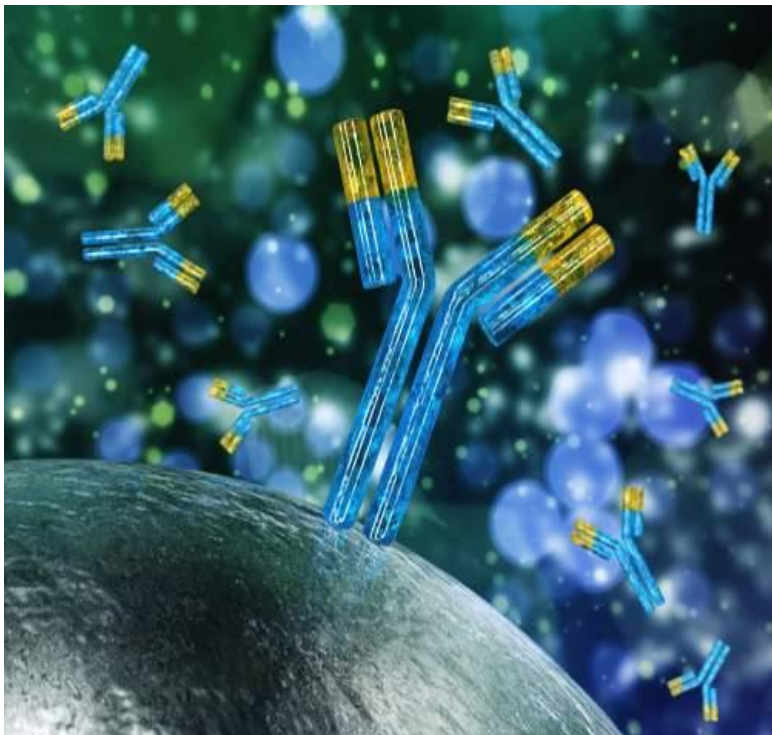


Simple Western 多因子检测 技巧和策略

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yanli.jin@bio-technie.com
18702126546
2020-04-17

为什么要多因子检测?

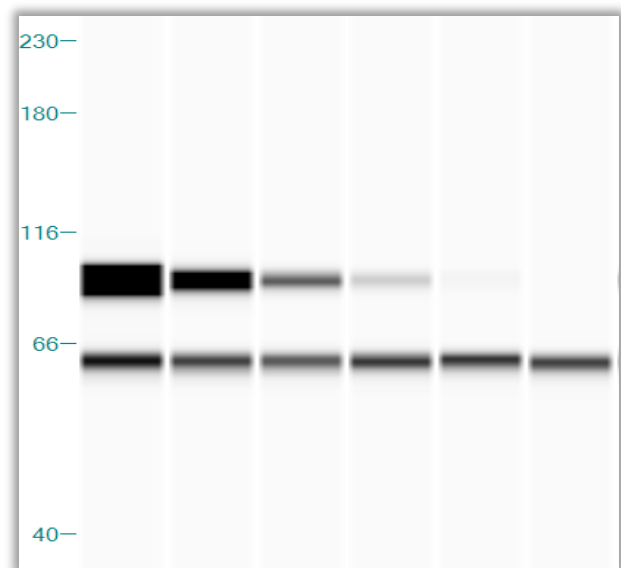


在一个毛细管中有更多相关数据点

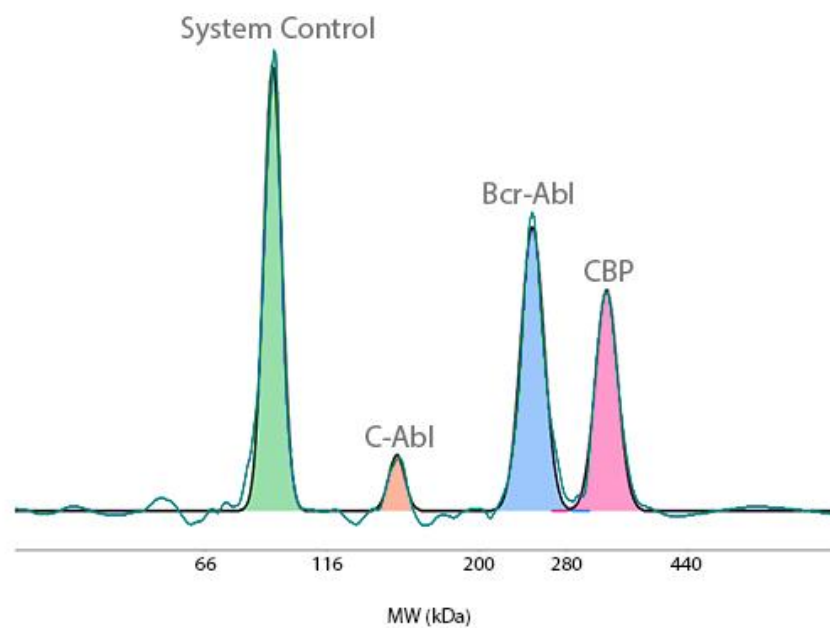
- 用内参蛋白归一化
- 同一样品检测多种靶蛋白
- 总蛋白和磷酸化蛋白

多因子检测例子:

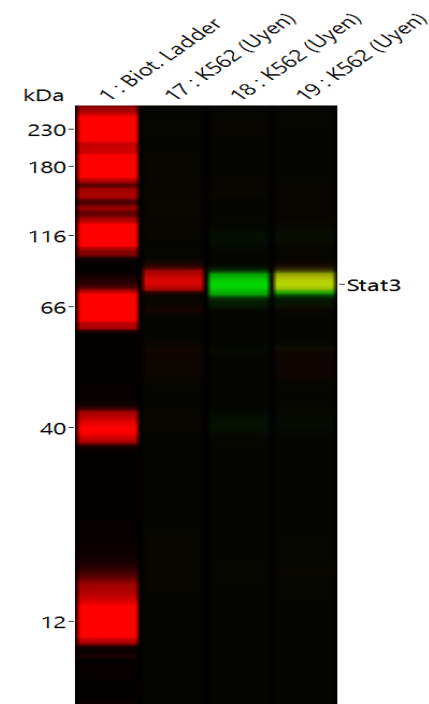
用内参蛋白归一化



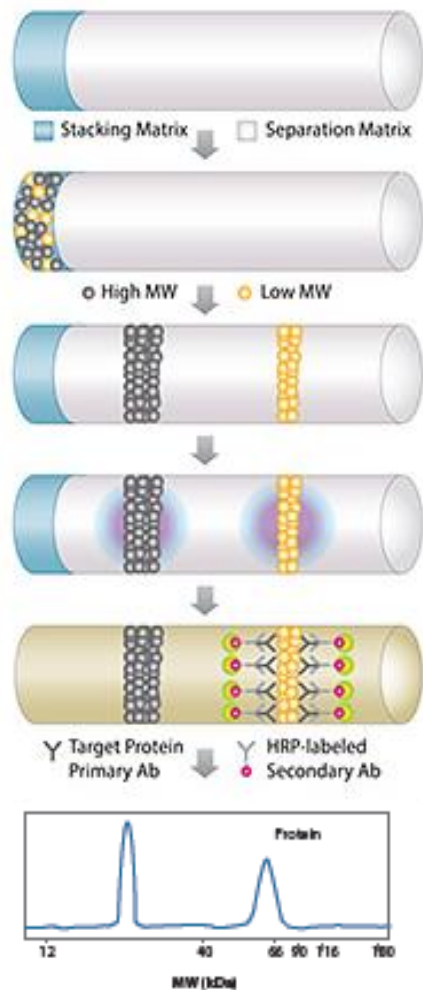
检测多个目标蛋白



磷酸化/总蛋白

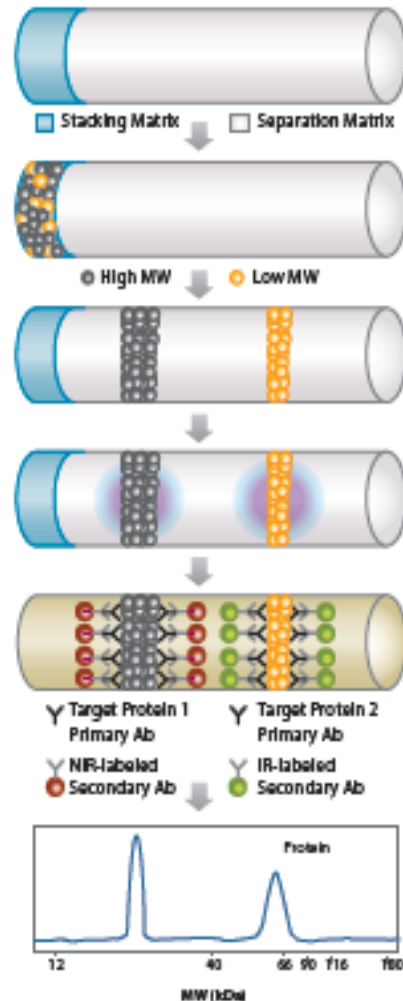


用 Simple Western 进行多因子检测

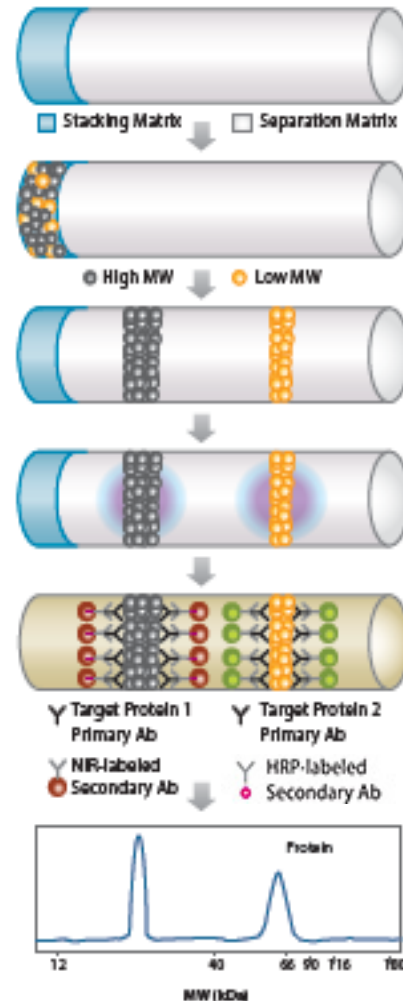


化学发光

Wes/Jess

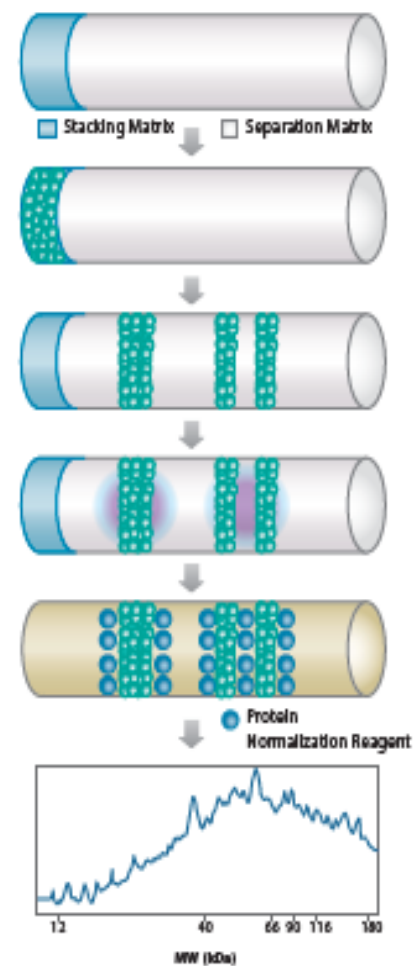


双色荧光



化学+荧光

Jess



化学+荧光+总蛋白检测

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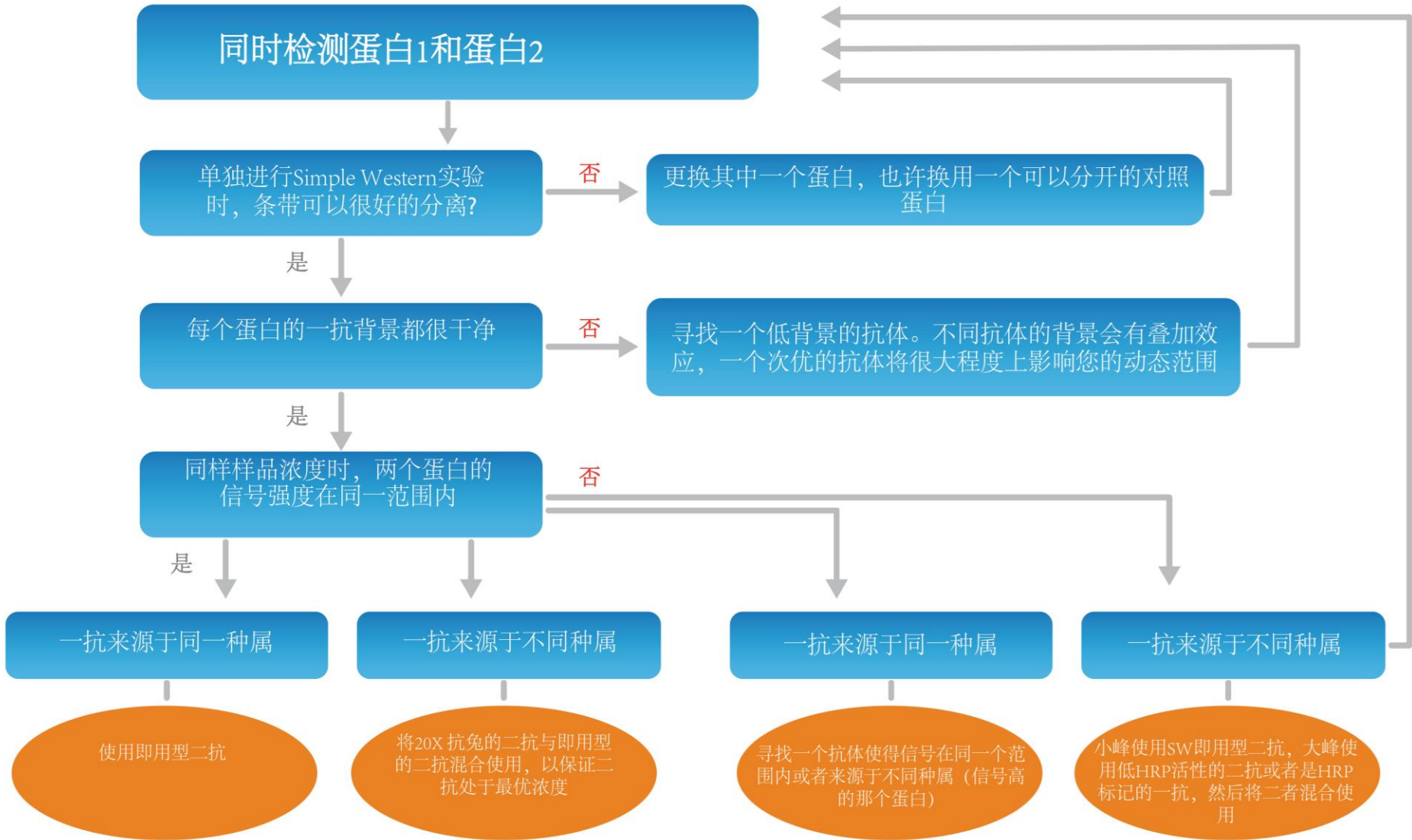
化学发光与荧光结合的多重检测

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化学+荧光+蛋白均一化的多重检测



化学发光多重检测工作流程



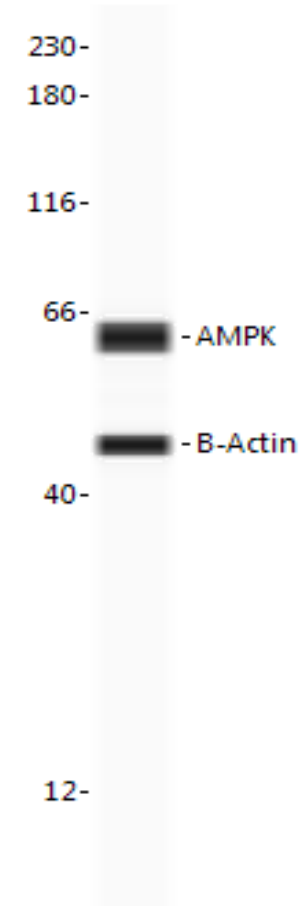
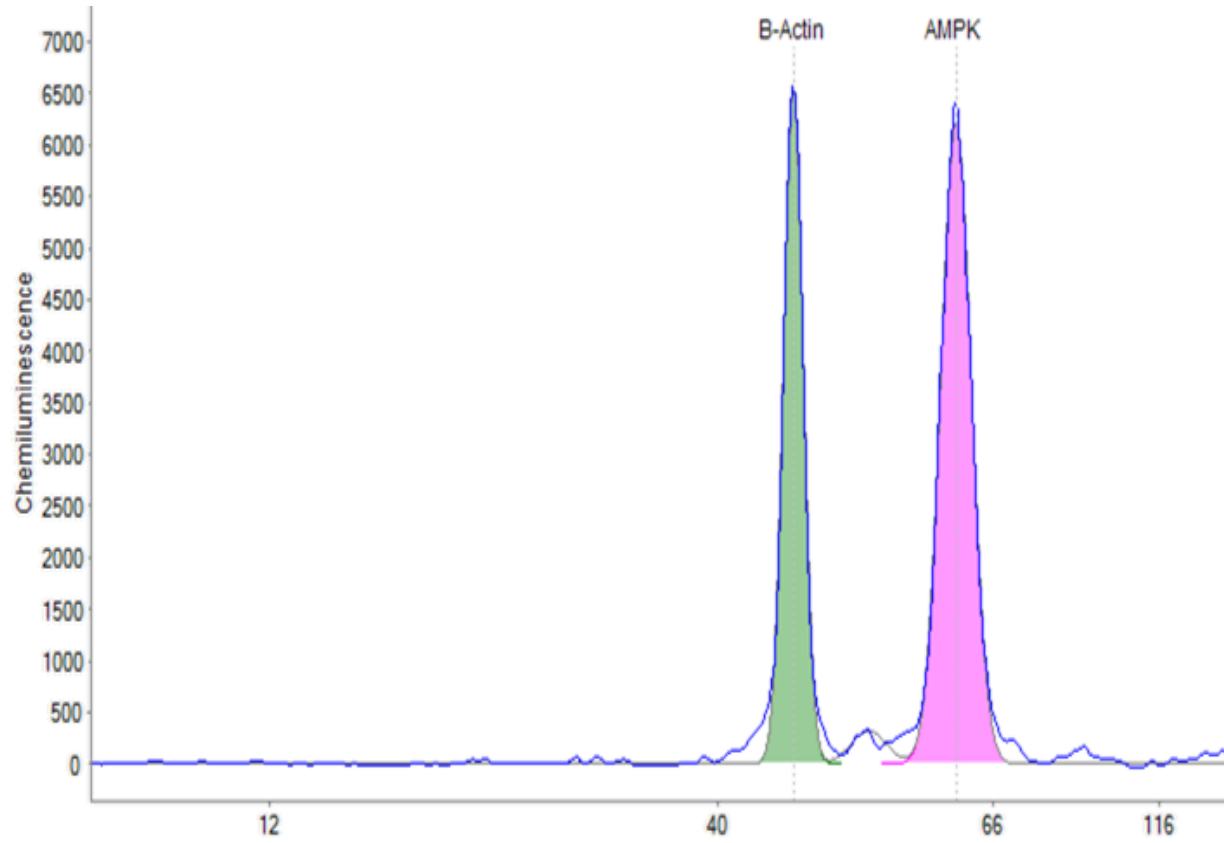
更换内参，使其可以完全分开

kDa	Whole Cell / Cytoplasmic	Nuclear	Mitochondrial	Cytoskeletal	Plasma Membrane	Serum & Extra- cellular Fluids
135					Cadherin	
124	Vinculin			Vinculin		
116		PARP				
100				α-Actinin	NaK-ATPase	
90	HSP 90					
89		PARP				
85					CD71	
77						Transferrin
74		Lamin A/C				
70	HSP 70					
68		Lamin B1				
63		Lamin A/C				
62		HDAC 1				
60	HSP 60		HSP 60			
55				β-Tubulin		
52				α-Tubulin		
45	MEK1/2	Lamin B1		β-Actin		
38		TBP				
37	GAPDH					
36		PCNA				
34			VDAC/Porin			
24					Caveolin-1	
21					Caveolin-1	
19				Cofilin		
17		Histone H3	COX IV			
9				Profilin 1		
5				Profilin 1		

分子量相差>10%

From: 《CST Guide: Pathways & Protocols》

对于丰度高的蛋白，可选择 HRP 直接标记一抗



对于不同宿主来源一抗，二抗饱和也很重要

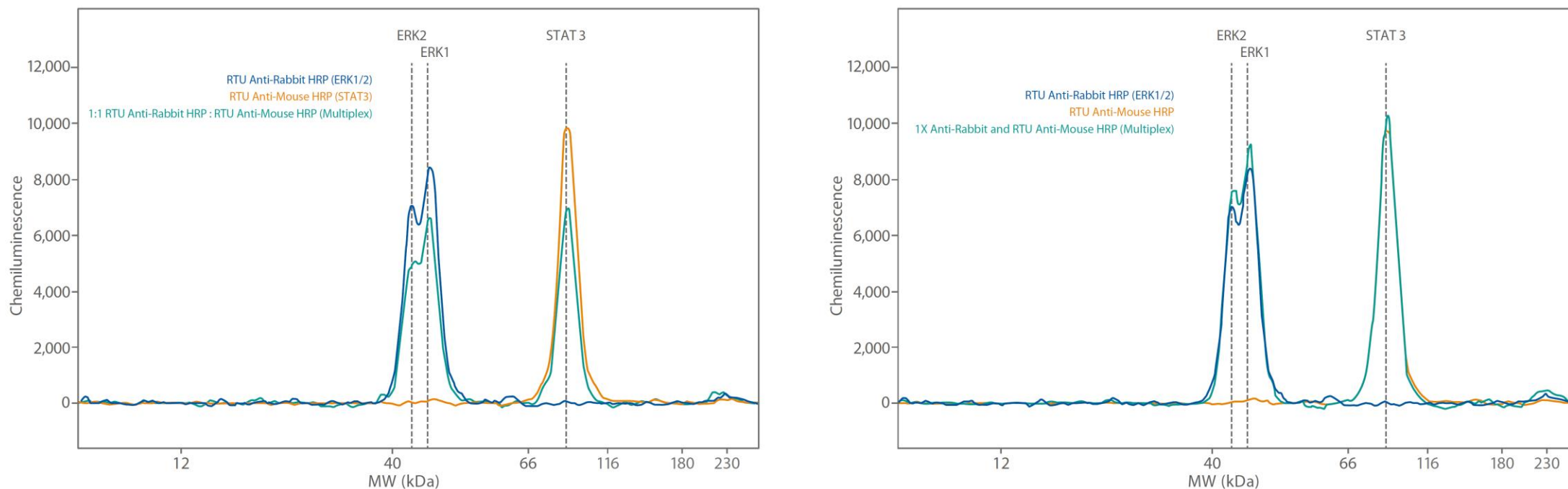


图 2. 多重检测的一抗来源于不同种属。(左)，将 RTU anti-mouse 和 RTU anti-rabbit 的二抗 1:1 混合，同时检测 ERK1 (rabbit anti-ERK1 RTU, PN 042-206) 和 STAT3 (mouse anti-Stat3, CST, 9139, 1:50)。(右)，将 20X anti-rabbit 二抗混入 RTU anti-mouse 二抗，不影响二抗的饱和度，和单独进行实验相比，实验结果一致。

抗兔和抗小鼠二抗 1:1 混合时，信号有明显下降，而使用 20X 抗兔二抗和 1X 抗小鼠二抗混合时，信号无明显下降。

二抗选择不当也会产生交叉反应

Product Name	Product Part Number (vial)	Minimal cross-reactivity against:	Host Species	Specificity
Anti-Rabbit HRP / 20X Anti-Rabbit HRP	042-206 / 043-426	None	Goat	Heavy and Light chains
Anti-Mouse HRP	042-205	None	Goat	Heavy and Light chains
Anti-Goat HRP	043-522	Chicken, Guinea Pig, Syrian Hamster, Horse, Human, Mouse, Rabbit, Rat	Donkey	Heavy and Light chains
Anti-Human IgG HRP	043-491	Human IgA, Human IgM, Bovine, Horse, Mouse, Rabbit	Goat	Fc region



注意事项	要求
分子量	>10%
抗体特异性	背景干净，在MW附近无杂带
线性范围	有交叉，可用同一上样浓度
一抗种属	尽量一致
二抗种属	尽量一致

推荐二抗	货号
Anti-Mouse Secondary HRP Antibody	PS 042-205
Anti-Rabbit Secondary HRP Antibody	PS 042-206
Anti-Human IgG Secondary HRP Antibody	PS 043-491-2
Anti-Goat Secondary HRP Antibody	PS 043-522-2
Anti-Rat Secondary HRP antibody	Abcam Ab6734

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荧光的多重检测

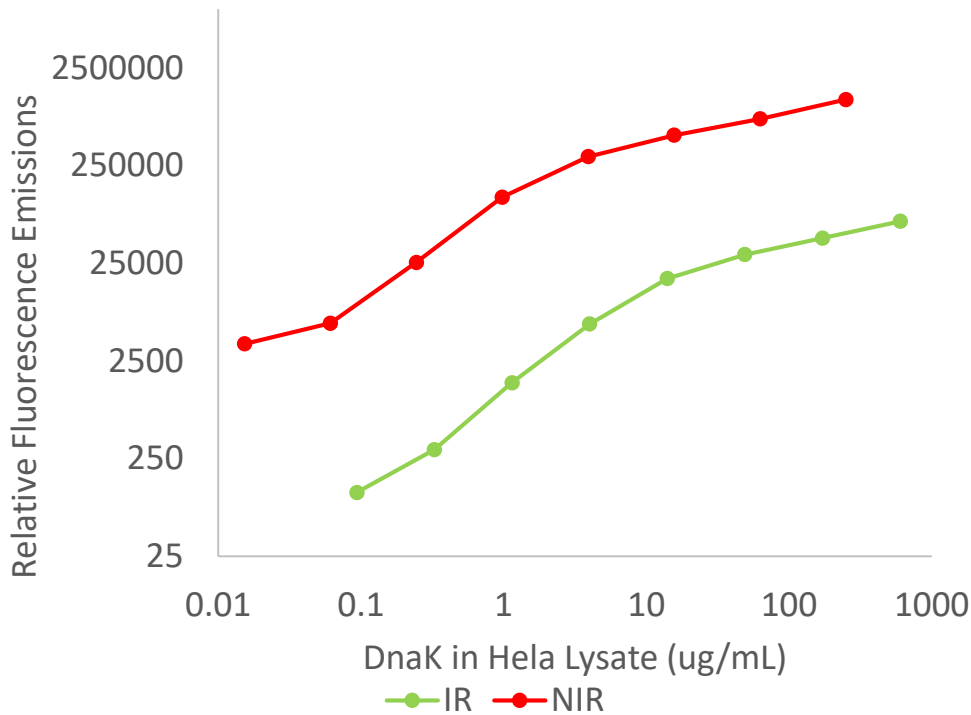
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化学发光与荧光结合的多重检测

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化学+荧光+蛋白均一化的多重检测

双色荧光的多重检测



IR 通常比 NIR 信号弱

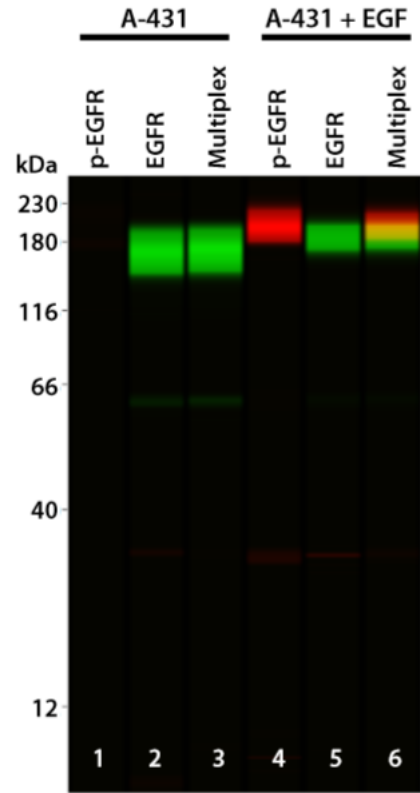


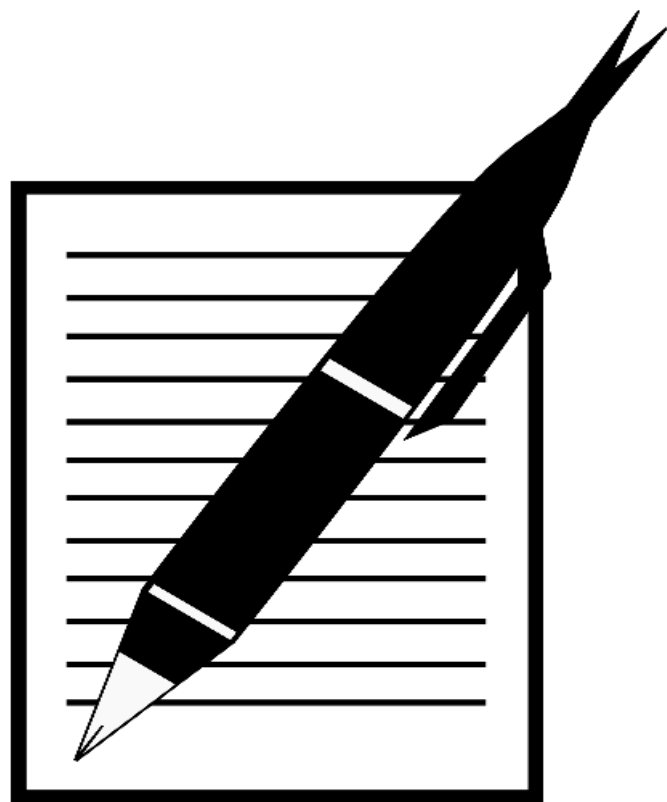
FIGURE 4. Lane view in Compass for Simple Western software showing p-EGFR and EGFR expression levels in unstimulated versus EGF-induced A-431 cells. Lane 6 shows the overlapping signal achievable with multiplexing on Jess.



Peaks															Capillaries			
Sample	Primary	Secondary	Cap	Peak	Name	Position	MW (kDa)	Height	Area	% Area	Corr. Area	Width	S/N	Baseline	Channel			
A431+ EGFR	EGFR	Anti-Rabbit IR	18	1	p-EGFR	523	183	37.4	79	100.0	76.7	2.0	3.6	149.8	NIR			
A431 Untrea...	p-EGFR	Anti-Mouse NIR	20	1	p-EGFR	521	182	105.1	1025	100.0	822.4	9.2	7.1	211.8	NIR			
A431 Untrea...	Multiplex	Multiplex	22	1	p-EGFR	519	184	79.8	868	100.0	710.9	10.2	5.0	204.1	NIR			
A431+ EGFR	EGFR	Anti-Rabbit IR	6	1	EGFR	517	182	170.3	3407	100.0	3156.6	18.8	94.1	9.5	IR			
A431+ EGFR	Multiplex	Multiplex	7	1	EGFR	518	183	192.6	3784	100.0	3613.4	18.5	131.8	10.9	IR			
A431 Untrea...	EGFR	Anti-Rabbit IR	9	1	EGFR	512	171	281.6	6759	100.0	5599.8	22.5	142.7	20.0	IR			

FIGURE 5. Peaks Table in Compass for Simple Western software showing quantitative target protein expression data for each capillary.

双色荧光的多重检测总结



注意事项	要求
抗体特异性	背景干净
线性范围	有交叉，可用同一上样浓度
荧光通道	低丰度蛋白用NIR Channel 高丰度蛋白用IR Channel
一抗种属	尽量不同
二抗种属	尽量一致

推荐二抗	货号
Anti-Rabbit Secondary NIR Antibody	043-819
Anti-Rabbit Secondary IR Antibody	043-820
Anti-Mouse Secondary NIR Antibody	043-821
Anti-Mouse Secondary IR Antibody	043-822

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荧光的多重检测

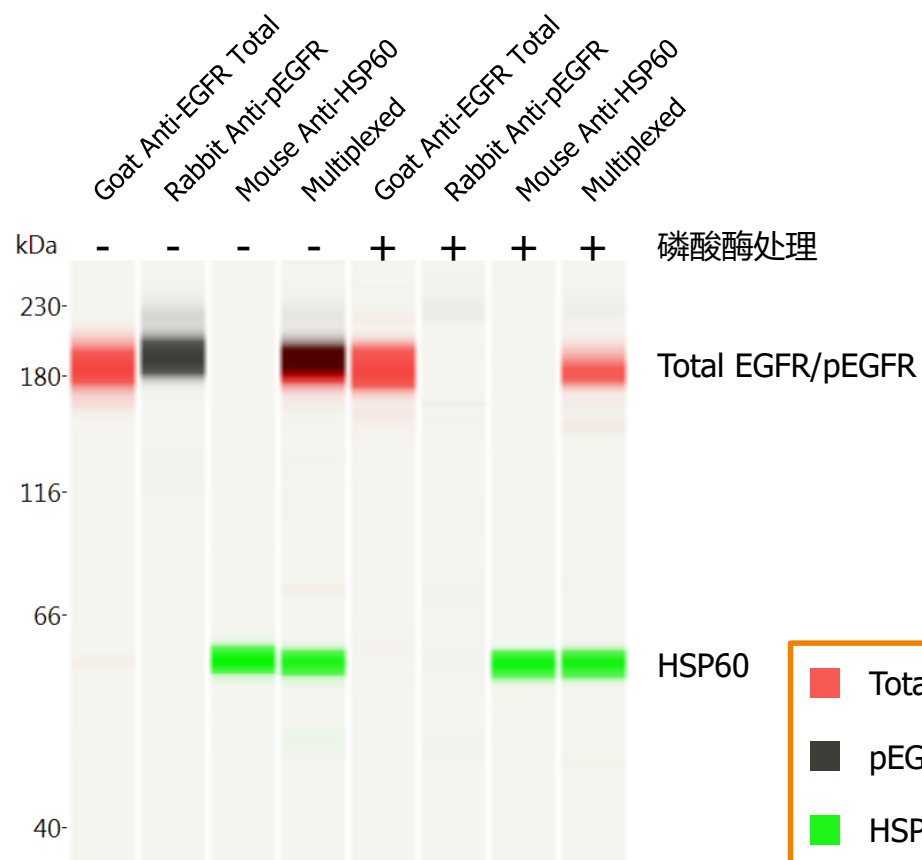
3

化学发光与荧光结合的多重检测

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化学+荧光+蛋白均一化的多重检测

荧光和化学发光相结合的多重检测



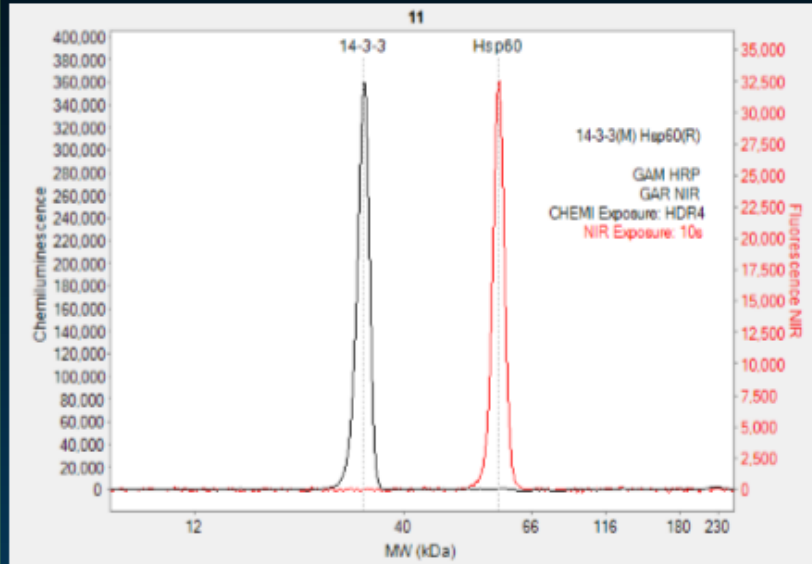
- Total EGFR (R&D AF231, Goat)
 - pEGFR (PA5-17848, Rabbit)
 - HSP60 (R&D AF1800-SP, Mouse)
- Anti-Goat HRP + Anti-Rabbit
NIR + Anti-Mouse IR (RTU;
1:20; 1:20)



MULTIPLEX 1: MOUSE-HRP & RABBIT-NIR

All goat combination

Highest signal in both channels
No Cross-reactivity observed

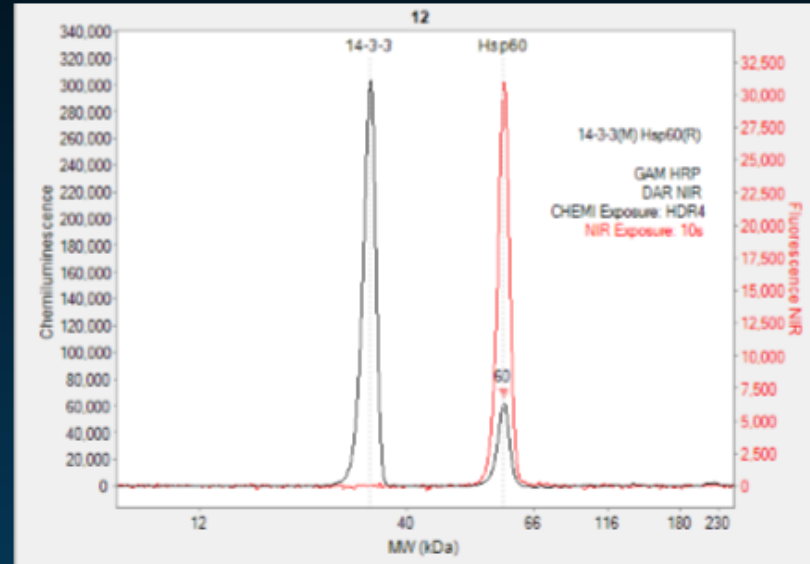


Conjugate	Antibody	Dilution
HRP	Goat anti-Mouse <i>ProteinSimple</i> (042-205)	RTU
NIR	Goat-anti-Rabbit 650 <i>Novus</i> (NBP1-72732C)	1:500

Note: NIR antibody was diluted directly in the RTU HRP antibody

ProteinSimple combination

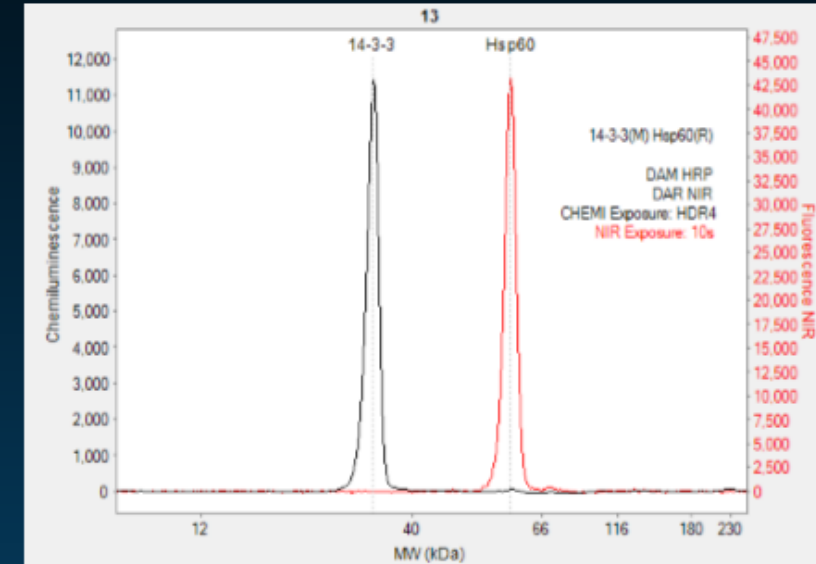
High Signal
Cross-reactivity observed



Conjugate	Antibody	Dilution
HRP	Goat anti-Mouse <i>ProteinSimple</i> (042-205)	RTU
NIR	Donkey anti-Rabbit <i>ProteinSimple</i> (043-819)	1:20

All donkey combination

Resulted in low signal
No Cross-reactivity observed



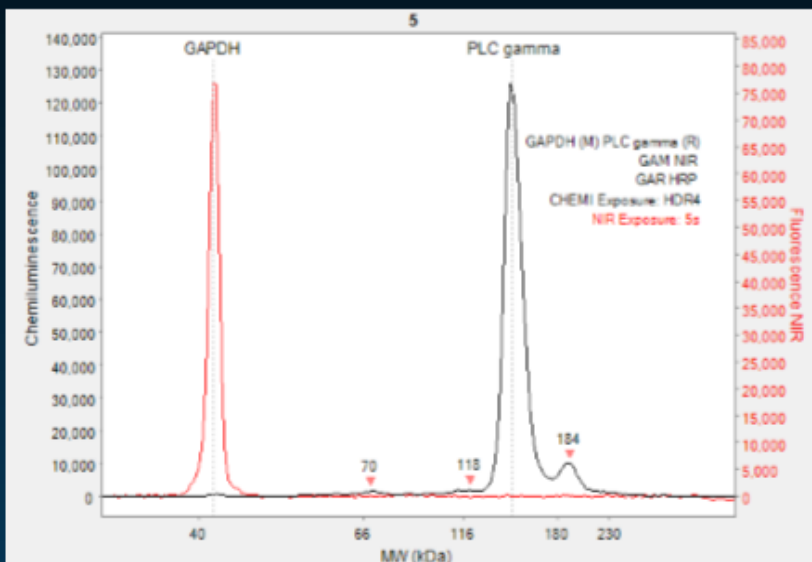
Conjugate	Antibody	Dilution
HRP	Donkey anti-Mouse <i>R&D Systems</i> (HAF018)	1:100
NIR	Donkey anti-Rabbit <i>ProteinSimple</i> (043-819)	1:20

Sample (Hela Lysate-0.1 mg/mL); Targets (14-3-3, HSP60); Replicates (n=8)

MULTIPLEX 2: RABBIT-HRP & MOUSE-NIR

All goat combination

Highest signal in both channels
No Cross-reactivity observed

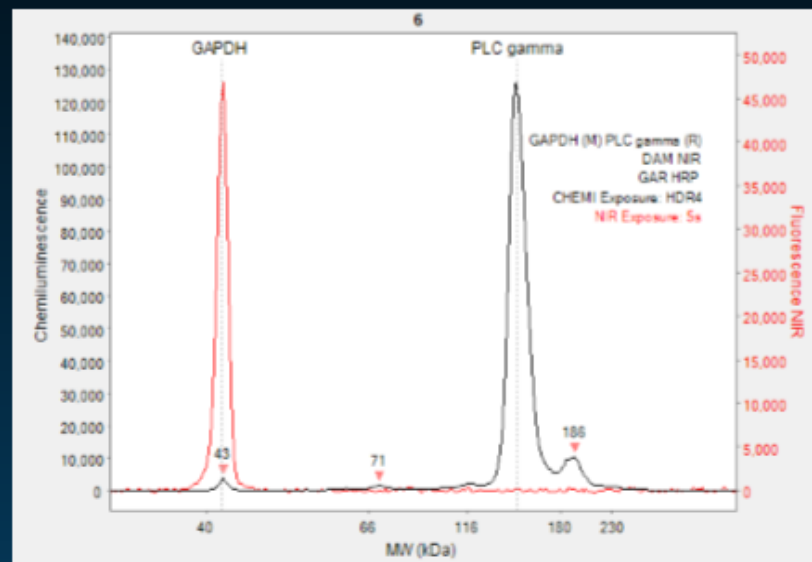


Conjugate	Antibody	Dilution
HRP	Goat anti-Rabbit <i>ProteinSimple</i> (042-206)	RTU
NIR	Goat anti-Mouse 650 <i>Novus</i> (NBP1-75147C)	1:50

Note: NIR antibody was diluted directly in the RTU HRP antibody

ProteinSimple combination

High Signal
Cross-reactivity observed

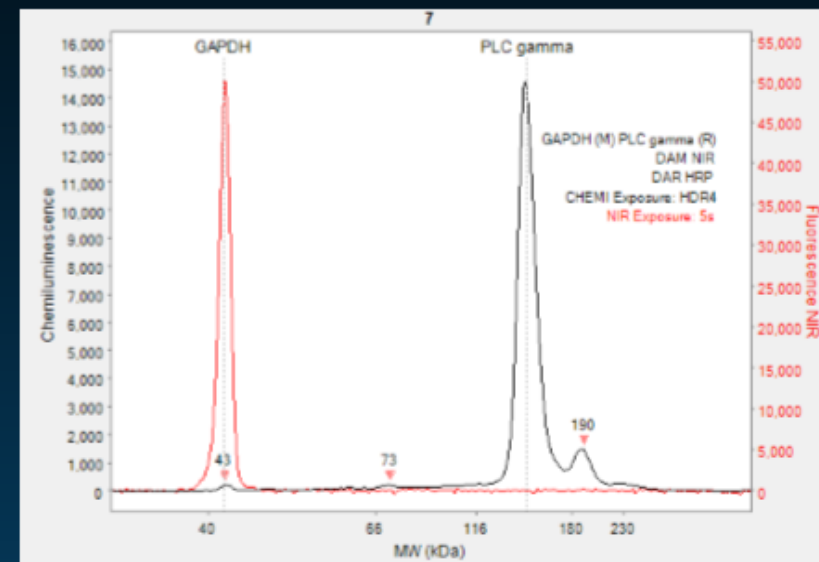


Conjugate	Antibody	Dilution
HRP	Goat anti-Rabbit <i>ProteinSimple</i> (042-206)	RTU
NIR	Donkey anti-Mouse <i>ProteinSimple</i> (043-821)	1:20

Note: cross-reactivity was not detected for lower signal NIR targets

All donkey combination

Low signal
slight cross-reactivity



Conjugate	Antibody	Dilution
HRP	Donkey anti-Rabbit <i>Novus</i> (NB7185)	1:50
NIR	Donkey anti-Mouse <i>ProteinSimple</i> (043-821)	1:20

Sample (Hela Lysate-0.1 mg/mL); Targets (GAPDH, PLC gamma); Replicates (n=8)

荧光和化学发光相结合的多重检测总结



Emission Wavelength

NIR = ~700 nm

IR = ~800 nm

注意事项	要求
抗体特异性	背景干净
线性范围	有交叉，可用同一上样浓度
灵敏度	CHEMI>NIR>IR Channel
通道选择	低丰度尽量用化学发光
一抗种属	尽量不同
二抗种属	尽量一致

推荐二抗	货号
Anti-Mouse Secondary HRP Antibody+ Anti-Rabbit 650 Antibody	PS 042-205 NBP1-72732C
Anti-Rabbit Secondary HRP Antibody + Anti-Mouse 650 Antibody	PS 042-206 NBP1-75147C
Anti-Goat HRP + Anti-Rabbit NIR + Anti-Mouse IR	PS 043-522-2 043-819 043-822

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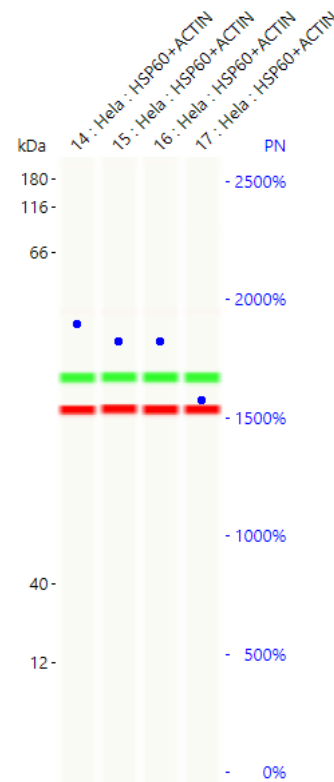
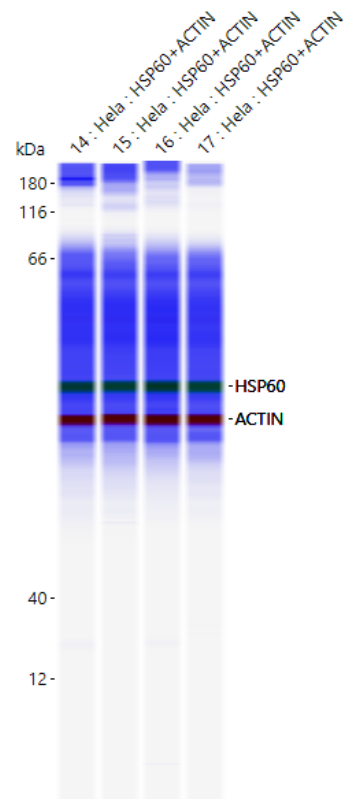
3

化学发光与荧光结合的多重检测

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化学+荧光+蛋白均一化的多重检测

荧光+化学发光+总蛋白均一化的多重检测



Peaks		Capillaries					
Sample	Prima...	Second...	Capillary	PN Corr. Area	PN (%)	HSP60	ACTIN
Hela	HSP6...	IR+NIR	14	101979.4	1896.5	3865.6	243233.7
Hela	HSP6...	IR+NIR	15	97765.7	1818.1	3858.4	257460.3
Hela	HSP6...	IR+NIR	16	97916.9	1820.9	4222.4	277877.8
Hela	HSP6...	IR+NIR	17	84675.4	1574.7	4118.9	251866.2



荧光/化学+蛋白均一化的多重检测总结



注意事项	要求
分子量大小	12-230kD
抗体特异性	背景干净
线性范围	0.2-1.2 mg/ml
灵敏度	CHEMI>NIR>IR Channel
通道选择	低丰度尽量用化学发光
一抗种属	尽量不同
二抗种属	尽量一致

Q&A

