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SIMPLE WESTERN
常见问题及解决方法

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PROTEIN SIMPLE -- 创新蛋白质分析技术专家

Jess/Abby/Wes

全自动 Western

FluorChem

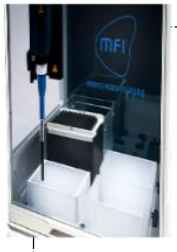
多功能成像

Ella

微流控全自动 ELISA

Milo

单细胞 Western



MFI

微流成像颗粒计数分类仪

Maurice

CE-SDS + icIEF 双功能 CE

MEET SIMPLE WESTERN

JESS ABBY WES

超微量样品+自动化+定量

GEL-RUNNING AUTO BLOT-FREE

TRANSFER-FREE HANDS-FREE

√ 原理及优势

√ 数据分析

√ 高级实验优化

常见问题及解决方法

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分离视频异常

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表观分子量

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荧光背景噪音

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抗体和荧光内参
交叉反应

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抗体和荧光内参
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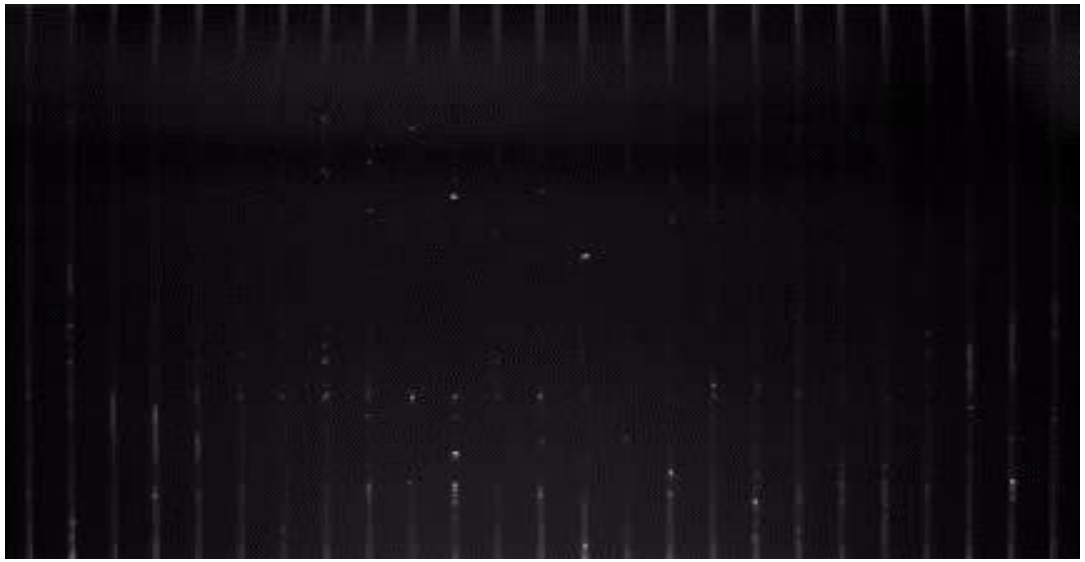
分离视频异常

1. 气泡
2. 毛细管未载入样品
3. 加样板内 Running Buffer 不足
4. 毛细管表面有污染

Separation (for 12-230 kDa)

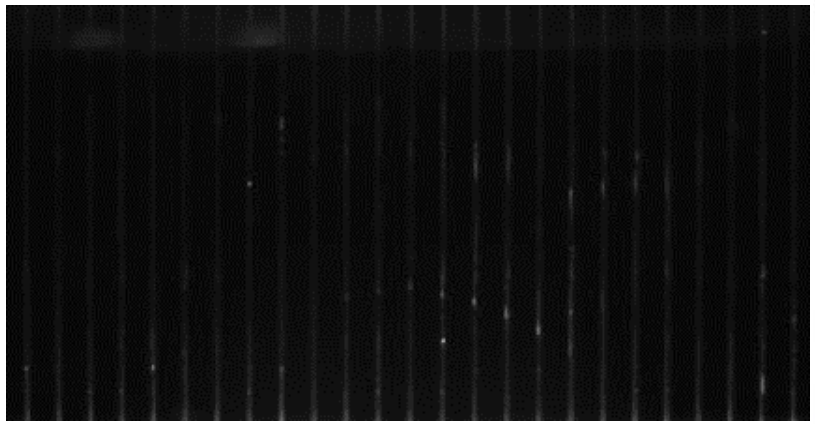


* 运行结束后请先回看分离视频检查是否正常

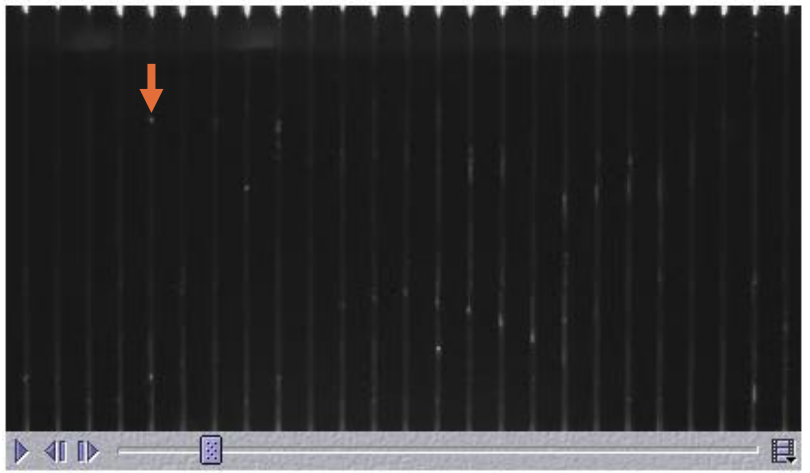
1. 气泡

- 一两个小白点出现并迅速移动
- 移动很快，在其余荧光内参前面
- 通常，荧光内参迁移在分离开始时是正常的
- 气泡离开毛细管后，荧光内参逐渐弥散

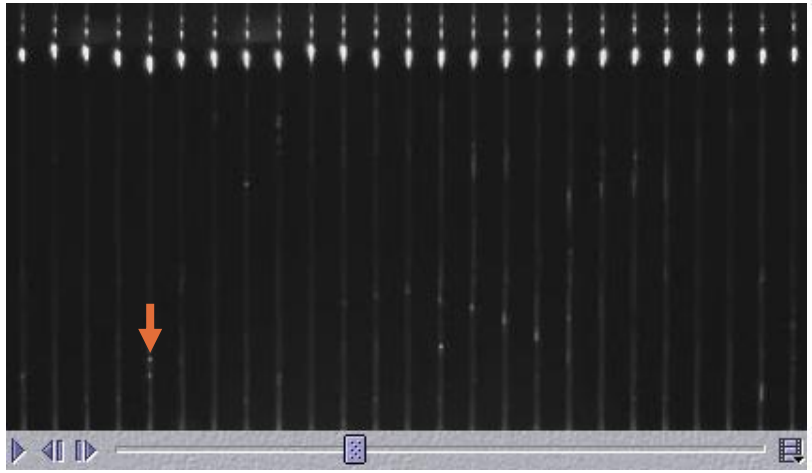
Separation Movie



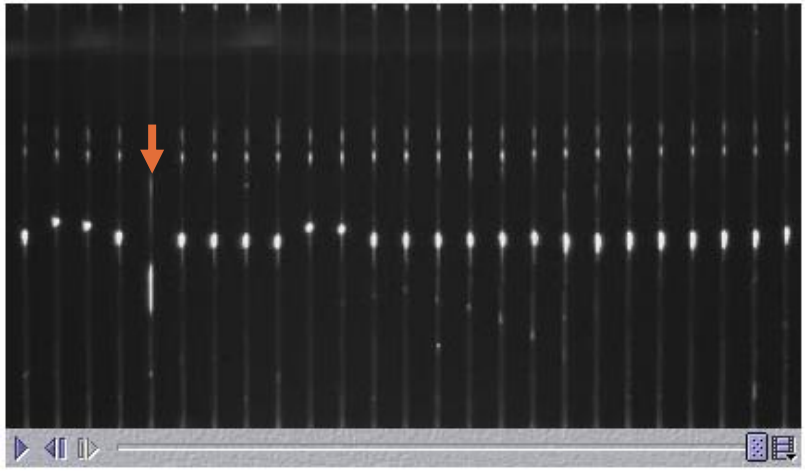
Beginning of the Separation



Middle of the Separation



End of the Separation

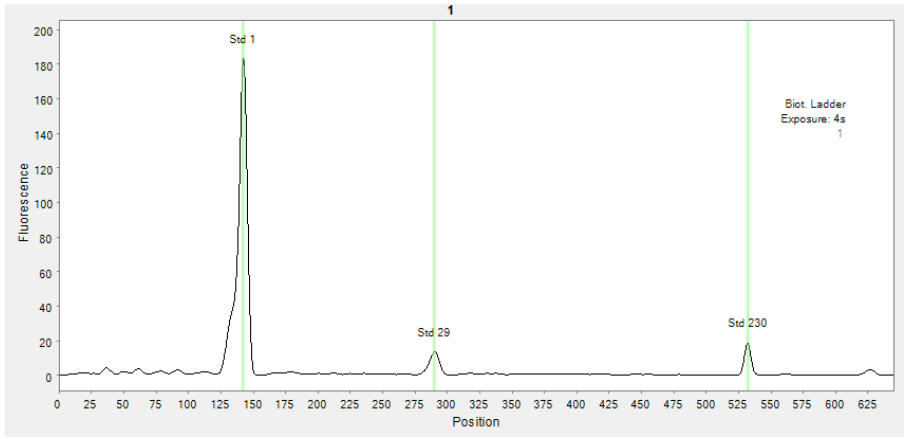


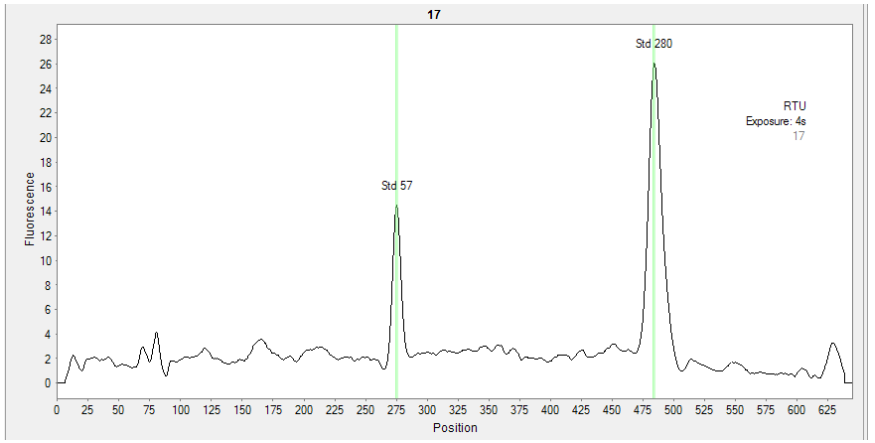
1. 气泡

12-230kDa：Std 1, 29 & 230 KD

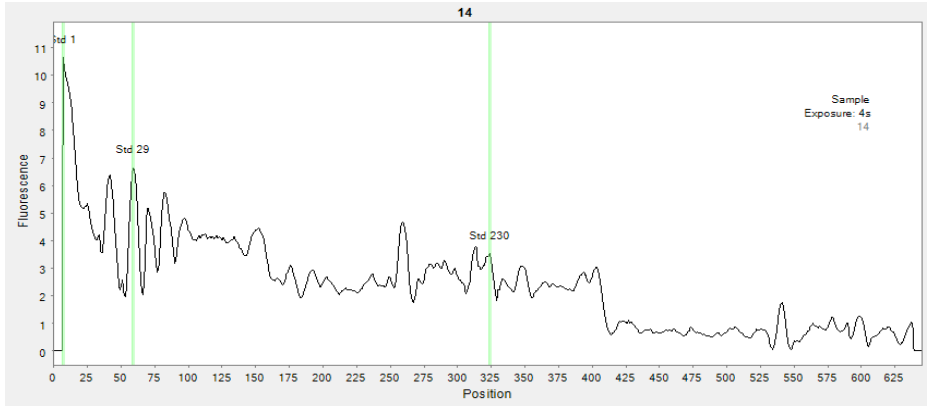
66-440kDa：Std 57 & 280 KD

good

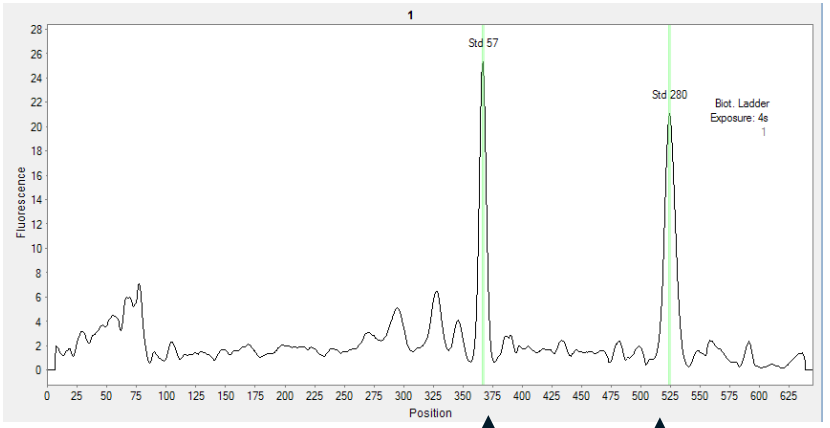




bad



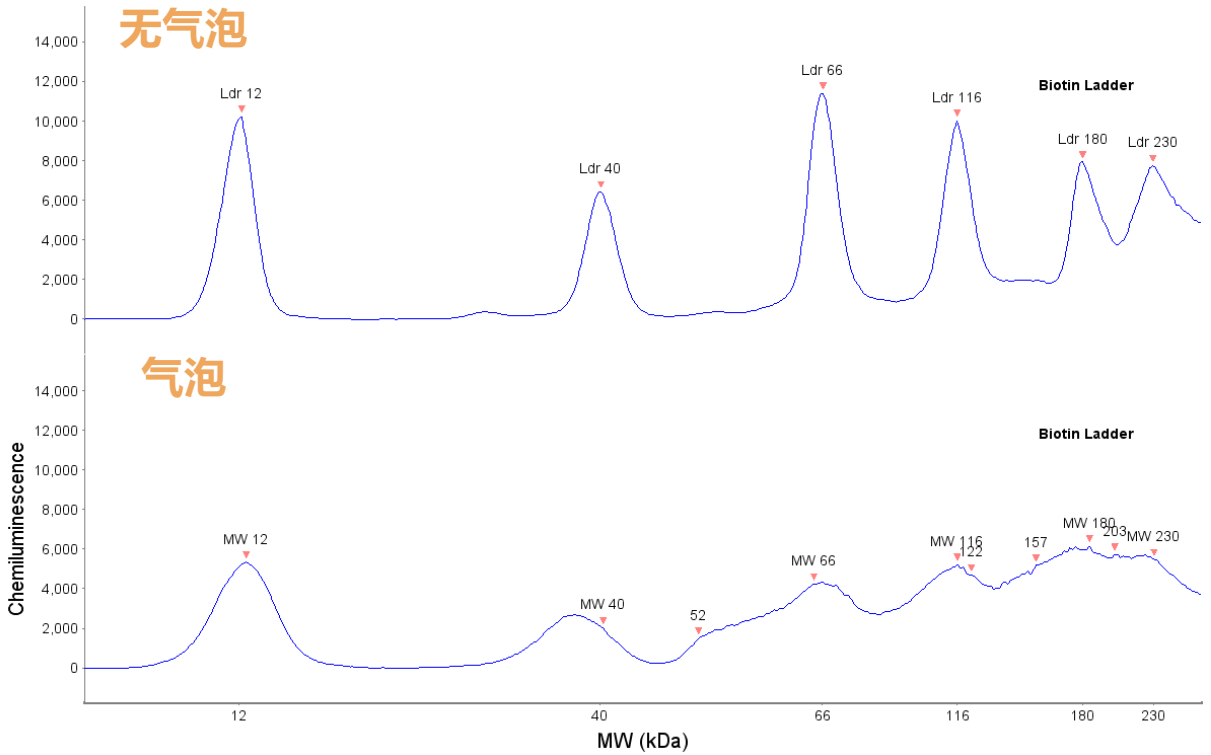
毛细管中有气泡



错误位置

1. 气泡

Analysis 视图:



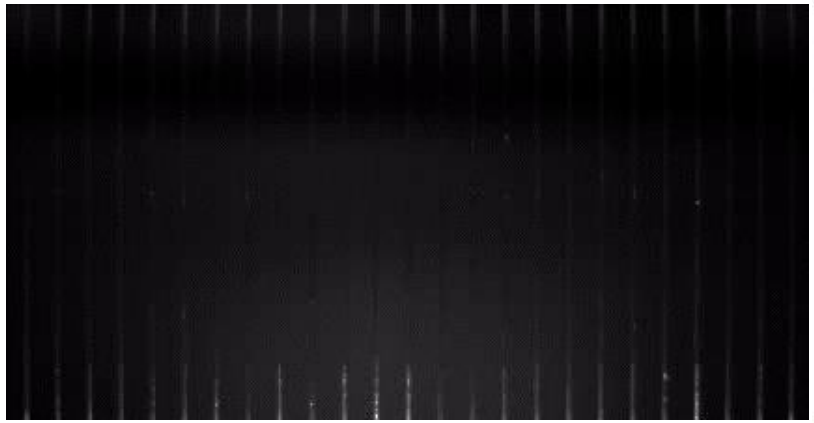
解决:

- 保证加样板 1,000xg 离心 5 分钟
- 如果还有气泡，延长离心时间至10分钟

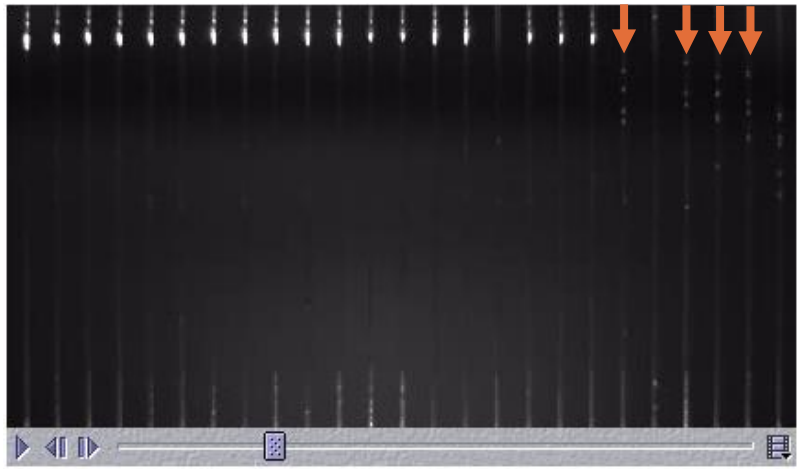
2. 毛细管未载入样品

- 许多小白点
- 以一组点的形式出现在每一根相近的毛细管中
- 非常迅速地移动到毛细管的末端
- 在空毛细管中没有荧光内参出现

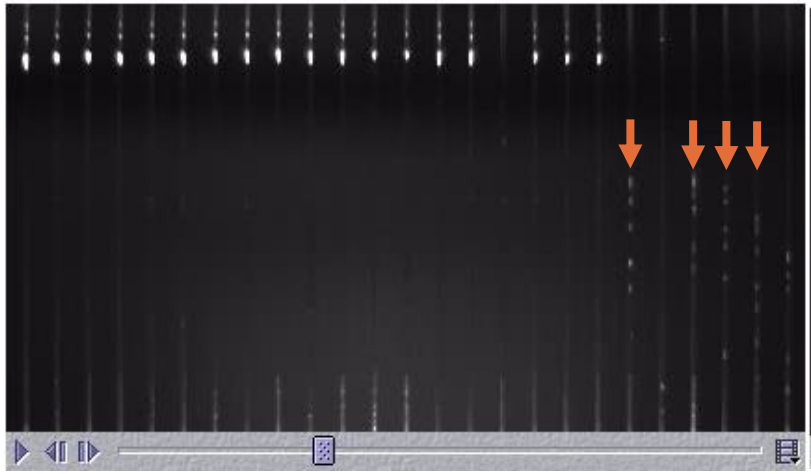
Separation Movie



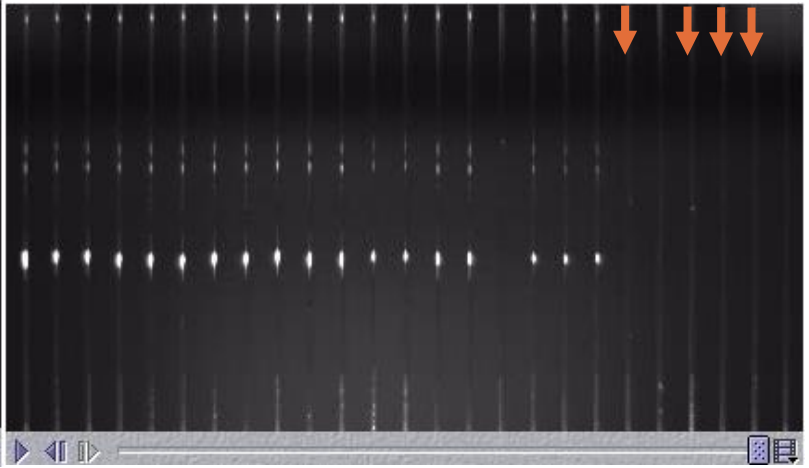
Beginning of the Separation



Middle of the Separation



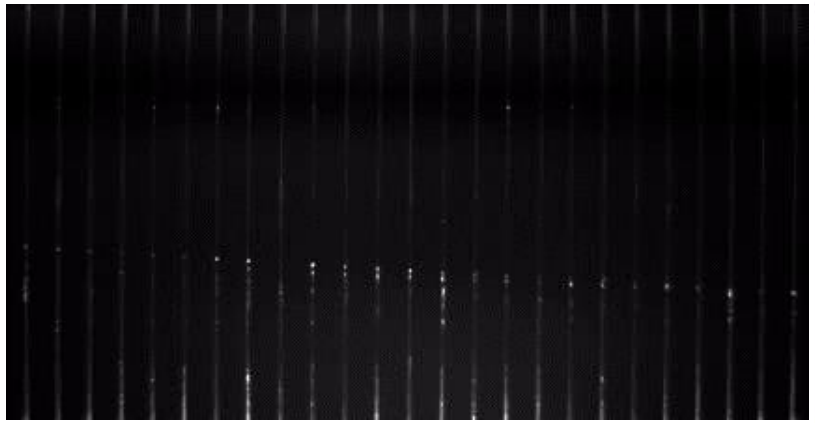
End of the Separation



3. 加样板内 RUNNING BUFFER 不足

- 内参没有一起分离，规律性出现无内参情况
- 在荧光内参 Image 窗格能看到样品塞，表明样品被加载到毛细管中，但荧光内参分离胶中没有迁移

Separation Movie



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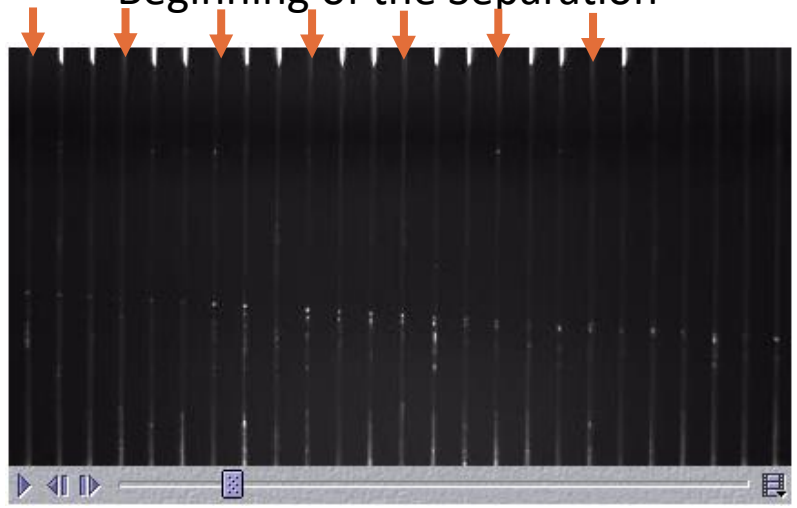

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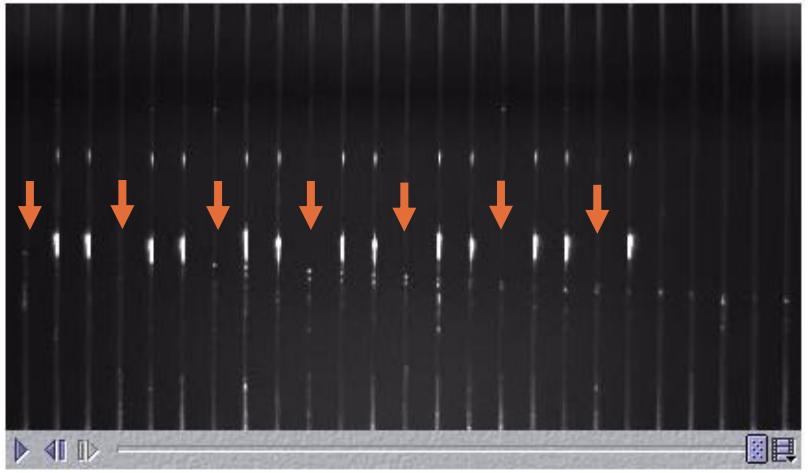
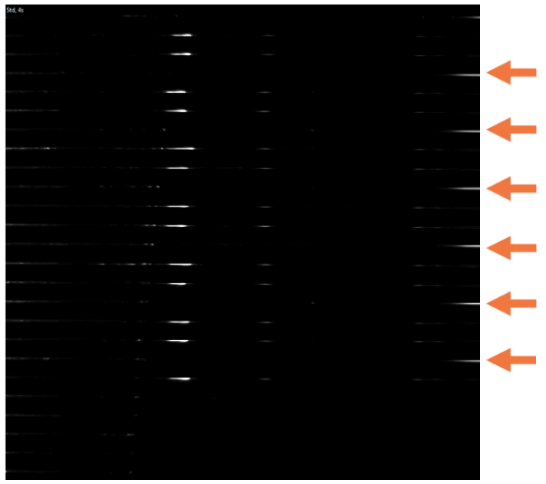


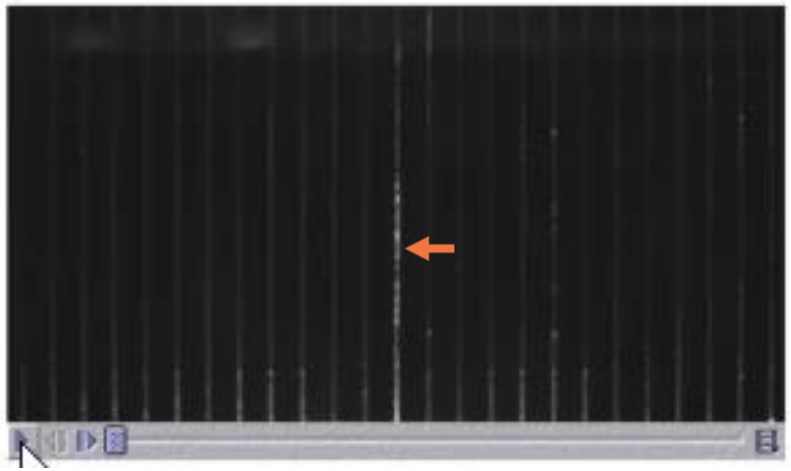
Standards Image View



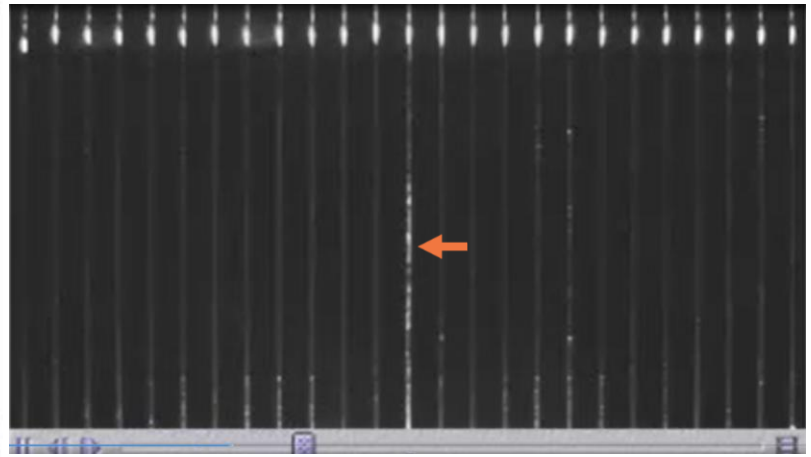
4. 毛细管表面有污染

- 静止的荧光伪影
- 伪影位于毛细管外侧
- 荧光内参迁移不受影响

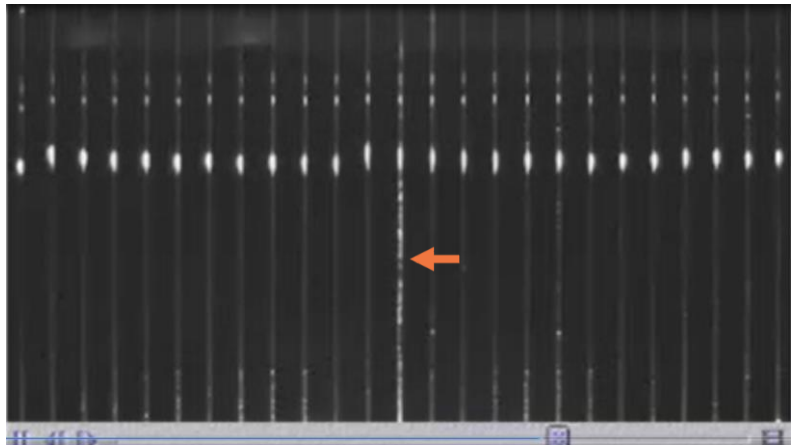
Separation Movie



Beginning of the Separation



Middle of the Separation



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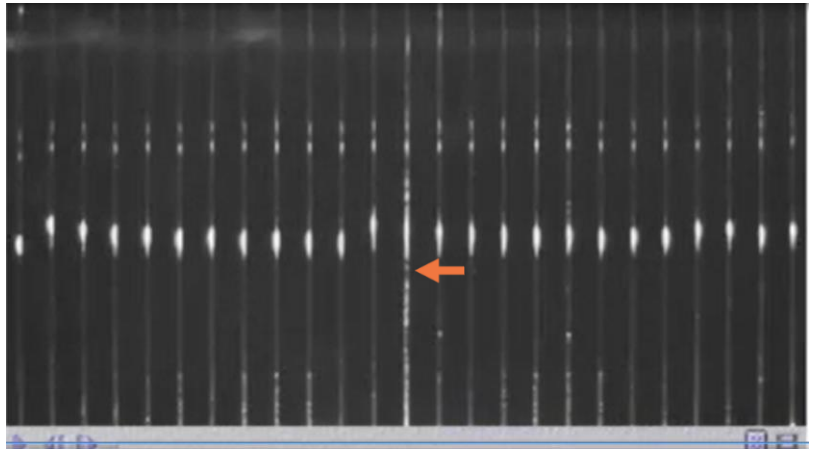

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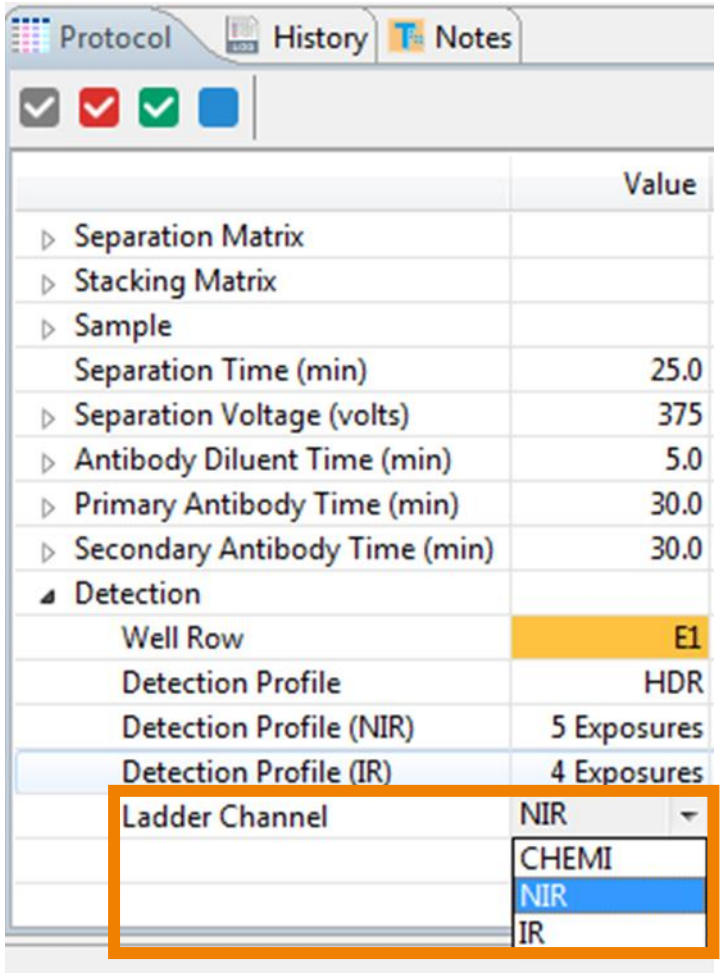
抗体和荧光内参交叉反应

LADDER

COMPASS 软件提供多种LADDER 检测方式

- **Compass 默认 ladder 设置**
  - 只有化学发光的实验:
    - Streptavidin-HRP
  - 只有荧光检测的实验:
    - Streptavidin-NIR
  - 化学发光和荧光均检测的实验:
    - Streptavidin-NIR

Assay Tab:



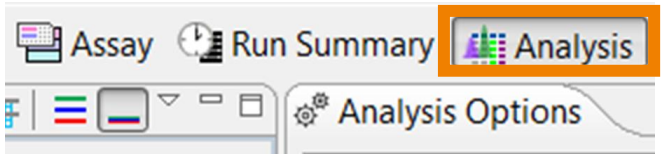
LADDER

如果实验前忘记更改 LADDER 检测通道，运行后在软件里还能更改

步骤:

1. 单击“Analysis” 窗格
2. 点击菜单栏的 “Edit” → “Analysis…”
3. 点击“Ladders”
4. 在 “Channel”中:
   - 设置正确的检测通道
5. 点击 “OK”

1



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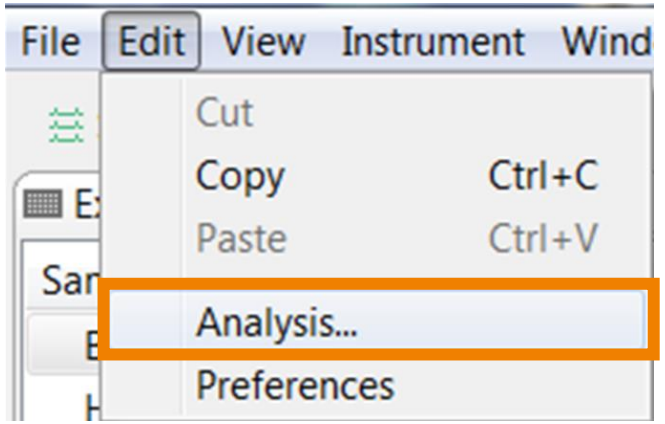

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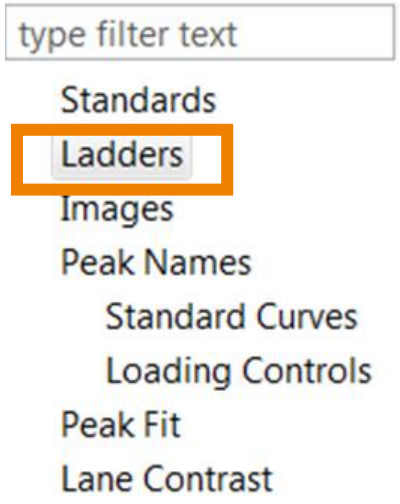

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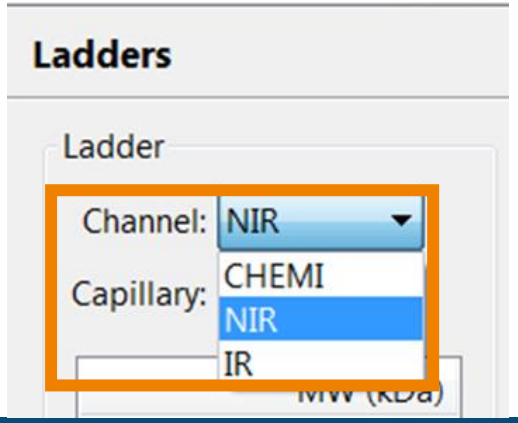
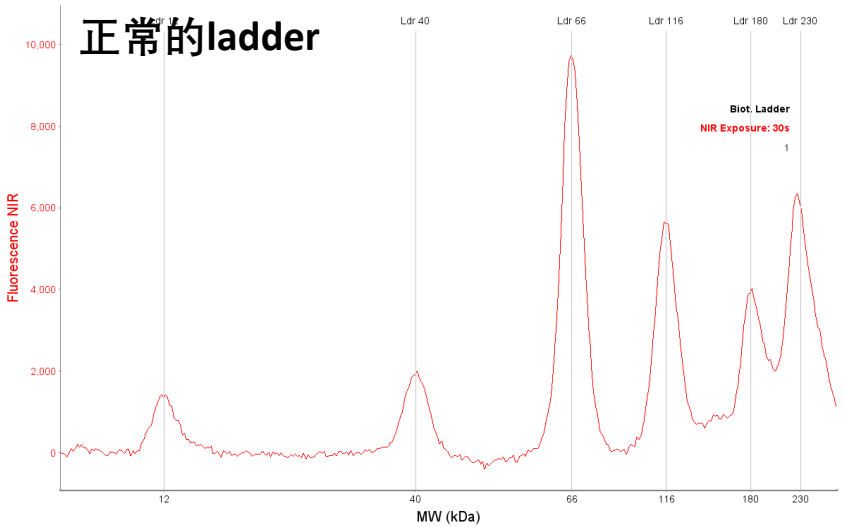
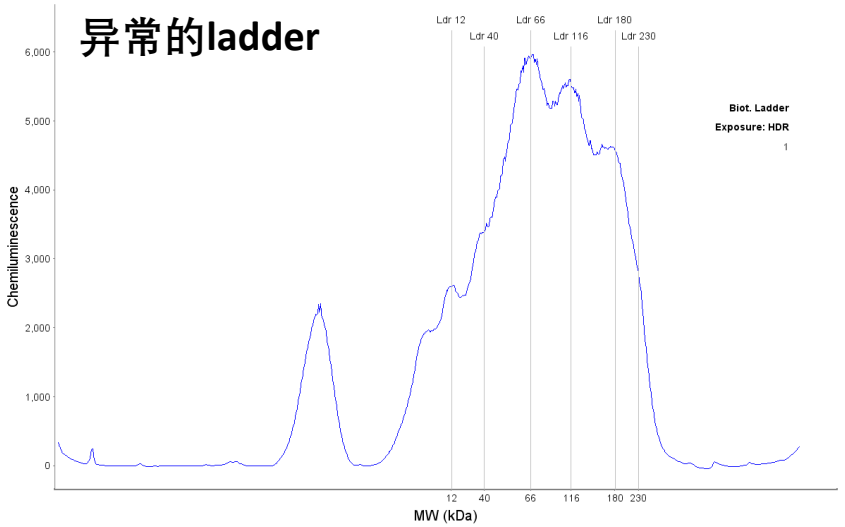


LADDER

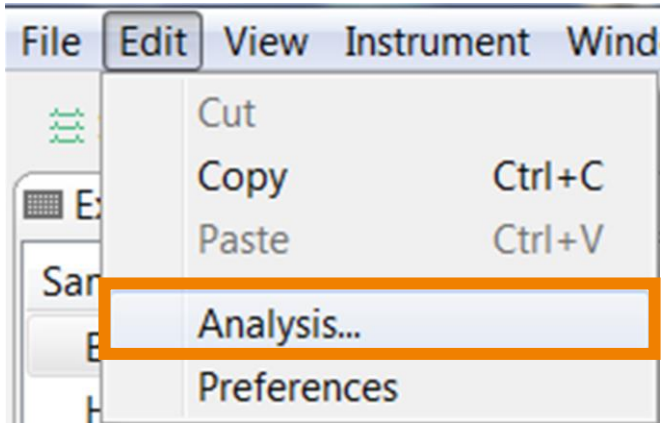




分子量 LADDER 如果有问题，可以关闭

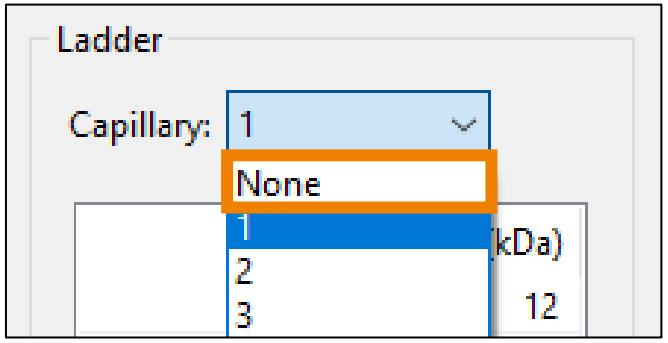
步骤:

1. 点击“Edit” → “Analysis…”
2. 点击“Ladders”
3. 在“Capillary” 中：
   - 选择“None”
4. 点击 “OK”



在没有 ladder 情况下，软件根据每个毛细管中的荧光内参来计算分子量

- 此时计算的分子量准确度不高



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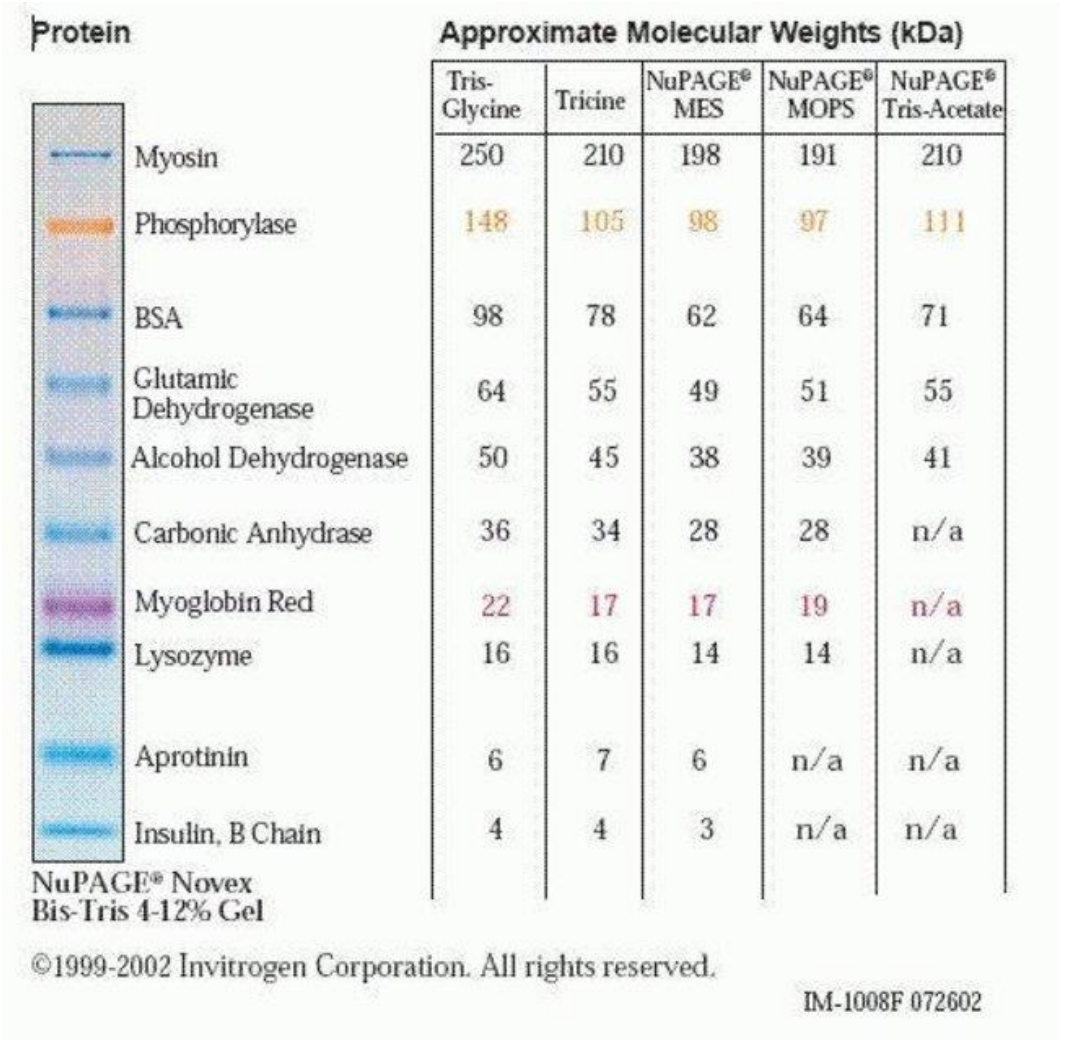
表观分子量

同一蛋白的表观分子量根据 SDS-PAGE 缓冲液系统的不同有所差异



Protein	Approximate Molecular Weights (kDa)				
	Tris-Glycine	Tricine	NuPAGE® MES	NuPAGE® MOPS	NuPAGE® Tris-Acetate
Myosin	250	210	198	191	210
Phosphorylase	148	105	98	97	111
BSA	98	78	62	64	71
Glutamic Dehydrogenase	64	55	49	51	55
Alcohol Dehydrogenase	50	45	38	39	41
Carbonic Anhydrase	36	34	28	28	n/a
Myoglobin Red	22	17	17	19	n/a
Lysozyme	16	16	14	14	n/a
Aprotinin	6	7	6	n/a	n/a
Insulin, B Chain	4	4	3	n/a	n/a

NuPAGE® Novex
Bis-Tris 4-12% Gel

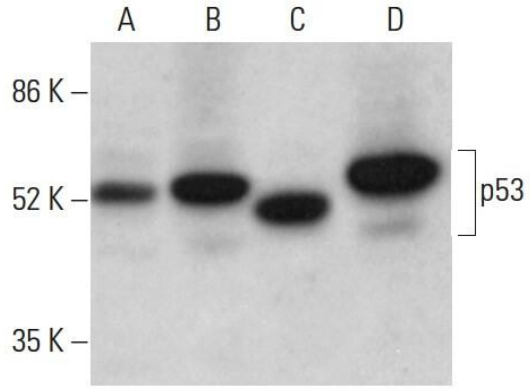
IM-1008F 072602

摘自 ThermoFisher SeeBlue Plus2 Ladder

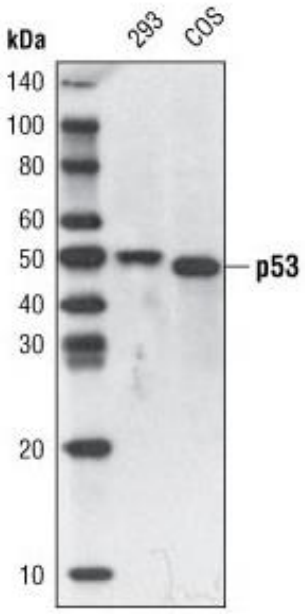
表观分子量

例：在抗体品牌不同时，P53 表观分子量不同

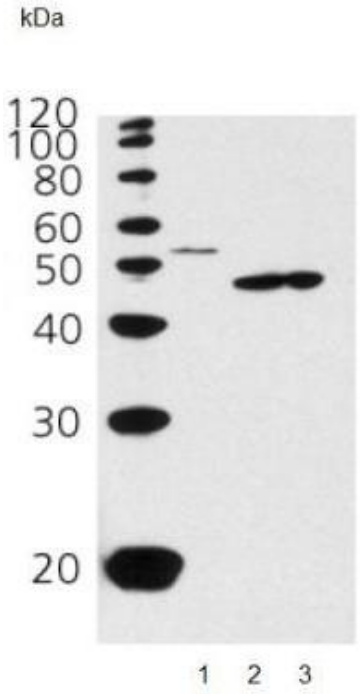
Santa Cruz Biotechnology
sc-126



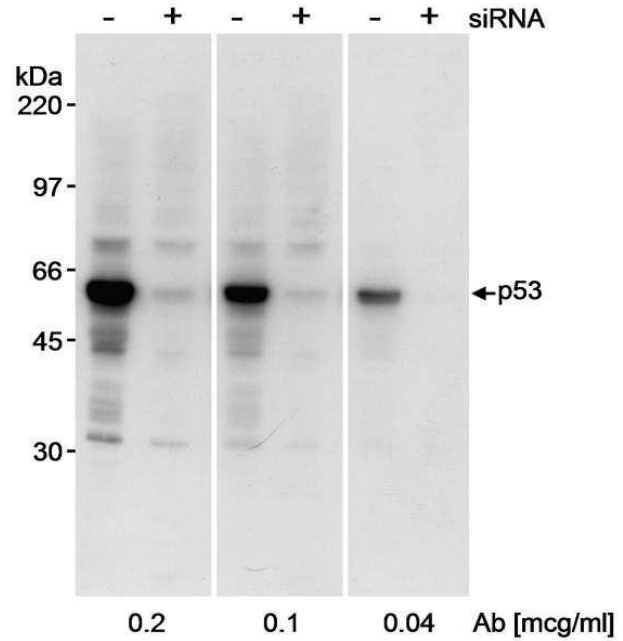
Cell Signaling Technology
4667



Abcam
ab90363



Novus Biologicals
NB200-171



p53 – 准确分子量为 43.65 kDa

表观分子量

多种因素影响表观分子量大小

abcam®
discover more

Why is the actual band size different from the predicted?

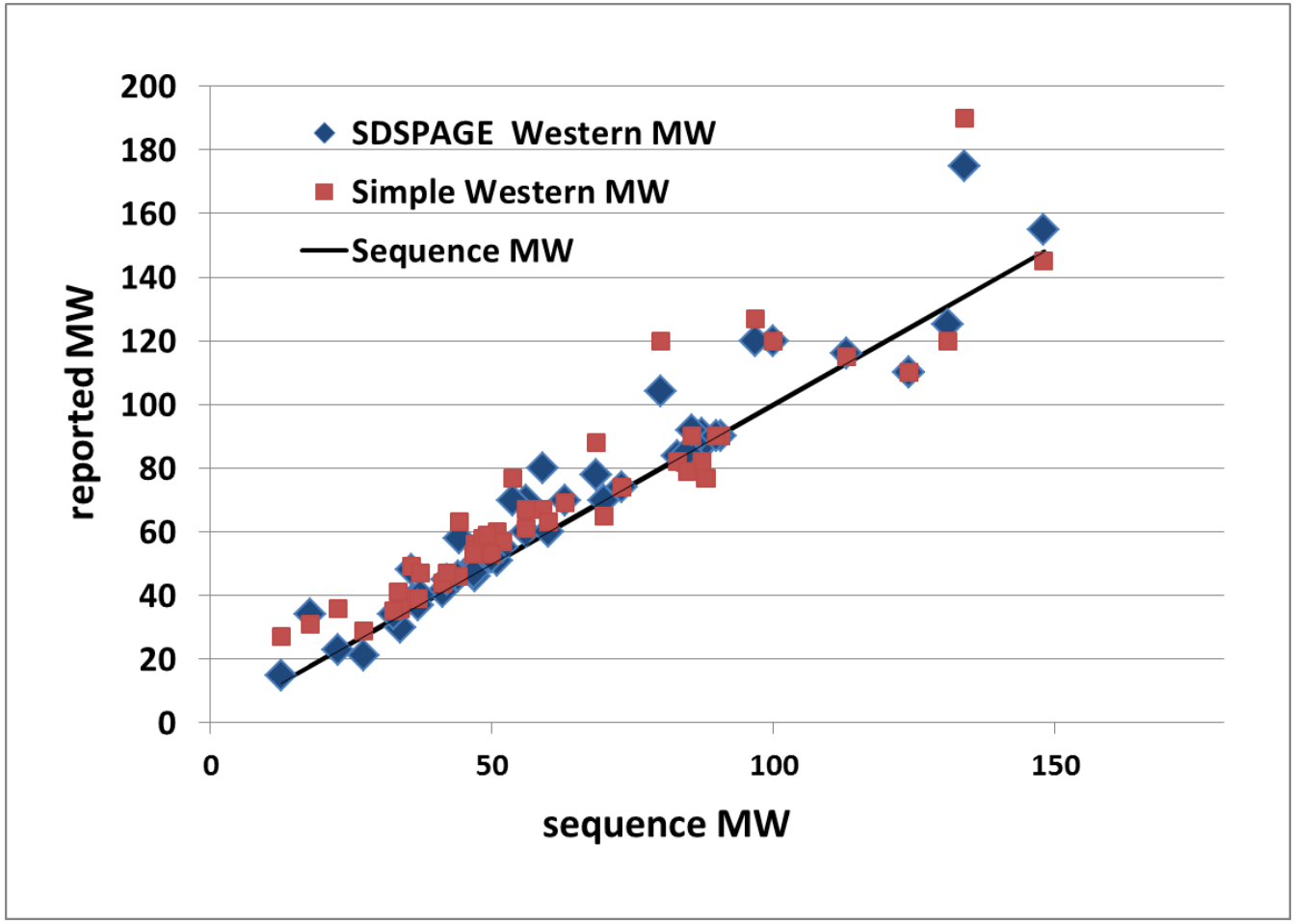
Western blotting is a technique that separates proteins based on size - in general, the smaller the protein the faster it migrates through the gel. However, migration is also affected by other factors and so the actual band size observed may differ from that predicted. Common factors include...

- **post-translational modification** - e.g. phosphorylation, glycosylation etc which increases the size of the protein
- **post-translation cleavage** - e.g. many proteins are synthesized as pro-proteins, and then cleaved to give the active form, e.g. pro-caspases
- **splice variants** - alternative splicing may create different sized proteins from the same gene
- **relative charge** - the composition of amino acids (charged vs non-charged)
- **multimers** - e.g. dimerisation of a protein. This is usually prevented in reducing conditions, although strong interactions can result in the appearance of higher bands

表观分子量

SIMPLE WESTERN 与传统 WB 的表观分子量，以及准确分子量

详见：MW Determination App Note



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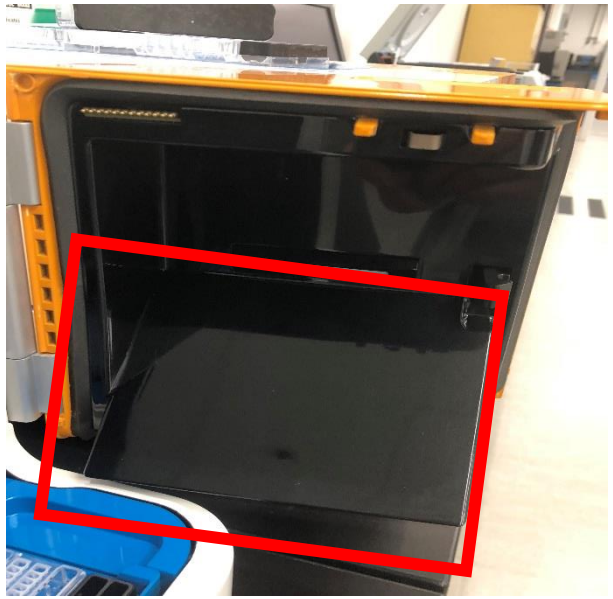
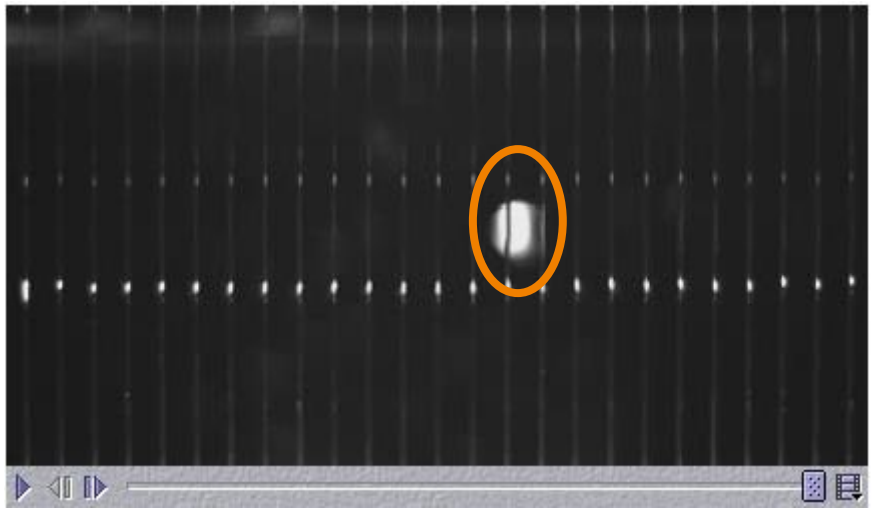
荧光背景噪音

门内侧的脏污导致荧光背景，影响数据（背景或峰形）



门内侧的指纹



解决：

用70% 异丙醇（或70%乙醇）蘸湿不掉屑的无尘纸/布，轻轻擦拭门内衬，自然干燥即可

建议使用前先进行清洁

在清洁门内侧之前自检测试失败

```
Green Background and Filter Check       18:43:01        68.617   FAILED
        21891 pixels (2.7612% ) are above 5500, limit is 2.0000%
```

* Self-test 程序用时 30 mins

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表观分子量

04

荧光背景噪音

05

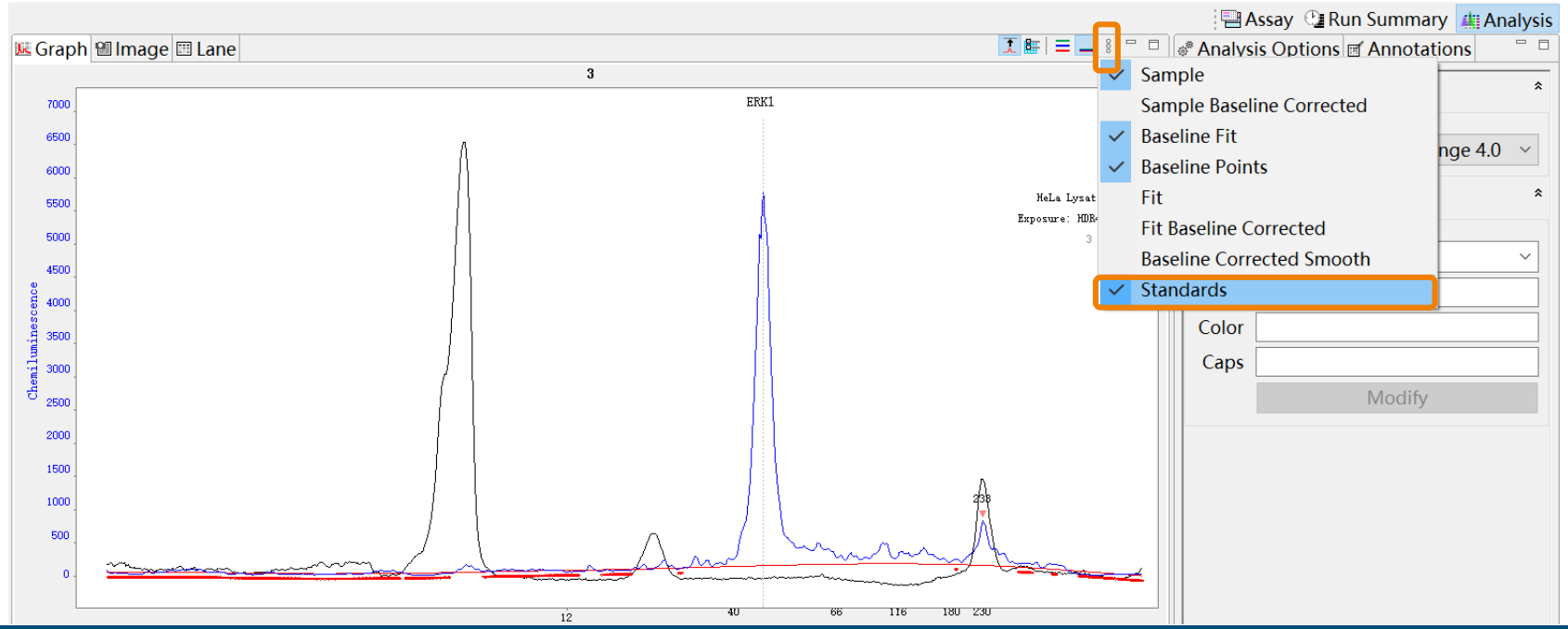
抗体和荧光内参
交叉反应

抗体和荧光内参交叉反应

怎么鉴定?

1. 预期外的峰，更荧光内参分子量吻合
2. 荧光内参和这些峰高度重叠
3. 无样品阴性对照中有对应峰
4. 样品稀释但该峰高不变

- 12–230 kit：1 kDa，29 kDa ，230 kDa
- 66–440 kit：57 kDa， 280 kDa
- 2–40 kit：1 kDa， 26 kDa



抗体和荧光内参交叉反应

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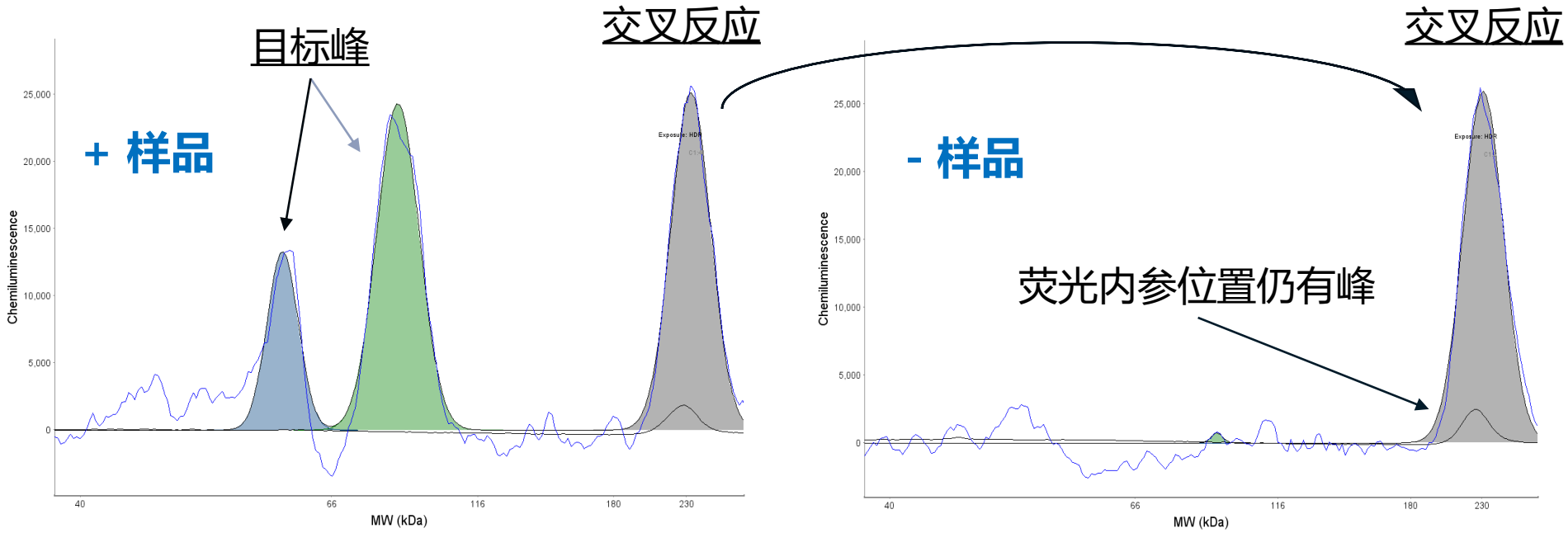


抗体和荧光内参交叉反应

如果一抗和荧光内参交叉反应了，怎么办？

1. **如果这些峰跟您的目的蛋白峰互不干扰:**
   - 可不处理

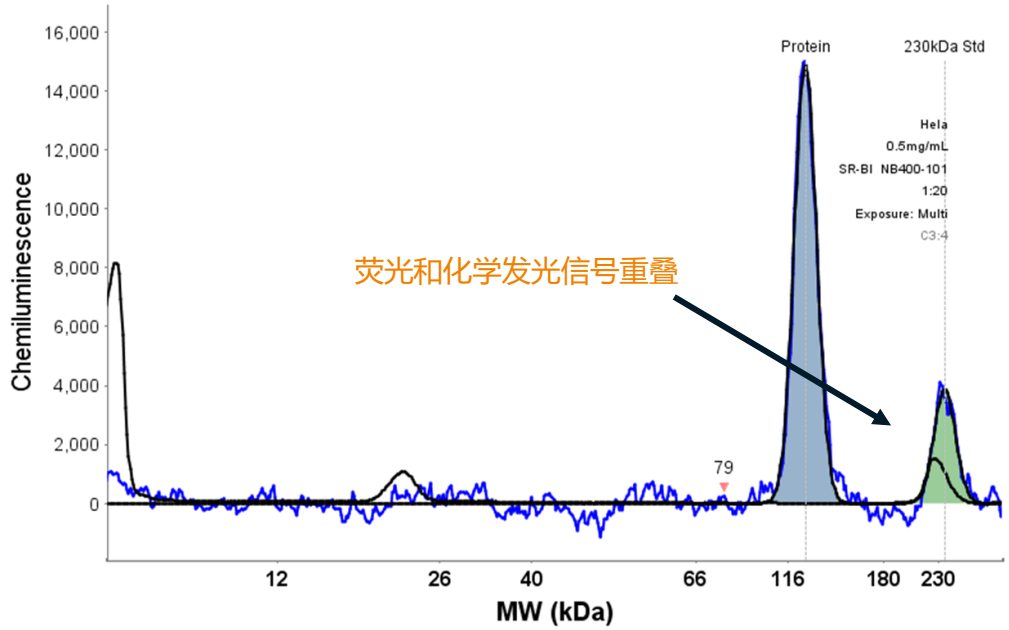
2. **如果这些峰跟您的目的蛋白距离很近或者互相干扰:**
   - 更换与荧光内参不结合的抗体
   - 对于 230kDa 荧光内参的交叉反应 (最常见):
     a. 使用 2-40kDa 里的 5X FL-Standard pack (Part# PS-FL05-8)
     b. 预计会有轻微的失真

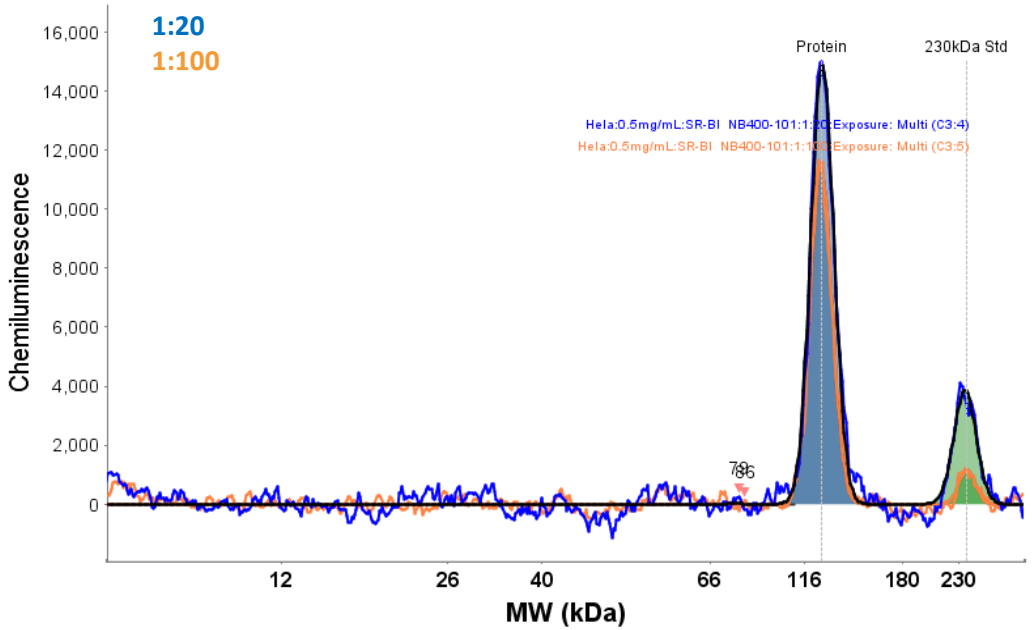
     或者

     c. 在保证抗体饱和的情况下，稀释抗体浓度

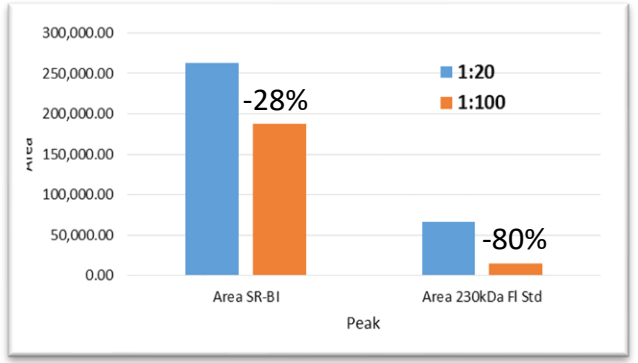
抗体和荧光内参交叉反应

例：通过稀释一抗，降低一抗于荧光内参的非特异结合





抗体5倍稀释后，荧光内参信号下降相比于目的信号下降，更显著



0.5mg/mL HeLa, SR-B1 抗体 (Novus, NB-400-101)

简明用户手册

现象	可能原因	解决方法
实验结束后没有或找不到运行结果文件（run file）	Wes 和电脑主机的通讯连接断开	确认Wes处于开机状态，打开Compass软件，确认连接Wes，点击Instrument > Runs...，在出现的窗口内找到运行结果文件，点击Save As保存
3个荧光内参全部弥散无法辨认	Wes 板子没有在室温保存，造成试剂成分沉淀析出	Wes 板子需要在实验开始前室温（18 – 24 ℃）放置至少24小时以上
荧光内参分离谱图与预期不符	使用了错误的荧光内参	请核对白色纸包上的标记，Standard Pack 1为12-230kD，Standard Pack 2为66-440kD
荧光内参跑出了毛细管	板子上机前没有离心。板子在运输过程中或者上样时有气泡	上机前室温离心，确保各孔内无气泡。如果再离心后看到有气泡，请用枪头将其戳破
荧光内参进入了毛细管，但是没有进一步分离	Wes 板子被冷藏或冷冻过，造成试剂成分析出	Wes 板子需要在实验开始前室温（18 – 24 ℃）放置至少24小时以上
Graph View 发现靶蛋白分子量位置有凹坑	化学发光信号淬灭	选择短的曝光时间（例如55）查看结果。可减少样品浓度
化学发光信号与蛋白量不成线性	不在检测的线性范围内	通过样品梯度稀释确定实验的检测上限和下限
化学发光信号重复性差	抗体不够量	通过抗体梯度稀释确定最佳的抗体稀释度
Total Protein 试剂盒批次内重复性差	Biotin Labeling Reagent 没有充分重悬 Biotin Labeling Reagent不是新鲜制备	Reconstitution Agent 1 和 Reconstitution Agent 2 混合后上下吸打 6 – 10 次，保证充分混匀 在上机前 30 分钟内制备 Biotin Labeling Reagent

公众号报修流程

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