WB   6+5+5 =   2小时

Western Blot Protocol all on ice!!!

- Cell collection (6-well)

1. Wash cell with cold PBS rm dead cell!
2. Add 200 uL of RIPA +PIC+phosphatase inhibitor or 300 ul R buff
   *RIPA = radioimmunoprecipitation assay buffer (lysis)
   *PIC = protease inhibitor cocktail ; stock 25x (1 tablet in 2 mL water or PBS)
   *phosphatase inhibitor stock 100x
   All these buffer on ice
3. Collect cell pellets with cell scraper in 1.5 mL tubes
4. Spin and rotate in cold room 30 mins
   *can add buffer mix and bring the plate on the shaker in cold room and collect the cell pellet later
5. Spin maximum speed 10 mins 4°c 21000 rcf
6. Collect supernatant
   *Pause point: keep supernatant in -20*c

- BCA rapid

*96-well transparent plate not for PCR. Albumin standard
*duplicate or triplicate
<Preparing at least 5 standard BSA (Bovine serum albumin) Stock BSA at 2 mg/mL, linear working range for BSA of 20 to 2000 μg/mL >

Microplate Standard Assay

Tube #	Standard Volume (μl)	Source of Standard	Diluent Volume (μl)	Final [Protein] (μg/ml)
1	20	2 mg/ml stock	0	2,000
2	30	2 mg/ml stock	10	1,500
3	20	2 mg/ml stock	20	1,000
4	20	Tube 2	20	750
5	20	Tube 3	20	500
6	20	Tube 5	20	250
7	20	Tube 6	20	125
8 (blank)	-	-	20	0

2.5ml : 0.05ml
5ml : 0.1ml fresh mixture!

1. Load 5 uL samples/std in the 96-well plate on ice
2. Prepare BCA rapid gold solution reagent A:B = 50 : 1 big brown A : small blue B
3. Add 100 uL BCA solution using multichannel, pipetting, beware of bubbles (multi-pipet)
   *the color change according to vol, time of reaction
5. Incubate at RT for at least 5 mins
6. Measure Absorbance 480 nm concentration of protein
7. Back calculate protein concentration from the standard curve

setting BCA_rapid Gold_ 480nm

5+20
125

- SDS-Gel running

SDS gel protocol-Biorad 14 max

1. Choose the smallest concentration as the baseline; good range is 20-25 ug/well and dilute with RIPA buffer to make all samples have equal protein 20-30

E.g.

Sample	Conc (ug/mL)	Vol(uL)	RIPA(uL)	Protein(ug)
1	600	30	0	18
2	900	20	10	18

100ul x 10 x 3

WB

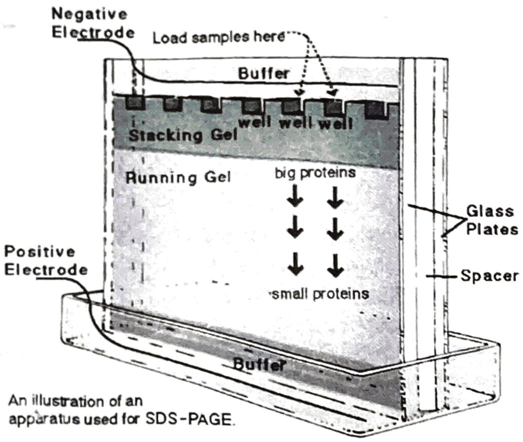
3	1630	11	19	18

4x Laemmli blue
DTT white powder

2. Prepare Laemmli sample buffer by adding a reducing agent (1:19)
   5 uL DTT + 95 uL 4xLaemmli
3. Dilute sample with 4xLaemmli (3:1)
   E.g. 1 lane = 30 uL sample + 10uL 4x Laemmli
4. Spin down and incubate at 99*c for 10 mins   linear protein
5. Choose appropriate pre-cast SDS gel concentration 4-20%, fill in some running buffer   8%-16% Gel, Tris-glds
6. Remove the strip on the lower end, put in the dock (the shorter plate go in front), fill the buffer to the level, remove the comb

running buff: 100 ml Tris-Glycine-SDS-Running buffer + 900 ml milliQ

7.



An illustration of an apparatus used for SDS-PAGE.

注意:
1. 撕封带
2. buffer 覆盖电极
3. 看气泡

8. Load 25-30 (max35) uL samples, 5 uL protein ladder   3ul 2.5ul
   Don't leave empty wells; add loading buffer to prevent skew protein migration
8. Set up : 150 V for 90 mins or until the loading dye go to the end (time not really matter)   170V 70V

- Transfer (Dry with iBlot)
1. Keep gel in buffer at all time
2. Soak the membrane in a milliQ
3. Crack gel open carefully, remove stacking part, cut gel from all margins
4. Put gel on a membrane without touching the protein in order (see instruction), use roller to eliminate air bubbles
   *arrange the top of the gel (higher kDa) toward the center of the membrane (higher electricity)
5. Turn the iBlot on :P0 protocol 7 mins

order: cover, top layer, filter, gel, bottom layer

Ragwan: 70V, 30 min start line; 120V 80 min; 170V 5min

- Antibody staining   antibody 1:1000 blocking buff
  *do not touch the blot, non-specific background
1. Cut membrane according to interested protein size
2. Blocking with 5% BSA/Milk/blocking buffer for 30-60 mins on rocker at RT or overnight at 4°c   16hr
3. Incubate the membrane with primary Ab in blocking buffer overnight at 4°c on rocker
   * 1* Ab can be reused, common concentration = 1:1000 default   1:2000 Jarid2   block buff
4. Wash with TBST 5 mins x3   milliQ 10 times
5. Incubate the membrane with fluorescent secondary Ab in blocking buffer at RT 1hr
   Commonly use Rabbit 800, Mouse 680 at 1:2000-4000   1:4000 weak   1:5000 default
6. Wash with TBST 5 mins x3   4
8. Image blot with Odyssey

Just cut
Don't discard

1ml primary
1ml second

首次 分 cut 太 小

3.34ul/10ml

PSI. admin admin   84 Res medium, 10 x 15
settings

blocking buffer

see anti:
Donkey anti-Rabbit
1:1000 → 1:3000 1:5000 blocking buff

MSI 5-10%

Ls180				HT115			
1	2	3	4	5	6	7	8
NTC	NA	g2-2	mix-1	NTC	NA	g1-2	g2-1

Stripping 15-30min

settings

BCA - rapid gold. ultransparent

480 wavelength

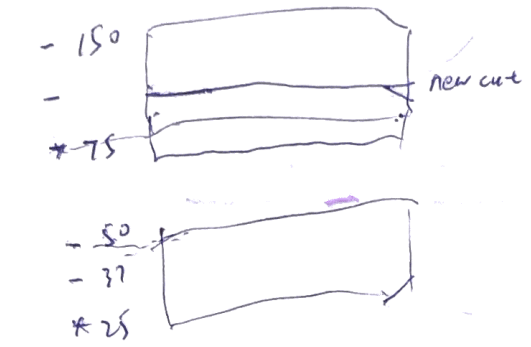
GREiner 96 U-Bottom Microplate PCR → V

shake
- Frequence 600 rpm
- Time 15 s

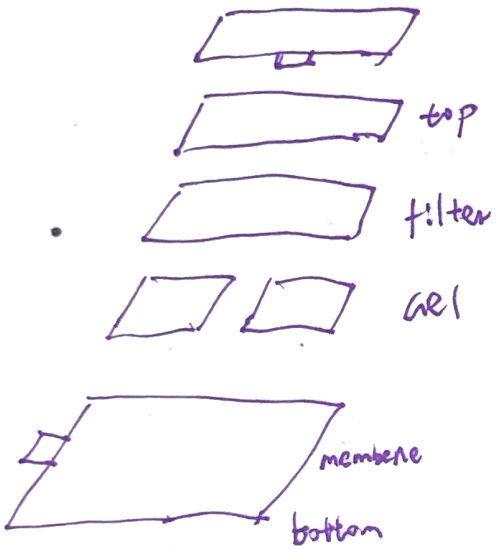
precision plus protein ladder

band

- Jarid 1 140 kda
- Sox9 72 kda
- Krt20 48 kda
- GAPDH 32 kda



order.



reuse WB

TBST wash 5min x 3 times