ Cycles ✓ 1 ✓ 2 ✓ 3 ✓ 4 ✓ 5 ✓ 6 ✓ 7 ✓ 8 	✓ Capillaries ✓ 1 ✓ 2 ✓ 3 ✓ 4 ✓ 5 ✓ 6 ✓ 7 ✓ 8 ✓ 9 ✓ 10 ✓ 11 ✓ 12			
Show named peaks only				
OK Cancel				



Compass will hide peak data in the electropherogram and results tables for all unnamed peaks, and instead only display data for named peaks in the electropherogram and results tables.

Changing the Virtual Blot View

Options in the lane pane let you change the contrast or invert the virtual blot, remove baseline noise, change lane labels or overlay standards data on sample lanes.

The lane pane toolbar has the following options:



Adjusting the Contrast

1. Click the **Contrast Adjustment** button. The contrast tool will display:



- 2. Click the bar and drag it up or down to adjust the contrast.
- 3. When finished, click **X** to close the tool.

Inverting the Virtual Blot

1. Click the **Invert** button. The virtual blot image will invert:



2. Click the **Invert** button again to return to the default view.

Selecting Lane Labels

The labels shown above the lanes in the virtual blot can be customized. To do this:

1. Click the **Edit Labels** button. The label box will display:



- 2. Check one or multiple label boxes, and uncheck those you don't want to display. To remove labels completely, uncheck all boxes. The following label options are available:
 - **Sample** Sample name. If sample names were entered in the assay template (Assay screen), those names will display here. Otherwise, Sample (default name) will display.
 - **Blocking** Blocking reagent name. If a name was entered in the assay template (Assay screen), that name will display here. Otherwise, Blocking (default name) will display.
 - **Primary** Primary antibody name. If primary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Primary (default name) will display.
 - Secondary Ab Secondary antibody name. If a secondary antibody name was entered in the assay template (Assay screen), those names will display here. Otherwise, Secondary (default name) will display.
 - Capillary Cycle and capillary number. For example, C1:3 indicates cycle 1, capillary 3

NOTE: Sally Sue and Peggy Sue run up to eight cycles (12 capillaries at a time), so the cycle number displayed will always be C1-C8 depending on the number of cycles programmed.

• Attributes - Attribute text. If attribute information was entered for any of the rows or wells in the assay template (Assay screen), up to four can be selected and displayed as lane labels.

Viewing the Uncorrected Sample Baseline

1. Click the **Remove Baseline** button (active for sample data only). This will remove the automatic baseline correction.



2. Click the **Remove Baseline** button again to return to the default, baseline corrected view.

Overlaying Standards Data on Sample Lanes

Data for the standards can be overlaid on the sample data in the virtual blot so you can view the raw, unaligned (uncorrected) lane data. To do this:

1. Click the **Overlay Standards Data** button (active for sample data only). An overlay of the raw sample and standards data will display:


2. Click the **Overlay Standards Data** button again to return to the default, auto-aligned view.

Moving Lanes in the Virtual Blot View

Lanes in the virtual blot view can be moved to make it easier to compare specific lanes of data. To do this:

1. Click a lane in the virtual blot. Hold the mouse button down and drag the lane to a new position.



2. Release the mouse button. The lane will now be repositioned in the virtual blot view.

Changing the Electropherogram View

Options in the graph pane let you zoom and scale electropherograms, overlay or stack plots and change the peak and plot information displayed.

The graph pane toolbar has the following options:



Auto Scale



Graph Options

Stack the Plots

Overlay the Plots

Autoscaling the Electropherogram

Click the **Autoscale** button to scale the y-axis to the largest peak in the electropherogram.



Click the Autoscale button again to return to default scaling.



Stacking Multiple Electropherograms

Electropherogram data for multiple capillaries can be stacked vertically in the graph pane for comparison. To do this:

- 1. Click Single View.
- 2. Select multiple rows in the experiment pane.
- 3. Click the **Stack the Plots** button. The individual electropherograms for each row selected will stack in the graph pane.



You can customize the colors used for the stacked plot display. For more information see "Selecting Custom Plot Colors for Graph Overlay" on page 414.

Overlaying Multiple Electropherograms

The electropherogram data for multiple capillaries can also be overlaid on top of each other for comparison in the graph pane. To do this:

- 1. Click Single View.
- 2. Select multiple rows in the experiment pane.
- 3. Click the **Overlay the Plots** button. The individual electropherograms for each row selected will overlay in the graph pane.



You can customize the colors used for the overlay plot display. For more information see "Selecting Custom Plot Colors for Graph Overlay" on page 414.

Zooming

To zoom in on a specific area of the electropherogram, hold the mouse button down and draw a box around the area with the mouse:





To return to default scaling, right click in the electropherogram and click **Zoom Out**.

Customizing the Data Display

You can customize electropherogram peak labels, plot labels and display options. To do this, select the **Graph Options** button.



Peak Labels

You can customize the labels used to identify peaks in the electropherogram with these options:



Matching Peak Names - Checking this box will draw vertical lines through each named peak. Using this option with Stack the Plots or Overlay the Plots features is useful for visual comparison of named peaks across multiple capillaries.



• **Peak Names** - Checking this box will display peak name labels on all named peaks in the electropherogram.

NOTE: If more than one peak label option is selected, peak name labels will always be used for named peaks.



Peak Values - Checking this box will display the molecular weight labels on all peaks in the electropherogram.

NOTES:

When viewing standards and registration data, this option is called Peak Positions. Labels displayed are peak positions rather than molecular weight.

If more than one peak label option is selected, peak name labels will always be used for named peaks.

The reported molecular weight for immunodetected and total protein sample proteins in Compass may vary slightly from predicted molecular weights based on sample and assay conditions.



Baseline and Grid Options

You can view the calculated baseline fit, peak integration and show grid lines with these options.



- Fitted peaks Checking this box will display how the peaks were fit by the software.
 - For Immunoassays, the software uses Gaussian fit by default:

NOTE: This option is only available for sample data.



For Total Protein Assays, the software uses Dropped Lines fit by default:



Baseline Fit - Checking this box will display the calculated baseline for the peaks. Baseline points will also display for regions of the electropherogram considered to be at baseline.

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NOTE: This option is only available for sample data.





Grid Lines - Checking this box will display grid lines in the graph area.

Plot Labels

You can customize the plot labels displayed on the electropherogram with these options.

Plot Label	
🔽 Sample	Attribute
Primary	Attribute
Capillary	Exposure

Plot labels are shown on the right side of the graph pane.

- **Sample** Checking this box will display the sample name. If sample names were entered in the assay template (Assay screen), those names will display here. Otherwise, Sample (default name) will display.
- **Primary** Checking this box will display the primary antibody name. If primary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Primary (default name) will display.
- **Capillary** Checking this box will display the cycle and capillary number. For example, C1:3 indicates cycle 1, capillary 3.
- Attributes Checking this box will display attribute text. If attribute information was entered for samples or primary antibodies in the assay template (Assay screen), they can be selected as plot labels.

• **Exposure** - Checking this box will display the exposure time(s) used for the data. The following example shows an electropherogram with all plot labels selected:



Selecting Data Viewing Options

The graph view menu provides you with multiple options for changing what type of electropherogram data is displayed. To access the view menu, click the down arrow next to the graph pane toolbar:



A check mark next to the menu option indicates it is currently selected. Multiple options can be selected at a time.

NOTE: Unless otherwise noted, graph view menu options are available for sample data only.



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Sample - Clicking this option will display raw, uncorrected sample data.

Sample Baseline Corrected - Clicking this option will display sample data with the baseline subtracted (zeroed). This is the default view.



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Baseline Fit - Clicking this option will display the calculated baseline for the raw sample data. In the example that follows, both Baseline fit and Sample are selected.

NOTE: This option is selected automatically when Baseline Fit is selected in graph options.



Baseline Points - Clicking this option will display regions of the electropherogram considered to be at baseline. In the example that follows, both Baseline Points and Sample are selected.



NOTE: This option is selected automatically when Baseline Fit is selected in graph options.

Fit - Clicking this option will display the bounding envelope of the fitted peaks as calculated by the software for the raw sample data. In the example that follows, both Fit and Sample are selected.



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Fit Baseline Corrected - Clicking this option will display the fitted peaks as calculated by the software for the sample baseline corrected data. In the example that follows, both Fit Baseline Corrected and Sample are selected, the fit plot is on the bottom.



NOTE: When viewing standards or registration data, this option is called Standards Fluorescence or Registration Fluorescence, respectively.



Standards - Checking this box aligns the molecular weight of the raw standards data to the sample data and overlays both electropherograms in the graph pane.

Adding and Removing Baseline Points

Points in the baseline can be added or removed as needed. To do this:

- 1. Click the **Graph Options** button in the graph pane toolbar and check **Baseline Fit**. This will display baseline points for the raw sample data.
- 2. Use the mouse to draw a box around the area you want to correct. This will zoom in on the area.



3. Right click a baseline point and click Add Baseline Point or Remove Baseline Point.

NOTE: To clear the manual addition or removal of baseline points and go back to the original view of the data, right click in the electropherogram and click **Clear All**.

Selecting the X-Axis Molecular Weight Range

The molecular weight range used for the x-axis can be changed. To do this, Select **View** in the main menu and click **View Region**. The following pop-up window will display:

🛞 View Region		×
Range Analysis Full	Custom	
Lower: 10.0	Upper:	350.0
ОК	Cancel	

•

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Selecting **Analysis** will match the range of the run data that was selected in the Peak Fit Analysis Settings in both the electropherogram and virtual blot view.

Selecting **Full** will display the range of the run data for the entire capillary in both the electropherogram and virtual blot view. (Insert View region Full)



Selecting **Custom** allows you to manually enter the Lower and Upper ranges of the run data to be displayed in both the electropherogram and virtual blot view.(Insert View region Custom)



NOTE: You can change the default x-axis range that Compass uses. For more information, see "Peak Fit Analysis Settings" on page 244.

Closing Run Files

If more than one run file is open, you can close just one file or all the open files at the same time.

- To close one of multiple open run files In the experiment pane, click on one of the sample rows in the file. Next click File from the main menu and click Close.
- To close all open run files Select File from the main menu and click Close All.

Compass Analysis Settings Overview

Compass has a variety of analysis features and settings that you can modify as needed to enhance run data.

To access these settings, select **Edit** in the main menu and click **Analysis**. If more than one run file is open, select the run file you want to view analysis settings for from the list:

Edit	View Instrum	ent Wind	w Help
	Cut		
	Сору	Ctrl+C	
	Paste	Ctrl+V	Craph 📷 Image 🖽 Lane
	Analysis	•	Simple Western ERK Demo
	Preferences		2011-07-13_16-55-20_7-12-2011

The following screen will display:

er text	Advanced		
anced ges	Analysis Settings	Analysis Settings: Advanced	
Fit	Advanced	Standards	
dards		Peak Width	15
		Allowable Drift	100
		Sample	
		Peak Fit Starting Width Ratio	0.5
	Add Remov	e Image	
	Apply Default:	Median Filter Threshold Ratio	0.1
	Advanced	 Median Filter Threshold Limit 	10
	Apply Override:		
	Apply To Settings		
	Add Remov	e	
			Restore

To move between analysis pages in this window, click on an option in the list on the left. The following analysis settings can be user-customized in Compass:

- Advanced Lets you customize analysis settings for samples, standards and image data.
- **Images** Lets you view the chemiluminescent exposures taken during the run and view data for different exposures in the Analysis screen.
- Peak Fit Lets you customize peak fit settings for sample data.
- **Peak Names** Lets you enter custom naming settings for sample proteins associated with specific blocking reagents, primary antibodies or attributes and have Compass automatically label the peaks in the run data.
- Standards Lets you customize the molecular weight and positions Compass uses to identify ladder standards, fluorescent standards and registration peaks, as well as change the capillary used for the ladder.

Advanced Analysis Settings

The advanced analysis settings page lets you view and change analysis settings for samples, standards and image data. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Advanced** in the options list.

NOTE: Settings can be modified in an assay prior to starting a run, or in a run file once the run has finished executing. Analysis changes made to an executing run will not be saved to the final run file.

Advanced		<-> <
Analysis Settings	Analysis Settings: Advanced	
Advanced	Standards	
\$	Peak Width	15
	Allowable Drift	100
	Sample	
Add	Peak Fit Starting Width Ratio	0.5
	Image	
Apply Default:	Median Filter Threshold Ratio	0.1
Advanced	 Median Filter Threshold Limit 	10
Apply Override:		
Apply To Settings		
Add Remo	ve	
		-

- Click **Import** to import an analysis settings file. This will be explained in more detail in "Importing Analysis Settings" on page 284.
- Click **Export** to export the current analysis settings file. This will be explained in more detail in "Exporting Analysis Settings" on page 284.
- Click Restore Original to restore Compass default settings.
- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

Standards Settings

- **Peak Width** The approximate width (at full width half max) used to filter out fluorescence artifacts which improves recognition of standards.
- Allowable Drift The distance in pixels that standards are expected to move compared to their entered positions in the standards analysis page. This setting assists in recognition of standards.

Sample Settings

• **Peak Fit Starting Width Ratio** - Focuses the peak fit towards the peak center and aids the overall peak fitting.

Image Settings

- Median Filter Threshold Ratio Pixel ratio used to filter out camera artifacts.
- Median Filter Threshold Limit Pixel threshold value used to filter out camera artifacts.

Advanced Analysis Settings Groups

Advanced analysis settings are saved as a group, and multiple settings groups can be created. Specific group settings can then be applied to individual capillaries, sample names or attributes in the run data.

NOTES:

We recommend using the Compass default values for advanced analysis settings. These settings are included in the default Advanced group.

Analysis settings are run-file specific. However, settings can be imported or exported for use with other run files. For more information see "Importing and Exporting Analysis Settings" on page 284.

Analysis groups are displayed in the analysis settings box:

Analysis Settin	gs		
Advanced			
			_
A	dd	Remove	

The Advanced group shown contains the Compass default analysis settings. You can make changes to this group and create new groups.

To view settings for a group, click on the group name in the analysis settings box.

Creating a New Analysis Group

- 1. Select Edit in the main menu and click Analysis, then click Advanced in the options list.
- 2. Click Add under the analysis settings box. A new group will be created:

Advanced		
Advanced 2		

3. Click on the new group and enter a new name.

Analysis Settings	
Advanced	
STAT analysis	
Add	Remove

- 4. Modify standards, sample or image parameters as needed.
- 5. To use the new group as the default analysis settings for the run file data, click the arrow in the drop down list next to Default, then click the new group from the list. Analysis settings in the new group will then be applied to the run data.

Analysis Settings	
Advanced	
STAT analysis	
Add	Remove
Default	Advanced
Override	STAT analysis

6. Click **OK** to save changes.

Changing the Default Analysis Group

- 1. Select Edit in the main menu and click Analysis, then click Advanced in the options list.
- 2. Click the arrow in the drop down list next to Default, then click a new default group from the list.

Analysis Settings			
Advanced			
STAT analysis			
Add	Remove		
Default	Advanced 👻		
	Advanced		
Override	STAT analysis		

3. Click **OK** to save changes. Analysis settings in the group selected will be applied to the run data.

Modifying an Analysis Group

- 1. Select Edit in the main menu and click Analysis, then click Advanced in the options list.
- 2. Click on the group in the analysis settings box you want to modify.

Analysis Settings	
Advanced	
STAT analysis	
Add Remove	

- 3. Modify standards, sample or image parameters as needed.
- 4. Click OK to save changes. The new analysis settings will be applied to the run data.

Deleting an Analysis Group

- 1. Select Edit in the main menu and click Analysis, then click Advanced in the options list.
- 2. Click on the group in the analysis settings box you want to delete and click **Remove**.

Analysis Settings
Advanced
STAT analysis
Add Remove

3. Click **OK** to save changes.

Applying Analysis Groups to Specific Run Data

- 1. Select Edit in the main menu and click Analysis, then click Advanced in the options list.
- 2. Click on the group in the analysis settings box you want to apply to specific run data.

Analysis Settings	
Advanced	
STAT analysis	
Add Remove	

3. Application of analysis groups to specific run data is done in the override box. Click **Add** under the override box. A default override data set will be created from sample information found in the run file.

Override	
Apply To	Settings
Biotinylated Ladder	STAT analysis
Add	Remove

4. Click the cell in the **Apply To** column, then click the down arrow.

Override		
Apply To		Settings
biotin ladder	-	Advanced 2
biotin ladder		
hela		
Cycle 1		
Cycle 2		
Cycle 3		
Cycle 4		
Cycle 5		
Cycle 6		
Cycle 7		
Cycle 8		Permana
Custom Setting	s	Kemove

- 5. Select an option from the drop down list. This will apply the settings group selected to specific run data as follows:
 - **Sample names** All sample names entered in the assay template (Assay screen) will display in the drop down list, otherwise the default name of Sample will be shown. Select a sample name to apply group settings to all capillaries that use this sample name in the run file.
 - Attributes All sample attributes entered in the assay template (Assay screen) will display in the drop down list. Select an attribute to apply group settings to all capillaries that use this attribute in the run file.

- **Cycle 1-8** When this option is selected, group settings will be applied to all capillaries in the cycle.
- **Custom settings** Lets you choose specific capillaries to apply the group settings to. When this option is selected, the following pop-up box appears to let you enter a specific capillary number or range of capillaries:

🍪 Custom Settings 📃 🛋	
Enter cycle and capillary descriptor	
] [
Examples: 2:3, 1:1-12 Cycle 2 capillary 3, and cycle 1 capillaries 1 through 12 1-3:6+8+10 Cycles 1 through 3, capillaries 6, 8 and 10	
OK Cancel]

6. If you need to change the analysis group used for a data set, click the cell in the **Settings** column and click the down arrow. Select a group from the drop down list.

Apply To	Settings
K562	Advanced K
	Advanced STAT analysis

- 7. Repeat the previous steps to apply other groups to specific run data.
- 8. To remove a data set, click on its cell in the **Apply To** column, then click **Remove**.
- 9. Click **OK** to save changes.

Images Analysis Settings

The Images analysis settings page lets you see what chemiluminescent exposures were taken during the run, and view data for different exposures in the Analysis screen. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Images** in the options list.

e filter text	Images		← → →
Advanced Images	Cycle	Luminescence	
Peak Names Standards	All	Multi-Image Analysis 🔹	
	1	Multi-Image Analysis 🔹	
	2	Multi-Image Analysis	
	3	Multi-Image Analysis	
	4	Multi-Image Analysis	
	6	Multi-Image Analysis	
	7	Multi-Image Analysis	
	8	Multi-Image Analysis 🗸	
			Restore Origin
			instance origin

- Click **Import** to import an analysis settings file. This will be explained in more detail in "Importing Analysis Settings" on page 284.
- Click **Export** to export the current analysis settings file. This will be explained in more detail in "Exporting Analysis Settings" on page 284.
- Click Restore Original to restore Compass default settings.
- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

Exposure Settings

The exposure used for the sample data displayed in the Analysis screen is shown in the All box:

Cycle	Luminescence
All	Multi-Image Analysis
1	Multi-Image Analysis 👻
2	Multi-Image Analysis 🔹
3	Multi-Image Analysis 🔹
4	Multi-Image Analysis 🔹
5	Multi-Image Analysis 🔹
6	Multi-Image Analysis 🔹
7	Multi-Image Analysis 🔹
8	Multi-Image Analysis 🔹

NOTE: Sally Sue and Peggy Sue run up to eight cycles (12 capillaries per cycle). The individual exposure setting selected for a specific cycle is applied for all 12 capillaries in that cycle unless selected for All cycles.

- **Multi-Image Analysis** Sample data displayed in the Analysis screen is compiled from all exposures taken during the run and utilized to calculate the chemiluminescent signal output at time zero of the chemiluminescent reaction. This calculation represents the kinetics of the chemiluminescent reaction and helps to eliminate signal burn out that may occur with stronger signals and longer exposure times.
- **Exposure #** Sample data displayed in the Analysis screen is for this specific exposure only.

To see the number of exposures and exposure times used for the run data, click the arrow in the drop down list next to **All**.
Cycle	Luminescence		
All	Multi-Image Analysis 🔹		
1	Multi-Image Analysis 🔹		
2	Multi-Image Analysis 🔹		
3	Multi-Image Analysis 🔹		
4	Multi-Image Analysis 🔹		
5	Multi-Image Analysis 🔹		
6	Multi-Image Analysis 🔹		
7	Multi-Image Analysis 🔹		
8	Multi-Image Analysis 🔹		
	Multi-Image Analysis		
	Exposure 2 30 seconds		
	Exposure 3 60 seconds		
	Exposure 4 120 seconds		
	Exposure 5 240 seconds		
	Exposure 6 480 seconds		

NOTE: The number of exposures taken and exposure times shown were specified in the assay protocol (Assay screen) prior to executing the run and cannot be modified.

Changing the Sample Data Exposure Displayed

The exposure used for the sample data displayed in the Analysis screen can be changed. To do this:

- 1. Select Edit in the main menu and click Analysis, then click Images in the options list.
- 2. Click the arrow in the drop down list next to **All** and select an exposure setting:

Cycle	Luminescence		
All	Multi-Image Analysis 🔹		
	Multi-Image Analysis		
	Exposure 1 15 seconds		
	Exposure 2 30 seconds		
1	Exposure 3 60 seconds		
	Exposure 4 120 seconds		
2	Exposure 5 240 seconds		
	Exposure 6 480 seconds		
3	Multi-Image Analysis		

3. Click **OK** to save changes and exit. Sample data for the exposure selected will display in the Analysis screen.

Peak Fit Analysis Settings

The peak fit analysis settings page lets you view and change peak fit settings for sample data. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Peak Fit** in the options list.

NOTE: Settings can be modified in an assay prior to starting a run, or in a run file once the run has finished executing. Analysis changes made to an executing run will not be saved to the final run file.

t Analysis: Sally Sue Siz	e		
r text	Peak Fit		
nced es	Analysis Settings	Analysis Settings: fit	
Fit	fit	Range	
lards		Minimum	1.0
		Maximum	250.0
		Baseline	
		Threshold	1.0
	Add	Window	15.0
	Apply Default:	Stiffness	1.0
	fit	Peak Find	
	Apply Override:	Threshold	10.0
	Apply To Settings	Width	9.0
		Area Calculation	Gaussian Fit 🔹
	Add Remove		
			Restore Origin
art Euroat			OK Carrel
ort Export			OK Can

Click **Import** to import an analysis settings file. This will be explained in more detail in "Importing Analysis Settings" on page 284.

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- Click **Export** to export the current analysis settings file. This will be explained in more detail in "Exporting Analysis Settings" on page 284.
- Click Restore Original to restore Compass default settings.
- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

Range Settings

- **Minimum** The molecular weight value (in kDa) below which peaks will not be identified. This value will also be used as the default lower MW range for the data displayed in the electropherogram and virtual blot.
- **Maximum**: The molecular weight value (in kDa) above which peaks will not be identified. This value will also be used as the default upper MW range for the data displayed in the electropherogram and virtual blot.

Baseline Settings

- **Threshold** The variance, or roughness, in a baseline data segment below which a point will be called part of the baseline.
- **Window** How long baseline data segments are expected to be in pixels. Shorter segments will allow the baseline to follow plateau sections of the signal.
- Stiffness The amount the baseline is allowed to vary from a straight line. Settings between 0.1 and 1.0 make the baseline fit closer to a straight line. Settings from 1.0 to 10.0 will make the baseline fit follow the data more closely.

Peak Find Settings

- **Threshold** The minimum signal to noise ratio required for a peak to be identified. A setting of 1.0 will detect many peaks, a setting of 10.0 will detect fewer peaks.
- Width The approximate peak width (at full width half max) in pixels used to detect peaks. The minimum value for this setting is 3.0. Larger widths help to eliminate the detection of shoulder peaks and noise peaks.
- Area Calculation Two fits are used, either Gaussian Fit or Dropped Lines. These settings can be changed before or after the run is finished.
 - For Immunoassays, peak area is calculated using Gaussian distribution by default:



• For Total Protein Assays, peak area is calculated using Dropped Lines. This type of area calculation is also often called the perpendicular drop method. This method is preferred when peaks overlap or are close to each other as they are in Total Protein Assays. This method draws two vertical lines from the left and right bounds of the peak down to the x-axis and then measures the total area bounded by the signal curve, the x-axis (y=0 line), and the two vertical lines.



Peak Fit Analysis Settings Groups

Peak fit settings are saved as a group, and multiple settings groups can be created. Specific group settings can then be applied to individual capillaries, sample names or attributes in the run data.

NOTES:

We recommend using the Compass default values for peak fit analysis settings. These settings are included in the default Peak Fit group.

Analysis settings are run-file specific. However, settings can be imported or exported for use with other run files. For more information see "Importing and Exporting Analysis Settings" on page 284.

Peak fit groups are displayed in the analysis settings box:

Analysis Settings	
Peak Fit	
Add	Remove

The Peak Fit group shown contains the Compass default analysis settings. You can make changes to this group and create new groups.

To view settings for a group, click on the group name in the analysis settings box.

Creating a New Peak Fit Group

- 1. Select Edit in the main menu and click Analysis, then click Peak Fit in the options list.
- 2. Click Add under the analysis settings box. A new group will be created:

Peak Fit		
Peak Fit 2		

3. Click on the new group and enter a new name.

Analysis Set	tings			
Peak Fit	C 1			
STAT peak	: fit			
_			_	

- 4. Modify range, baseline or peak find parameters as needed.
- 5. To use the new group as the default peak fit settings for the run file data, click the arrow in the drop down list next to Default, then click the new group from the list. Peak fit settings in the new group will then be applied to the run data.

Peak Fit	
STAT peak fit	
Add	Remove
Default	Peak Fit
Default	Peak Fit Peak Fit

6. Click **OK** to save changes.

Changing the Default Peak Fit Group

- 1. Select Edit in the main menu and click Analysis, then click Peak Fit in the options list.
- 2. Click the arrow in the drop down list next to Default, then click a new default group from the list.

Analysis Settings	
Peak Fit	
STAT peak fit	
Add	Remove
Default	Peak Fit 💌
	Peak Fit
Override	STAT peak fit

3. Click OK to save changes. Peak fit settings in the group selected will be applied to the run data.

Modifying a Peak Fit Group

- 1. Select Edit in the main menu and click Analysis, then click Peak Fit in the options list.
- 2. Click on the group in the analysis settings box you want to modify.



- 3. Modify range, baseline or peak find parameters as needed.
- 4. Click **OK** to save changes. The new peak fit settings will be applied to the run data.

Deleting a Peak Fit Group

- 1. Select Edit in the main menu and click Analysis, then click Peak Fit in the options list.
- 2. Click on the group in the analysis settings box you want to delete and click **Remove**.

Peak Fit			
STAT pe	ak fit		

3. Click **OK** to save changes.

Applying Peak Fit Groups to Specific Run Data

- 1. Select Edit in the main menu and click Analysis, then click Peak Fit in the options list.
- 2. Click on the group in the analysis settings box you want to apply to specific run data.

Analysis	Settings		
Peak Fit	:		
STAT p	eak fit		
ſ	Add	Remove	

3. Application of peak fit groups to specific run data is done in the override box. Click **Add** under the override box. A default override data set will be created from sample information found in the run file.

Override	
Apply To	Settings
Biotinylated Ladder	STAT peak fit
Add	Remove

4. Click the cell in the **Apply To** column, then click the down arrow.

Override	
Apply To	Settings
tinylated Ladder 🔨	STAT peak fit
Biotinylated Ladden K562 RTU K562 1mg/ml Cycle 1 Cycle 2 Cycle 3 Cycle 3 Cycle 4 Cycle 5 Cycle 5 Cycle 6 Cycle 7 Cycle 8 Custom Settings	Remove

- 5. Select an option from the drop down list. This will apply the settings group selected to specific run data as follows:
 - **Sample names** All sample names entered in the assay template (Assay screen) will display in the drop down list, otherwise the default name of Sample will be shown. Select a sample name to apply group settings to all capillaries that use this sample name in the run file.

- Attributes All sample attributes entered in the assay template (Assay screen) will display in the drop down list. Select an attribute to apply group settings to all capillaries that use this attribute in the run file.
- **Cycle 1-8** When this option is selected, group settings will be applied to all capillaries in the cycle.
- **Custom settings** Lets you choose specific capillaries to apply the group settings to. When this option is selected, the following pop-up box appears to let you enter a specific capillary number or range of capillaries:

🛞 Custom Settings
Enter cycle and capillary descriptor
Examples: 2:3, 1:1-12 Cycle 2 capillary 3, and cycle 1 capillaries 1 through 12 1-3:6+8+10 Cycles 1 through 3, capillaries 6, 8 and 10
OK Cancel

6. If you need to change the peak fit group used for a data set, click the cell in the **Settings** column and click the down arrow. Select a group from the drop down list.

Override	
Apply To	Settings
Biotinylated Ladder	STAT peak fit 🛛 👻
	Peak Fit STAT peak fit
Add	Remove

- 7. Repeat the previous steps to apply other groups to specific run data.
- 8. To remove a data set, click on its cell in the **Apply To** column, then click **Remove**.
- 9. Click **OK** to save changes.

Peak Names Settings

The peak names analysis settings page lets you enter custom naming settings for sample proteins. Compass can automatically label sample peaks in the run data associated with specific blocking reagent names, primary antibody names or attributes. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Peak Names** in the options list.

NOTE: Settings can be modified in an assay prior to starting a run, or in a run file once the run has finished executing. Analysis changes made to an executing run will not be saved to the final run file.

	Peak Names						¢	• 🜩
Advanced Images	Analysis Settings		Analysis	Settings:	Peak Nam	es 1		
Peak Fit Peak Names	Peak Names 1		Name	MW	Color	Range (%)	Control	Show
Standards			Peak 1	110	Color	10		V
	Add	Remove						
	Apply Settings	Settings						
	All	Peak Names 1						
							Add	Remov
			Control	I				
			Control Referen	l ce Capill trol Area	ary H	eLa 000.0		
	Add	Remove	Control Referen	l ce Capill trol Area Standard	ary H	eLa 000.0 Define		

Click **Import** to import an analysis settings file. This will be explained in more detail in "Importing Analysis Settings" on page 284.

•

- Click Export to export the current analysis settings file. This will be explained in more detail in "Exporting Analysis Settings" on page 284.
- Click Restore Original to restore Compass default settings.
- Click **OK** to save changes and exit.
- Click Cancel to exit without saving changes.

Peak Names Analysis Settings Groups

Peak name settings are saved as a group, and multiple settings groups can be created. Specific group settings can then be applied to individual capillaries, blocking reagent names or primary antibody names and attributes in the run data.

NOTE: Analysis settings are run-file specific. However, settings can be imported or exported for use with other run files. For more information see "Importing and Exporting Analysis Settings" on page 284.

Peak name groups are displayed in the analysis settings box:

Analysis Settings	
Peak Names 1]
	1
Add Remove	

The Peak Names group shown is a Compass template group. You can make changes to this group and create new groups.

To view settings for a group, click on the group name in the analysis settings box.

Creating a Peak Names Group

- 1. Select Edit in the main menu and click Analysis, then click Peak Names in the options list.
- 2. Click on the Peak Names 1 template group in the analysis settings box.

Analysis Settings Peak Names 1		 	

3. Enter a new name for the group.

Analysis Settings	
ERK1/2	
Add	Remove

4. Click in the first cell in the **Name** column in the analysis settings peak table.

Name	MW	Color	Range (%)	Control	Show
Peak1	100		10		1

5. Enter a sample protein name associated with the primary antibody used in the run.

Analysis Set	ttings: ERK1	/2			
Name	MW	Color	Range (%)	Control	Show
ERK2	100		10		V

6. Click in the first cell in the **MW** column.

tings: ERK1	/2			
MW	Color	Range (%)	Control	Show
100		10		v
	MW	MW Color	MW Color Range (%)	MW Color Range (%) Control

7. Enter the molecular weight (in kDa) for the sample protein.

Name	MW	Color	Range (%)	Control	Show
ERK2	42		10		V

8. Click in the first cell in the **Color** column, then click the button.

Name	MW	Color	Range (%)	Control	Show
ERK2	42	🗖 🖉 🛄	10		V

The color selection box will display:

Color			×	J
Basic colors:				
🔳 📕 🛢				
Custom colors	:			
Defir	ne Custor	n Colors >	>	
ОК	Cano	cel		

The color selected will be used to identify the sample protein peak in the peaks and capillaries table in the Analysis screen.

9. Click a color or define a custom color and click **OK**. The color selection will update in the table:

lame	MW	Color	Range (%)	Show
2K2	42		10	

10. Click in the first cell in the **Range (%)** column.

inalysis Set	tings: ERK1	/2			
Name	MW	Color	Range (%)	Control	Show
			200.00	-	-

11. Enter a range window for the MW entered. Compass will automatically name peaks found within this percent of the molecular weight. For example, if the molecular weight entered is 40 kDa and a 10% range is used, all peaks between 36 and 44 kDa will be identified with this peak name.

NOTE: The reported molecular weight for immunodetected and total protein sample proteins in Compass may vary slightly from predicted molecular weights based on sample and assay conditions.

12. Select the checkbox in the first cell of the **Show** column. This will turn peak naming on for the sample protein.

Analysis	Settings:	Protein			
Name	MW	Color	Range (%)	Control	Show
ERK2	42		10		~

To turn peak naming off for a particular sample protein, deselect the checkbox in the Show column.

13. To add another sample protein, click **Add** under the analysis settings peak table:

Name	MW	Color	Range (%)	Control	Show
ERK2	42		10		~
Peak2	100		10		~

Name	MW	Color	Range (%)	Control	Show
ERK2	42		10		 Image: A start of the start of
ERK1	44		10		V

14. Repeat the previous steps to enter information for other sample proteins. In the following example, two sample proteins were entered:

To remove a sample protein, select its row and click **Remove**.

15. Click **OK** to save changes.

Adding Peak Names Groups

- 1. Select Edit in the main menu and click Analysis, then click Peak Names in the options list.
- 2. Click Add under the analysis settings box. A new group will be created:

Analysis Settings		
ERK1/2		
ERK1/2 2		
Add	Remove]

3. Click on the new group and enter a new name.

Analysis Settings	
ERK1/2	
STAT	
Add	Remove

- 4. Enter information in the analysis settings peak table as described in "Creating a Peak Names Group" on page 253.
- 5. Click **OK** to save changes.

Modifying a Peak Names Group

- 1. Select Edit in the main menu and click Analysis, then click Peak Names in the options list.
- 2. Click on the group in the analysis settings box you want to modify.

Analysis Settings	
ERK1/2	
STAT	
Add Remove	

- 3. Change the information in the analysis settings peak table as described in "Creating a Peak Names Group" on page 253.
- 4. Click **OK** to save changes.

Deleting a Peak Names Group

- 1. Select Edit in the main menu and click Analysis, then click Peak Names in the options list.
- 2. Click on the group in the analysis settings box you want to delete and click **Remove**.



3. Click **OK** to save changes.

Applying Peak Names Groups to Run Data

- 1. Select Edit in the main menu and click Analysis, then click Peak Names in the options list.
- 2. Click on the group in the analysis settings box you want to apply to specific run data.

Analysis Settings	
ERK1/2	
STAT	
Add Remove	

3. Application of peak names groups to specific run data is done in the override box. Click **Add** under the override box. A default override data set will be created.

Apply Settings	
Apply To	Settings
All	ERK1/2
Add	Remove

4. Click the cell in the **Apply To** column, then click the down arrow.

Apply Settings	
Apply To	Settings
All	ERK1/2
All	NT.
Blocking	
anti-ERK1/2	
Cycle 1	
Cycle 2	
Cycle 3	
Cycle 4	
Cycle 5	
Cycle b	
Cycle /	Remove
Cycle o	
Custom Settings	

- 5. Select an option from the drop down list. This will apply the settings group selected to specific run data as follows:
 - All When this option is selected, peak names group settings will be applied to all capillaries in the run file.
 - **Blocking reagent** When this option is selected, group settings will be applied to all capillaries that use this reagent name in the run file.
 - **Primary antibody names** All primary antibody names entered in the assay template (Assay screen) will display in the drop down list, otherwise the default name of Primary will be shown. Select a name to apply group settings to all capillaries that use the primary antibody name in the run file.
 - Attributes All primary antibody attributes entered in the assay template (Assay screen) will display in the drop down list. Select an attribute to apply group settings to all capillaries that use this attribute in the run file.
 - **Cycle 1-8** When this option is selected, group settings will be applied to all capillaries in the cycle.
 - **Custom settings** Lets you choose specific capillaries to apply the group settings to. When this option is selected, the following pop-up box appears to let you enter a specific capillary number or range of capillaries:

Section Settings	x
Enter cycle and capillary descriptor	
Examples: 2:3, 1:1-12 Cycle 2 capillary 3, and cycle 1 capillaries 1 through 12 1-3:6+8+10 Cycles 1 through 3, capillaries 6, 8 and 10	
OK	el

6. If you need to change the peak names group used for a data set, click the cell in the **Settings** column and click the down arrow. Select a group from the drop down list.

Apply Settings	
Apply To	Settings
All	ERK1/2
	ERK1/2
Ad	d Remove

- 7. Repeat the previous steps to apply other groups to specific run data.
- 8. To remove a data set, click on its cell in the **Apply To** column, then click **Remove**.

9. Click **OK** to save changes. Named peaks will be identified with a peak name label in the electropherogram and virtual blot, and will be color coded in the peaks and capillaries tables:



Standard Curve Settings

To use a standard curve to quantitate the concentration of a target protein detected either by an Immunoassay or a Total Protein Assay, first create peak names groups as described earlier for your standard curve protein and the target protein. In the example below, GST-AKT1 is the standard curve protein and AKT1 is the target protein.

NOTE: Analysis settings are run file-specific. However, settings can be imported or exported for use with other run files. For more information see "Importing and Exporting Analysis Settings" on page 284.

e filter text	Peak Names						¢	• 🔶 •
Advanced Images	Analysis Settings		Analysis Setti	ngs: AKT	L			
Peak Fit Peak Names Standards	AKT1		Name	MW	Color	Range (%)	Control	Show
			GST-AKT	99		10		
	A	dd Remove						
	Apply Settings	Settings						
	All	AKT1						
							Add	Remove
			Control Reference C	apillary Area	Jurk	at 0.0		~
	A	dd Remove	St	andard C	urve: D	efine	Rest	ore Origir

To set up a standard curve:

1. Click the **Define...** button.

🛞 Standard	Curve		×
Peak: Fit:	GST-AKT Linear	•	
Capilla	ry	Concentration	
Jurkat		62.5	
Jurkat		31.2	
Units:	Add pg/uL	Remove	
	OK	Can	cel

- 2. From the Peak drop down list, select the peak name for your standard curve protein.
- 3. Choose either a Linear or 4 Parameter (4PL) curve fit from the Fit drop down list.
- 4. Select the checkbox in the first cell of the **Capillary** column. Sample names and attributes will be displayed in the list. Any number included in the name will auto-populate the Concentration column when selected.
- 5. To add another concentration, click **Add** under the Standard Curve table.
- 6. Repeat the previous steps to enter information for other concentrations. In the following example, six concentrations were entered:

۵ ک	tandard Curve	X
	Peak: GST-AKT	-
	Fit: Linear	•
	Capillary	Concentration
	62.5 pg/uL	62.5
	31.2 pg/uL	31.2
	15.6 pg/uL	15.6
	7.8 pg/uL	7.8
	3.9 pg/uL	3.9
	0 pg/uL	0.0
	Add	Remove
	Units: pg/uL	
	ОК	Cancel

To remove a concentration, select its row and click **Remove**.

- 7. Enter the concentration units in the box (for example, $pg/\mu L$).
- 8. Click **OK** to save changes.

Applying Peak Names Groups to Run Standard Curve

- 1. Select Edit in the main menu and click Analysis, then click Peak Names in the options list.
- 2. Click on the group in the analysis settings box you want to apply to specific run data.

Analysis Settings
AKT1
GST-AKT
Add Remove

3. Application of peak names groups to specific run data is done in the override box. Click **Add** under the override box. A default override data set will be created.

Apply To	Settings
All	AKT1

4. Click the cell in the Apply To column, then click the down arrow.

Apply To		Settings
All	÷	AKT1
All None Anti-AKT1 5%DEX Cycle 1 Custom Settings		

- 5. Select an option from the drop down list. This will apply the settings group selected to specific run data as follows:
 - a. **All** When this option is selected, peak names group settings will be applied to all capillaries in the run file.
 - b. **Blocking reagent** When this option is selected, group settings will be applied to all capillaries that use this reagent name in the run file.
 - c. **Primary antibody names** All primary antibody names entered in the assay template (Assay screen) will display in the drop down list, otherwise the default name of Primary will be shown. Select a name to apply group settings to all capillaries that use the primary antibody name in the run file.
 - d. **Attributes** All primary antibody attributes entered in the assay template (Assay screen) will display in the drop down list. Select an attribute to apply group settings to all capillaries that use this attribute in the run file.
 - e. Cycle 1-8 When this option is selected, group settings will be applied to all capillaries in the cycle.

f. **Custom settings** - Lets you choose specific capillaries to apply the group settings to. When this option is selected, the following pop-up box appears to let you enter a specific capillary number or range of capillaries:

Sustom Settings	X
Enter cycle and capillary descriptor	
Examples: 2:3, 1:1-12 Cycle 2 capillary 3, and cycle 1 capillaries 1 throu 1-3:6+8+10 Cycles 1 through 3, capillaries 6, 8 and 10	ugh 12 OK Cancel

6. If you need to change the peak names group used for a data set, click the cell in the Settings column and click the down arrow. Select a group from the drop down list.

Apply To	Settings	
All	AKT1	-
	AKT1 GST-AKT	

- 7. Repeat the previous steps to apply other groups to specific run data.
- 8. To remove a data set, click on its cell in the **Apply To** column, then click **Remove**.
- 9. Click **OK** to save changes. The curve fit will be visible on the Std Curve tab and the concentration of the proteins will appear in the Peaks table:



System or Loading Control Settings

It is possible to use a system or loading control protein to normalize data between capillaries, between runs and between instruments. First create peak names groups as described earlier for your control protein and the target protein. In the example below, System Control is the control protein and ERK1 is the target protein.

NOTE: Analysis settings are run file-specific. However, settings can be imported or exported for use with other run files. For more information see "Importing and Exporting Analysis Settings" on page 284.

filter text	008_Hela_ERK1-DIG_c12	51				-	6	• = •
Advanced mages	Analysis Settings		Analysis Settings: Pr	rotein				~
eak Fit	Protein					-		
Yeak Names			Name	MW	Color	Range (%)	Control	Show
stanuarus			System Control	26		10		
			ERK1	45		10		
	Apply Settings Apply To All	Settings Protein						
			Control			A	dd	Remove
			Control Reference Capillar	у	HeLa	A	dd	Remove
			Control Reference Capillar V Control Area	у	HeLa 100000	0	dd	Remove
		Add Remove	Control Reference Capillar I Control Area Stand	y lard Curve	HeLa 100000 :: Defin	0 e	dd	Remove
		Add Remove	Control Reference Capillar I Control Area Stand	y lard Curve	HeLa 100000 :: Defin	.0	dd Res	Remove tore Origin

To identify the control protein:

- 1. Click the **Control** box.
- 2. Select a Reference Capillary from the pull-down menu. The peak area of this capillary will be used to normalize the peak area of the named peaks.
- 3. To manually assign the Control Area, click the box and enter the peak area of your control protein.
- 4. Click **OK** to save changes. Compass will automatically normalize the peak area of the target protein against the control protein. The corrected area appears in the Capillaries table:



Standards Settings

The standards analysis settings page lets you view and change the molecular weight and position for ladder and fluorescent standards, set the registration peak, and change the capillary used for the ladder. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Standards** in the options list. The default standard settings for both the 12-230 kDa and 66-440 kDa size ranges are shown in the following examples:

NOTE: Settings can be modified in an assay prior to starting a run, or in a run file once the run has finished executing. Analysis changes made to an executing run will not be saved to the final run file.

filter text	Standards						⇔ ◄ ⇔
Advanced mages	Analysis Settings		Biotinylated	l Ladder (12	kDa-2	30kDa)	
Peak Fit	Biotinylated Lad	der (12kDa-230kDa)	Fluoresce	nt Peaks			
Peak Names		. ,				1	
standards			MW	Position	Fit	Registration	
			1	170			
			29	350			
			230	650			
	Add	Remove					
	Apply Default:		_		Add	Remove	
	Biotinylated Lado	der (12kDa-230kDa)	-				
	Apply Override:		Ladder C	apillary 1		•	
	Apply To	Settings				1	
			MW	Position	Fit		
			12	250			
			40	400			
			116	500			
			110	600			
			230	650			
					Add	Remove	
	Add	Remove					
							Restore Orig

ext	Standards					\diamond
ed	Analysis Settings	Biotinylated	d Ladder (66	kDa-4	40kDa)	
	Biotinylated Ladder (66kDa-440kDa)	Fluoresce	ent Peaks			
)		MW	Position	Fit	Registration	
		57	400			
		280	650	•		
	Add Remove					
	Apply Default:					۲. ۲
			Δ	dd	Remove	
	Biotinylated Ladder (66kDa-440kDa)	•	A	dd	Remove	
	Biotinylated Ladder (66kDa-440kDa) Apply Override:	▼ Ladder C	Capillary 1	dd	Remove	
	Biotinylated Ladder (66kDa-440kDa) Apply Override: Apply To Settings	▼ Ladder C	Capillary 1	dd	Remove]
	Biotinylated Ladder (66kDa-440kDa) Apply Override: Apply To Settings	Ladder C MW	Capillary 1 Position	Fit	Remove	
	Biotinylated Ladder (66kDa-440kDa) Apply Override: Apply To Settings	Ladder C MW 66	Capillary 1 Position 500	dd Fit V	Remove	
	Biotinylated Ladder (66kDa-440kDa) Apply Override: Apply To Settings	 Ladder C MW 66 116 200 	Position 500 550	fit V	Remove	
	Biotinylated Ladder (66kDa-440kDa) Apply Override: Apply To Settings	 Ladder C MW 66 116 200 280 	Capillary 1 Position 500 550 600 650	fit V V	Remove	
	Biotinylated Ladder (66kDa-440kDa) Apply Override: Apply To Settings	 Ladder C MW 66 116 200 280 440 	Capillary 1 Position 500 550 600 650 700	fit V V V	Remove	
	Biotinylated Ladder (66kDa-440kDa) Apply Override: Apply To Settings	 Ladder C MW 66 116 200 280 440 	A Capillary 1 Position 550 650 650 650 700	dd Fit V V V V	Remove	
	Biotinylated Ladder (66kDa-440kDa) Apply Override: Apply To Settings Add Remove	 Ladder C MW 66 116 200 280 440 	A Capillary 1 Position 550 600 650 700	fit V V V dd	Remove]

- Click **Import** to import an analysis settings file. This will be explained in more detail in "Importing Analysis Settings" on page 284.
- Click **Export** to export the current analysis settings file. This will be explained in more detail in "Exporting Analysis Settings" on page 284.
- Click **Restore Original** to restore Compass default settings.
- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

Standards Analysis Settings Groups

Standards settings are saved as a group, and multiple settings groups can be created. Specific group settings can then be applied to individual capillaries, sample names or attributes in the run data.

NOTES:

We recommend using the Compass default values for standards analysis settings. These settings are included in the default Standards group.

Analysis settings are run-file specific. However, settings can be imported or exported for use with other run files. For more information see "Importing and Exporting Analysis Settings" on page 284.

Standards groups are displayed in the analysis settings box:

Analysis Settings	
Standards	
Add	Remove

The Standards group shown contains the Compass default settings. You can make changes to this group and create new groups.

To view settings for a group, click on the group name in the analysis settings box.

Changing the Capillary Used for the Ladder

Known ladder standards are used to calculate the molecular weights of unknown sample proteins. As noted in each of the Master Kit Product Inserts, we strongly recommend that you use capillary 1 for the ladder. However, you can change the ladder capillary as needed, or opt to not use a ladder at all.

NOTES:

When the ladder capillary is set to none, fluorescent standards information is used to calculate sample protein molecular weight instead of the ladder.

The reported molecular weight for immunodetected and total protein sample proteins in Compass may vary slightly from predicted molecular weights based on sample and assay conditions.

To change the ladder capillary:

1. Select Edit in the main menu and click Analysis, then click Standards in the options list.

- page 274
- 2. Click the arrow in the drop down list next to Ladder Capillary, then click a capillary number or none from the list.



Compass will use the data in the selected capillary to recalculate molecular weights for sample proteins in the run data using the information in the ladder table. If none is selected, Compass will instead use the information in the fluorescent peaks table (fluorescent standards) to calculate molecular weight for sample proteins.

NOTE: When the ladder capillary is set to none, the ladder table becomes inactive and cannot be modified.

Creating a New Standards Group

- 1. Select Edit in the main menu and click Analysis, then click Standards in the options list.
- 2. Click Add under the analysis settings box. A new group will be created:

Analysis Settings
Standards
Standards 2
Add Remove

3. Click on the new group and enter a new name.

Analysis S	ettings		
Standar	ds		
New Sta	indards		
	Add	Remove	

4. Click in the first cell in the **MW** column in the Fluorescent Peaks table.

MW	Position	Fit	Registr	ation	
1	200			ation	
29	400		Ë		
230	900	v			

5. Enter the molecular weight (in kDa) for the fluorescent standard.

	200		V	
29	400	¥		
30	900	~		

6. Click in the first cell in the **Position** column.

MW	Position	Fit	Registration
2	200		 Image: A start of the start of
29	400	V	
230	900	~	

7. Enter the position of the fluorescent standard peak.

VIV	Position	Fit	Registration
2	115		
29	400	¥	
230	900	~	

NOTE: Standards peak positions are relative to each other. Only the difference in their position is used to help identify the standard peaks. When entering standard peak information for the first time, review the standards data in the Analysis screen to find the correct peak position.

8. Repeat the steps above for the remaining standards in the table.

- To add another standard Click Add under the peak table, then modify the information in the new row.
- To remove a standard Select its row and click Remove.
- 9. Select which standard should be used for capillary registration by clicking the checkbox in the **Registra**tion column. The first standard is typically used for the registration.

WN	Position	Fit	Registration	
2	115		V	
29	400	¥		
230	900	~		

NOTE: In order for Compass to perform data analysis, at least one peak must be selected for registration.

10. Select which standards should be used for molecular weight determination of sample proteins by clicking the checkbox in the **Fit** column. The standards not used for registration are typically used for fit.

WM	Position	Fit	Registration	
2	115		V	
29	400	¥		
230	900	~		

11. Click the arrow in the drop down list next to Ladder Capillary, then click a capillary number or none from the list. Capillary 1 is typically used for the ladder.



Compass will use the data in the selected capillary to calculate the molecular weights for sample proteins using the information in the ladder table. If none is selected, Compass will instead use the information in the fluorescent peaks table (fluorescent standards) to calculate molecular weight for sample proteins.

NOTES:

When the ladder capillary is set to none, the ladder table becomes inactive and cannot be modified.

The reported molecular weight for immunodetected and total protein sample proteins in Compass may vary slightly from predicted molecular weights based on sample and assay conditions.

12. If a ladder capillary was selected, click in the first cell in the **MW** column in the ladder table.

MW	Position	Fit
2	350	V
40	520	¥
66	550	~
116	700	¥
180	850	¥
230	900	 Image: A start of the start of

Enter the molecular weight (in kDa) for the ladder standard.
MW	Position	Fit
15	350	~
40	520	~
66	550	~
116	700	~
180	850	~
230	900	~

13. Click in the first cell in the **Position** column.

Ladder Capillary 3					
MW	Position	Fit			
15	200	V			
40	520	~			
66	550	~			
116	700	~			
180	850	~			
230	900	~			
		Add			

Enter the position of the ladder standard peak.

1W	Position	Fit
15	205	~
40	520	~
66	550	~
116	700	~
180	850	~
230	900	~

NOTE:

Ladder peak positions are relative to each other. Only the difference in their position is used to help identify the ladder peaks. When entering ladder peak information for the first time, review the ladder data in the Analysis screen to find the correct peak position.

14. Repeat the steps above for the remaining ladder standards in the table.

- To add another ladder standard Click Add under the table, then modify the information in the new row.
- To remove a ladder standard Select its row and click Remove.
- 15. Select which ladder standards should be used for molecular weight determination of sample proteins by clicking the checkbox in the **Fit** column. All ladder standards are typically used for fit.

1W	Position	Fit	
15	205	V	
45	515	~	
70	615	 Image: A set of the set of the	
.00	715	~	
.20	815	~	
95	920	 Image: A start of the start of	

16. To use the new group as the default analysis settings for the run file data, click the arrow in the drop down list next to Default, then click the new group from the list. Analysis settings in the new group will then be applied to the run data.

Analysis Settings	
Standard	
New Standards	
Add	Remove
Default	Standard 👻
	Standard
	New Standards

17. Click **OK** to save changes.

Changing the Default Standards Group

- 1. Select Edit in the main menu and click Analysis, then click Standards in the options list.
- 2. Click the arrow in the drop down list next to Default, then click a new default group from the list.

Standard	
New Standards	
Add	Remove
efault	Standard
	Standard
	NL CL L

3. Click **OK** to save changes. Analysis settings in the group selected will be applied to the run data.

Modifying a Standards Group

- 1. Select Edit in the main menu and click Analysis, then click Standards in the options list.
- 2. Click on the group in the analysis settings box you want to modify.

	s		
New Star	ndards		

- 3. Modify fluorescent standards and ladder standards information as described in "Creating a New Standards Group" on page 275.
- 4. Click **OK** to save changes. The new analysis settings will be applied to the run data.

Deleting an Analysis Group

- 1. Select Edit in the main menu and click Analysis, then click Standards in the options list.
- 2. Click on the group in the analysis settings box you want to delete and click **Remove**.

Analysis Settings		
Standards		
New Standards		
Add	Remove	

3. Click **OK** to save changes.

Applying Analysis Groups to Specific Run Data

- 1. Select Edit in the main menu and click Analysis, then click Standards in the options list.
- 2. Click on the group in the analysis settings box you want to apply to specific run data.

Analys	is Settings			
Stand	dards			
New	Standards			
				
	Add	Rem	ove	

3. Application of analysis groups to specific run data is done in the override box. Click **Add** under the override box. A default override data set will be created from sample information found in the run file.

Override	
Apply To	Settings
Biotinylated Ladder	Standard
Add	Remove

4. Click the cell in the **Apply To** column, then click the down arrow.

Override	
Apply To	Settings
tinylated Ladder 👻	Standard
Biotinylated Ladde	3
K562	
RTU K562	
1mg/ml	
Cycle 1	
Cycle 2	
Cycle 3	
Cycle 4	
Cycle 5	
Cycle b	
Cycle /	
Cycle 8	
Custom Settings	L Damana
Add	Kemove

- 5. Select an option from the drop down list. This will apply the settings group selected to specific run data as follows:
 - **Sample names** All sample names entered in the assay template (Assay screen) will display in the drop down list, otherwise the default name of Sample will be shown. Select a name to apply group settings to all capillaries that use this sample name in the run file.
 - Attributes All sample attributes entered in the assay template (Assay screen) will display in the drop down list. Select an attribute to apply group settings to all capillaries that use this attribute in the run file.
 - **Cycle 1-8** When this option is selected, group settings will be applied to all capillaries in the cycle.
 - **Custom settings** Lets you choose specific capillaries to apply the group settings to. When this option is selected, the following pop-up box appears to let you enter a specific capillary number or range of capillaries:

🛞 Custom Settings
Enter cycle and capillary descriptor
Examples: 2:3, 1:1-12 Cycle 2 capillary 3, and cycle 1 capillaries 1 through 12 1-3:6+8+10 Cycles 1 through 3, capillaries 6, 8 and 10
OK Cancel

6. If you need to change the analysis group used for a data set, click the cell in the **Settings** column and click the down arrow. Select a group from the drop down list.

Override	
Apply To	Settings
Biotinylated Ladder	Standard
	Standard V New Standards
Add	Remove

- 7. Repeat the previous steps to apply other groups to specific run data.
- 8. To remove a data set, click on its cell in the **Apply To** column, then click **Remove**.
- 9. Click **OK** to save changes.

Importing and Exporting Analysis Settings

The analysis settings in a run file can be exported as a separate file. This allows the same analysis settings to be imported into other assays or run files at a later time, rather than having to re-enter settings manually.

Importing Analysis Settings

NOTE: Importing an analysis settings file populates the settings in all Analysis pages.

- 1. Open the run file or assay you want to import analysis settings to.
- 2. Select Edit in the main menu and click Default Analysis (Assay screen) or Analysis (Analysis screen).
- 3. Click **Import** on any page.
- 4. Select a settings file (*.settings) and click **OK**. The imported settings will display in all analysis pages.

Exporting Analysis Settings

NOTE: Exporting an analysis settings file exports the settings in all Analysis pages.

- 1. Open the run file or assay you want to export analysis settings from.
- 2. Select Edit in the main menu and click Default Analysis (Assay screen) or Analysis (Analysis screen).
- 3. Click **Export** on any page. The following window displays:



- 4. The default directory is Compass/Assays. Change the directory if needed.
- 5. Enter a file name and click **Save**. The settings will be saved as a *.settings file.

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Chapter 9: Charge Assay Data Analysis

Chapter Overview

- Analysis Screen Overview
- Opening Run Files
- How Run Data is Displayed in the Analysis Screen
- Viewing Run Data
- Compass Run Data Notifications and Warnings
- Checking Your Results
- Group Statistics
- Copying Data Views and Results Tables
- Exporting Run Files
- Changing Sample Protein Identification
- Changing the Virtual Blot View
- Changing the Electropherogram View
- Closing Run Files
- Compass Analysis Settings Overview
- Advanced Analysis Settings
- Images Analysis Settings
- Peak Fit Analysis Settings
- Peak Names Settings
- Standards Settings
- Importing and Exporting Analysis Settings

Analysis Screen Overview

The Analysis screen is used to view run data including electropherograms, capillary images, lane view data and tabulated results. Any post-run analysis is also done in this screen. To access this screen, click **Analysis** in the screen tab:



Analysis Screen Panes

The Analysis screen has six panes, each displays the following data for up to 96 capillaries per experimental run:

- Experiment Lists the assay protocol steps and assay template information.
- **Graph** Displays electropherogram data for immunodetected sample proteins, fluorescent standards or capillary registrations.
- **Image** Displays a 12-capillary image of the separated immunodetected sample proteins, fluorescent standards or capillary registrations.
- Lane Displays data for immunodetected sample proteins, fluorescent standards or capillary registrations as bands in individual lanes. This virtual blot-like image is similar to traditional blot results.
- **Peaks** Lists the tabulated results for immunodetected sample proteins and information for identified fluorescent standards and capillary registrations.
- **Capillaries** Displays a list of the immunodetected sample proteins Compass named automatically using the user-defined peak name analysis parameters.



NOTE: The reported pl for immunodetected sample proteins in Compass may vary slightly from predicted pls based on sample, buffer and assay conditions.

Software Menus Active in the Analysis Screen

The following software menus are available:

- File
- Edit
- View
- Instrument (when Compass is connected to Peggy Sue)
- Window
- Help

The File, Edit and View menu options specific to the Analysis screen are described next.

File Menu

The following File menu options are active:



- Open Run Opens a run file.
- Add Run Opens and views other run files in addition to those that are already open.
- **Close** Closes the run file currently being viewed.
- Close All Closes all open run files.
- Save Saves changes to the open run file.
- Save As Saves changes to the open run file under a different file name.
- Export Tables Exports the results for all capillaries in the run in .txt format.
- Export Spectra Exports the raw data traces for each capillary in the run in .txt or .cdf format.
- Exit Closes Compass.

Edit Menu

The following Edit menu options are active:



- **Copy** Lets you copy data shown in the graph, lane, peaks or capillaries panes. See "Copying Data Views and Results Tables" on page 334 for more information.
- **Analysis** Displays the analysis settings used to analyze the run data and lets you change them as needed. See "Compass Analysis Settings Overview" on page 372 for more information.
- **Preferences** Lets you set and save custom preferences for data export, plot colors in the graph and Peggy Sue's Twitter settings. See Chapter 10, *"Setting Your Preferences"* for more information.

View Menu

The following View menu options are active:



- Single View Displays data in a per capillary (single) view format.
- Multiple View Displays data in a per 12-capillary (multiple) view format.
- Standards Lets you change the data view to show only the fluorescent standards.
- **Registration** Lets you change the data view to show only the capillary registrations.
- Samples Lets you change the data view to show only immunodetected sample proteins.
- **Grouping** Lets you analyze replicates by calculating the mean, standard deviation and CV of named proteins.

- Filter Lets you display data only for specific capillaries or named proteins.
- View Region Lets you change the pl (x-axis) range of the data displayed.
- Show Hidden- Shows capillaries that are hidden from the data view.

Opening Run Files

You can open one or multiple run files at a time to compare data between runs.

Opening One Run File

NOTE: If you need to open a run file when another run is executing, launch another instance of Compass first, then open the file.

1. Select File in the main menu and click Open Run.

File	Edit View Instrumen	t W	indow Help
	Open Run 🕨		DemoData
	Add Run		2012-03-05_11-51-19_HelaControlERKassay
	Close		Simple Western ERK Demo
	Close All		2012-02-29_18-08-50_2012Feb29_as1Ab_25min
	Close All		2012-02-29_11-51-19_2012Feb29_las1Ab_25min
	Save		3 ab run
	Save As		Simple Western
	Export Tables		2011-08-31_16-38-23_test
	Export Spectra •		2011-09-01_16-41-02
	Exit		Browse

2. A list of the last 10 runs opened will display. Select one of these runs or click **Browse** to open the Runs folder and select a different file.

Opening Multiple Run Files

NOTE: If you need to open a run file when another run is executing, launch another instance of Compass first, then open the file.

1. To open the first run file, select File in the main menu and click Open Run.

File Edit View Instrument	t W	indow Help
Open Run 🕨		DemoData
Add Run 🕨		2012-03-05_11-51-19_HelaControlERKassay
Close		Simple Western ERK Demo
Close All		2012-02-29_18-08-50_2012Feb29_as1Ab_25min
		2012-02-29_11-51-19_2012Feb29_as1Ab_25min
Save		3 ab run
Save As		Simple Western
Export Tables		2011-08-31_16-38-23_test
Export Spectra 🕨		2011-09-01_16-41-02
Exit		Browse

- 2. A list of the last 10 runs opened will display. Select one of these runs or click **Browse** to open the Runs folder and select a different file.
- 3. To open another run file, select **File** in the main menu and click **Add Run**.

File	Edit View Instrumer	t V	Vindow Help				
	Open Run 🔶						
	Add Run		2011-08-31_16-38-23_test				
	Close		3 ab run				
	Close All		2011-09-01_16-41-02				
	Save	L	Browse				
	Save As	1	5000 -				
	Export Tables	1	4500				
	Export Spectra •	1	4000-				
	Exit	1					

4. A list of the last 10 runs opened will display. Select one of these runs or click **Browse** to open the Runs folder and select a different file. When a run is added, its data will append to the open run file, and will display as a second set of up to 96 capillaries in all screen panes. The second run file name will also appear in the Compass title bar:



5. Repeat the last two steps to open additional runs.

How Run Data is Displayed in the Analysis Screen

Data in the run file is compartmentalized for easy review in one of six panes in the Analysis screen.

Experiment Pane: Assay and Capillary Information

The experiment pane displays assay and capillary information for each of the 96 capillaries in the run. A maximized view of the experiment pane is shown below.

Experiment										
Sample	Primary	Cycle	Сар	S	1	2	3			
High phospho HeLa in 5-8	ERK1/2	1	1	A1	11	A				
Low phospho HeLa in 5-8	ERK1/2	1	2	A2	12	A				
High phospho HeLa in 5-8	ERK1/2	1	3	A3	в	A				
Low phospho HeLa in 5-8	ERK1/2	1	4	A4	I4	A				
High phospho HeLa in 5-8	ERK1/2	1	5	A5	15	A				
Low phospho HeLa in 5-8	ERK1/2	1	6	A6	16	A				
High phospho HeLa in 5-8	ERK1/2	1	7	A7	17	A				
Low phospho HeLa in 5-8	ERK1/2	1	8	A8	18	A				
High phospho HeLa in 5-8	ERK1/2	1	9	A9	19	A				
Low phospho HeLa in 5-8	ERK1/2	1	10	A	110	A				
High phospho HeLa in 5-8	ERK1/2	1	11	A	111	A				
Low phospho HeLa in 5-8	ERK1/2	1	12	A	112	A				

- To view all columns Click the Experiment tab, then use the scroll bar or click Maximize in the upper right corner.
- To resize columns Click the Experiment tab. Roll the mouse over a column border until the sizing arrow appears, then click and drag to resize.

Experiment pane column descriptions for the Peggy Sue Charge default assay are as follows:

• **Sample** - Sample name. If sample names were entered in the assay template (Assay screen), those names will display here. Otherwise, Sample (default name) will display.

NOTE: Data notification icons will display in the sample column if Compass detected a potential analysis issue or data was manually modified by the user. For more information see "Compass Run Data Notifications and Warnings" on page 318.

- **Primary** Primary antibody name. If primary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Primary (default name) will display.
- Cycle Run cycle number. There are 12 capillaries in one cycle.

NOTE: Peggy Sue can run up to eight cycles (12 capillaries per cycle) in an experiment. Each cycle is designated in the Experiment summary.

- **Cap** Capillary number.
- **S** Well on the assay plate used for sample.
- 1 Well on the assay plate used for primary antibody.
- 2 Well on the assay plate used for secondary antibody.
- **3** Well on the assay plate used for tertiary antibody (if used).

Graph Pane: Electropherogram Data

Click the **Graph** tab to view data for immunodetected sample proteins, fluorescent standards or capillary registrations. Data for samples is shown in the following example, and immunodetected proteins are displayed as peaks:



More Graph view options will be described in more detail in "Changing the Electropherogram View" on page 349.

Image Pane: Capillary Separation Image Data

Click the **Image** tab to view final separation images of immunodetected sample proteins, fluorescent standards or capillary registrations for up to 96 capillaries per experimental run. Image data for samples is shown in the following example:



Lane Pane: Virtual Blot-Like Image Data

Click the **Lane** tab to view data for immunodetected sample proteins, fluorescent standards or capillary registrations as bands in individual lanes. This virtual blot-like image is similar to traditional blot results. Data for samples in the lane view is shown in the following example, and immunodetected proteins are displayed as bands.



To view information for a band, roll the mouse over a band until the info box appears.

NOTE: The reported pl for immunodetected sample proteins in Compass may vary slightly from predicted pls based on sample, buffer and assay conditions.

Lane data displayed in the virtual blot is automatically aligned by Compass. To view raw, unaligned lane data and learn more about virtual blot viewing options, see "Changing the Virtual Blot View" on page 343.

Peaks Pane: Calculated Results

Click the **Peaks** tab to view tabulated results for immunodetected sample proteins, fluorescent standards or capillary registrations. Each row in the table shows the individual results for each peak detected in each capillary. Data for samples in the peaks table is shown in the following example:

III Peaks	Capillari	es										, 🗆
Sample	Primary	Cap	Peak	Name	Position	pI	Height	Area	% Area	Width	S/N	
High pho	ERK1/2	C1:1	1	ppErk1	499	5.468	394.8	6337	58.7	0.0464	517.9	
High pho	ERK1/2	C1:1	2	pErk1	583	5.735	153.9	1411	13.1	0.0265	321.1	
High pho	ERK1/2	C1:1	3	ppERK2	607	5.805	711.2	5864	62.4	0.0239	1538.7	
High pho	ERK1/2	C1:1	4	Erk1	639	5.904	401.0	3054	28.3	0.0220	879.5	
High pho	ERK1/2	C1:1	5	pERK2	693	6.129	75.8	701	7.5	0.0469	159.2	
High pho	ERK1/2	C1:1	6	ERK2	783	6.581	219.5	2831	30.1	0.0562	352.9	
Low phos	ERK1/2	C1:2	1	ppErk1	505	5.504	45.4	477	3.5	0.0300	75.9	
Low phos	FRK1/2	C1:2	2	nErk1	582	5,739	74.9	492	3.6	0.0188	137.0	-

NOTES:

Peaks that Compass names automatically using the user-defined peak name analysis parameters are color-coded.

The reported pl for immunodetected sample proteins in Compass may vary slightly from predicted pls based on sample, buffer and assay conditions.

- To view all rows Click the Peaks tab, then use the scroll bar or click Maximize in the upper right corner.
- To resize columns Click the Peaks tab. Roll the mouse over a column border until the sizing arrow appears, then click and drag to resize.

Peak table column descriptions are as follows:

- **Sample** Sample name. If sample names were entered in the assay template (Assay screen), those names will display here. Otherwise, Sample (default name) will display.
- **Primary** Primary antibody name. If primary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Primary (default name) will display.
- Cap Cycle and capillary number. For example, C1:3 indicates cycle 1, capillary 3.

NOTE: Peggy Sue runs up to eight cycles in an experiment, 12 capillaries at a time. Capillary and cycle numbers are displayed in the Experiment tab.

• **Peak** - Peak number. Peaks are numbered in order of detection.

- **Name** Peak name. Displays peaks that Compass named automatically using the user-defined peak name analysis parameters. Cells will be blank if Compass was not able to name the peak or if naming parameters were not entered.
- **Position** Displays the pixel position of a peak in the image.
- **pl** Displays the calculated pl for the peak.
- Height Displays the calculated peak height.
- Area Displays the calculated peak area.
- **% Area** Displays the calculated percent area for the named peak compared to all named peaks. This value results from dividing the individual peak area by the sum of all named peak areas for the capillary and multiplying by 100 (shown for named peak sample data only).
- Width Displays the calculated peak width (shown for sample data only).
- **S/N** Displays the calculated signal to noise ratio for the peak.

Capillaries Pane: User-Specified Peak Names

Click the **Capillaries** tab to view tabulated results for sample proteins associated with specific primary antibodies in the run data. Compass labels these sample peaks automatically using user-defined peak name settings. Each row in the table shows the individual results for the named peaks detected in each capillary. Data for samples in the capillaries table is shown in the following example.

III Peaks III Capill Vies										
% ~										
Sample	Primary	Capillary	ppErk1	pErk1	Erk1	ppERK2	pERK2	ERK2	*	
High pho	ERK1/2	C1:1	6337	1411	3054	5864	701	2831		
Low phos	ERK1/2	C1:2	477	492	12533	440	275	13959	=	
High pho	ERK1/2	C1:3	7333	1672	3272	6400	833	3457		
Low phos	ERK1/2	C1:4	591	577	13618	589	388	16427		
High pho	ERK1/2	C1:5	8556	1533	3320	6819	921	3667		
Low phos	ERK1/2	C1:6	710	596	14005	561	330	17124	-	
Law a la	5510 (S		0000	4000	2025	7101		1007	· ·	

NOTES:

Peaks that Compass names automatically with user-defined peak name settings are color-coded.

Information displayed for fluorescent standards and capillary registration data will be for identified standards or registration peaks.

• To view all rows - Click the Capillaries tab, then use the scroll bar or click Maximize in the upper right corner.

 To resize columns - Click the Capillaries tab. Roll the mouse over a column border until the sizing arrow appears, then click and drag to resize.

Capillaries table column descriptions are as follows:

- **Sample** Sample name. If sample names were entered in the assay template (Assay screen), those names will display here. Otherwise, Sample (default name) will display.
- **Primary** Primary antibody name. If primary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Primary (default name) will display.
- **Capillary** Cycle and capillary number. For example, C1:3 indicates cycle 1, capillary 3.

NOTE: Peggy Sue runs12 capillaries at a time in a cycle and is able to run up to eight cycles in an experiment. The Information on cycle and capillary number is displayed in the Experiment tab.

- **Peak Name Columns** An individual column per peak name will display for every peak identified by name in the run data. Cells for capillaries in these columns will be blank if Compass did not find peaks automatically using the user-defined peak name analysis parameters (or none were entered).
 - To view % area in the peak name columns Select % in the upper right corner of the pane. This displays the calculated percent area for the named peak compared to all named peaks. This value results from dividing the individual peak area by the sum of all named peak areas for the capillary and multiplying by 100.

Peaks	🗰 Peaks 🗰 Capillaries 👘 🗖										
									K√		
Sample	Primary	Capillary	ppErk1	pErk1	Erk1	ppERK2	pERK2	ERK2	15 -		
High pho	ERK1/2	C1:1	58.7	13.1	28.3	62.4	7.5	30.1			
Low phos	ERK1/2	C1:2	3.5	3.6	92.8	3.0	1.9	95.1	=		
High pho	ERK1/2	C1:3	59.7	13.6	26.7	59.9	7.8	32.3			
Low phos	ERK1/2	C1:4	4.0	3.9	92.1	3.4	2.2	94.4			
High pho	ERK1/2	C1:5	63.8	11.4	24.8	59.8	8.1	32.1			
Low phos	ERK1/2	C1:6	4.6	3.9	91.5	3.1	1.8	95.0	-		
and a second	COLOR IO			40.0							

• To view peak area in the peak name columns (default) - Deselect % in the upper right corner of the pane. This displays calculated peak area for the individual peak only.

Viewing Run Data

Each run file contains the following data for up to 96 capillaries:

- Sample data For the immunodetected proteins in the sample.
- Standards data For the fluorescent standards run with each sample.
- Registration data For tracking capillaries as they are moved for various assay steps.

Data can be viewed for one, all, or individually selected capillaries.

Switching Between Sample, Standards and Registration Data Views

You can switch between viewing sample, standards and registration data in a run file using the View bar or View menu:



Data buttons in the View bar:





Show Registrations



Show Samples



To view sample data - Click Show Samples in the View bar or select View in the main menu and click Samples:

- Data in this view is for immunodetected sample proteins only.
- Graph view data displays electropherograms in chemiluminescence units (y-axis) and pl (x-axis).
- Lane view data displays immunodetected sample proteins only.
- Image view data displays immunodetected sample proteins only.
- Results for each immunodetected protein are shown in the peaks and capillaries tables.

NOTE: The reported pl for immunodetected sample proteins in Compass may vary slightly from predicted pls based on sample, buffer and assay conditions.

For information on checking and identifying sample peaks, see "Step 4 – Checking Samples" on page 325.

🛞 DemoData_Charge_HeLa_ERK12, Demo data_Hi-Low ERK (old) - Compass - • • File Edit View Instrument Window Help Assay 🖓 Run Summary 🚛 Analysis 🚊 Standards 🛧 Reg. 🚊 Samples 🛛 🔳 🏭 🗖 🗖 🗽 Graph 🛛 🔛 Image 🖽 Lane Experiment I 🖩 🗖 🗸 🗖 🖬 Sample Primary Cycle ^ C1:1 280 High ... ERK1/2 1 Std 7.3 Low p... ERK1/2 1 260 High ... ERK1/2 1 Std 4.9 240 Low p... ERK1/2 1 220 Hi-phospho HeLa ✓ High ... ERK1/2 1 200 Low p... ERK1/2 1 180 High ... ERK1/2 1 160 140 120 100 Low p... ERK1/2 1 High ... ERK1/2 1 Low p... ERK1/2 1 High ... ERK1/2 1 100 Low p... ERK1/2 1 Hi-ph... ERK1/2 1 80 Low-p... ERK1/2 1 60 Hi-ph... ERK1/2 1 Std 6.0 Std 6.4 40 Low-p... ERK1/2 Std 7.0 1 . Hi-ph... ERK1/2 1 20 Low-p... ERK1/2 1 0 Hi-ph... ERK1/2 1 100 200 300 400 500 600 700 800 900 1,000 0 Low-p... ERK1/2 1 Position Hi-ph... ERK1/2 1 Low-p... ERK1/2 1 - -Peaks Capillaries Hi-ph... ERK1/2 1 * Sample Primary Cap Peak Position Height Low-p... ERK1/2 1 Hi-ph... ERK1/2 C1:1 10 475 5.2 Low-p... ERK1/2 2 S Hi-ph... ERK1/2 22.4 C1:1 11 552 Hi-ph... ERK1/2 2 S Hi-ph... ERK1/2 Low-p... ERK1/2 2 C1:1 12 620 23.6 Hi-ph... ERK1/2 Hi-ph... ERK1/2 C1:1 13 666 10.5 2 Low-p... ERK1/2 S Hi-ph... ERK1/2 C1:1 14 738 17.9 Е 2 Hi-ph... ERK1/2 2 Hi-ph... ERK1/2 C1:1 15 772 8.9 16 S Hi-ph... ERK1/2 C1:1 783 250.2 Þ III

To view standards data - Click Show Standards in the View bar or select View in the main menu and click Standards:

- Data in this view is for the fluorescent standards only. These standards are premixed with each sample prior to analysis.
- Graph view data displays electropherograms in fluorescence (y-axis) and position (x-axis).
- Lane view data displays fluorescent standards only.
- Image view data displays fluorescent standards only.
- Standards are highlighted in both the peaks and capillaries tables and marked with an S.

For information on checking and identifying standards peaks, see "Step 2 – Checking Fluorescent Sizing Standards" on page 320.

To view registration data - Click Show Registrations in the View bar or select View in the main menu and click Registration:



- Data in this view shows capillary registration only.
- Graph view data displays electropherograms in fluorescence (y-axis) and position (x-axis).
- Lane view data displays capillary registration only.
- Image view data displays capillary registration only.
- Registrations are highlighted in both the peaks and capillaries tables and marked with an R.

Because capillaries must be moved multiple times during the run, their positions are tracked to account for potential changes in imaging position as they are moved to and from the separation block. To do this, one of the standards is used as a capillary registration. An image of the standard used for registration is taken at the end of the separation step prior to chemiluminescent imaging. The position of this peak in the registration image is then compared to the position of the same standard peak in the standards image. The shift value listed in the peaks table is the change in pixel position between the two images. Compass data is then aligned to account for any changes in capillary position.

For information on checking and identifying registration peaks, see "Step 3 – Checking Capillary Registrations" on page 324.

Selecting and Displaying Capillary Data

You can choose to view data from one, multiple, or all capillaries at once.

• To look at data for one capillary - Click a row in the experiment pane. Data for just the row selected will display in all data views and tables. The following example shows sample data displayed in the lane and peaks panes when one sample is selected:



To look at data for multiple non-sequential capillaries - Hold the **Ctrl** key and select the rows you want to view in the experiment pane. Data for only the rows selected will display in all data views and results tables. The following example shows sample data displayed in the graph (in multiple view) and capillaries panes when multiple rows are selected:



To look at data for multiple sequential capillaries - Hold the **Shift** key and select the first and last rows you want to view in the experiment pane. Data for only the selected rows will display in all data views and results tables. The following example shows standards data displayed in the image and peaks panes when multiple sequential rows are selected:



To look at data for all capillaries - Hold the **Shift** key and select the first and last rows in the experiment pane. Data for all rows will display in all data views and results tables. The following example shows sample data displayed in the lane and peaks panes when all rows are selected. The pane scroll bars can be used to view all 8 cycles:



Switching Between Single and Multiple Views of the Capillaries

You can switch between displaying run data in the graph, image and lane panes in a per capillary (single) format or up to 96 capillary format. This view is selected in the View bar or the View menu.

🗮 Sta	indai	rds 🛧 Reg. 🚖 Sampl	es 🔳 🔛
	View	Instrument Window Single View Multiple View	
	۹	Standards Registration Samples	
		Grouping Filter View Region Show Hidden	

Capillary view buttons in the View bar:



Single View



Multiple View

To view data in a per capillary format - Click Single View in the View bar or select View in the main menu and click Single View:



Data for the row(s) selected in the experiment pane will display as follows:

- Electropherograms in the graph pane display overlaid or stacked (depending on the graph option chosen).
- Results for only the selected row(s) will display in the peaks and capillaries tables.
- The selected row(s) are highlighted in the image pane:



• Lanes for only the selected row(s) are displayed in the lane pane:





To view data up to 96-capillary format - Click Multiple View in the View bar or select View in the main menu and click Multiple View:

Data for the row(s) selected in the experiment pane will display as follows:

- Electropherograms corresponding to the selected row(s) are highlighted and displayed in the graph pane.
- Results for selected capillaries display in the peaks and capillaries tables and are highlighted.
- The selected row(s) are highlighted in the image pane:


• All selected lanes display in the lane pane, and lanes corresponding to the selected row(s) are highlighted.



Hiding Capillary Data

Capillary data can be hidden from view if needed. To do this:

1. Click the **Experiment** tab.

- 8 Experiment Sample Primary Cycle * Sample Primary 1 Primary Sample 1 Sample Driman 1 Sample P × Hide Clear All Sample P
- 2. Select the rows you want to hide, then right click on one of the selected rows and click Hide:

Data for selected rows will be hidden in all data views and results tables (except for the image pane).

- **To view hidden rows** Select **View** in the main menu and click **Show Hidden**. Hidden rows will become visible again in all panes, and hidden rows will be marked with an X in the experiment pane:
 - Experiment
 Image: Cycle

 Sample
 Primary
 1

 Sample
 Primary
 1
- To unhide rows Select the hidden row(s). Right click on one of the selected rows and click Unhide.

Setting Run Data Display Filters

The filter lets you auto-hide specific capillaries in the run file data, or only show data for peaks that Compass identified automatically using the user-defined peak name analysis parameters.

NOTE: When more than one run file is open, filter settings will be applied to all files.

• To filter data to show specific capillaries only - Select View in the main menu and click Filter. Uncheck the boxes for the capillaries you do not want shown, then click OK.

🛞 Filter	—					
Cycles 1	Capillaries					
 ✓ 2 ✓ 3 ✓ 4 	 ✓ 2 ✓ 3 ✓ 4 					
 ✓ 4 ✓ 5 ✓ 6 	▼ 5 ▼ 6					
✓ 7✓ 8	 ✓ 7 ✓ 8 ✓ 0 					
	▼ 10 ▼ 11					
12						
Show named peaks only						
OK Cancel						

Only data for the checked capillaries will display in data views and results tables, unchecked capillaries will be hidden.

• To filter data to show named peaks only - Select View in the main menu and click Filter. Select Show named peaks only then click OK. Only data for peaks that Compass identified automatically using the user-defined peak name analysis parameters will display in data views and results tables, all other peak data will be hidden.

Compass Run Data Notifications and Warnings

If Compass detects a potential data issue, a notification or warning will display next to the row in the experiment pane. A list of warnings, notifications and corrective actions are as follows:



• **Manual correction of sample data notification** - Indicates the user modified sample data manually, such as adding or removing a sample peak. Rolling the mouse over the icon displays the type of data modification made.

Sample	Primary	Cycle	C
🗸 High	ERK1/2	1	1
Low p	ERK1/2	1	-
High	ERK1/2 Ba	iseline M	anual

Standards warning - Indicates that one of the standards may not be identified properly. This can be resolved by manually identifying the standards. Please refer to "Step 2 – Checking Fluorescent Sizing Standards" on page 320 for details. Rolling the mouse over the icon displays warning details.



- Manual correction of standards data notification Indicates the user modified the standards data manually. Rolling the mouse over the icon displays the type of data modification made.
- Registrations warning Indicates that a capillary registration was not identified properly. This can be resolved by manually identifying the registration peak. Please refer to "Step 3 Checking Capillary Registrations" on page 324 for details. Rolling the mouse over the icon displays warning details.



- Manual correction of registrations notification Indicates the user modified the capillary registration data manually. Rolling the mouse over the icon displays the type of data modification made.
- Peak fit warning Indicates that a peak cannot be fit properly. This is typically caused either by a very small peak in the electropherogram or a peak that is very close to the end of the pl range. This can be resolved by removing the peak. Please refer to "Step 4 Checking Samples" on page 325 or "Step 4 Checking Samples" on page 325 for details. Rolling the mouse over the icon displays warning details.

🛦 Kit low-pho	anti-H	2	4			
Kit low-pho	anti-E	2	5			
Peak Fit Warning: Too many iterations 5						

Checking Your Results

Compass detects immunodetected proteins, fluorescent standards and capillary registrations and reports results automatically with no user-interaction. However, we recommend you review their data as outlined in this section as good general practice to ensure accurate results. If a data warning is presented in the experiment pane, the following steps will also help you identify and correct any issues.

Step 1 – Review the Fluorescent Sizing Standards Movie

All capillaries should have three fluorescent sizing standards. To verify the standards separated in the capillary correctly:

- 1. When the run has completed, click the **Run Summary** screen tab.
- 2. Click the **Separation** tab and play the movie (this will be the fluorescent standards separation).

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3. For each capillary, verify that the standards visibly separated. Each fluorescent point in the capillary represents one of the three sizing standards.

Step 2 – Checking Fluorescent Sizing Standards

To verify the standards are identified correctly:

- 1. Click the **Analysis** screen tab.
- 2. Click **Show Standards** in the View bar. Verification that the standards have been correctly identified can be done in either the graph or lane panes.

Graph Pane:

- a. Click **Single View** in the View bar.
- b. Click on the first row in the experiment pane, then click the **Graph** tab. Check that the electropherogram has the appropriate number of fluorescent pl standard peaks for the pl Standard Ladder you are using. They will also be identified with a green **S** in the peaks table. The pl standards at the low and high end of the pl range in the electropherogram are at higher concentrations as they are also used for capillary registration. In the following example, the pl standards shown are those for pl Standard Ladder 3 (P/N 040-646).



If standards are not identified correctly, they can be manually corrected as follows:

• If an incorrect peak is identified as a standard - Right click the peak in the electropherogram or peaks table and select Not a Standard. Compass should correctly reassign the remaining peaks as standards, and update the peaks table.

• To set an unidentified peak as a standard - Right click the peak in the electropherogram or peaks table and select Force Standard. Compass will assign the peak as a standard, and correctly reassign the remaining standard peaks. A lock icon indicating the standard was set manually will display next to the peak in the peaks table.

NOTE: To remove standards peak assignments that were made manually, right click on the peak in the electropherogram or peaks table and click **Clear**.

c. Repeat the previous steps for the remaining rows in the experiment pane to make sure all standards are identified correctly.

Lane Pane:

- a. Click Multiple View in the View bar.
- b. Click on the first row in the experiment pane, then click the Lane tab. Standards will be bands and identified with a green outline. Check that each lane has the appropriate number of fluorescent pl standard bands for the pl Standard Ladder you are using. They will also be identified with a green S in the peaks table. The pl standard bands at the low and high end of the pl range in each lane will display greater intensity as they are at higher concentrations and are also used as registration standards. In the following example, the pl standards shown are those for pl Standard Ladder 3 (P/N 040-646). To view band labels, roll the mouse over the individual bands.

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If standards are not identified correctly, they can be manually corrected as follows:

- If an incorrect band is identified as a standard Right click the band in the lane or peaks table and select Not a Standard. Compass should correctly reassign the remaining bands as standards.
- To set an unidentified band as a standard Right click the band in the lane or peaks table and select Force Standard. Compass will assign the band as a standard, and correctly reassign the remaining standard bands.
- c. Repeat the previous steps for the remaining rows in the experiment pane to make sure all standards are identified correctly.

Step 3 – Checking Capillary Registrations

All capillaries should have a single capillary registration peak. To verify the registrations are identified correctly:

- 1. Click the **Analysis** screen tab.
- 2. Click Show Registrations and Single View in the View bar.
- 3. Click the **Graph** tab.
- 4. Click on the first row in the experiment pane. The registration peak will be the first and largest peak in the electropherogram. Check that the two registration peaks are identified and labeled Reg 1 and Reg 2 in the electropherogram. They will also be identified with a purple **R** in the peaks table.



5. If the registration peak is not identified correctly, right click the correct registration peak in the electropherogram or peaks table and select **Registration Force**. Compass will assign the new peak as the capillary registration. A lock icon indicating the registration was set manually will display next to the peak in the peaks table. NOTE: To remove registration peak assignments that were made manually, right click on the peak in the electropherogram or peaks table and click **Registration Clear**.

6. Repeat the previous steps for the remaining rows in the experiment pane to make sure all registrations are identified correctly.

Step 4 – Checking Samples

All immunodetected sample proteins in the graph and lane panes will be labeled automatically with the calculated protein pl.

NOTE: The reported pl for immunodetected sample proteins in Compass may vary slightly from predicted pls based on sample, buffer and assay conditions.

To verify that sample proteins are identified correctly:

- 1. Click the **Analysis** screen tab.
- 2. Click **Show Samples** in the View bar. Verification that sample proteins have been correctly identified can be done in either the graph or lane panes, but manual corrections must be done in the graph pane.

Graph Pane:

- a. Click **Single View** in the View bar.
- b. Click on the row in the experiment pane that contains the sample you wish to check, then click the **Graph** tab.



If sample peaks are not identified correctly, they can be manually corrected as follows:

- If an incorrect peak is identified as a sample peak Right click the peak in the electropherogram or peaks table and select **Remove peak**. Compass will no longer identify it as a sample peak in the electropherogram and the peak data will be removed in the results tables.
- To set an unidentified peak as a sample peak Right click the peak in the electropherogram or peaks table and select Add Peak. Compass will calculate and display the results for the peak in the results tables and identify the peak in the electropherogram.

NOTE: To remove sample peak assignments that were made manually and go back to the original view of the data, right click in the electropherogram and click **Clear All**.

c. Repeat the previous steps for the remaining sample rows in the experiment pane to make sure all sample proteins are identified correctly.

Lane Pane:

b. Click on the row in the experiment pane that contains the sample you wish to check, then click the **Lane** tab. To view sample protein band labels, roll the cursor over the individual bands. If sample bands are not identified correctly, they must be corrected in the graph pane as described in the previous section.



Step 5 – Assigning Peak Names (Optional)

Compass can identify and automatically name sample proteins associated with specific primary antibodies in the run data using user-specified peak name settings. For more information on how to do this see "Peak Names Settings" on page 391.

Group Statistics

The Grouping View is used to analyze replicates by calculating the mean, standard deviation, %CV and SEM for named proteins (see "Peak Names Settings" on page 391 for detailed information on entering named proteins). Statistics for each protein are also plotted for easy comparison between samples, antibodies, and proteins.

Using Groups

- 1. A group is automatically created for capillaries with the same sample and primary antibody name, so to use this feature, this information must first be entered into the Assay template. To do this:
 - a. Click the **Assay** tab and go to the Template pane.
 - b. Enter sample names and primary antibody names as described in "Step 6 Add Assay Plate Annotations (Optional)" on page 90. Be sure to enter the same sample and/or primary antibody names for the groups of samples you want to calculate statistics for.

In this example there are two sample types, **High phospho HeLa** and **Low phospho HeLa** which were run with two different antibodies, **ERK 1/2 Primary 1** and **ERK 1/2 Primary 2**.



Each of the two samples were run with the two antibodies in every cycle, and the ERK1/2 antibody generates 6 named peaks.

2. Select the **Analysis** tab. Group your results and view the associated statistics by selecting **View** and clicking on **Grouping**. In the box, click **Enable Grouping**.



3. Select a grouping option by clicking the box next to the option. These options allow you to group capillaries in multiple ways:



- **Group across runs** Groups capillaries from multiple runs. This option creates fewer groups and generates statistics across multiple runs.
- Group across cycles Groups capillaries run in different cycles.
- **No option selected** When only one run is open, groups will only contain capillaries from the same cycle. When more than one run is open, groups are created for each run.

Viewing Statistics

Peak and Capillary Groups

The Peak Groups pane reports statistics for each named protein in every group. Each group shows the statistics for named proteins which includes average area, standard deviation, %CV and SEM. The number in parenthesis after the sample name indicates the number of capillaries in the group.

ample	Primary	Cap	Name	Area	Std.Dev	CV	SEM
Hi-phospho HeLa (24)	ERK1/2 Primary 1		ERK2	3204	972	5.3	198
Hi-phospho HeLa (24)	ERK1/2 Primary 1		Erk1	2841	516	8.2	105
Hi-phospho HeLa (24)	ERK1/2 Primary 1		pERK2	1308	256	9.5	52
Hi-phospho HeLa (24)	ERK1/2 Primary 1		pErk1	887	766	6.4	156
Hi-phospho HeLa (24)	ERK1/2 Primary 1		ppERK2	5105	1368	6.8	279
Hi-phospho HeLa (24)	ERK1/2 Primary 1		ppErk1	8716	1438	6.5	293
Hi-phospho HeLa (24)	ERK1/2 Primary 2		ERK2	2625	1157	4.1	236
Hi-phospho HeLa (24)	ERK1/2 Primary 2		Erk1	2597	680	6.2	139
Hi-phospho HeLa (24)	ERK1/2 Primary 2		pERK2	1116	372	3.3	76
Hi-phospho HeLa (24)	ERK1/2 Primary 2		pErk1	678	396	8.5	81
Hi-phospho HeLa (24)	ERK1/2 Primary 2		ppERK2	3973	1839	6.3	375
Hi-phospho HeLa (24)	ERK1/2 Primary 2		ppErk1	8109	2309	8.5	471
Low-phospho HeLa (24)	ERK1/2 Primary 1		ERK2	9745	1538	5.8	314
Low-phospho HeLa (24)	ERK1/2 Primary 1		Erk1	7707	1444	8.7	295
Low-phospho HeLa (24)	ERK1/2 Primary 1		pERK2	1879	306	6.3	62
Low-phospho HeLa (24)	ERK1/2 Primary 1		pErk1	248	71	8.5	14
Low-phospho HeLa (24)	ERK1/2 Primary 1		ppERK2	397	92	3.2	19
Low-phospho HeLa (24)	ERK1/2 Primary 1		ppErk1	710	194	7.3	40
Low-phospho HeLa (24)	ERK1/2 Primary 2		ERK2	9120	2117	3.2	432
Low-phospho HeLa (24)	ERK1/2 Primary 2		Erk1	7774	1601	6.6	327
Low-phospho HeLa (24)	ERK1/2 Primary 2		pERK2	1878	419	2.3	86
Low-phospho HeLa (24)	ERK1/2 Primary 2		pErk1	225	83	6.9	17
Low-phospho HeLa (24)	ERK1/2 Primary 2		ppERK2	326	139	2.7	28
Low-phospho HeLa (24)	ERK1/2 Primary 2		ppErk1	693	202	9.2	41

Clicking the arrow next to a group lists the individual capillaries in the group and reported data for each capillary:

Sample	Primary	Cap	Name	Area	Std.Dev	CV	SEM
b Hi-phospho HeLa (24)	ERK1/2 Primary 1		ERK2	3204	972	5.3	198
⊿ Hi-phospho HeLa (24)	ERK1/2 Primary 1		Erk1	2841	516	8.2	105
Hi-phospho HeLa	ERK1/2 Primary 1	C1:1	Erk1	2792			
Hi-phospho HeLa	ERK1/2 Primary 1	C1:3	Erk1	4132			
Hi-phospho HeLa	ERK1/2 Primary 1	C1:5	Erk1	3735			
Hi-phospho HeLa	ERK1/2 Primary 1	C2:2	Erk1	3115			
Hi-phospho HeLa	ERK1/2 Primary 1	C2:4	Erk1	3704			
Hi-phospho HeLa	ERK1/2 Primary 1	C2:6	Erk1	3397			
Hi-phospho HeLa	ERK1/2 Primary 1	C3:1	Erk1	2565			
Hi-phospho HeLa	ERK1/2 Primary 1	C3:3	Erk1	3428			
Hi-phospho HeLa	ERK1/2 Primary 1	C3:5	Erk1	3034			
Hi-phospho HeLa	ERK1/2 Primary 1	C4:2	Erk1	2663			
Hi-phospho HeLa	ERK1/2 Primary 1	C4:4	Erk1	3015			
Hi-phospho HeLa	ERK1/2 Primary 1	C4:6	Erk1	2821			
Hi-phospho HeLa	ERK1/2 Primary 1	C5:1	Erk1	2230			
Hi-phospho HeLa	ERK1/2 Primary 1	C5:3	Erk1	2695			
Hi-phospho HeLa	ERK1/2 Primary 1	C5:5	Erk1	2569			
Hi-phospho HeLa	ERK1/2 Primary 1	C6:2	Erk1	2536			
Hi-phospho HeLa	ERK1/2 Primary 1	C6:4	Erk1	2762			
Hi-phospho HeLa	ERK1/2 Primary 1	C6:6	Erk1	2713			
Hi-phospho HeLa	ERK1/2 Primary 1	C7:1	Erk1	2352			
Hi-phospho HeLa	ERK1/2 Primary 1	C7:3	Erk1	2278			
Hi-phospho HeLa	ERK1/2 Primary 1	C7:5	Erk1	2419			
Hi-phospho HeLa	ERK1/2 Primary 1	C8:2	Erk1	2122			
Hi-phospho HeLa	ERK1/2 Primary 1	C8:4	Erk1	2639			
Hi-phospho HeLa	ERK1/2 Primary 1	C8:6	Erk1	2474			
b Hi-phospho HeLa (24)	ERK1/2 Primary 1		pERK2	1308	256	9.5	52
b Hi-phospho HeLa (24)	ERK1/2 Primary 1		pErk1	887	766	6.4	156
b Hi-phospho HeLa (24)	ERK1/2 Primary 1		ppERK2	5105	1368	6.8	279
b Hi-phospho HeLa (24)	ERK1/2 Primary 1		ppErk1	8716	1438	6.5	293
b Hi-phospho HeLa (24)	ERK1/2 Primary 2		ERK2	2625	1157	4.1	236
b Hi-phospho HeLa (24)	ERK1/2 Primary 2		Erk1	2597	680	6.2	139
b Hi-phospho HeLa (24)	ERK1/2 Primary 2		pERK2	1116	372	3.3	76
b Hi-phospho HeLa (24)	ERK1/2 Primary 2		pErk1	678	396	8.5	81
 11: a b a sub a 11 a b a 7940 	EDI/1 /0 D-1		50/2	2022	1000	6.2	775

The **Capillary Groups** pane pivots the **Peak Groups** results to show statistics for named protein peaks in individual columns.

Peak Groups 🛄 Capillar	y Groups	oup Plot												
Sample	Primary	Capillary	ppErk1	Std.Dev	% CV	SEM	pErk1	Std.Dev	% CV	SEM	Erk1	Std.Dev	% CV	SEM
b Hi-phospho HeLa (24)	ERK1/2 Primary 1		8716	1438	6.5	293	887	766	6.4	156	2841	516	8.2	105
b Hi-phospho HeLa (24)	ERK1/2 Primary 2		8109	2309	8.5	471	678	396	8.5	81	2597	680	6.2	139
b Low-phospho HeLa (24)	ERK1/2 Primary 1		710	194	7.3	40	248	71	8.5	14	7707	1444	8.7	295
b Low-phospho HeLa (24)	ERK1/2 Primary 2		693	202	9.2	41	225	83	6.9	17	7774	1601	6.6	327

Group Plots

The mean values for named peaks in each group are plotted in bar graphs with error bars showing the standard deviation. The plots compare different antibodies for the same sample and different samples for the same antibody to allow a choice of presentation.



Hiding or Removing Capillaries in Group Analysis

Hidden capillaries are not included in groups. However, hiding capillaries provides an easy way to reject individual capillaries from the statistical analysis. See "Hiding Capillary Data" on page 315 for details on how to do this.

Larger groups of capillaries can also be excluded from analysis. To do this, select View and click Filter.

😻 Filter 🛛 🔀							
 ♥ Filter ♥ Cycles ♥ 1 ♥ 2 ♥ 3 ♥ 4 ♥ 5 ♥ 6 ♥ 7 ♥ 8 	Capillaries 1 2 3 4 5 6 7 8						
₩ 8	8 9 10 11 12						
Show named peaks only OK Cancel							

Uncheck the box next to the cycles or capillaries you wish to remove and click **OK**. This data will now be removed from the grouped view statistics.

Copying Data Views and Results Tables

You can copy and paste data and results tables into other documents, or save a data view as a graphic file.

Copying Data Views

- 1. Click in the graph or lane pane.
- 2. Select Edit in the main menu and click Copy, or right click and select Copy.
- 3. If you selected copy from the graph pane the following window will display, click **Copy**. If you selected copy from the lane pane skip to the next step.

Copy Graph	×
Graph title:	Samples
 Metafile (El 	MF)
🔘 Bitmap (PN	IG)
O Portable Do	ocument Format (PDF)
Save	Copy Cancel

4. Open a document (Microsoft[®] Word[®], Excel[®], PowerPoint[®], etc.). Right click in the document and select **Paste**. A graphic of the copied data view will be pasted into the document.

Copying Results Tables

- 1. Click in the peaks or capillaries pane.
- 2. Select one or multiple rows.
- 3. Select Edit in the main menu and click Copy, or right click and select Copy.
- 4. Open a document (Microsoft[®] Word[®], Excel[®], PowerPoint[®], etc.). Right click in the document and select **Paste**. Data for the rows selected will be pasted into the document.

Saving the Graph View as an Image File

- 1. Click in the graph pane.
- 2. Select Edit in the main menu and click Copy, or right click and select Copy.
- 3. Select an image option (EMF, PNG or PDF) in the pop-up window, then click Save.

Copy Graph							
Graph title:	Samples						
🔘 Metafile (EN	Metafile (EMF)						
Bitmap (PN)	G)						
Portable Document Format (PDF)							
Save	Copy Cancel						

4. Select a directory to save the file to and enter a file name, then click **OK**.

Exporting Run Files

Results tables and raw plot data can be exported for use in other applications.

Exporting Results Tables

To export the information in the peaks and capillaries tables:

- 1. Click File in the main menu and click Export Tables.
- 2. Select a directory to save the files to and click **OK**. Data will be exported in .txt format.

NOTE: To exclude export of standards data or export results table data in .csv format, see "Setting Data Export Options" on page 413.

Exporting Raw Sample Electropherogram Data

To export raw sample plot data:

1. Click File in the main menu and click Export Spectra.

File	Edit View Instrumen	t W	indow Help
	Open Run 🕨		
	Add Run 🕨		Granh 99 Image
	Close	E	Jac oraphi mage
	Close All		
	Save	2	5500
	Save As	1	5000
	Export Tables		4500
	Export Spectra •		Text Format
	Exit		Andi Format
		5	= <u>acon</u>

- To export data in .txt format Select Text Format. Plots will be exported in one file for all capillaries.
- To export data in .cdf format Select Andi Format. Plots will be exported in one file per capillary.
- 2. Select a directory to save the files to and click **OK**. Data will be exported in the selected format.

Changing Sample Protein Identification

Compass allows you to customize what sample proteins are reported in the results tables by making manual adjustments in the electropherogram or peaks table.

Adding or Removing Sample Data

- 1. Click **Show Samples** in the View bar.
- 2. Click **Single View** in the View bar.
- 3. Click on the row in the experiment pane that contains the sample you wish to correct, then click the **Graph** tab.
 - To remove a peak from the data Right click the peak in the electropherogram or peaks table and select **Remove peak**. Compass will no longer identify it as a sample peak in the electropherogram and the peak data will be removed in the results tables.



• To add an unidentified peak to the data - Right click the peak in the electropherogram or peaks table and select Add Peak. Compass will calculate and display the results for the peak in the results tables and identify the peak in the electropherogram.

NOTES:

To remove sample peak assignments that were made manually and go back to the original view of the data, right click in the electropherogram and click **Clear All**.

Virtual blot data in the lane pane will also update to reflect changes made in the graph pane.

Hiding Sample Data

You can hide the results for a sample protein in the results tables without completely removing it from the reported results. To to this:

- 1. Click **Show Samples** in the View bar.
- 2. Click **Single View** in the View bar.
- 3. Click on the row in the experiment pane that contains the sample you wish to correct, then click the **Graph** tab.
- 4. Right click the peak in the electropherogram or peaks table and select **Hide peak**. Compass will hide the peak data in the results tables.





5. To view hidden peak data, click **View** in the main menu and click **Show Hidden**. Hidden peak data will display in the results table and be marked with an **X**.

6. To unhide a peak, right click on the peak in the electropherogram or peaks table and select **Unhide Peak**.

Changing Peak Names for Sample Data

If Compass did not automatically name a sample protein associated with a specific primary antibody, this can be adjusted manually. To do this:

- 1. Click **Show Samples** in the View bar.
- 2. Click **Single View** in the View bar.
- 3. Click on the row in the experiment pane that contains the sample you wish to correct, then click the **Graph** tab.

4. Right click the peak in the electropherogram or peaks table and click **Name**, then click a name in the list. Compass will change the peak name in the electropherogram and results tables, and adjust peak names for other sample proteins accordingly.



NOTES:

For details on how to specify peak name settings, see "Peak Names Settings" on page 391.

Virtual blot data in the lane pane will also update to reflect changes made in the graph pane.

Displaying Sample Data for Named Peaks Only

You can adjust the sample data to display results only for user-specified named peaks. To do this:

- 1. Click **Show Samples** in the View bar.
- 2. Click **View** in the main menu and click **Filter**.
- 3. Check the Show Named Peaks only box and click OK.

😵 Filter	x
 ✓ Cycles ✓ 1 ✓ 2 ✓ 3 ✓ 4 ✓ 5 ✓ 6 ✓ 7 ✓ 8 	✓ Capillaries ✓ 1 ✓ 2 ✓ 3 ✓ 4 ✓ 5 ✓ 6 ✓ 7 ✓ 8 ✓ 9 ✓ 10 ✓ 11 ✓ 12
Show named peaks only	
OK Cancel	



Compass will hide peak data in the electropherogram and results tables for all unnamed peaks, and instead only display data for named peaks in the electropherogram and results tables.

Changing the Virtual Blot View

Options in the lane pane let you change the contrast or invert the virtual blot, remove baseline noise, change lane labels or overlay standards data on sample lanes.

The lane pane toolbar has the following options:



Adjusting the Contrast

1. Click the **Contrast Adjustment** button. The contrast tool will display:



- 2. Click the bar and drag it up or down to adjust the contrast.
- 3. When finished, click **X** to close the tool.

Inverting the Virtual Blot

1. Click the **Invert** button. The virtual blot image will invert:



2. Click the **Invert** button again to return to the default view.

Selecting Lane Labels

The labels shown above the lanes in the virtual blot can be customized. To do this:

1. Click the **Edit Labels** button. The label box will display:



- 2. Check one or multiple label boxes, and uncheck those you don't want to display. To remove labels completely, uncheck all boxes. The following label options are available:
 - **Sample** Sample name. If sample names were entered in the assay template (Assay screen), those names will display here. Otherwise, Sample (default name) will display.
 - **Primary** Primary antibody name. If primary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Primary (default name) will display.
 - Secondary Ab Secondary antibody name. If a secondary antibody name was entered in the assay template (Assay screen), those names will display here. Otherwise, Secondary (default name) will display.
 - Capillary Cycle and capillary number. For example, C1:3 indicates cycle 1, capillary 3

NOTE: Peggy Sue runs up to eight cycles (12 capillaries at a time), so the cycle number displayed will always be C1-C8 depending on the number of cycles programmed.

• **Attributes** - Attribute text. If attribute information was entered for any of the rows or wells in the assay template (Assay screen), up to four can be selected and displayed as lane labels.

Viewing the Uncorrected Sample Baseline

1. Click the **Remove Baseline** button (active for sample data only). This will remove the automatic baseline correction.



2. Click the **Remove Baseline** button again to return to the default, baseline corrected view.

Overlaying Standards Data on Sample Lanes

Data for the standards can be overlaid on the sample data in the virtual blot so you can view the raw, unaligned (uncorrected) lane data. To do this:

1. Click the **Overlay Standards Data** button (active for sample data only). An overlay of the raw sample and standards data will display:



2. Click the **Overlay Standards Data** button again to return to the default, auto-aligned view.

Moving Lanes in the Virtual Blot View

Lanes in the virtual blot view can be moved to make it easier to compare specific lanes of data. To do this:

1. Click a lane in the virtual blot. Hold the mouse button down and drag the lane to a new position.



2. Release the mouse button. The lane will now be repositioned in the virtual blot view.

Changing the Electropherogram View

Options in the graph pane let you zoom and scale electropherograms, overlay or stack plots and change the peak and plot information displayed.

The graph pane toolbar has the following options:



Auto Scale



Graph Options

Stack the Plots

Overlay the Plots

Autoscaling the Electropherogram

Click the **Autoscale** button to scale the y-axis to the largest peak in the electropherogram.



Click the Autoscale button again to return to default scaling.


Stacking Multiple Electropherograms

Electropherogram data for multiple capillaries can be stacked vertically in the graph pane for comparison. To do this:

- 1. Click Single View.
- 2. Select multiple rows in the experiment pane.
- 3. Click the **Stack the Plots** button. The individual electropherograms for each row selected will stack in the graph pane.



You can customize the colors used for the stacked plot display. For more information see "Selecting Custom Plot Colors for Graph Overlay" on page 414.

Overlaying Multiple Electropherograms

The electropherogram data for multiple capillaries can also be overlaid on top of each other for comparison in the graph pane. To do this:

- 1. Click Single View.
- 2. Select multiple rows in the experiment pane.
- 3. Click the **Overlay the Plots** button. The individual electropherograms for each row selected will overlay in the graph pane.



You can customize the colors used for the overlay plot display. For more information see "Selecting Custom Plot Colors for Graph Overlay" on page 414.

Zooming

To zoom in on a specific area of the electropherogram, hold the mouse button down and draw a box around the area with the mouse:





To return to default scaling, right click in the electropherogram and click **Zoom Out**.

Customizing the Data Display

You can customize electropherogram peak labels, plot labels and display options. To do this, select the **Graph Options** button.



Peak Labels

You can customize the labels used to identify peaks in the electropherogram with these options:



Matching Peak Names - Checking this box will draw vertical lines through each named peak. Using this option with Stack the Plots or Overlay the Plots features is useful for visual comparison of named peaks across multiple capillaries.



• **Peak Names** - Checking this box will display peak name labels on all named peaks in the electropherogram.

NOTE: If more than one peak label option is selected, peak name labels will always be used for named peaks.



Peak Values - Checking this box will display the pl labels on all peaks in the electropherogram.

NOTES:

When viewing standards and registration data, this option is called Peak Positions. Labels displayed are peak positions rather than pl.

If more than one peak label option is selected, peak name labels will always be used for named peaks.

The reported pl for immunodetected sample proteins in Compass may vary slightly from predicted pls based on sample, buffer and assay conditions.



Baseline and Grid Options

You can view the calculated baseline fit, peak integration and show grid lines with these options.



• Fitted peaks - Checking this box will display how the peaks were fit by the software.

NOTE: This option is only available for sample data.



Baseline Fit - Checking this box will display the calculated baseline for the peaks. Baseline points will also display for regions of the electropherogram considered to be at baseline.

•







Grid Lines - Checking this box will display grid lines in the graph area.

Plot Labels

You can customize the plot labels displayed on the electropherogram with these options.

Plot Label	
🔽 Sample	Attribute
Primary	Attribute
Capillary	Exposure

Plot labels are shown on the right side of the graph pane.

- **Sample** Checking this box will display the sample name. If sample names were entered in the assay template (Assay screen), those names will display here. Otherwise, Sample (default name) will display.
- **Primary** Checking this box will display the primary antibody name. If primary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Primary (default name) will display.
- **Capillary** Checking this box will display the cycle and capillary number. For example, C1:3 indicates cycle 1, capillary 3.
- **Attributes** Checking this box will display attribute text. If attribute information was entered for samples or primary antibodies in the assay template (Assay screen), they can be selected as plot labels.

• **Exposure** - Checking this box will display the exposure time(s) used for the data. The following example shows an electropherogram with all plot labels selected:



Selecting Data Viewing Options

The graph view menu provides multiple options for changing what type of electropherogram data is displayed. To access the view menu, click the down arrow next to the graph pane toolbar:



A check mark next to the menu option indicates it is currently selected. Multiple options can be selected at a time.

NOTE: Unless otherwise noted, graph view menu options are available for sample data only.

•



Sample - Clicking this option will display raw, uncorrected sample data.

Sample Baseline Corrected - Clicking this option will display sample data with the baseline subtracted (zeroed). This is the default view.



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NOTE: This option is selected automatically when Baseline Fit is selected in graph options.



Baseline Points - Clicking this option will display regions of the electropherogram considered to be at baseline. In the example that follows, both Baseline Points and Sample are selected.

NOTE: This option is selected automatically when Baseline Fit is selected in graph options.



- 1 🖩 🗐 🗕 🔍 🗖 📲 Image 🖽 Lane 🛵 Graph Samples 1700 ERK2 rk1 Erk1 ppERK2 pERK2 ppErk1 pErk1 1600 1500 1400 Low-phospho HeLa 1300 1200 1200 1100 1000 900 C1:10 900 800 700 600 500 5.2 5.3 5.4 5.5 5.6 5.7 5.8 5.9 6.0 6.1 6.2 6.3 6.4 6.5 6.6 6.7 6.8 6.9 7.0 pl
- **Fit** Clicking this option will display the bounding envelope of the fitted peaks as calculated by the software for the raw sample data. In the example that follows, both Fit and Sample are selected.

Fit Baseline Corrected - Clicking this option will display the fitted peaks as calculated by the software for the sample baseline corrected data. In the example that follows, both Fit Baseline Corrected and Sample are selected, the fit plot is on the bottom.



NOTE: When viewing standards or registration data, this option is called Standards Fluorescence or Registration Fluorescence, respectively.



Standards - Checking this box aligns the pl of the raw standards data to the sample data and overlays both electropherograms in the graph pane.

Adding and Removing Baseline Points

Points in the baseline can be added or removed as needed. To do this:

- 1. Click the **Graph Options** button in the graph pane toolbar and check **Baseline Fit**. This will display baseline points for the raw sample data.
- 2. Use the mouse to draw a box around the area you want to correct. This will zoom in on the area.



3. Right click a baseline point and click Add Baseline Point or Remove Baseline Point.

NOTE: To clear the manual addition or removal of baseline points and go back to the original view of the data, right click in the electropherogram and click **Clear All**.

Selecting the X-Axis pl Range

The pl range used for the x-axis can be changed. To do this, Select **View** in the main menu and click **View Region**. The following pop-up window will display:

💿 View Reg	jion		×
🔲 Full Rar	nge		
Lower:	5.0	Upper:	7.0
	ОК	Cancel	

To change the x-axis pl range displayed for the run data - Enter new values in the Lower or Upper range in pl and click OK. Electropherogram and virtual blot data will update to display only the data in the entered range.

•



To see the full x-axis pl range included in the run data - Check Full Range. Electropherogram and virtual blot data will update to display the full range of available data.

NOTE: You can change the default x-axis range that Compass uses. For more information, see "Peak Fit Analysis Settings" on page 384.

Closing Run Files

If more than one run file is open, you can close just one file or all the open files at the same time.

- To close one of multiple open run files In the experiment pane, click on one of the sample rows in the file. Next click File from the main menu and click Close.
- To close all open run files Select File from the main menu and click Close All.

Compass Analysis Settings Overview

Compass has a variety of analysis features and settings that you can modify as needed to enhance run data.

To access these settings, select **Edit** in the main menu and click **Analysis**. If more than one run file is open, select the run file you want to view analysis settings for from the list:

File	Edit View Instrum	ment Wind	ow Help
	Cut Copy Paste	Ctrl+C Ctrl+V	Graph 🛛 Image 🖽 Lane
0	Analysis	+	Training data_Hi-Low ERK.cbz
w	Preferences		DemoData_Charge_HeLa_ERK12.cbz

The following screen will display:

🛞 Analysis: Simple Western ERK D	Demo			
type filter text	Advanced			⇔ • ⇔ • •
Advanced	Applysis Settings		Applysic Settings, Advanced	
Peak Fit	Advanced		Analysis Settings: Advanced	
Peak Names	Auvanceu		Standards	
Standards			Peak Width	15
			Allowable Drift	100
			Sample	
	Add	Remove	Peak Fit Starting Width Ratio	0.5
			Image	
	Default	Advanced	Median Filter Threshold Ratio	0.5
	Override		Median Filter Threshold Limit	100
	Apply To	Settings		
	Add	Remove		
				Restore Original
Import Export			ОК	Cancel

To move between analysis pages in this window, click on an option in the list on the left. The following analysis settings can be user-customized in Compass:

- Advanced Lets you customize analysis settings for samples, standards and image data.
- **Images** Lets you view the chemiluminescent exposures taken during the run and view data for different exposures in the Analysis screen.
- **Peak Fit** Lets you customize peak fit settings for sample data.
- **Peak Names** Lets you enter custom naming settings for sample proteins associated with specific primary antibodies or attributes and have Compass automatically label the peaks in the run data.
- **Standards** Lets you customize the pl and positions Compass uses to identify fluorescent standards and registration peaks.

Advanced Analysis Settings

The advanced analysis settings page lets you view and change analysis settings for samples, standards and image data. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Advanced** in the options list.

NOTE: Settings can be modified in an assay prior to starting a run, or in a run file once the run has finished executing. Analysis changes made to an executing run will not be saved to the final run file.

🛞 Analysis: Simple Western ER	(Demo		
type filter text	Advanced		↓ ↓ ↓ ▼
Advanced Images	Analysis Settings	Analysis Settings: Advanced	
Peak Fit	Advanced	Standards	
Peak Names Standards		Book Width	15
Standards		Peak width	15
		Allowable Drift	100
		Sample	
	Add	Peak Fit Starting Width Ratio	0.5
		Image	
	Default Advanced	Median Filter Threshold Ratio	0.5
			0.0
	Override	Median Filter Threshold Limit	100
	Apply To Settings		
	Add Remove		
			Restore Original
Import Export			Cancel

- Click **Import** to import an analysis settings file. This will be explained in more detail in "Importing Analysis Settings" on page 409.
- Click **Export** to export the current analysis settings file. This will be explained in more detail in "Exporting Analysis Settings" on page 410.
- Click Restore Original to restore Compass default settings.
- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

Standards Settings

- **Peak Width** The approximate width (at full width half max) used to filter out fluorescence artifacts which improves recognition of standards. The default value is 15.
- Allowable Drift The distance in pixels that standards are expected to move compared to their entered positions in the standards analysis page. This setting assists in recognition of standards. The default value is 100.

Sample Settings

• **Peak Fit Starting Width Ratio** - Focuses the peak fit towards the peak center and aids the overall peak fitting. The default value is 0.5.

Image Settings

- Median Filter Threshold Ratio Pixel ratio used to filter out camera artifacts. The default value is 0.5.
- **Median Filter Threshold Limit** Pixel threshold value used to filter out camera artifacts. The default value is 100.

Advanced Analysis Settings Groups

Advanced analysis settings are saved as a group, and multiple settings groups can be created. Specific group settings can then be applied to individual capillaries, sample names or attributes in the run data.

NOTES:

We recommend using the Compass default values for advanced analysis settings. These settings are included in the default Advanced group.

Analysis settings are run-file specific. However, settings can be imported or exported for use with other run files. For more information see "Importing and Exporting Analysis Settings" on page 409.

Analysis groups are displayed in the analysis settings box:

Ashuranaad	 	
Advanced		
		_

The Advanced group shown contains the Compass default analysis settings. You can make changes to this group and create new groups.

To view settings for a group, click on the group name in the analysis settings box.

Creating a New Analysis Group

- 1. Select Edit in the main menu and click Analysis, then click Advanced in the options list.
- 2. Click **Add** under the analysis settings box. A new group will be created:

Advanced			
Advanced 2			

3. Click on the new group and enter a new name.

Analysis Settings	
Advanced	
STAT analysis	
Add	Remove

- 4. Modify standards, sample or image parameters as needed.
- 5. To use the new group as the default analysis settings for the run file data, click the arrow in the drop down list next to Default, then click the new group from the list. Analysis settings in the new group will then be applied to the run data.

Analysis Settings	
Advanced	
STAT analysis	
Add	Remove
Default	Advanced -
Override	STAT analysis

6. Click **OK** to save changes.

Changing the Default Analysis Group

- 1. Select Edit in the main menu and click Analysis, then click Advanced in the options list.
- 2. Click the arrow in the drop down list next to Default, then click a new default group from the list.

Analysis Settings				
Advanced				
STAT analysis				
Add	Remove			
Default	Advanced 👻			
	Advanced			
Override	STAT analysis			

3. Click OK to save changes. Analysis settings in the group selected will be applied to the run data.

Modifying an Analysis Group

- 1. Select Edit in the main menu and click Analysis, then click Advanced in the options list.
- 2. Click on the group in the analysis settings box you want to modify.

Analysis Settings	
Advanced	
STAT analysis	
Add Remove	

- 3. Modify standards, sample or image parameters as needed.
- 4. Click OK to save changes. The new analysis settings will be applied to the run data.

Deleting an Analysis Group

- 1. Select Edit in the main menu and click Analysis, then click Advanced in the options list.
- 2. Click on the group in the analysis settings box you want to delete and click **Remove**.

Analysis Settings
Advanced
STAT analysis
Add Remove

3. Click **OK** to save changes.

Applying Analysis Groups to Specific Run Data

- 1. Select Edit in the main menu and click Analysis, then click Advanced in the options list.
- 2. Click on the group in the analysis settings box you want to apply to specific run data.

nalysis Set	tings		
Advanced <mark>HeLa</mark>			
	Add	Remove	

3. Application of analysis groups to specific run data is done in the override box. Click **Add** under the override box. A default override data set will be created from sample information found in the run file.

Override	
Apply To	Settings
Hi-phospho HeLa	HeLa
Add	Remove

4. Click the cell in the **Apply To** column, then click the down arrow.

Override	
Apply To	Settings
i-phospho HeLa 👻	HeLa
Hi-phospho HeLa	
Low-phospho HeLa	
Cycle 1	
Cycle 2	
Cycle 3	
Cycle 4	
Cycle 5	
Cycle 6	
Cycle 7	
Cycle 8	D
Custom Settings	Kemove

- 5. Select an option from the drop down list. This will apply the settings group selected to specific run data as follows:
 - **Sample names** All sample names entered in the assay template (Assay screen) will display in the drop down list, otherwise the default name of Sample will be shown. Select a sample name to apply group settings to all capillaries that use this sample name in the run file.
 - Attributes All sample attributes entered in the assay template (Assay screen) will display in the drop down list. Select an attribute to apply group settings to all capillaries that use this attribute in the run file.

- Cycle 1-8 When this option is selected, group settings will be applied to all capillaries in the cycle.
- **Custom settings** Lets you choose specific capillaries to apply the group settings to. When this option is selected, the following pop-up box appears to let you enter a specific capillary number or range of capillaries:

🗞 Custom Settings 📃 💌
Enter cycle and capillary descriptor
Examples: 2:3, 1:1-12 Cycle 2 capillary 3, and cycle 1 capillaries 1 through 12 1-3:6-8+10 Cycles 1 through 3, capillaries 6, 8 and 10
OK Cancel

6. If you need to change the analysis group used for a data set, click the cell in the **Settings** column and click the down arrow. Select a group from the drop down list.

Override	
Apply To	Settings
Hi-phospho HeLa	HeLa 👻
	Advanced HeLa
Add	Remove

- 7. Repeat the previous steps to apply other groups to specific run data.
- 8. To remove a data set, click on its cell in the **Apply To** column, then click **Remove**.
- 9. Click **OK** to save changes.

Images Analysis Settings

The Images analysis settings page lets you see what chemiluminescent exposures were taken during the run, and view data for different exposures in the Analysis screen. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Images** in the options list.

😻 Analysis: Training data_Hi-Lo	w ERK.cbz	
type filter text	Images	
Advanced Images Peak Fit	Cycle Luminescence	
Peak Names Standards	All Exposure 3 240 seconds -	
	1 Exposure 3 240 seconds	
	2 Exposure 3 240 seconds 3 Exposure 3 240 seconds	
	4 Exposure 3 240 seconds	
	6 Exposure 3 240 seconds	
	7 Exposure 3 240 seconds	
	8 Exposure 3 240 seconds	
		Restore Original
Import Export		OK Cancel

- Click **Import** to import an analysis settings file. This will be explained in more detail in "Importing Analysis Settings" on page 409.
- Click **Export** to export the current analysis settings file. This will be explained in more detail in "Exporting Analysis Settings" on page 410.
- Click Restore Original to restore Compass default settings.
- Click **OK** to save changes and exit.
- Click Cancel to exit without saving changes.

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Exposure Settings

The exposure used for the sample data displayed in the Analysis screen is shown in the All box:

Cycle		Luminescence
All	Exposure 3	240 seconds 🔹
1	Exposure 3	240 seconds 👻
2	Exposure 3	240 seconds 🔹
3	Exposure 3	240 seconds 🔹
4	Exposure 3	240 seconds 🔹
5	Exposure 3	240 seconds 🔹
6	Exposure 3	240 seconds 🔹
7	Exposure 3	240 seconds 🔹
8	Exposure 3	240 seconds 👻

NOTE: Peggy Sue runs up to eight cycles (12 capillaries per cycle). The individual exposure setting selected for a specific cycle is applied for all 12 capillaries in that cycle unless selected for All cycles.

• Exposure # - Sample data displayed in the Analysis screen is for this specific exposure only.

To see the number of exposures and exposure times used for the run data, click the arrow in the drop down list next to **All**.



NOTE: The number of exposures taken and exposure times shown were specified in the assay protocol (Assay screen) prior to executing the run and cannot be modified.

Changing the Sample Data Exposure Displayed

The exposure used for the sample data displayed in the Analysis screen can be changed. To do this:

- 1. Select Edit in the main menu and click Analysis, then click Images in the options list.
- 2. Click the arrow in the drop down list next to All and select an exposure setting:

Cycle	Luminescence		
All	Exposure 3 240 seconds 👻		
	Exposure 1 60 seconds		
1	Exposure 2 240 seconds Exposure 3 240 seconds Exposure 3 240 seconds		

3. Click **OK** to save changes and exit. Sample data for the exposure selected will display in the Analysis screen.

Peak Fit Analysis Settings

The peak fit analysis settings page lets you view and change peak fit settings for sample data. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Peak Fit** in the options list.

NOTE: Settings can be modified in an assay prior to starting a run, or in a run file once the run has finished executing. Analysis changes made to an executing run will not be saved to the final run file.

ype filter text	Peak Fit			(
Advanced Images Peak Fit Peak Names	Analysis Settings Peak Fit		Analysis Settings: Peak Fit Range	
Standards			Minimum Maximum	7.0
	Add	Remove	Baseline Threshold	1.0
	Default	Peak Fit 👻	Window Stiffness	15.0 1.0
	Override		Peak Find	
	Apply To	Settings	Threshold Width	20.0 7.0
	Add	Remove		Restore Origin

- Click **Import** to import an analysis settings file. This will be explained in more detail in "Importing Analysis Settings" on page 409.
- Click **Export** to export the current analysis settings file. This will be explained in more detail in "Exporting Analysis Settings" on page 410.
- Click **Restore Original** to restore Compass default settings.
- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

Range Settings

- **Minimum** The pl value below which peaks will not be identified. This value will also be used as the default lower pl range for the data displayed in the electropherogram and virtual blot. The default value is 5.
- **Maximum**: The pl value above which peaks will not be identified. This value will also be used as the default upper pl range for the data displayed in the electropherogram and virtual blot. The default value is 7.

Baseline Settings

- **Threshold** The variance, or roughness, in a baseline data segment below which a point will be called part of the baseline. The default value is 1.0.
- Window How long baseline data segments are expected to be in pixels. Shorter segments will allow the baseline to follow plateau sections of the signal. The default value is 15.
- **Stiffness** The amount the baseline is allowed to vary from a straight line. Settings between 0.1 and 1.0 make the baseline fit closer to a straight line. Settings from 1.0 to 10.0 will make the baseline fit follow the data more closely. The default value is 1.0.

Peak Find Settings

- **Threshold** The minimum signal to noise ratio required for a peak to be identified. A setting of 1.0 will detect many peaks, a setting of 30.0 will detect fewer peaks. The default value is 20.0.
- Width The approximate peak width (at full width half max) in pixels used to detect peaks. The minimum value for this setting is 3.0. Larger widths help to eliminate the detection of shoulder peaks and noise peaks. The default value is 7.0.

Peak Fit Analysis Settings Groups

Peak fit settings are saved as a group, and multiple settings groups can be created. Specific group settings can then be applied to individual capillaries, sample names or attributes in the run data.

NOTES:

We recommend using the Compass default values for peak fit analysis settings. These settings are included in the default Peak Fit group.

Analysis settings are run-file specific. However, settings can be imported or exported for use with other run files. For more information see "Importing and Exporting Analysis Settings" on page 409.

Peak fit groups are displayed in the analysis settings box:

Analysis Settings	
Peak Fit	
Add	Remove

The Peak Fit group shown contains the Compass default analysis settings. You can make changes to this group and create new groups.

To view settings for a group, click on the group name in the analysis settings box.

Creating a New Peak Fit Group

- 1. Select Edit in the main menu and click Analysis, then click Peak Fit in the options list.
- 2. Click Add under the analysis settings box. A new group will be created:

Peak Fit		
Peak Fit 2		

3. Click on the new group and enter a new name.

Analysis Settings	
Peak Fit	
STAT peak fit	
Add	Remove

- 4. Modify range, baseline or peak find parameters as needed.
- 5. To use the new group as the default peak fit settings for the run file data, click the arrow in the drop down list next to Default, then click the new group from the list. Peak fit settings in the new group will then be applied to the run data.
| Analysis Settings | |
|-------------------|------------|
| Peak Fit | |
| STAT peak fit | |
| | |
| | |
| | |
| | |
| | |
| Add | Remove |
| Default | Peak Fit 🔹 |
| | Deak Fit |
| | PEAKTIL |

6. Click **OK** to save changes.

Changing the Default Peak Fit Group

- 1. Select Edit in the main menu and click Analysis, then click Peak Fit in the options list.
- 2. Click the arrow in the drop down list next to Default, then click a new default group from the list.

Analysis Settings	
Peak Fit	
STAT peak fit	
Add	Remove
Default	Dools Eit
	Peak Fit
Override	STAT peak fit
ovenue	

3. Click OK to save changes. Peak fit settings in the group selected will be applied to the run data.

Modifying a Peak Fit Group

- 1. Select Edit in the main menu and click Analysis, then click Peak Fit in the options list.
- 2. Click on the group in the analysis settings box you want to modify.

Peak Fit			
STAT pea	ık fit		

- 3. Modify range, baseline or peak find parameters as needed.
- 4. Click **OK** to save changes. The new peak fit settings will be applied to the run data.

Deleting a Peak Fit Group

- 1. Select Edit in the main menu and click Analysis, then click Peak Fit in the options list.
- 2. Click on the group in the analysis settings box you want to delete and click **Remove**.

Peak Fit			
STAT pe	ak fit		

3. Click **OK** to save changes.

Applying Peak Fit Groups to Specific Run Data

- 1. Select Edit in the main menu and click Analysis, then click Peak Fit in the options list.
- 2. Click on the group in the analysis settings box you want to apply to specific run data.

Peak Fit			
STAT peak	fit		

3. Application of peak fit groups to specific run data is done in the override box. Click **Add** under the override box. A default override data set will be created from sample information found in the run file.

Override	
Apply To	Settings
Hi-phospho HeLa	STAT peak fit
Add	Remove

4. Click the cell in the **Apply To** column, then click the down arrow.

Override		
Apply To	Se	ettings
i-phospho HeLa		AT peak fit
Hi-phospho HeLa	3	
Low-phospho He	La	
1 mg/mL		
Cycle 1		
Cycle 2		
Cycle 3		
Cycle 4		
Cycle 5		
Cycle 6		
Cycle 7		
Cycle 8		Kemove
Custom Settings.		

- 5. Select an option from the drop down list. This will apply the settings group selected to specific run data as follows:
 - **Sample names** All sample names entered in the assay template (Assay screen) will display in the drop down list, otherwise the default name of Sample will be shown. Select a sample name to apply group settings to all capillaries that use this sample name in the run file.
 - Attributes All sample attributes entered in the assay template (Assay screen) will display in the drop down list. Select an attribute to apply group settings to all capillaries that use this attribute in the run file.
 - **Cycle 1-8** When this option is selected, group settings will be applied to all capillaries in the cycle.
 - **Custom settings** Lets you choose specific capillaries to apply the group settings to. When this option is selected, the following pop-up box appears to let you enter a specific capillary number or range of capillaries:

🛞 Custom Settings	X
Enter cycle and capillary descriptor	
[
Examples: 2:3, 1:1-12 Cycle 2 capillary 3, and cycle 1 capillaries 1 throug 1-3:6+8+10 Cycles 1 through 3, capillaries 6, 8 and 10	jh 12
	OK Cancel

6. If you need to change the peak fit group used for a data set, click the cell in the **Settings** column and click the down arrow. Select a group from the drop down list.

Apply To	Settings	
Hi-phospho HeLa	STAT peak fit	-
	Peak Fit	
	STAT peak fit	

- 7. Repeat the previous steps to apply other groups to specific run data.
- 8. To remove a data set, click on its cell in the **Apply To** column, then click **Remove**.
- 9. Click **OK** to save changes.

Peak Names Settings

The peak names analysis settings page lets you enter custom naming settings for sample proteins. Compass can automatically label sample peaks in the run data associated with specific blocking reagent names, primary antibody names or attributes. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Peak Names** in the options list.

NOTE: Settings can be modified in an assay prior to starting a run, or in a run file once the run has finished executing. Analysis changes made to an executing run will not be saved to the final run file.

Analysis: DemoData_Char	ge_HeLa_ERK12.cbz						
ype filter text	Peak Names						$\Leftrightarrow \bullet \Rightarrow \bullet \bullet \bullet$
Advanced Images	Analysis Settings		Analysis S	ettings: ER	кı		
Peak Fit Peak Names Standards	ERK1 ERK2		Name	pI	Color	Range	Show
Standards			ppErk1	5.5		0.1	
			Erk1	5.9		0.1	
	Add	Remove					
	Apply Settings		_				
	Apply To	Settings					
	AII AII	ERK1 ERK2					
							Add Remove
	Add	Remove					
							Restore Origina
Import Expo	ort					ОК	Cancel

- Click **Import** to import an analysis settings file. This will be explained in more detail in "Importing Analysis Settings" on page 409.
- Click **Export** to export the current analysis settings file. This will be explained in more detail in "Exporting Analysis Settings" on page 410.
- Click Restore Original to restore Compass default settings.
- Click **OK** to save changes and exit.

Click **Cancel** to exit without saving changes.

Peak Names Analysis Settings Groups

Peak name settings are saved as a group, and multiple settings groups can be created. Specific group settings can then be applied to individual capillaries, blocking reagent names or primary antibody names and attributes in the run data.

NOTE: Analysis settings are run-file specific. However, settings can be imported or exported for use with other run files. For more information see "Importing and Exporting Analysis Settings" on page 409.

Peak name groups are displayed in the analysis settings box:

Analysis Settings	
Peak Names 1	
Add Remove	

The Peak Names group shown is a Compass template group. You can make changes to this group and create new groups.

To view settings for a group, click on the group name in the analysis settings box.

Creating a Peak Names Group

- 1. Select Edit in the main menu and click Analysis, then click Peak Names in the options list.
- 2. Click on the **Peak Names 1** template group in the analysis settings box.

Analysis Settings	
Peak Names 1	
Add Remove	

3. Enter a new name for the group.

•

Analysis Settings	
ERK1	
Add Remove	

4. Click in the first cell in the **Name** column in the analysis settings peak table.

Name	pI	Color	Range	Show
Peak1	6		0.05	 Image: A set of the set of the

5. Enter a sample protein name associated with the primary antibody used in the run.

Analysis S	ettings: El	RK1		
Name	pI	Color	Range	Show
ppErk1	6		0.05	

6. Click in the first cell in the **pl** column.

nalysis S	ettings: E	RK1		
Name	pI	Color	Range	Show
ppErk1	6		0.05	 Image: A set of the set of the

7. Enter the pl for the sample protein.

alysis S	ettings: EF	RK1		
Name	pI	Color	Range	Show
ppErk1	5.5		0.05	 Image: A start of the start of
pperkt	5.0		0.05	V

8. Click in the first cell in the **Color** column, then click the button.

nalysis S	ettings: EF	RK1		
Name	pI	Color	Range	Show
ppErk1	5.5	(0,1]	0.05	V
		l	à	

The color selection box will display:

Color
Basic colors:
Custom colors:
Define Custom Colors >> OK Cancel

The color selected will be used to identify the sample protein peak in the peaks and capillaries table in the Analysis screen.

9. Click a color or define a custom color and click **OK**. The color selection will update in the table:

nalysis Se	ettings: EF	RK1		
Name	pI	Color	Range	Show
ppErk1	5.5		0.05	V

10. Click in the first cell in the **Range (%)** column.

Analysis So	ettings: EF	RK1		
Name	pI	Color	Range	Show
ppErk1	5.5		0.05	 Image: A set of the set of the

11. Enter a range window for the pl entered. Compass will automatically name peaks found within this percent of the pl. For example, if the pl entered is 5.5 and a 0.1 pl range is used, all peaks between pl 5.4 and 5.6 will be identified with this peak name.

NOTE: The reported pl for immunodetected sample proteins in Compass may vary slightly from predicted pls based on sample, buffer and assay conditions.

12. Select the checkbox in the first cell of the **Show** column. This will turn peak naming on for the sample protein.

Analysis Se	ettings: Ef	RK1			
Name	pI	Color	Range	Show	
ppErk1	5.5		0.1	V	

To turn peak naming off for a particular sample protein, deselect the checkbox in the Show column.

13. To add another sample protein, click **Add** under the analysis settings peak table:

Name	pI	Color	Range	Show	
ppErk1	5.5		0.1	V	
Peak2	6		0.05	 Image: A set of the set of the	

Name	pI	Color	Range	Show	
ppErk1	5.5		0.1	~	
pErk1	5.75		0.05	 Image: A set of the set of the	
Erk1	5.9		0.1	~	

14. Repeat the previous steps to enter information for other sample proteins. In the following example, three sample proteins were entered:

To remove a sample protein, select its row and click **Remove**.

15. Click **OK** to save changes.

Adding Peak Names Groups

- 1. Select Edit in the main menu and click Analysis, then click Peak Names in the options list.
- 2. Click Add under the analysis settings box. A new group will be created:

Analysis Settings
ERK1
ERK1 2
Add Remove

3. Click on the new group and enter a new name.

Analysis Settings	
ERK1	
ERK2	
Add Remove	

- 4. Enter information in the analysis settings peak table as described in "Creating a Peak Names Group" on page 392.
- 5. Click **OK** to save changes.

Modifying a Peak Names Group

- 1. Select Edit in the main menu and click Analysis, then click Peak Names in the options list.
- 2. Click on the group in the analysis settings box you want to modify.

Analysis Settings	
ERK1/2	٦
STAT	
Add Remove	

- 3. Change the information in the analysis settings peak table as described in "Creating a Peak Names Group" on page 392.
- 4. Click **OK** to save changes.

Deleting a Peak Names Group

- 1. Select Edit in the main menu and click Analysis, then click Peak Names in the options list.
- 2. Click on the group in the analysis settings box you want to delete and click **Remove**.

Analysis Settings	
ERK1	
STAT	
Add Remove	

3. Click **OK** to save changes.

Applying Peak Names Groups to Run Data

- 1. Select Edit in the main menu and click Analysis, then click Peak Names in the options list.
- 2. Click on the group in the analysis settings box you want to apply to specific run data.

Analy	sis Settings		
ERK			
ERK2	2		
	Add	Remove	

3. Application of peak names groups to specific run data is done in the override box. Click **Add** under the override box. A default override data set will be created.

Apply Settings	
Apply To	Settings
All	ERK1
Add	Remove

4. Click the cell in the **Apply To** column, then click the down arrow.

Apply Settings		
Apply To		Settings
All	Ŧ	ERK1
All ERK1/2 Cycle 1 Cycle 2 Cycle 3 Cycle 4 Cycle 5 Cycle 5 Cycle 6 Cycle 7		
Cycle 8 Custom Setti	ngs	Remove

- 5. Select an option from the drop down list. This will apply the settings group selected to specific run data as follows:
 - All When this option is selected, peak names group settings will be applied to all capillaries in the run file.
 - **Primary antibody names** All primary antibody names entered in the assay template (Assay screen) will display in the drop down list, otherwise the default name of Primary will be shown. Select a name to apply group settings to all capillaries that use the primary antibody name in the run file.
 - Attributes All primary antibody attributes entered in the assay template (Assay screen) will display in the drop down list. Select an attribute to apply group settings to all capillaries that use this attribute in the run file.
 - **Cycle 1-8** When this option is selected, group settings will be applied to all capillaries in the cycle.
 - **Custom settings** Lets you choose specific capillaries to apply the group settings to. When this option is selected, the following pop-up box appears to let you enter a specific capillary number or range of capillaries:

Sustom Settings	X
Enter cycle and capillary descriptor	
Examples: 2:3, 1:1-12 Cycle 2 capillary 3, and cycle 1 capillaries 1 thro 1-3:6+8+10 Cycles 1 through 3, capillaries 6, 8 and 10	ugh 12
	OK Cancel

6. If you need to change the peak names group used for a data set, click the cell in the **Settings** column and click the down arrow. Select a group from the drop down list.

Apply Settings	
Apply To	Settings
All	ERK1 +
	ERK1 ERK2
Ad	d Remove

- 7. Repeat the previous steps to apply other groups to specific run data.
- 8. To remove a data set, click on its cell in the **Apply To** column, then click **Remove**.
- 9. Click **OK** to save changes. Named peaks will be identified with a peak name label in the electropherogram and virtual blot, and will be color coded in the peaks and capillaries tables:



Standards Settings

The standards analysis settings page lets you view and change the pl and position for fluorescent standards and set the registration peaks. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Standards** in the options list.

NOTE: Settings can be modified in an assay prior to starting a run, or in a run file once the run has finished executing. Analysis changes made to an executing run will not be saved to the final run file.

:	Standards						⇔ • ⇔
	Analysis Settings Std. Ladder 1 (premix pI 3-10)						
	Std. Ladder 1 (prem	ix pI 3-10)	Fluorescent Peaks				
ies	Std. Ladder 2 (prem	ix pI 4-7)	In	Desition	Pagistration	Ci+	
U	Std. Ladder 3 (premix pI 5-8)		pi	Position	Registration	FIL	
	Std. Ladder 4 (prem	ix pI 5-6)	4	200			
	Standard		4.5	520			
			6.4	550	Π		
	Add	Remove	7.3	650			
	Override Apply To	Settings (Std. Ladder 2 (Std. Ladder 3 (Std. Ladder 4 (Standard			Add Rem	love	
	Add	Remove				ſ	Restore Ori

- Click **Import** to import an analysis settings file. This will be explained in more detail in "Importing Analysis Settings" on page 409.
- Click **Export** to export the current analysis settings file. This will be explained in more detail in "Exporting Analysis Settings" on page 410.
- Click Restore Original to restore Compass default settings.
- Click **OK** to save changes and exit.

•

Click **Cancel** to exit without saving changes.

Standards Analysis Settings Groups

Standards settings are saved as a group, and multiple settings groups can be created. Specific group settings can then be applied to individual capillaries, sample names or attributes in the run data.

NOTES:

We recommend using the Compass default values for standards analysis settings. These settings are included in the default Standards group.

Analysis settings are run-file specific. However, settings can be imported or exported for use with other run files. For more information see "Importing and Exporting Analysis Settings" on page 409.

Standards groups are displayed in the analysis settings box:

	(premix pr 5-10)
td. Ladder 2	(premix pI 4-7)
Std. Ladder 3	(premix pI 5-8)
Std. Ladder 4	(premix pI 5-6)
Standard	

The Std. Ladder groups shown contains the Compass default analysis settings for pl Standard Ladders used with each of the premixes (separation gradients) for charge assays on Peggy Sue. You can select and use one of these default groups, make changes to groups or create new groups.

To view settings for a group, click on the group name in the analysis settings box.

Creating a New Standards Group

- 1. Select Edit in the main menu and click Analysis, then click Standards in the options list.
- 2. Click Add under the analysis settings box. A new group will be created:

Analysis Settings	
Standards	1
Standards 2	
Add Remove	

3. Click on the new group and enter a new name.

Analysis S	Settings			
Standar	ds			
New Sta	andards			
	Add	Remov	e	

4. Click in the first cell in the **pl** column in the Fluorescent Peaks table.

pI	Position	Registration	Fit	
9	200	~	V	
6	550		V	
6.4	625		 Image: A set of the set of the	
7	740		 Image: A set of the set of the	
7.3	780	 Image: A start of the start of	V	

5. Enter the pl for the fluorescent standard.

pI	Position	Registration	Fit	
4.0	200	~		
6	550		 Image: A set of the set of the	
6.4	625		 Image: A set of the set of the	
7	740		V	
7.3	780	~	V	

6. Click in the first cell in the **Position** column.

4 200 Image: Constraint of the second secon	pI	Position	Registration	Fit	
6 550 V 4 625 V 7 740 V	4	200	V	~	
.4 625 🗌 🗹 7 740 🗌	6	550			
7 740	6.4	625		V	
	7	740		~	
.3 780 🗸 🖌	7.3	780	 Image: A start of the start of	~	

7. Enter the position of the fluorescent standard peak.

pI	Position	Registration	Fit
4	210	~	
6	550		~
6.4	625		 Image: A set of the set of the
7	740		~
7.3	780	~	~

NOTE: Standards peak positions are relative to each other. Only the difference in their position is used to help identify the standard peaks. When entering standard peak information for the first time, review the standards data in the Analysis screen to find the correct peak position.

- 8. Repeat the steps above for the remaining standards in the table.
 - To add another standard Click Add under the peak table, then modify the information in the new row.
 - To remove a standard Select its row and click Remove.
- 9. Select which standard should be used for capillary registration by clicking the checkbox in the **Registra-tion** column. The first and last standards are typically used for the registration.

pI	Position	Registration	Fit	
4	210	V		
4.9	350		 Image: A set of the set of the	
6	520		 Image: A set of the set of the	
5.4	550		~	
7.3	650	\checkmark	~	

NOTE: In order for Compass to perform data analysis, at least one peak must be selected for registration.

10. Select which standards should be used for pl determination of sample proteins by clicking the checkbox in the **Fit** column. The standards not used for registration are typically also used for fit.

pI	Position	Registration	Fit	
4	210	~		
4.9	350			
6	520		 Image: A start of the start of	
6.4	550		 Image: A set of the set of the	
7.3	650	 Image: A start of the start of	 Image: A set of the set of the	
	650			

11. To use the new group as the default analysis settings for the run file data, click the arrow in the drop down list next to Default, then click the new group from the list. Analysis settings in the new group will then be applied to the run data.

Analysis Settings	
Standard	
New Standards	
Add	Remove
Default	Standard 🔹
	Standard
	New Standards

12. Click **OK** to save changes.

Changing the Default Standards Group

- 1. Select Edit in the main menu and click Analysis, then click Standards in the options list.
- 2. Click the arrow in the drop down list next to Default, then click a new default group from the list.

Analysis Settings	
Standard	
New Standards	
Add	Remove
Default	Standard 👻
	Standard
	New Standards

3. Click **OK** to save changes. Analysis settings in the group selected will be applied to the run data.

Modifying a Standards Group

- 1. Select Edit in the main menu and click Analysis, then click Standards in the options list.
- 2. Click on the group in the analysis settings box you want to modify.

Standards			
New Stan	dards		

- 3. Modify fluorescent standards information as described in "Creating a New Standards Group" on page 403.
- 4. Click **OK** to save changes. The new analysis settings will be applied to the run data.

Deleting an Analysis Group

- 1. Select Edit in the main menu and click Analysis, then click Standards in the options list.
- 2. Click on the group in the analysis settings box you want to delete and click **Remove**.

Standard	ds		
New Sta	ndards		

3. Click **OK** to save changes.

Applying Analysis Groups to Specific Run Data

- 1. Select Edit in the main menu and click Analysis, then click Standards in the options list.
- 2. Click on the group in the analysis settings box you want to apply to specific run data.

Standard	5		
New Star	ndards		

page 408

3. Application of analysis groups to specific run data is done in the override box. Click **Add** under the override box. A default override data set will be created from sample information found in the run file.

Override	
Apply To	Settings
Hi-phospho HeLa	New Standards
Add	Remove

4. Click the cell in the **Apply To** column, then click the down arrow.

Override	
Apply To	Settings
i-phospho HeLa 👻	New Standards
Hi-phospho HeLa Low-phospho HeLa Cycle 1 Cycle 2 Cycle 3 Cycle 4 Cycle 5 Cycle 6 Cycle 6 Cycle 7	
Custom Settings	Remove

- 5. Select an option from the drop down list. This will apply the settings group selected to specific run data as follows:
 - **Sample names** All sample names entered in the assay template (Assay screen) will display in the drop down list, otherwise the default name of Sample will be shown. Select a name to apply group settings to all capillaries that use this sample name in the run file.
 - Attributes All sample attributes entered in the assay template (Assay screen) will display in the drop down list. Select an attribute to apply group settings to all capillaries that use this attribute in the run file.
 - **Cycle 1-8** When this option is selected, group settings will be applied to all capillaries in the cycle.
 - **Custom settings** Lets you choose specific capillaries to apply the group settings to. When this option is selected, the following pop-up box appears to let you enter a specific capillary number or range of capillaries:

🕲 Custom Settings	×
Enter cycle and capillary descriptor	
[
Examples: 2:3, 1:1-12 Cycle 2 capillary 3, and cycle 1 capillaries 1 through 12 1-3:6+8+10 Cycles 1 through 3, capillaries 6, 8 and 10	
ОК	Cancel

6. If you need to change the analysis group used for a data set, click the cell in the **Settings** column and click the down arrow. Select a group from the drop down list.

Settings
New Standards 📼
Standards New Standards

- 7. Repeat the previous steps to apply other groups to specific run data.
- 8. To remove a data set, click on its cell in the **Apply To** column, then click **Remove**.
- 9. Click **OK** to save changes.

Importing and Exporting Analysis Settings

The analysis settings in a run file can be exported as a separate file. This allows the same analysis settings to be imported into other assays or run files at a later time, rather than having to re-enter settings manually.

Importing Analysis Settings

NOTE: Importing an analysis settings file populates the settings in all Analysis pages.

1. Open the run file or assay you want to import analysis settings to.

- 2. Select Edit in the main menu and click Default Analysis (Assay screen) or Analysis (Analysis screen).
- 3. Click **Import** on any page.
- 4. Select a settings file (*.settings) and click OK. The imported settings will display in all analysis pages.

Exporting Analysis Settings

NOTE: Exporting an analysis settings file exports the settings in all Analysis pages.

- 1. Open the run file or assay you want to export analysis settings from.
- 2. Select Edit in the main menu and click Default Analysis (Assay screen) or Analysis (Analysis screen).
- 3. Click Export on any page. The following window displays:



- 4. The default directory is Compass/Assays. Change the directory if needed.
- 5. Enter a file name and click **Save**. The settings will be saved as a *.settings file.

Chapter 10: Setting Your Preferences

Chapter Overview

- Custom Preference Options
- Setting Data Export Options
- Selecting Custom Plot Colors for Graph Overlay
- Setting Up Wes, Sally Sue and Peggy Sue to Send Tweets

Custom Preference Options

You can set and save custom preferences for data export, plot colors in the graph and Twitter communication. To access these settings, select **Edit** in the main menu and click **Preferences**.

(Preferences		_ D X
type filter text Analysis Graph Twitter	Analysis Export Standards 📝 Export using a comma as the column deliminator 🗖	\$ • \$ • •
	Restore Defaul	ts Apply
	ОК	Cancel

To move between preferences pages in this window, click on any option in the list on the left. The following items can be user-customized in Compass:

- Analysis Lets you customize data export options.
- **Graph** Lets you customize graph color displays.
- **Twitter** Lets you configure Wes, Sally Sue or Peggy Sue to Tweet run status.

Setting Data Export Options

Select Edit in the main menu and click Preferences, then click Analysis in the options list.

Preferences		_ D X
type filter text Analysis Graph Twitter	Analysis Export Standards	
	OK	Cancel

- **Export Standards** Selecting this option includes data for the standards in each sample when run data is exported. When this option is deselected, only sample data will be exported. This option is selected by default.
- **Export using a comma as the column delimiter -** Selecting this option exports run data in .csv format. When this option is deselected, the data is exported in .txt format.
- Click **Apply** to apply changes to any open run files in Compass.
- Click **Restore Defaults** to restore Compass default settings.
- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

Selecting Custom Plot Colors for Graph Overlay

Select Edit in the main menu and click Preferences, then click Graph in the options list.

Preferences		
type filter text	Graph	↓ ↓ ↓ ↓
Analysis Graph Twitter	Apply colors to stacked plots Plot color 1 Plot color 2 Plot color 3 Plot color 3 Plot color 4 Plot color 5 Plot color 5 Plot color 6 Plot color 7 Plot color 7 Plot color 8 Plot color 9	
		Restore Defaults Apply OK Cancel

• **Apply colors to stacked plots** - Selecting this option applies the color scheme to individual electropherograms when the Stack the Plots option is selected in the Analysis screen graph pane.

NOTE: If Apply colors to stack plots is not checked, the colors shown in the preferences screen will be applied only to overlaid electropherograms when the Overlay the Plot option is selected in the graph pane.

- When this option is deselected, plots will use Compass default colors.
- Click **Apply** to apply changes to any open run files in Compass.
- Click Restore Defaults to restore Compass default settings.
- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

Changing Plot Colors

- 1. Select Edit in the main menu and click Preferences, then click Graph in the preferences list.
- 2. Click the color button next to a plot number. The color selection box displays:



- 3. Select a color or define a custom color and click **OK**. The color button will update to the new color selected.
- 4. Repeat the steps above for any other plot colors.
- 5. Check **Apply Colors to Stacked Plots** if you want the new color scheme to also be used for the Stack the Plots option in the graph pane.
- 6. Click **Apply** to apply changes to plots currently displayed in the graph pane.
- 7. Click **OK** to save changes and exit. When the Overlay the Plots option is selected in the graph pane, the new color scheme will be used.

Setting Up Wes, Sally Sue and Peggy Sue to Send Tweets

Select Edit in the main menu and click Preferences, then click Twitter in the options list.

Preferences			
type filter text	Twitter		⇔ • ⇔ • •
Analysis Graph	Twitter User Name:		
Twitter			Set Account
	-		Clear
	I weet When :		
	Run is completed Errors		
	Tweet Message		
		Restore D	Defaults Apply
		ОК	Cancel

- Click Apply to apply changes.
- Click Restore Defaults to restore Compass default settings.
- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

To have Wes, Sally Sue or Peggy Sue tweet a Twitter account:

NOTES:

To set Wes, Sally Sue or Peggy Sue up to tweet, the computer you are using must have an internet connection.

To tweet, Wes, Sally Sue or Peggy Sue must be connected to the internet through a network connection or via the local lab computer.

We recommend setting up a separate Twitter account for Wes, Sally Sue or Peggy Sue. This lets multiple people in the lab follow run progress. It also lets you send tweets directly from Wes, Sally Sue or Peggy Sue to all users, for example to notify others when the instrument is available or when an error has been reset, etc.

1. Click **Set Account**. A set account window will display in Compass and the following browser window will open:



- 2. Enter a user name or email and password, then click **Authorize app**. A new page will display in the browser with a PIN number.
- 3. Enter the PIN number in the set account window in Compass and click OK:

Set Account	×
Enter PIN given by Twitter Web Site	
	OK Cancel

4. The user name will now appear in the Twitter User Name box. Select one or all of the tweet options in the Tweet When box, then click **Apply**.

(Preferences			
type filter text	Twitter		
Analysis Graph Twitter	Twitter User Name: Tweet When : Run is started Run is completed Errors Tweet Message	Test	Set Account Clear
	'		OK Cancel

5. To confirm the Twitter account is receiving messages, click **Tweet Message**. Enter a test message and click **OK**.

Sending Tweet Message	x
Message to send to Twitter	
Compass Test Message at 20120605 170630	
	OK Cancel

6. If the test Tweet was successful, Compass will display the following message:



7. Click **OK** to save changes and exit. Wes, Sally Sue or Peggy Sue will automatically tweet as the selected options occur, as shown below:

@ Test Gel Free, Hands Free, Blot Free		4 TWEETS	
Santa Cla	ra http://www.pro	leinsimple.com	0 FOLLOWING 0 FOLLOWERS
Tweets	>	Tweets	
Following Followers	>	PL0006 @Test Compass Test Message at 20120615 201241	1m
Favorites	>	Expand PL0006 @Test Compass Test Message at 20120615 201146	2m
© 2012 Twitter About Help Terms Blog Status Apps Resources Job Advertisers Businesses Media De	Privacy Is velopers	Expand PL0006 @Test @Test Hi, Thanks for the free time, GREAT Data View conversation	5 Jun
		PL0006 @Test Compass Test Message at 20120605 170630 Expand	5 Jun

Changing the Twitter Account

To change the Twitter account Wes, Sally Sue or Peggy Sue uses:

- 1. Select Edit in the main menu and click Preferences, and click Twitter in the preferences list.
- 2. Click **Clear**.
- 3. Set up the new account as described in "Setting Up Wes, Sally Sue and Peggy Sue to Send Tweets" on page 416.

Sending Manual Tweets from Wes, Sally Sue and Peggy Sue

You can send tweets directly from Wes, Sally Sue or Peggy Sue. For example, you may want to notify other users that the instrument is available, being serviced or when an error has been cleared. To do this:

- 1. Select **Edit** in the main menu and click **Preferences** and click **Twitter** in the preferences list.
- 2. Click Tweet Message.
- 3. Enter a test message and click **OK**. The tweet will be received by any users following the Twitter account Wes, Sally Sue or Peggy Sue uses.

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Chapter 11:

Compass Access Control and 21 CFR Part 11 Compliance

Chapter Overview

- Overview
- Enabling Access Control
- Logging In to Compass
- Saving Changes
- Signing Files
- Instrument Command Log
- Run File History
- Troubleshooting Problems and Suggested Solutions
- Authorization Server



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Overview

The Compass Access Control feature can be used to help satisfy the 21CFR Part 11 data security requirements when using Simple Western instruments. When Access Control is enabled and the Authorization Server has been installed (see "Authorization Server" on page 432):

- Users are required to log in to Compass when the software is launched
- A history of all actions is maintained
- Data files are signed and encrypted to prevent unauthorized changes (e.g., all files are controlled)
- Each instrument maintains a history of user commands
- Each assay and data file includes a history of signed changes to the file

Compass can be run with or without Access Control enabled. When Access Control is disabled, no user log in is required and files are not encrypted or signed. The instrument history and file history are still maintained but the entries are not signed.

Enabling Access Control

Access Control is enabled in **Preferences**. Select **Edit** in the main menu, click **Preferences**, then select **Access Control**.

۲	Preferences	- 🗆 🗙
type filter text Access Control Analysis Graph Twitter	Access Control Enable I 0.1.2.18 Port 8000	\$ • \$ • •
		Restore Defaults Apply
		OK Cancel

To enable Access Control:

- 1. Check the **Enable** box.
- 2. Enter the IP address of the Authorization server. Use format X.X.X.X or LocalHost if installing the server on the local machine.

NOTE: Always use the default port setting of 8000, this should not be changed.

3. Close Compass. The next time Compass is launched, a user log in will be required.

NOTE: Access Control can only be disabled by logging into Compass and deselecting the **Enable** box in the Access Control page of Preferences.

Logging In to Compass

With Access Control enabled, all users must log in to Compass whenever the software is launched.

۲	Compass Login				
1.1					
User:]			
Password:					
	Login Cancel				

Enter your user name and password previously setup by your Compass Administrator.

A successful log in will display the Compass main window with the user information in the lower status bar. The full user name is displayed with the unique user ID in parenthesis:

۲					Com	pass						- 🗆	x
File Edit View Instrument V	Window Hel	þ											
🕴 🗮 🛧 🚉 🔳 👪 🍇	•								📑 Assay	🕘 Run Sur	mmary 📘	Analy	is
Experiment 🗖 🗖	J. Graph	🕲 Image 🗉	Lane							1	F = -	~ -	•
Sample Primary Cycle													
	Peaks	Capillaries)										
	Sample	Primary	Cap	Peak	Name	Position	MW (kDa)	Height	Area	% Area	Width	S/N	В
< >	<	Steve	n Gallagh	er (steve	:g) is logge	d in.							>

If there is no activity in Compass for 20 minutes, the user must re-enter their password to perform any controlled actions:

۲	Compass Login					
Inactivity	timeout please re-enter password.					
User	steveg					
Password						
	Login Logout					

Resolving Log In Issues

Log in failures may occur when:

- The server is temporarily unavailable
- Compass is using the wrong IP address

When this happens, the following message displays:



Click **Disable** to restart Compass with Access Control disabled. Verify or correct the server IP address then close and restart Compass to log in with Access Control enabled.

Saving Changes

When **Save** is selected from the **File** menu, a dialog box will display to allow you to enter a comment before saving the signed file:



The comment is added to the signature entry in the file History:

🕑 Status 📙	History				
Date		User Name	Message		Comment
04/15/20	13 9:48 AM	b	RunStart	2013-04-15_09-49-28_Simon_short	
05/10/20	13 4:22 PM	steveg	Saved an	alysis changes	Changed Baseline fr.
<					>
Time	05/10/2013	4·22 PM	User	steven	
Message	Saved analy	isis changes	0.00	storeg	
Comment	Changed B	aseline from 1.0	to 2.0		
comment	changeab	usenne monti no	10 2.0		

Signing Files

Select **e-Signature** from the **File** menu to add an electronic signature to a file.

۲	e-Signature	×
	Add your e-signature to this file. Comment:	
	Approved	
	e-Sign Cancel	

The signed entry will be added to the file History with the meaning of the signature entered in the comment, such as *Approved* or *Verified*.

🕑 Status 🔚 History			
Date	User Name	Message	Comment
04/15/2013 9:48 AM	b	RunStart2013-04-15_09-49-28_Simon_short	
05/10/2013 4:22 PM	steveg	Saved analysis changes	Changed Baseline fr
05/10/2013 4:44 PM	steveg	e-Signature applied	Approved
<			>
Time 05/10/2013	4:44 PM	User steveg	
Message e-Signatur	e applied		
Comment Approved			

Instrument Command Log

The Instrument Command Log can be viewed at any time by selecting the **Instrument** menu and clicking **Properties**, and then clicking the **Command Log** button:

© 2013-05-10_15-28-43_EZassay_Peggysize - Compa	iss ×
Name: Peggy PL0005 Location:	
Type: Peggy Network Name: p10005.local. Serial Number: PL0005 Network Address: 10.1.2.173 Instrument Software: 2.1.18354	
Instrument Date and Time 2013-05-10 17:03:02 -07:00 Set to PC time	
Error Log Test Log OK	Cancel

The Command Log lists all the commands sent to the instrument that were signed by the user who sent the command. If you want to copy the Command Log at any time, right click in the table and select **Copy**, then paste into another document.

۲		Command Log	×
Date	User Name	Message	Comment
05/01/2013 4:07 PM	uyen	Started run Run: 2013-05-01_15-50-32_models_R	
05/02/2013 1:57 PM	uyen	Started Self-Test	
05/02/2013 3:46 PM	uyen	Started run Run: 2013-05-02_15-29-13_models	
05/03/2013 3:00 PM	uyen	Started run Run: 2013-05-03_14-43-31_Screening	
05/03/2013 3:00 PM	uyen	Reset after error	
05/03/2013 3:03 PM	uyen	Started run Run: 2013-05-03_14-45-57_Screening	
05/03/2013 3:03 PM	uyen	Stopped run	
05/03/2013 3:03 PM	uyen	Stopped run	
05/03/2013 3:03 PM	uyen	Stopped run	
05/03/2013 3:06 PM	uyen	Started run Run: 2013-05-03_14-49-52_Screening	
05/07/2013 3:31 PM	uyen	Started run Run: 2013-05-07_15-14-15_DExinpri	
05/08/2013 4:46 PM	uyen	Started run Run: 2013-05-08_16-29-35_TrisT22_2	
05/09/2013 4:25 PM	uyen	Started run Run: 2013-05-09_16-08-00_BGAR_Rb	
05/10/2013 3:46 PM	uyen	Started run Run: 2013-05-10_15-28-43_EZassay_P	
<			>
Time 05/01/2013 4 Message Started run Comment	07 PM Us Run: 2013-05-01_	er uyen 15-50-32_models_Rb3step_Peggysize_TrisTricine22 As OK C	say: ancel

Run File History

Select the **Run Summary** screen tab and then the **History** tab to see the file History. To copy the file History, right click in the table and select **Copy**, then paste into another document.

۲		2013-04-15_09-49-28_Sit	mon_short - Compass	- 🗆 🗙
File Edit Instrument V	Vindow Help			
				📑 Assay 💽 Run Summary 🚛 Analysis
Run: 2013-04-15_09-49-2	28_Simon_short			Separation 🛃 IV Plot
🕑 Status 📙 History				
Date	User Name	Message	Comment	
04/15/2013 9:48 AM	b	RunStart2013-04-15_09-49-28_Simon_short		
05/10/2013 4:22 PM	steveg	Saved analysis changes	Changed Baseline fr	
05/10/2013 4:44 PM	steveg	e-Signature applied	Approved	
· · · · · · · · · · · · · · · · · · ·				
-				
-				
<			>	
Time		User		
Message				
Comment				
comment				
l		Steven Gallagher (steveg) i:	s logged in.	

Troubleshooting Problems and Suggested Solutions

If any of the following error messages are encountered, follow the recommended steps below to resolve the issue.

- Unknown user name or password.
 - Check if the Caps Lock is on, user name and password are case sensitive.
 - Ask a Compass administrator to confirm your user name. If your password is unknown then the administrator can reset your password (see "Resetting User Passwords" on page 440 for more information).

Server not available.

- From the Edit menu, click Preferences and then Access Control to confirm the server address is set to the correct Authorization server address. Compass must be able to reach the server on the network.
- The server must have inbound access to port 8000 enabled.
- Controlled file cannot be opened without log in. To open a controlled Run file, enable Access Control by clicking Edit, then Preferences and Access Control. Select Enable, close Compass, then re-launch Compass with a valid log in.
- Uncontrolled file cannot be opened when logged in. To open an uncontrolled Run file, disable Access Control by clicking Edit, then Preferences and Access Control. Deselect Enable, close Compass then re-launch the software.

NOTE: Uncontrolled Assay files can be opened when Compass Access Control is enabled (controlled mode).

- **Command disabled.** Certain commands are only available when a user with the correct permissions is logged in. To change user permissions, use a web browser to log in to the Authorization server web interface at the address shown on the **Access Control** page in **Preferences**, such as: 10.1.3.231:8000.
- Compass does not prompt for log in. Compass will only prompt for a log in on launch when Access
 Control is enabled in Preferences. Enable Access Control by clicking Edit, then Preferences and
 Access Control. Select Enable, close Compass, then re-launch the software. You should now be
 prompted for a log in.

Authorization Server

The Authorization Server controls the log in access to Compass. In the simplest configuration, the server is run on the same computer as Compass and only that copy of Compass is controlled. A single server can also be used to control access to multiple copies of Compass running on different computers, so long as they have network access to the server. Multiple copies of the server may be run on the same network, and each server will have its own user database.

To enable Compass to use a particular Authorization Server, click **Edit**, then **Preferences** and **Access Control** and enter the server IP address using format X.X.X.X.

.

NOTES:

Always use the default port setting of 8000, this should not be changed.

If the server is installed on the same computer as Compass (e.g., the local machine), enter LocalHost instead of the IP address. Contact your local IT Administrator to assist with installing the Authorization Server in your preferred format.

Server Administration

The Authorization Server is configured through a web interface at the IP address of the server on port 8000. To access the Server home page, open any browser and type the IP address on port 8000 in a X.X.X.X8000 or http://X.X.X.X8000 format. Use LocalHost instead of the IP address if the Server is installed on the local machine.

The default server administrator is:

- User: admin
- Password: admin

After installing the Authorization Server, the administrator user name and password can be changed.

🕒 Log in ProteinSimple site 🗙 📃		X
← → C 🗋 10.1.2.18:8000/admin	v/	☆ 🔘 🗉
	ProteinSimple Administration	
	Username: admin	
	Password: •••••	
	Log in	

Adding Non-admin Users

Add a user to the server to allow that user to log in to Compass. To do this:

1. Select **Users** from the Site Administration home page:



2. From the Users page, select Add User:

Select user to change Prc ×					- • ×			
← → C 🗋 10.1.2.18:80	← → C 🗋 10.1.2.18:8000/admin/auth/user/ ☆ 🔘 =							
ProteinSimple Admin	ProteinSimple Administration Welcome, k. Documentation / Change password / Log out							
Home > Auth > Users								
Select user to cha	ange				Add user +			
۹.	Search				Filter			
Action:	Go 0 of 5 sele	cted			By staff status			
Username 🔺	Email address	First name	Last name	Staff status	Yes			
🔲 fay		fay	deng	•				
🗆 k	k@k.com			0	All			
🔲 dc				•	Yes			
steveg		Steven	Gallagher	•	By active			
uyen uyen		Uyen	Nguyen	•	All			
5 users	Yes No							
5 00010					By groups			
					All			
					Administrator			

3. Fill in the fields to create a new user:

Add user ProteinSimple 5 ×				
← → C [10.1.2.18:8000/admin/auth/user/add/ ☆ 0 =				
ProteinSimple Admin	istration	Welcome, ${f k}.$ Documentation / Change password / Log out		
Home > Auth > Users > Add user				
Add user				
First, enter a username and p	bassword. Then, you'll be able to edit	more user options.		
Username:	Required, 30 characters or fewer. Lette	rs, digits and $(./+/-/_ only)$.		
Password:				
Password confirmation:	Enter the same password as above, for	· verification.		
		Save and add another Save and continue editing Save		

After adding a new user more information can be added:

Change user ProteinSim _F ×					
← → C 🗋 1	0.1.2.18:8000/admin/auth/user/3/	☆ 🖸 🗉			
ProteinSimpl	e Administration	Welcome, k . Documentation / Change password / Log out			
Home > Auth > Users	> steveg				
Change us	er	History View on site 🔸			
Username:	steveg Required. 30 characters or fewer. Letters, digits and @/./+/	-/_ only.			
Password:	algorithm: pbkdf2_sha256 iterations: 10000 salt: RJk6 /T3qfU************************************	oj****** hash: is user's password, but you can change the password using			
Personal info					
First name:	Steven				
Last name:	Gallagher				
Email address:		~			

Permissions

All users can log in to Compass, but the commands available within Compass are controlled by Permission settings. Commands a user does not have permission to use will be disabled. After user permissions have been changed on the server the user must close and re-open Compass to use the new permissions.

Users can belong to groups that have multiple permissions such as Manager or Scientist:

→ C 🗋	10.1.2.18:8000/admin/auth/user/10/	☆ O
ermissions		
✔ Active	Designates whether this user should be treated as	artive. Uppelost this instead of deleting assounts
	Designates whether this user should be treated as	active, onsered this instead of dereting accounts.
Staff status	Designates whether the user can log into this admi	n site.
Superuser s	tatus	
	Designates that this user has all permissions witho	ut explicitly assigning them.
	The groups this user belongs to. A user will get all "Control", or "Command" on a Mac, to select more	permissions granted to each of his/her group. Hold down than one.
Groups:	Available groups @	Chosen groups 🖗 🕈
	Q Filter	^
	Reviewer ^	
	Scientist	
	6	
	6	
	Choose all 📀	Remove all
	Specific permissions for this user. Hold down "Contr	ol", or "Command" on a Mac, to select more than one.
User	Available user permissions @	Chosen user permissions @
permissions:	Q Filter	^
	auth user Deny analysis editing	
	auth user Deny instrument administrati	
	auth user Deny plate editing	
	auth user Deny sign off of data	
	auth user Allow analysis editing auth user Allow copy, export of data	
	auth user Allow instrument administration auth user Allow instrument control	
	auth user Allow plate editing auth user Allow protocol editing	~
	Choose all 🕥	Remove all
nnortant date		
last login:	Date: 2013-06-11 Today 📖	
cust login.	Time: 08:19:34 Now 🔘	
Date joined:	Date: 2013-06-11 Today	

Use the Groups page to change the permissions in a group or create new groups:

🕒 Site administration Prote 🗙 💶			- □ ×
← → C [] 10.1.2.18:8000/admin/		5	20 =
ProteinSimple Administration		Welcome, admin . Change password	/ Log out
Site administration			
Auth		Recent Actions	
Groups	🗣 Add 🛛 🧷 Change	My Actions	
Users	🖶 Add 🛛 🖉 Change	 steveg User typeScientist User TypeScientist User TypeScientist User TypeReviewer User TypeReviewer User fay 	

To change permissions for a group click **Change**, then select a group:

Select group to change P ×			
← → C [] 10.1.2.18:8000/admin/	← → C [] 10.1.2.18:8000/admin/auth/group/		
ProteinSimple Administration		Welcome, admin. Change password / Log out	
Home > Auth > Groups			
Select group to change		Add group +	
٩	Search		
Action: Go	0 of 3 selected		
Group			
Operator			
Reviewer			
Scientist			
3 groups			

Move individual group permissions in or out of the Available Permissions and Chosen Permissions boxes by selecting a permission in either box. Click the **left** or **right** arrow button to move the permission into the other box.

🕒 Change group	ProteinSir ×		×
← → C 10.1.2.18:8000/admin/auth/group/3/		<u> か の で ま の で の で の の の の の の の の の の の の の</u>	Ξ
Reload this page ProteinSimple Administration		Welcome, admin . Change password / Log o	out ^
Home > Auth > Grou	ps > Scientist		- 1
Change gr	oup	History	
Name:	Scientist		
Permissions:	Hold down "Control", or "Command" on a Mac, to s	elect more than one.	
	Choose all 🔇	Remove all	
# Delete		Save and add another Save and continue editing Save	`

Adding Admin Users

To create a user with administrator permissions:

- 1. Follow the steps described in "Adding Non-admin Users" on page 434 to create the admin user.
- 2. Under permissions, select **Staff status** and **Superuser status**:

Permissions	
Active	
	Designates whether this user should be treated as active. Unselect this instead of deleting accounts.
Staff status	
	Designates whether the user can log into this admin site.
✓ Superuser st	atus
	Designates that this user has all permissions without explicitly assigning them.

3. Assign the admin user to a group.

NOTE: Selecting Superuser status enables server permissions only. Admin users must be also be assigned to a group to in order to have Compass permissions.

Resetting User Passwords

To reset a user password:

1. Select **Users** from the Site Administration home page, then select the user to change. The following screen displays:

🕒 Change user Pro	ProteinSim _F ×	-		×
← → C 🗋 1	10.1.2.18:8000/admin/auth/user/10/	\$	0	≡
ProteinSimpl	le Administration Welcome, admin. Change pa	assword /	Log o	ut î
Home > Auth > Users	rs > steveg			
Change us	History	/iew on si	te →	
Username:	steveg Required. 30 characters or fewer. Letters, digits and @/./+/-/_ only.			
Password:	algorithm: pbkdf2_sha256 iterations: 10000 salt: b083Ho****** hash: VsekSs***********************************	password		
Personal info				
First name:	Steve			
Last name:	Gallagher			
Email address:	:			
Permissions				i I
☑ Active	Designates whether this user should be treated as active. Unselect this instead of deleting accounts.			
□ Staff status	Designates whether the user can log into this admin site.			~

2. Raw passwords are not stored, they must be changed manually. Click the text link to access the password change form:

Change passwo	rd: steveg ×	- - ×
← ⇒ C 🗋	10.1.2.18:8000/admin/auth/user/10/password/	☆ 🗿 =
ProteinSimple Administration Welcome, admin. Change password /		
Home > Auth > User	s > steveg > Change password	
Change pa	sword: steveg.	
Password:		
Password (again):	Enter the same password as above, for verification.	
		Change password

3. Enter the new password, then click **Change password**.

Encryption Details

Compass uses the SHA1 hash algorithm to generate a 160 bit hash code that is unique for all files. All files saved by Compass are encrypted with a digital key. This key along with the hash codes guarantees the file history is correct and no other edits were made. All changes saved to a file have the electronic signature of the user who saved the file. The **e-Signature** command allows a user to sign off on a state such as approved or verified.

There is no individual ownership of files, all users who log into Compass can open any file.

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