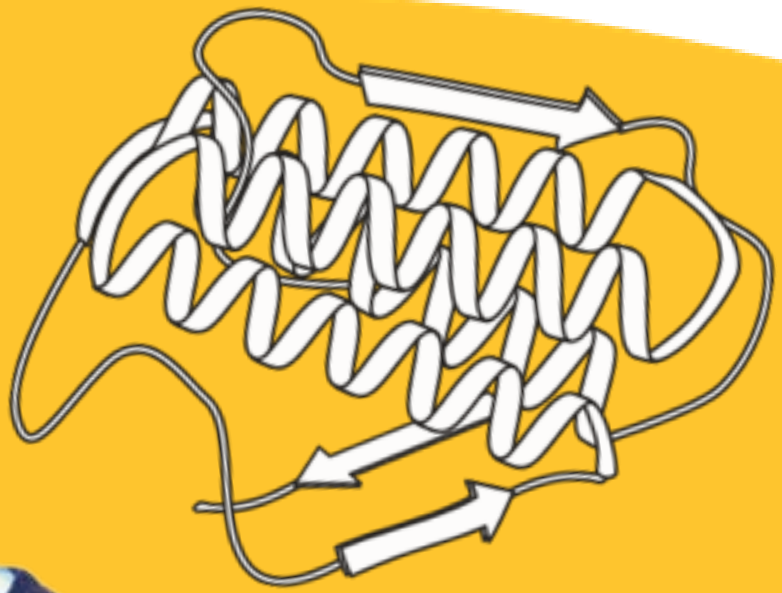
Wes 技术原理介绍



Wes

- 节省样品
  - 仅需 5 μL/孔样品
  - 0.2-1 μg/μL 样品
- 3 小时内获得结果
  - 最多可检测 75 个样品/天
- 加样流程简单
  - 预分装板
  - 一次性毛细管卡盒（cartridge）

工作原理

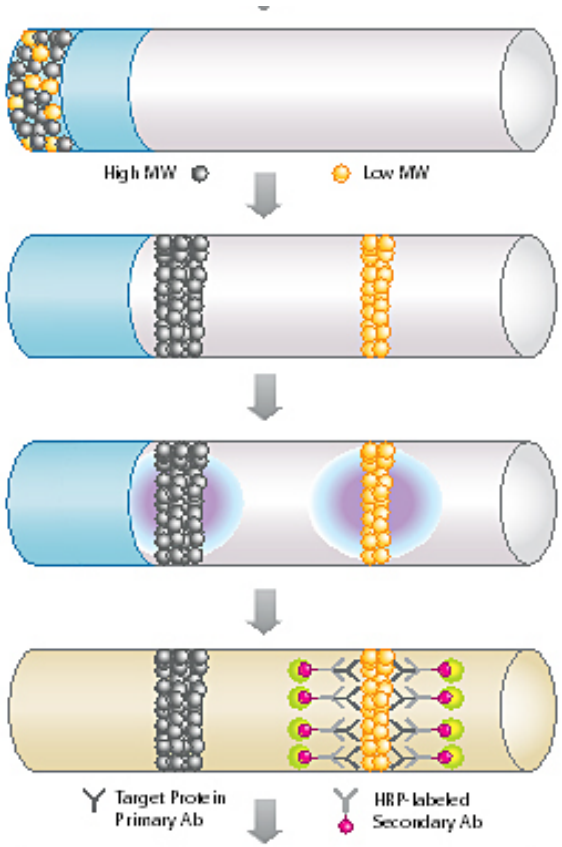
蛋白上样

蛋白分离

蛋白固定

免疫杂交

数据采集

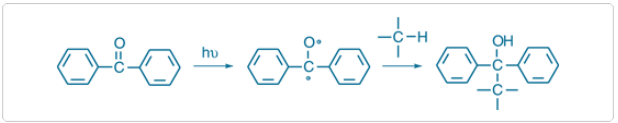


所有在毛细管内的步骤都自动完成!

与传统 Western *不同*:

无需制胶
（液态胶）

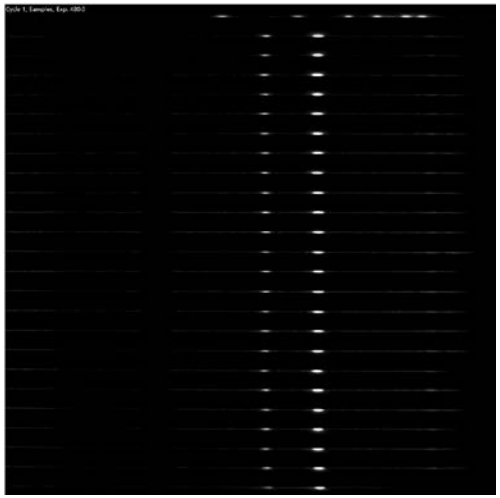
无需转膜（紫外激活交联）



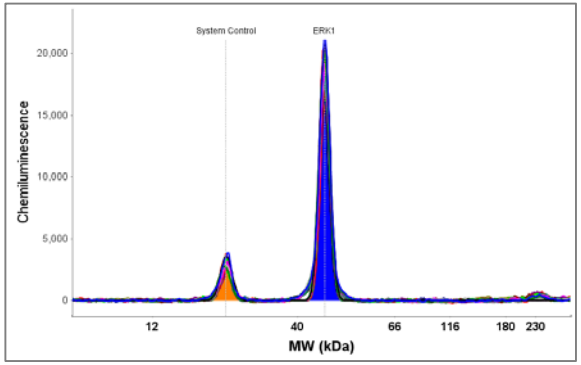
无需人工孵育/清洗/压片

无需人工数据分析

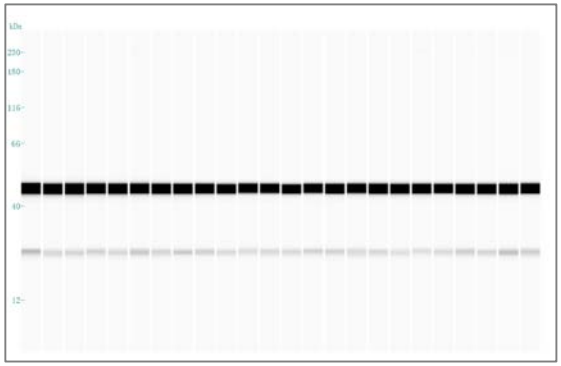
结果呈现方式



成像图



峰形图



泳道式

操作流程

传统 Western

样品制备

↓

制胶、上样和电泳：2 – 3h

↓

转膜：0.5 – 2h

↓

封闭：1 – 2h

↓

一抗孵育：1h

↓

清洗 (3x 5-10 min)

↓

二抗孵育：1h

↓

清洗 (3x 5-10 min)

↓

加入化学发光底物

↓

曝光：1-30 min

↓

人工数据分析

24 小时

Simple Western

样品制备

↓

把样品、封闭液、一抗、二抗、发光液、洗液加入预分装的板内

↓

将板子和 cartridge 放入 Wes

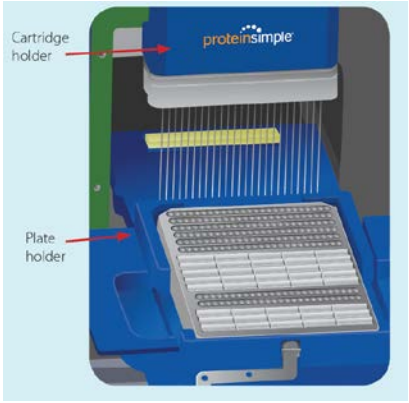
↓

点击 start 运行程序

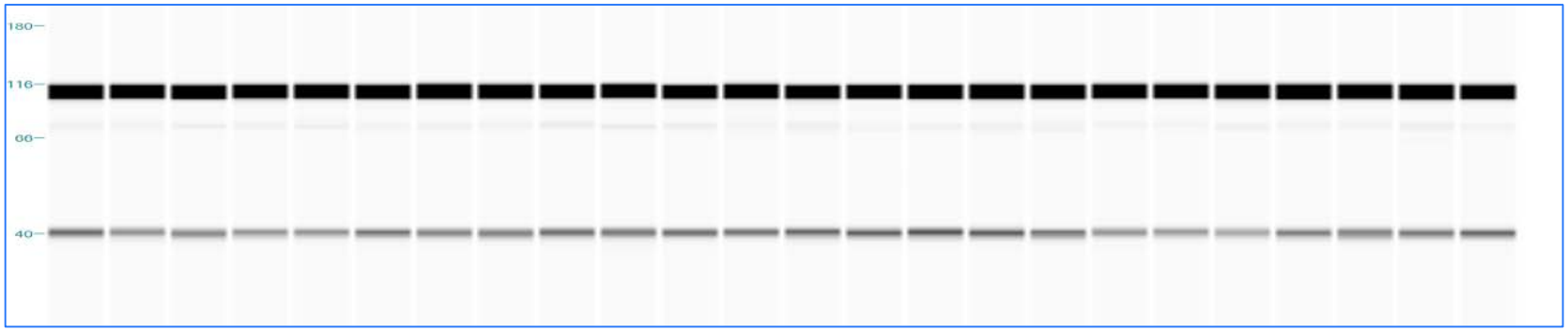
↓

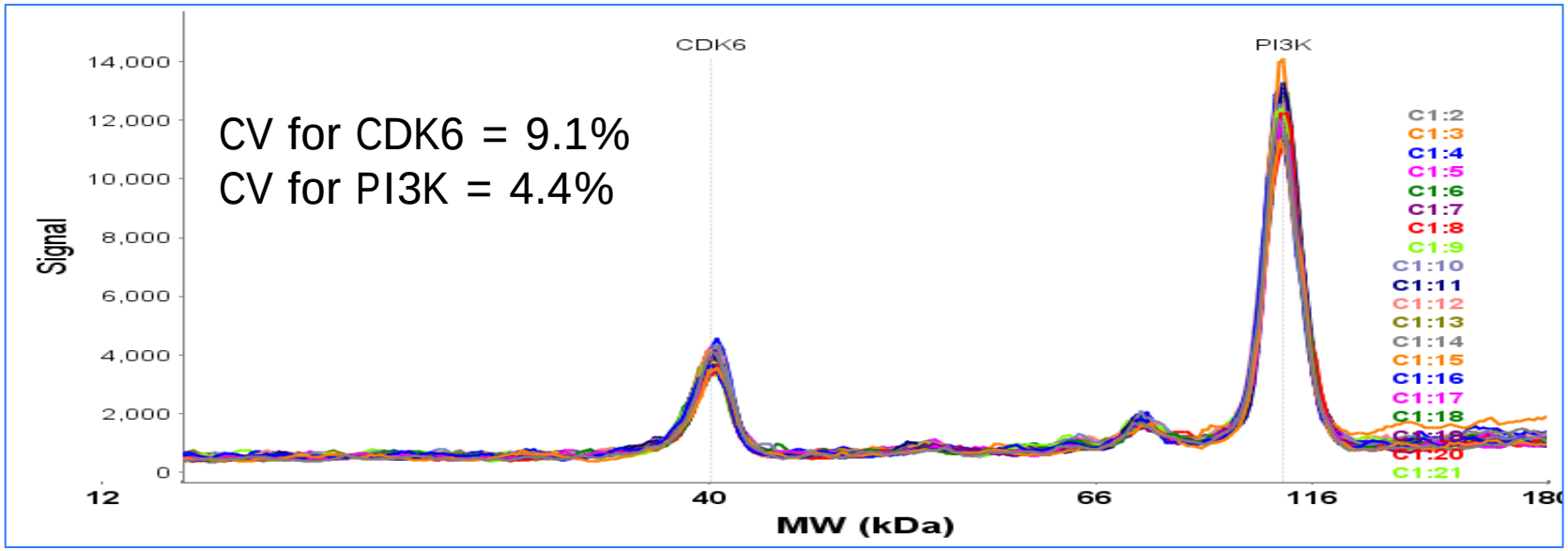
得到数据

3 小时

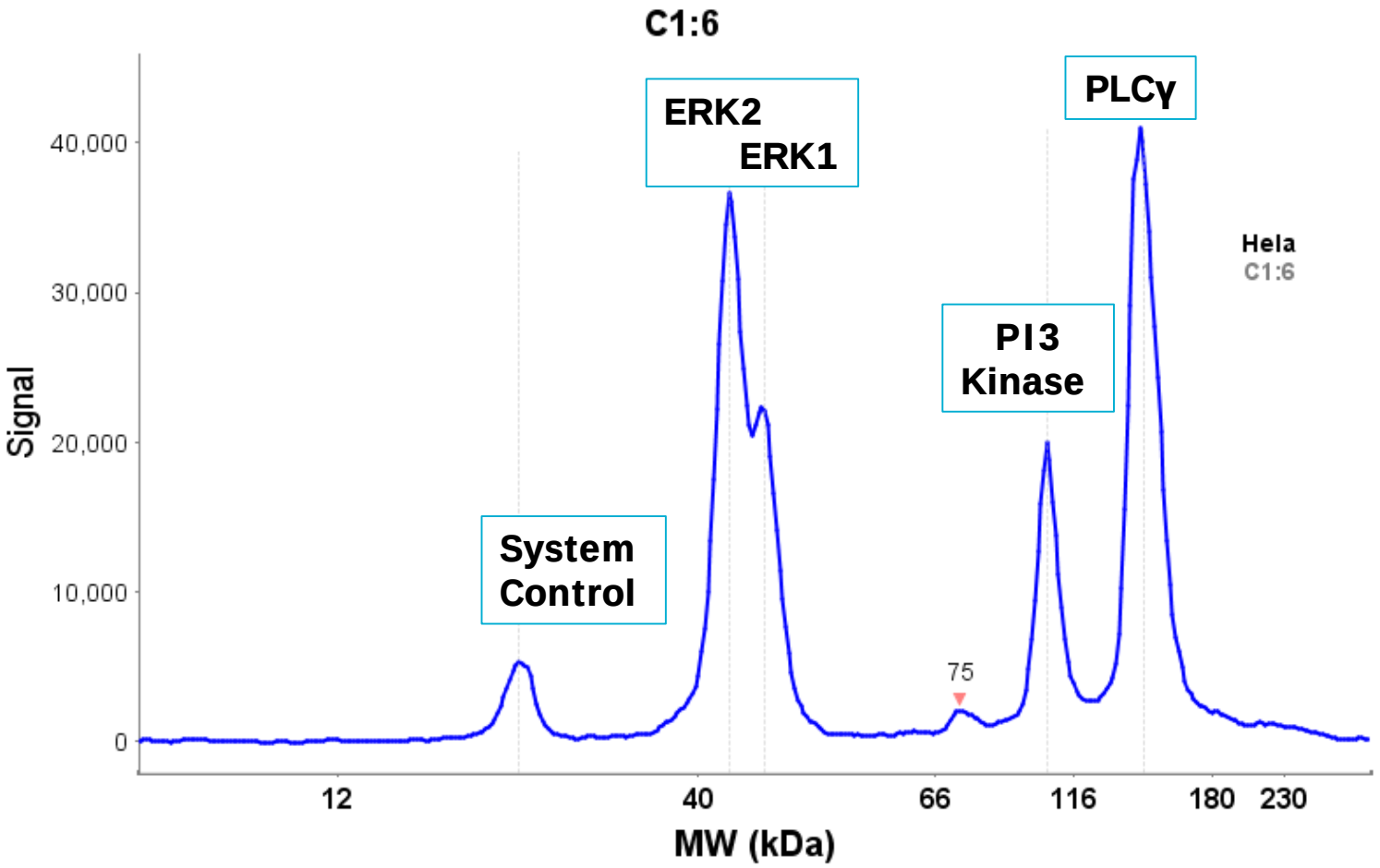


数据重复性好!

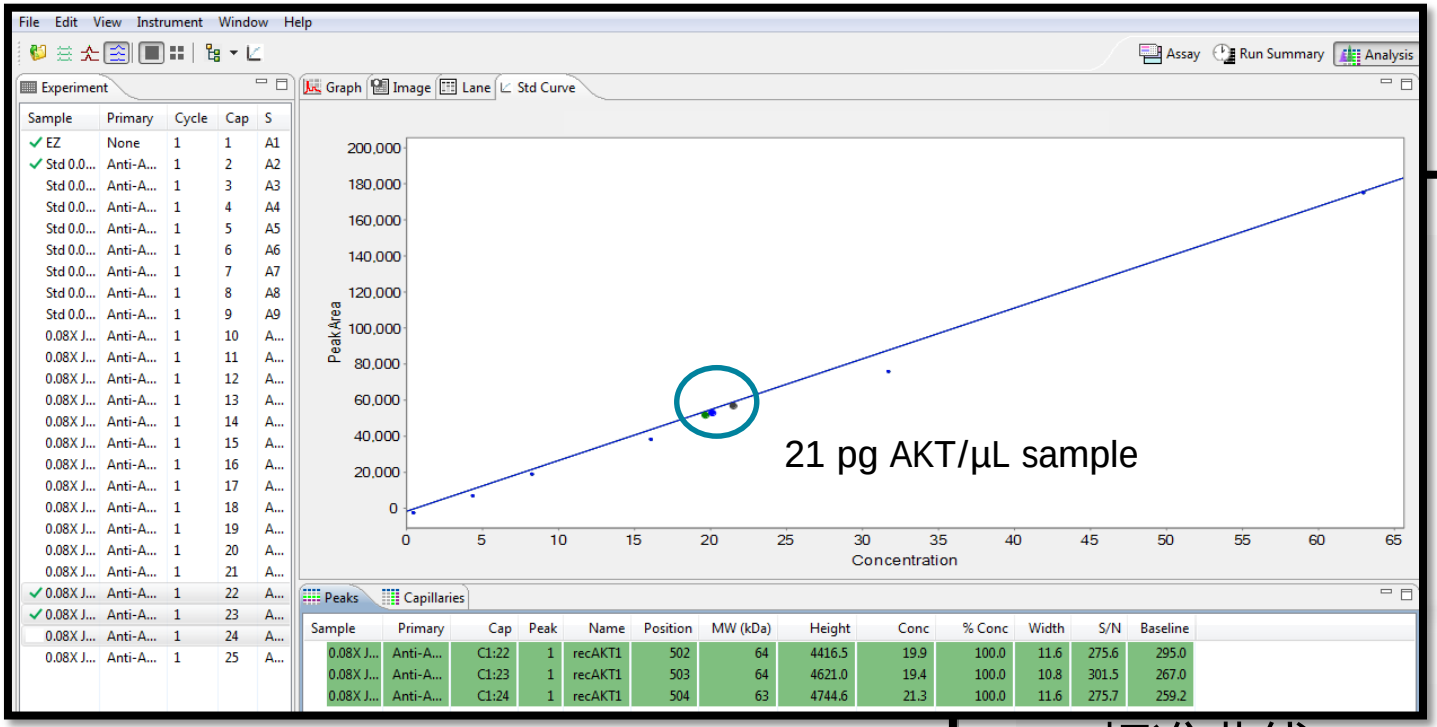

180
116
66
40


CV for CDK6 = 9.1%
CV for PI3K = 4.4%
CDK6
PI3K
Signal
14,000
12,000
10,000
8,000
6,000
4,000
2,000
0
12
40
66
116
MW (kDa)
C1:2
C1:3
C1:4
C1:5
C1:6
C1:7
C1:8
C1:9
C1:10
C1:11
C1:12
C1:13
C1:14
C1:15
C1:16
C1:17
C1:18
C1:20
C1:21

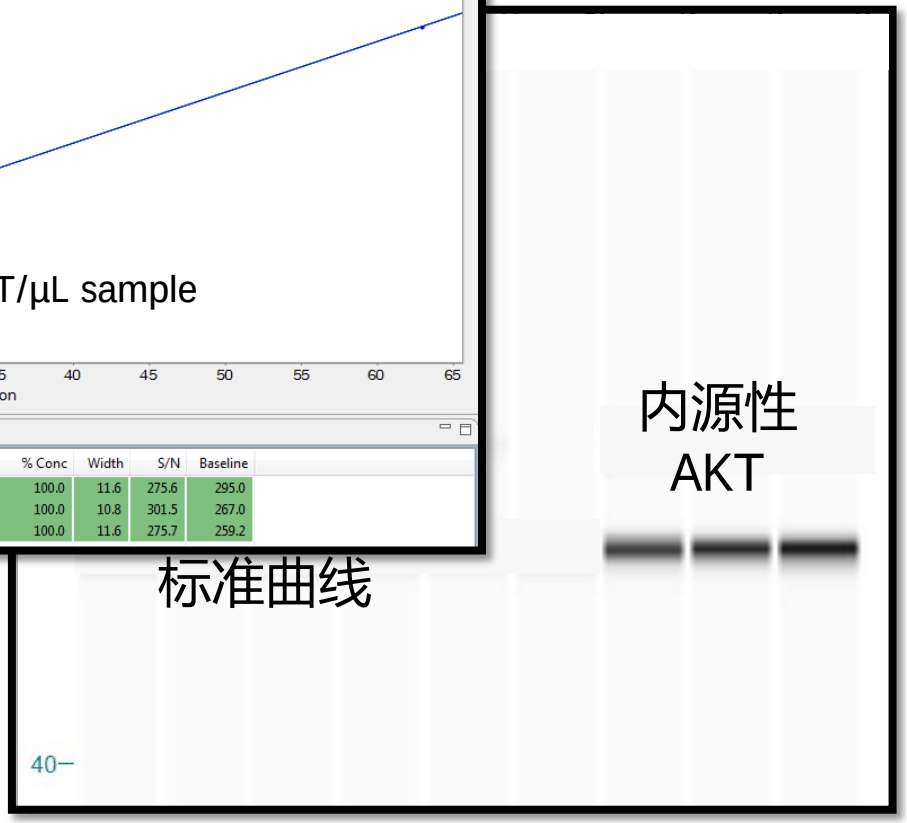
多重检测


C1:6
ERK2
ERK1
PLCγ
PI3
Kinase
System
Control
Hela
C1:6
75
Signal
40,000
30,000
20,000
10,000
0
12
40
66
116
180
230
MW (kDa)

真实定量


File Edit View Instrument Window Help
Assay
Run Summary
Analysis
Experiment
Graph
Image
Lane
Std Curve
Sample Primary Cycle Cap S
EZ None 1 1 A1
Std 0.0... Anti-A... 1 2 A2
Std 0.0... Anti-A... 1 3 A3
Std 0.0... Anti-A... 1 4 A4
Std 0.0... Anti-A... 1 5 A5
Std 0.0... Anti-A... 1 6 A6
Std 0.0... Anti-A... 1 7 A7
Std 0.0... Anti-A... 1 8 A8
Std 0.0... Anti-A... 1 9 A9
0.08X J... Anti-A... 1 10 A...
0.08X J... Anti-A... 1 11 A...
0.08X J... Anti-A... 1 12 A...
0.08X J... Anti-A... 1 13 A...
0.08X J... Anti-A... 1 14 A...
0.08X J... Anti-A... 1 15 A...
0.08X J... Anti-A... 1 16 A...
0.08X J... Anti-A... 1 17 A...
0.08X J... Anti-A... 1 18 A...
0.08X J... Anti-A... 1 19 A...
0.08X J... Anti-A... 1 20 A...
0.08X J... Anti-A... 1 21 A...
0.08X J... Anti-A... 1 22 A...
0.08X J... Anti-A... 1 23 A...
0.08X J... Anti-A... 1 24 A...
0.08X J... Anti-A... 1 25 A...
Peak Area
200,000
180,000
160,000
140,000
120,000
100,000
80,000
60,000
40,000
20,000
0
0 5 10 15 20 25 30 35 40 45 50 55 60 65
Concentration
21 pg AKT/µL sample
Peaks
Capillaries
Sample Primary Cap Peak Name Position MW (kDa) Height Conc % Conc Width S/N Baseline
0.08X J... Anti-A... C1:22 1 recAKT1 502 64 4416.5 19.9 100.0 11.6 275.6 295.0
0.08X J... Anti-A... C1:23 1 recAKT1 503 64 4621.0 19.4 100.0 10.8 301.5 267.0
0.08X J... Anti-A... C1:24 1 recAKT1 504 63 4744.6 21.3 100.0 11.6 275.7 259.2

标准曲线


内源性
AKT
40—

在 Wes 上验证过的抗体

http://www.proteinsimple.com/antibody/antibodies.html

Simple Western Antibody Database

The Simple Western Antibody Database is a user-interactive listing of antibodies that have been screened and tested in Simple Western Charge- and Size-based assays. This database is intended to provide assay general development guidance in identifying and selecting antibodies to test.

To bring you even more Simple Western-certified antibodies, we've partnered with our sister companies R&D Systems and Novus Biologicals so you don't have to do the work. You can find their antibodies listed below, or for more details visit their websites and search by Application (type "Simple Western"). We've given these antibodies the seal of approval they deserve after being certified in-house by our experienced scientists.

The performance of any antibody selected will need to be optimized for the test system being examined. We encourage users to contribute to the database by clicking the Submit New Antibody button and sharing specific information about the antibodies that have been tested. If you need help with or have questions about any of the information in this antibody database, please contact support@proteinsimple.com.

The database can be sorted by clicking on the column headings. In addition, keywords can be searched and identified from the database using the Search box.

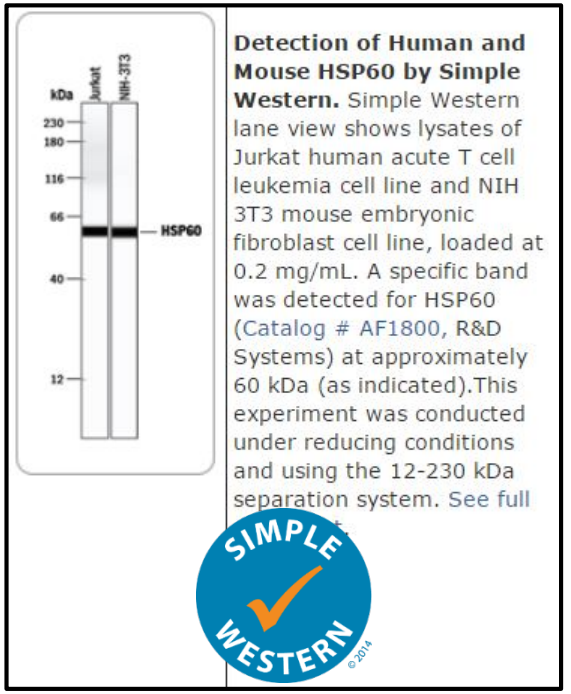
Antibodies (1662) Search: Go Reset Search Show All Submit New Antibody...

Page: << 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 26 27 28 29 30 31 32 33 34 35 36 37 38 39
40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59
60 61 62 63 64 65 66 67 > >>

Protein Target ▲	Antibody Type	Antibody Vendor	Product Number	Protein Isoform	Antibody Species	Cell Model(s)	Separated by Size or Charge	Antibody Dilution	Apparent MW in kDa on Simple Western	Matrix
AGXT	Primary	Novus Biologicals	NBP1-89200	AGXT	Rabbit Polyclonal	Liver (left), HepG2 (right)	Size-Wes, Sally Sue/Peggy Sue	1:20	47	12-230kD
ARG1	Primary	Novus Biologicals	NBP2-14787	ARG1	Mouse Monoclonal	Liver	Size-Wes, Sally Sue/Peggy Sue	1:20	42	12-230kD
ARG1	Primary	Novus Biologicals	NBP1-87490	ARG1	Rabbit Polyclonal	Liver (left), HepG2 (right)	Size-Wes, Sally Sue/Peggy Sue	1:50	41	12-230kD

来自 Bio-Techne 集团兄弟品牌 Novus Biologicals 和 R&D Systems

- “验证过” 意味着:
  - 有推荐的抗体稀释度
  - 有推荐的裂解物浓度
  - 有图片和数据
  - 有推荐的实验条件
  - 100% 品质保证



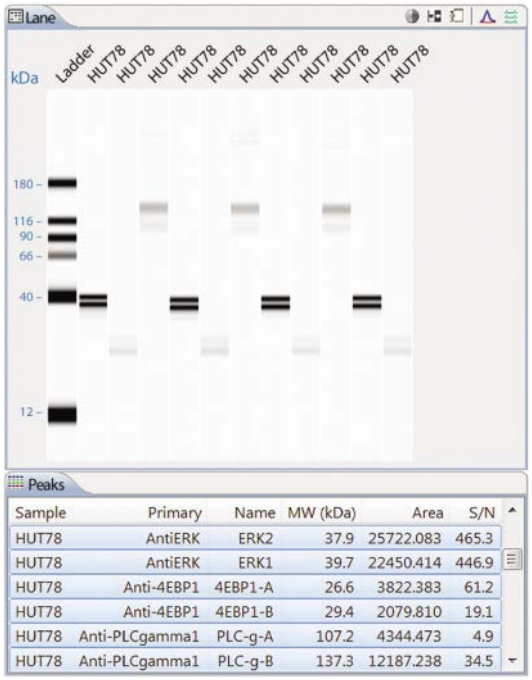
www.novusbio.com/proteinsimple
www.rndsystems.com/simplewestern

Wes 实验操作

Wes 试剂盒

- Mouse and Rabbit Master kits
  - 12-230kDa separation range
  - 66-440kDa separation range
- 25 and 13 capillary disposable cartridges

流程简单



Sample	Primary	Name	MW (kDa)	Area	S/N
HUT78	AntiERK	ERK2	37.9	25722.083	465.3
HUT78	AntiERK	ERK1	39.7	22450.414	446.9
HUT78	Anti-4EBP1	4EBP1-A	26.6	3822.383	61.2
HUT78	Anti-4EBP1	4EBP1-B	29.4	2079.810	19.1
HUT78	Anti-PLCgamma1	PLC-g-A	107.2	4344.473	4.9
HUT78	Anti-PLCgamma1	PLC-g-B	137.3	12187.238	34.5

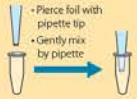
加样　　一键开始　　获得数据

操作说明书

1. Prepare your reagents

A PREPARE STANDARD PACK REAGENTS

DTT (Clear Tube)



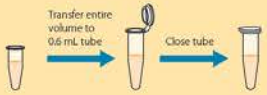
- Add 40 µL deionized water to make a 400 mM solution

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

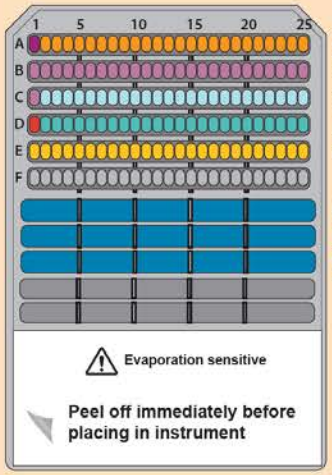
D PREPARE YOUR ANTIBODIES

- Primary Antibody: The ERK1 Primary Antibody is ready to use. 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 Antibody: The supplied antibody is ready to use without dilution.

E MIX LUMINOL-S AND PEROXIDE

- Combine 150 µL Luminol-S and 150 µL Peroxide in a microcentrifuge tube

2. Pipette your plate



Biotinylated Ladder, 5 µL; Prepared Samples, 5 µL
Wes Antibody Diluent II, 10 µL
Wes Antibody Diluent II, 10 µL; Primary Antibody, 10 µL
Streptavidin-HRP, 10 µL; Secondary Antibody, 10 µL
Luminol-Peroxide Mix, 10 µL

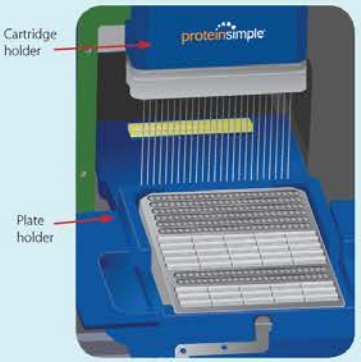
Wash Buffer
500 µL/compartment
2.5 mL/row

- 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.

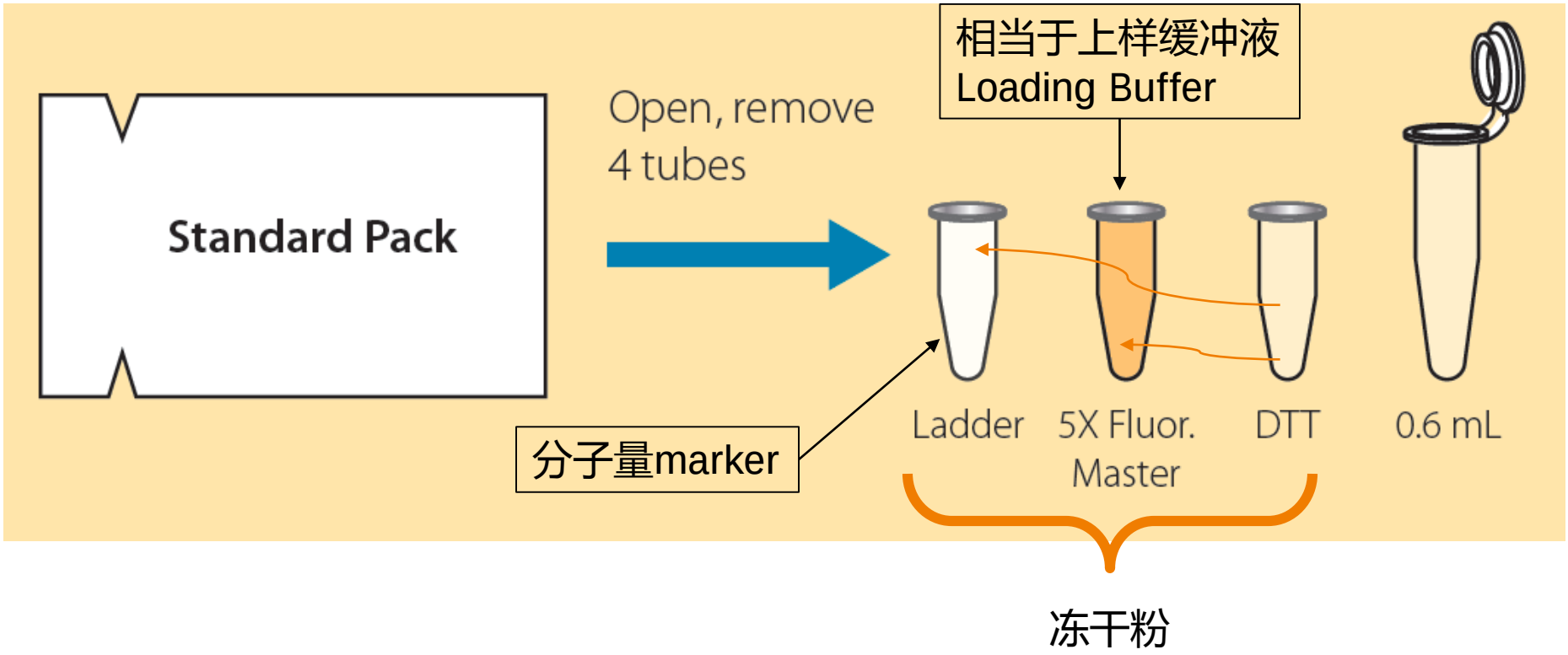
1. Dispense reagents into the assay plate using the volumes shown in the plate diagram.
2. Centrifuge the plate for 5 minutes at 2500 rpm (~1000 x *g*) at room temperature. Ensure liquid is fully down in all wells.

3. Start Wes

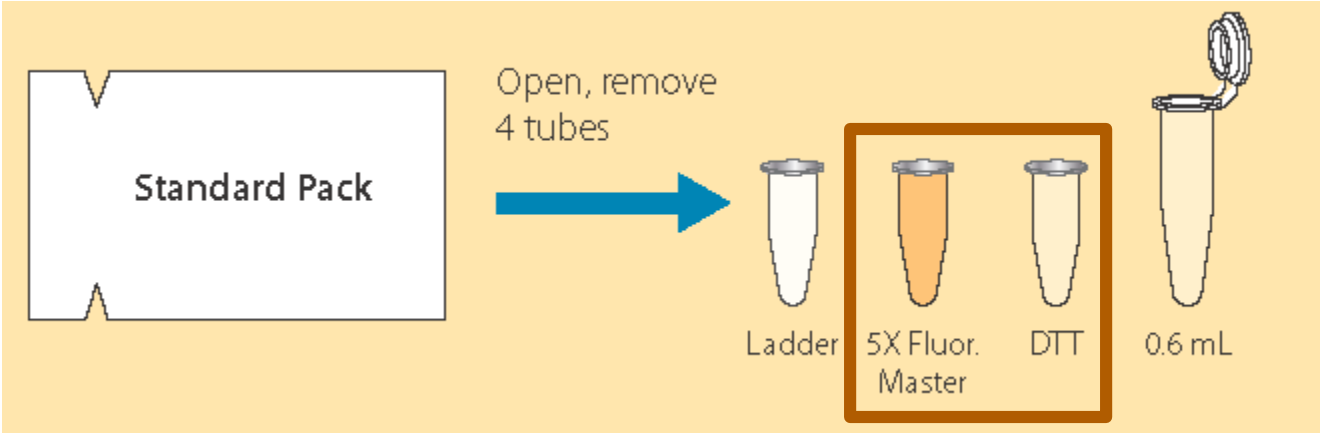
1. Load the desired assay in Compass software.
2. Open Wes' door.
3. Insert a capillary cartridge into the cartridge holder. The interior light will change from orange to blue.
4. Remove the assay plate lid. Hold plate firmly on bench and carefully peel off evaporation seal. Pop any bubbles observed 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.

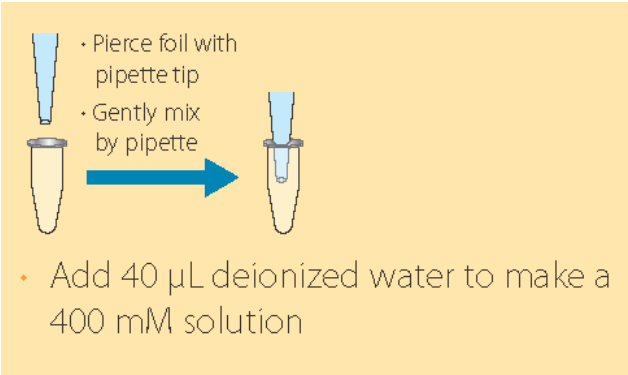


Biotin Ladder, 荧光内参和DTT

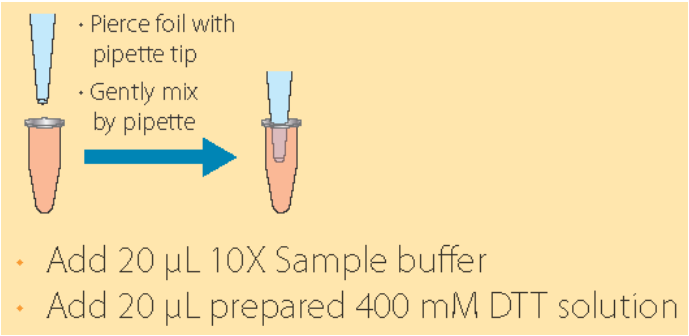


Sample Prep – Step 1:
配制 5X FL Master Mix





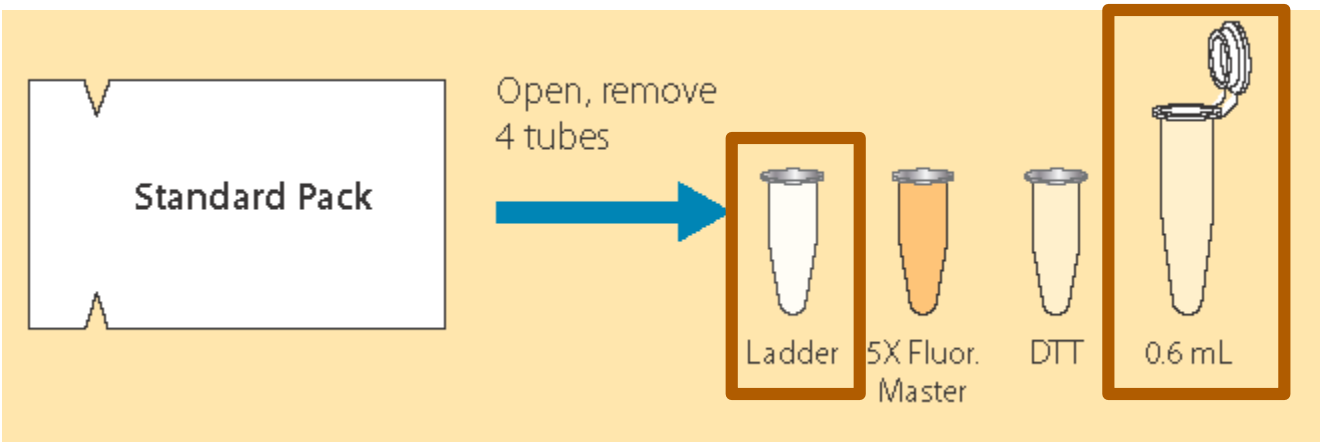
Reconstitute DTT

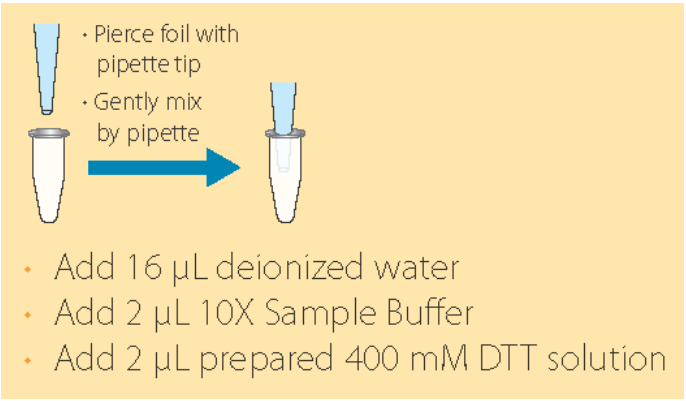


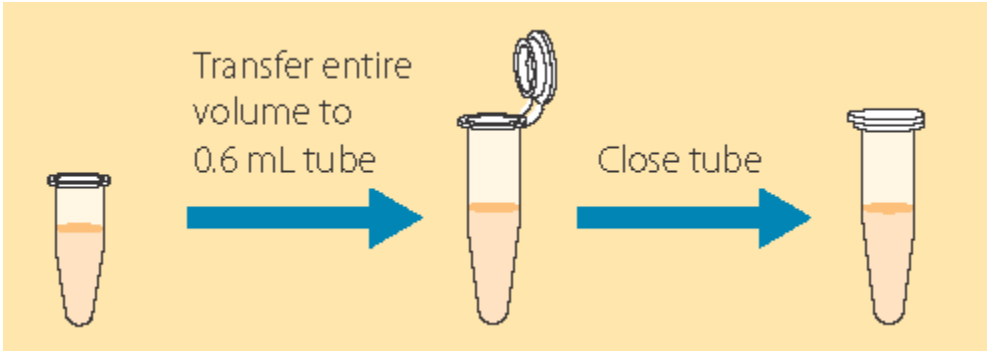
Add: DTT
10x Sample Buffer

终浓度: 5x

Sample Prep – Step 2:
配制分子量标准品（marker)





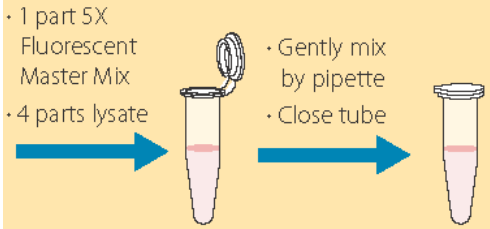


Add: H_2O
DTT
10x Sample Buffer

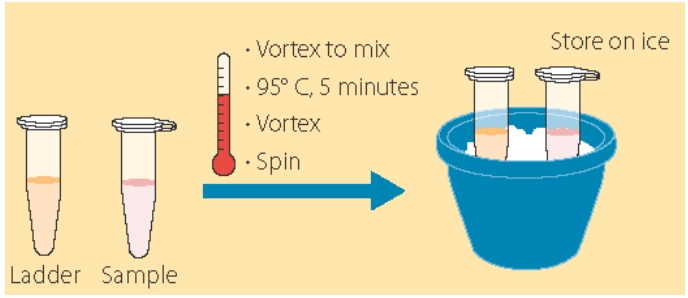
Sample Prep – Step 3:
稀释样品

When using lyates, we recommend a final lysate concentration of 0.2 mg/mL. The optimal protein concentration depends on the expression level of your protein. If needed, dilute your lysate with 0.1X Sample Buffer (dilute 10X Sample Buffer 1:100 with water).

Combine 1 part (e.g. 1 µL) 5X Fluorescent Master Mix with 4 parts (e.g. 4 µL) lysate in a microcentrifuge tube (final concentration 0.2 mg/mL).



样品用 **0.1X** Sample Buffer
混合: **4** 体积 样品
1 体积 FL Master Mix



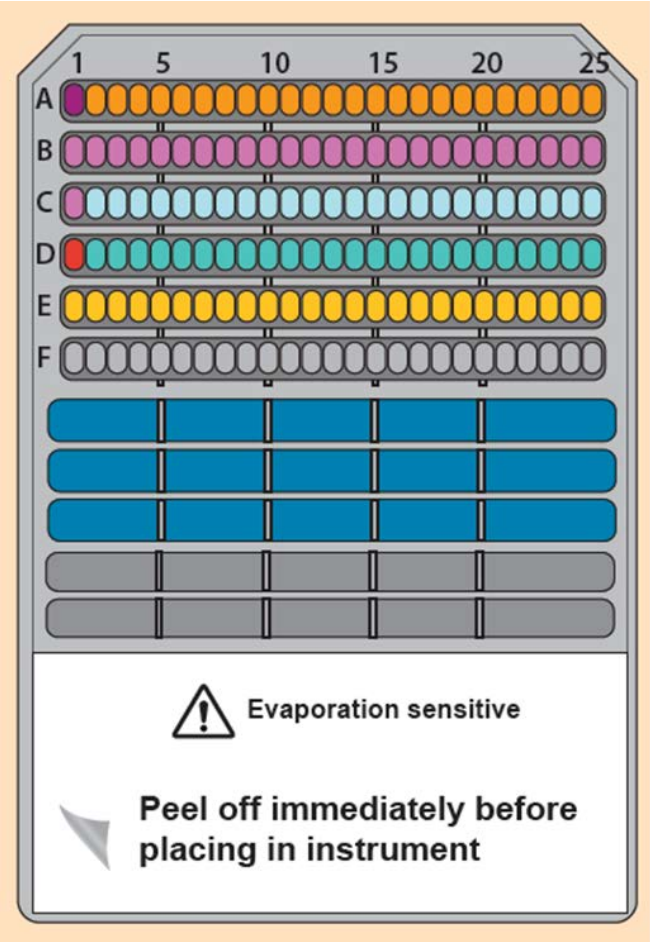
95°C 加热 5 分钟充分变性

变性时建议用小管并封口以减少蒸发造成样品终浓度改变

- 需两次离心，以保证充分混合
  ① 变性后先冰上冷却
  ② Vortex混匀
  ③ 短时离心
  ④ Vortex混匀
  ⑤ 短时离心

用户加样内容

第 1 个孔:
分子量标准品 5 µL
封闭液 10 µL
封闭液 10 µL
Streptavidin HRP
发光底物 10 µL



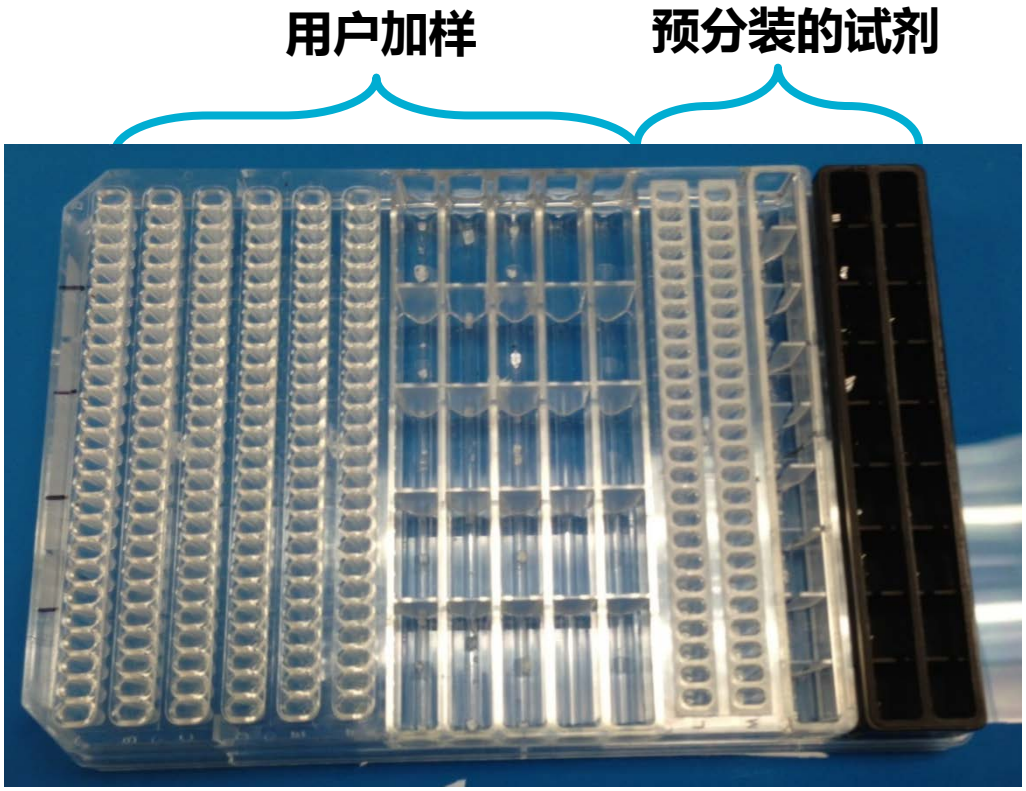
A：Sample 5 µL (0.2-1 µg/µL)
B：封闭液 10 µL
C：稀释后的一抗 10 µL
D：二抗 10 µL
E：发光底物 10 µL

洗液
洗液
洗液

分离胶
浓缩胶
洗胶液
电泳缓冲液
电泳缓冲液

预分装板实物

- 每排 25 孔
- 试剂包括:
  - 样品和免疫检测相关的试剂由用户加样
  - 分离相关的试剂出厂时已加入板内并封膜



实验注意事项

- 预分装板和10X Sample Buffer 使用前必须保证放在18-24°C 24小时以上，否则不能使用
- 避免孔内有气泡
- 加完样后板子要在室温离心
- 上机时才能撕开封膜

Simple Your Western
It’s time!

proteinsimple
a biotechne brand