

SDS-PAGE Gel and Western blotting

Solutions:

Acrylamide-Bisacrylamide (40% acryl., 1.5% bis)

40 g acrylamide / up to 100 ml in H₂O

1.5 g bis / (store in 4°C)

Stacking Gel Buffer (SGB, 0.5 M Tris)

12.1 g Tris up to 200 ml of H₂O, PH 6.8

(store in 4°C)

Lower Gel Buffer (LGB, 1.5 M Tris)

36.3 g Tris, up to 200 ml of H₂O, PH 8.8

(store in 4°C)

20% Sodium Dodecyl Sulfate (SDS)

20 g SDS, up to 100 ml in H₂O

1.5% Ammonium Persulfate (APS)

1.5 g APS, up to 100 ml in H₂O

(Aliquot and store in -20°C)

0.5% TEMED

1 ml TEMED up to 200 ml of H₂O

(store in 4°C)

Make SDS-PAGE gel:

Assemble the gel cassette (The key for no leaking is every thing need to be very clean!)

Lower Gel	<u>11%</u>	<u>9%</u>	<u>7.5%</u>
40% AcBis	8.25 ml	6.75 ml	5.6 ml
4xLGB	7.5 ml	7.5 ml	7.5 ml
DH ₂ O	11.1 ml	12.6 ml	13.8 ml
20% SDS	150 ul	150 ul	150 ul
1.5% APS	1.5 ml	1.5 ml	1.5 ml
0.5% TEMED	1.5 ml	1.5 ml	1.5 ml

(use 1.5 mm spacer, enough for 4 gels)

Upper Gel

DH₂O 14.5 ml

Stacking Gel Buffer 2.5 ml

40% AcBis 2.5 ml

20% SDS 100 ul

10% APS 200 ul

TEMED 16 ul

Gel can be used 1 hour after it permeabilized. Or take the gel off from cassette store in moist chamber (i.e., with wet paper towels) at 4oC up to 1 ~ 2 weeks.

Western Blotting

1. Add sample buffer (with BME) to each sample, boil the sample for 4 minutes.
2. load the sample to SDS-PAGE gel.
3. after blue line reach to bottom of the gel, stop.
4. activate PVDF membrane (Immobilon) with Methnol for 30 sec., rinse with H₂O, equilibrate with blotting buffer at least 10 min. (use gloves, do not touch it with your finger)
5. Equilibrate gel in transfer buffer 2 x 10 minutes on shaker.
6. Assemble cassette in order: sponge, filter paper, PVDF membrane, gel, filter paper, sponge. Make sure [-] electrode (black)/gel/PVDF/[+] electrode(red or white).
7. Start Transfer. 400mA for one hour in 4oC.
8. Block non-specific binding to the membrane in 5% milk or 3% BSA in TBST for one hour in RT.
9. Primary antibody in 3%BSA (most AB 1:1000 dilution), RT 1 hour (Depend on antibody, some need longer time, 3 hours in RT or over night in 4oC).
10. Wash the membrane with TBST 3 x 5 min on shaker.
11. Secondary antibody in 3%BSA, 20 ~ 30 min RT. (sigma 1:4000 dilution)
12. Wash the membrane 3 x 10 min with TBST. Rinse once with H₂O.
13. Develop with SuperSignal West Pico Chemiluminescent substrate (Pierce# 34080).
14. Detect signal by image reader or film.

Buffers:

Sample Buffer:

5 ml Stacking Gel buffer
2 ml 10 % SDS
1 ml 0.1% Bromophenol blue
5 ml 60% Glycerol
+/- 1ml b-mercaptoethanol
6 ml H₂O

1 x Running Buffer

14.4 g Glycine / up to 1 Liter
3 g Tris / pH should be 8.3
2.5 ml of 20% SDS /

10 x Running Buffer

144g Glycine / up to 1 liter
30 g Tris / pH should be 8.3
25 ml of 20% SDS /

1 x Wash Buffer (TBST)

2.5 mM Tris

15 mM NaCl (Sodium Chloride)

0.005% Tween 20

10 x Wash Buffer (TBST)

25 mM Tris (30.28g) / up to 1 Liter

150 mM NaCl (Sodium Chloride, 87.66g) / pH 7.4

0.05% tween 20 (5 ml) /

1 x Transfer Buffer(1)

100 ml 10 x CAPS / up to 1 liter in water

100 ml methanol /

10 x CAPS (100 mM CAPS)

22.13 g CAPS

up to 1 liter water, pH 11.0

Transfer Buffer (2)

1 x Transfer buffer

100 ml of 10x stock / up to 1 liter with water

200 ml Methanol /

10 X Tris/Glycine

Tris base 30g / up to 1 liter in water

Glycine 144g /