Wes

proteinsimple
a biotechne brand

Toll-free: (888) 607-9692
Tel: (408) 510-5500
Fax: (408) 510-5599
info@proteinsimple.com
proteinsimple.com

031-105 Rev H

Wes

Training Kit
Part# PS-T001

Let's get started!

Reagents and materials in this kit

ORANGE BOX - STORE AT 18-24 °C

INCLUDES	PART NO
Wash Buffer (60 mL)	042-202
10X Sample Buffer (440 µL)	042-195
Pre-Filled Microplates (8)	043-165
Capillary Cartridges (8)	009-050

CLAMSHELL 1 - STORE AT 2-8 °C

INCLUDES	PART NO
Standard Pack (8): Biotinylated Ladder, Fluorescent 5X Master Mix, DTT, and empty 0.6 mL tube	PS-ST01

CLAMSHELL 2 STORE AT 2-8 °C

INCLUDES	PART NO
Anti-Rabbit Secondary Antibody (2 mL)	042-206
Luminol (2 mL)	043-311
Peroxide (2 mL)	043-379
Antibody Diluent 2 (20 mL)	042-203
Strept-HRP (132 µL)	042-414

CLAMSHELL 3 STORE AT 2-8 °C

INCLUDES	PART NO
10X System Control Primary Antibody- Rabbit (250 µL)	042-196
ERK1 Primary Antibody- Rabbit (0.5 mL)	042-486
Anti-Mouse Secondary Antibody (2 mL)	042-205

CLAMSHELL 4 STORE AT -80 °C

INCLUDES	PART NO
HeLa Lysate Control for Size (2 x 20 µL)	042-488

Other things you'll need

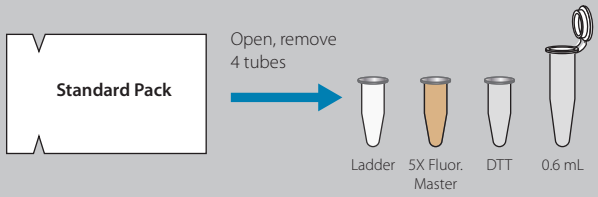
- Water, 0.22 µm-filtered and deionized (molecular biology grade or better)
- Pipettes and tips
- Microcentrifuge and tubes
- Ice and ice bucket
- Vortex
- Heat block
- Centrifuge with plate adapter

A few things you should know

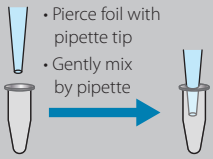
- Warm Wes' plates up to room temperature for at least 24 hours before you start the first assay.
- Capillaries are moisture- and light-sensitive.
- Store unopened cartridge packages and plates at room temperature and do not remove the seals until ready to use.
- The first capillary in the cartridge has been optimized for running the biotinylated ladder. Pipette the biotinylated ladder and samples only as shown in Step 2.
- Plate well evaporation dramatically affects experimental results. To prevent evaporation, keep the lid on the assay plate and do not remove the seal until you're ready to put the assay plate into Wes. Keep the lid on between reagent additions and post-preparation.
- The Antibody Diluent 2, Sample Buffer (10X), Streptavidin-HRP, Wash Buffer, and Secondary Antibody are ready-to-use reagents and should not be diluted.
- An optional System Control Primary Antibody (042-196 or 042-191) can be mixed with your primary antibody in the assay to calculate inter-assay and inter-instrument variability.
- You can use Bicine/CHAPS buffer (P/N: 040-764) or RIPA buffer (P/N: 040-483) to lyse your cells.

1. Prepare your reagents

A PREPARE STANDARD PACK REAGENTS

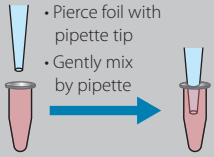


DTT (Clear Tube)



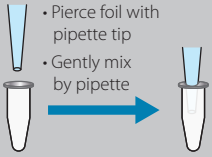
- Add 40 µL deionized water to make a 400 mM solution

Fluorescent 5X Master Mix (Pink Tube)

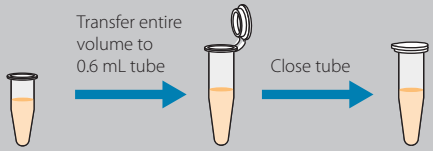


- Add 20 µL 10X Sample buffer
- Add 20 µL prepared 400 mM DTT solution

Biotinylated Ladder (White Tube)

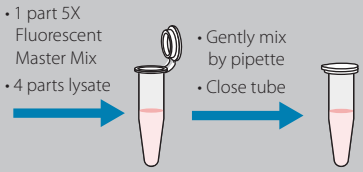


- Add 16 µL deionized water
- Add 2 µL 10X Sample Buffer
- Add 2 µL prepared 400 mM DTT solution

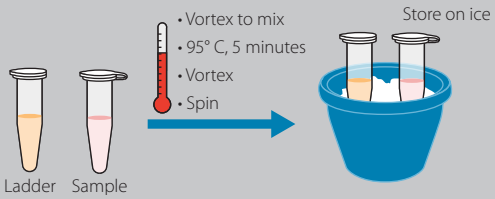


B PREPARE YOUR SAMPLES

- Prepare 0.1X Sample Buffer by mixing:
  - 1.5 µL 10X Sample Buffer
  - 148.5 µL deionized water
- Dilute the HeLa Lysate Control by mixing:
  - 12.5 µL stock HeLa lysate
  - 112.5 µL of 0.1X Sample Buffer
- Combine 1 part (28 µL) 5X Fluorescent Master Mix with 4 parts (112 µL) diluted lysate in a microcentrifuge tube.



C DENATURE YOUR SAMPLES AND BIOTINYLATED LADDER



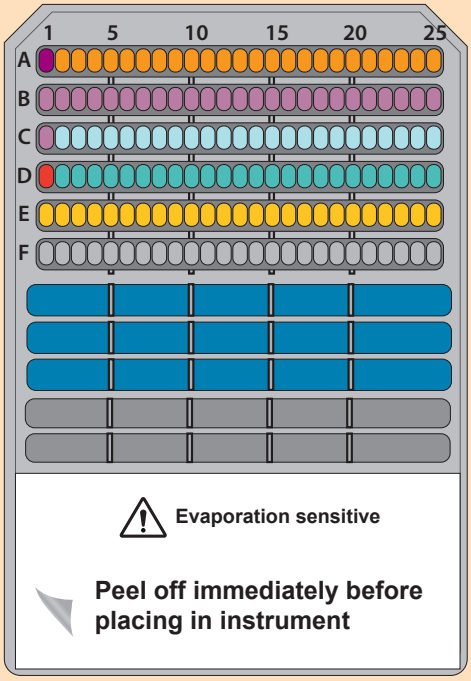
D PREPARE YOUR ANTIBODIES

- Primary Antibody: The Rabbit Anti-ERK1 Primary Antibody is ready to use. Optional: If you're using the System Control, add 25 µL of the System Control Primary Antibody to 225 µL of the ERK1 Primary Antibody. Pipette 10 µL of the mixture into each Primary Antibody well.
- Secondary HRP Conjugates: The supplied conjugates are ready to use without dilution. Use the Anti-Rabbit Secondary Antibody with the anti-ERK 1.

E MIX LUMINOL-S AND PEROXIDE

- Combine 200 µL Luminol-S and 200 µL Peroxide in a microcentrifuge tube

2. Pipette your plate



- For more consistent results, keep the lid on between reagent additions and minimize bubble formation when adding Wash Buffer to the troughs in the microplates.

1. Dispense reagents into the assay plate as follows:

WELLS	COLOR	COMPONENT	VOLUME
A1		Biotinylated ladder	5 µL
A2–A25		HeLa Lysate	3 µL
B1–B25		Wes Antibody Diluent 2	10 µL
C1		Wes Antibody Diluent 2	10 µL
C2–C25		Rabbit Anti-ERK Primary Antibody	10 µL
D1		Streptavidin-HRP	10 µL
D2–D25		Anti-Rabbit Secondary Antibody	10 µL
E1–E25		Luminol-Peroxide Mix	15 µL
F1–F25		Empty	—
Troughs, Top 3 Rows		Wash Buffer	500 µL/ compartment

2. Centrifuge the plate for 5 minutes at 2500 rpm (~1000 x *g*) at room temperature. Ensure liquid is fully down in all wells.

3. Start Wes

1. Launch Compass and go to File>Open Assay. Browse to Documents\Compass\Assays and open the assay file named "ERK1 Wes run".
2. Open Wes' door.
3. Insert a capillary cartridge into the cartridge holder. The interior light will change from orange to blue.
4. Remove the assay plate lid. Hold plate firmly on bench and carefully peel off evaporation seal. Pop any bubbles observed in the Separation Matrix wells with a pipette tip.
5. Place the assay plate on the plate holder.
6. Close Wes' door.
7. Click the Start button in Compass.
8. When the run is complete, discard the plate and cartridge.