SDS-PAGE－考马斯亮蓝染色

1、 实验器材

名称	厂家	型号
酶标仪	Rayto	RT-6100
台式高速冷冻离心机	DRAGONLAB	D3024R
掌上离心机	Wanwu	D1008E
涡旋混合器	Wanwu	MX-F
磁力搅拌器	Wanwu	MS-PB
脱色摇床	Wanwu	TSY-B
垂直电泳仪	Wanwu	BV-2
研磨仪	Wanwu	KZ-II

2、 主要实验试剂

试剂	厂家	货号
RIPA 裂解液	Wanwu	G2002
50*cooktail	Wanwu	G2006
PMSF（100mM）	Wanwu	G2008
磷酸化蛋白酶抑制剂	Wanwu	G2007
BCA 蛋白定量检测试剂盒	Wanwu	G2026
5*蛋白还原型上样缓冲液	Wanwu	G2013
SDS-PAGE 凝胶制备试剂盒	Wanwu	G2003
蛋白 Marker	Wanwu	26617
电泳缓冲液	Wanwu	G2018
考马斯亮蓝染液	Wanwu	G2021-250ML
考马斯亮蓝脱色液	Wanwu	G2022-250ML

3、实验步骤

（1） 细胞总蛋白提取

1.1 对于悬浮细胞: 2000rpm，4℃，5 min，离心收集细胞沉淀，每 10^6 细胞加 250 μL 左右 RIPA 裂解液（在使用前数分钟内加入蛋白酶抑制剂），振荡。如果需要提高蛋白浓度，可适当减少裂解液使用量。

1.2 对于贴壁细胞:

1.2.1 用 PBS 冲洗细胞 2-3 次，最后一次清洗完，倒掉 PBS，用移液器尽量吸干残留液体；

1.2.2 加入适当体积的 RIPA 裂解液（使用前数分钟内加入蛋白酶抑制剂）于培养板/培养瓶内 3-5min。期间反复晃动培养板/瓶，使试剂与细胞充分接触；

1.2.3 用细胞刮刀将细胞刮下，转移到 1.5ml 离心管中；

1.3 冰上裂解 30min，期间用移液器反复吹打，确保细胞完全裂解。

1.4 12000rpm,4℃，离心 10min，收集上清，即为总蛋白溶液。

（2） 组织总蛋白提取：

2.1 组织块用预冷的 PBS 洗涤 2-3 次，去除血污，剪成小块置于匀浆管中，加入 1~2 个 3mm 的匀浆珠，加入 10 倍组织体积的裂解液（使用前数分钟内加入蛋白酶抑制剂），设置匀浆程序进行匀浆；如果需要提高蛋白浓度，可以适量减少裂解液体积；

2.2 将匀浆完成的匀浆管取出，放置冰上裂解液 30min，每隔 5min 震荡一次确保组织完全裂解；

2.3 12000rpm，4℃，离心 10min，收集上清，即为总蛋白溶液。、

（3） SDS-PAGE 电泳

3.1 清洗玻璃板；

3.2 制胶与上样

3.3 将玻璃板对齐后放入制胶器中对齐，卡紧，以免漏胶；

3.4 按实验需要配制分离胶，加入 TEMED 后立即摇匀即可灌胶。大约 45min 后可倒去胶上层水并用吸水纸将剩余水吸干；

	分离胶配比					
试剂	8%	10%	12%	15%	18%	20%
H_2O mL	4.63	4	3.3	2.3	1.3	0.63
30%丙烯酰胺（29：1） mL	2.67	3.3	4	5	6	6.67
1.5M TRIS－HCl(PH 8.8) mL	2.5	2.5	2.5	2.5	2.5	2.5
10%SDS mL	0.1	0.1	0.1	0.1	0.1	0.1
AP mL	0.1	0.1	0.1	0.1	0.1	0.1
TEMED μL	5μ	5μL	5μL	5μL	5μL	5μL
总体积 mL	10mL					

试剂	5%浓缩胶配比
H_2O mL	2
30％丙烯酰胺（29：1）mL	0.5
1M TRIS－Hcl(PH 6.8) mL	0.5
10％SDS μL	40
AP μL	30
TEMED μL	4μL
总体积 mL	3mL

3.5 按前面方法配 5%的浓缩胶，加入 TEMED 后立即摇匀即可灌胶。将剩余空间灌满浓缩胶然后将梳子插入浓缩胶中；

3.6 加足够的电泳液后上样电泳。将样品加入电泳孔中，电泳；浓缩胶电压 75V，分离胶用 120V，电泳至溴酚蓝离玻璃板底部还有大约 1cm 时即可终止电泳。

(4)、考马斯亮蓝染色及后续实验步骤

4.1 将胶放入玻璃平皿中加入考马斯亮染色液后 70℃孵育 5min，置于摇床上染色 90min；

4.2 将考马斯亮染色液回收后用清水洗净胶，加入考马斯亮脱色夜，置于脱色摇床上脱色过夜；

4.3 将脱色液去除，用清水洗净胶后对目的条带进行拍照；

(5)、结果及分析（详见 Excel 灰度分析表格）

SDS-PAGE- coomassie blue staining

1、Experiment instruments

name	company	Model number
Microplate Reader	Rayto	RT-6100
Centrifuge	DRAGONLAB	D3024R
Mini centrifuge	Wanwu	D1008E
Vortex mixer	Wanwu	MX-F
Magnetic stirrers	Wanwu	MS-PB
Decolorizing shaker	Wanwu	TSY-B
Vertical electrophoresis apparatus	Wanwu	BV-2
Homogenizer	Wanwu	KZ-II

2、Reagents

Reagents	Company	Catlog
RIPA buffer	Wanwu	G2002
50* cooktail	Wanwu	G2006
PMSF（100mM）	Wanwu	G2008
Phosphatase inhibitor	Wanwu	G2007
BCA protein quantitative detection kit	Wanwu	G2026
5*loading buffer	Wanwu	G2013
SDS-PAGE kits	Wanwu	G2003
Protein Marker	Thermo	26617
Electrophoretic buffer	（WanwuFermentas）	G2018
Coomassie brilliant blue dye	Wanwu	G2021-250ML
Decolorizing solution of Coomassie Brilliant blue	Wanwu	G2022-250ML

3、coomassie blue staining protocol

（1） Sample Preparation (Total protein)

1.1 For suspension cells: Centrifuge at 2000rpm，4℃ for 5 min to collect cells, add RIPA buffer. (250μL RIPA buffer/10^6 cells（Protease and phosphatase inhibitors should be added before use）. If you wish to increase protein concentration，then add less lysis buffer.

1.2 For adherent cells

1.2.1 Place the cell culture dish on ice and wash the cells three times with ice-cold PBS.

1.2.2 Aspirate the PBS, then add ice-cold RIPA buffer (Protease and phosphatase inhibitors should be added before use).

1.2.3 Scrape adherent cells off the dish , then gently transfer the cell suspension into a 1.5mL microcentrifuge tube.

1.3 Maintain constant agitation for 30 min on ice bath, use pipette to mix it up.

1.4 Centrifuge at 12,000 rpm , 4°C for 10 min. Gently remove the tubes from the centrifuge and place on ice,tranfer the supernatant to a fresh tube.

（2） Preparation of lysate from tissues

2.1 Wash the sample with cold PBS for twice or three times， then homogenize with an electric homogenizer. Volumes of lysis buffer must be determined in relation to the amount of tissue.

2.2 Maintain constant agitation for 30 min on ice bath, use pipette to mix it up.

2.3 Centrifuge at 12,000 rpm ,4°C for 10 min . Gently aspirate the supernatant and place in a fresh tube kept on ice.

（3）SDS-PAGE

3.1 Washing glass plates.

3.2 Preparing and loading the gel.

3.3 Align the glass plate and put it into the gel maker to align and clamp tightly to avoid gel leakage

3.4 Different concentrations of separation gel were prepared according to the experimental requirements, and mixed evenly immediately after adding TEMED, and the separation gel was poured to an appropriate height. .After about 45min, pour the water from the upper layer of the separation glue and drain the remaining water with absorbent paper.

Reagents	8%	10%	12%	15%	18%	20%
H_2O mL	4.63	4	3.3	2.3	1.3	0.63
30％ acrylamide（29：1） mL	2.67	3.3	4	5	6	6.67
1.5M TRIS－HCl(PH 8.8) mL	2.5	2.5	2.5	2.5	2.5	2.5
10％SDS mL	0.1	0.1	0.1	0.1	0.1	0.1
AP mL	0.1	0.1	0.1	0.1	0.1	0.1
TEMED μL	5	5	5	5	5	
Total	10 mL					

Reagents	concentration 5%
H_2O mL	2
30% acrylamide（29：1） mL	0.5
1M TRIS－HCl(PH 6.8)mL	0.5
10%SDS μL	40
AP μL	30
TEMED μL	4
Total mL	3

3.5 Prepare 5% concentrated gel according to the above formula. After adding TEMED, mix well immediately, and then the gel can be filled. Fill the remaining space with the concentrate and insert the comb into the concentrate Make sure there are no bubbles under the comb.

3.6 After stacking gel has polymerized, remove comb carefully and begin the electrophoresis.

3.7 Add enough electrophoresis solution for sample electrophoresis., Concentrated gel voltage is 75V, and separation gel uses 120V. The electrophoresis was stopped when the bottom of bromophenol blue was about 1cm.

(4)、Coomassie brilliant blue staining and subsequent experimental steps

4.1 Put the gel into a glass plate, add Coomassie brilliant staining solution, and incubate at 70 ℃ for 5 min, and then place it on a shaker for 90 min;

4.2 After recovery, wash the gel with water, add the decolorizing solution, and put it on the decolorizing shaker overnight;

4.3 Remove the decolorizing solution, clean the gel with water and take photos of the target band.

(5)、Results and analysis (see excel gray analysis table for details)