

SDS Polyacrylamide Gel & Western Blot

An Introductory Protocol

Reagents for Gel

30% Bis/Acrylamide Mix (i.e., 29.2% acrylamide and 0.8% N,N'-methylene-bis-acrylamide)
1.5 M Tris, pH 8.8 (store at 4°C)
1.0 M Tris, pH 6.8 (store at 4°C)
10% Sodium Dodecyl Sulfate (SDS)
10% Ammonium Persulfate (APS)
TEMED (N,N,N',N'-tetramethylethylenediamine)

Water-Saturated n-Butanol

50 ml n-butanol
5 ml ddH₂O
Combine in bottle and shake. Use top phase to overlay gels. Store at room temperature (RT) indefinitely.

5X Tank Buffer

30.28 g Tris (FW 121.1)
144.13 g Glycine
10 g SDS (or 10 ml 10% SDS)
ddH₂O to **2 L**

10% APS

0.1 g Ammonium Persulfate
ddH₂O to 1.0 ml
Ideally use fresh. Can store at -20°C

2X Protein Loading Buffer

1.25 ml 1 M Tris pH 6.8
4 ml 10% SDS
2.0 ml glycerol
2.0 mg bromophenol blue
0.31 g dithiothreitol (DTT; FW 154.2)
ddH₂O to 10.0 ml
Store in 0.5 ml aliquots at -20°C for 6 months.

Equipment

Glass plates, casting stand, combs
Vertical gel unit & power supply
5 cc syringe with 27-gauge needle
100°C heat block

Reagents for Transfer to Nitrocellulose Membrane

Transfer buffer for SDS-proteins using nitrocellulose

3.03 g Tris
14.4 g Glycine
200 ml methanol
ddH₂O to 1 L

Equipment

Semi-dry Blot Transfer apparatus (BioRad) & power supply
Nitrocellulose (Schleicher & Schuell)
Whatman paper or blot transfer pads

Reagents for Western Blot

5X TBST Stock

25 ml 2 M Tris pH 8
150 ml 5 M NaCl
2.5 ml Tween-20
ddH₂O to 1 L

Working Solutions:

1X TBST + 5% milk (use Carnation Nonfat Evaporated Milk)

1X TBST + 0.5% milk

1X TBST

Ponceau Solution (ready made, stock solution)

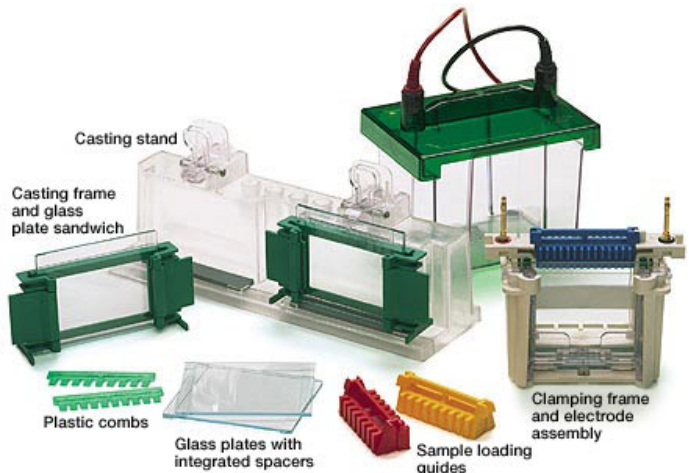
1° Antibody

2° Antibody (Horseradish peroxidase (HRP))

ECL reagent, i.e., LumiLight (Roche)

Protocol: SDS-polyacrylamide gel

1. Clean glass plates with ddH₂O & EtOH
2. Assemble glass plates in casting stand
3. Prepare Separating and Stacking, mixing all EXCEPT APS and TEMED (See page 5 for these solution recipes)



4. To separating mix, add APS & TEMED, immediately pipette into gel to about 1.5-2.0 cm below front/small glass plate
5. Add thin layer of H₂O-saturated n-butanol to aid edge polymerization and remove air bubbles
6. After polymerization (~15 min), pour off n-butanol (into sink)
7. To stacking mix, add APS & TEMED, immediately pipette onto gel until flash with top edge of small glass plate
8. Add comb, make sure it's centered. Allow stacking gel to polymerize
9. Once polymerized, remove comb, fill syringe and needle with 1X gel tank buffer and squirt into wells to remove bubbles. Make sure wells are clean with no residual acrylamide.
10. Meanwhile, add 2X protein loading buffer to protein samples (i.e., 10 μ l sample + 5 μ l buffer), mix, boil at 100°C for at least 5 min.
11. Plan loading order of samples; recommended: place molecular weight marker in non-equidistant well.
12. Place gel in electrode assembly; place gel into tank. Fill with 1X Gel Tank Buffer (inside electrode assembly and outside). Make sure interior of electrode assembly has equal or more buffer as outside.
13. Attach to electrode assembly/tank to power supply. Run at 110 V for ~1-2 hr or until you can see loading buffer reach bottom edge of separating gel.
14. Upon completion of gel run, disassemble. Carefully remove gel from between glass plates (use razor or ruler to separate glass plates). If gel sticks to glass plate, float off plate in Transfer Buffer.

Protocol: Transfer to Nitrocellulose

1. Place gel in dish containing Transfer Buffer. Allow to soak for ~10 min.
2. Per gel, cut to size: 1 piece of nitrocellulose and 6 pieces of Whatman paper or 2 thick blot pads. Place all in Transfer Buffer.
3. Layer onto Trans-Blot Electrophoretic Transfer cell from **bottom to top**:
 - a. 3 pieces Whatman (or 1 thick blot pad)
 - b. nitrocellulose
 - c. gel
 - d. 3 pieces of Whatman. (or 1 thick blot pad)
4. Make sure there are no air bubbles.

5. Run at 15V (small gels) for ~30-45 min.
6. Stain nitrocellulose with Ponceau to ensure protein transfer. Rinse with running water to remove Ponceau. Keep blot moist for storage (i.e., in blocking solution).

Protocol: Western Blot

1. Block in TBST + 5% milk overnight at 4°C
2. Incubate with primary antibody at appropriate dilution in TBST + 0.5% milk for at least 1 hr at RT. Use 3-5 ml.
3. Wash 3x ~15 min in TBST + 0.5% milk.
4. Incubate with secondary antibody at appropriate dilution in TBST + 0.5% milk at least 1 hr RT
5. Wash 2x 15 min ea in TBST + 0.5% milk
6. Wash 3x 15 min ea in TBST
7. Drain blot
8. Apply ECL reagent (i.e., LumiLight from Roche) per package instructions
9. Drain blot, place in sheet protector (keeping moist), bracket exposure times to film. I.e., develop film after 5-10 min exposure and 30-45 min exposure.

protein gels

10 ml separating
5 ml stacking

Separating Solution

s/Glycine SDS-Polyacrylamide Gel Electrophoresis

	5 ml	10 ml	15 ml	20 ml	25 ml	30 ml	40 ml	50 ml
6%								
H ₂ O	2.7	5.3	8.0	10.6	13.3	15.9	21.1	26.5
30% Acrylamide mix ^a	1.0	2.0	3.0	4.0	5.0	6.0	8.0	10.0
1.5 M Tris (pH 8.8)	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
10% SDS ^b	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
10% APS ^c	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED ^d	0.004	0.008	0.012	0.016	0.02	0.024	0.032	0.04
8%								
H ₂ O	2.3	4.6	7.0	9.3	11.6	13.9	18.6	23.2
30% Acrylamide mix	1.3	2.7	4.0	5.3	6.7	8.0	10.7	13.4
1.5 M Tris (pH 8.8)	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
10% SDS	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
10% APS (25%)	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED	0.003	0.006	0.009	0.012	0.015	0.018	0.024	0.03
10%								
H ₂ O	2.0	4.0	5.9	7.9	9.9	11.9	15.8	20
30% Acrylamide mix	1.7	3.3	5	6.7	8.3	10.0	13.3	16.6
1.5 M Tris (pH 8.8)	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
10% SDS	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
10% APS (25%)	0.05	0.1 (40 μ l)	0.15	0.2	0.25	0.3	0.4	0.5
TEMED	0.002	0.004	0.006	0.008	0.01	0.012	0.016	0.02
12%								
H ₂ O	1.7	3.3	5.0	6.6	8.3	9.9	13.2	16.4
30% Acrylamide mix	2.0	4.0	6.0	8.0	10.0	12.0	14.0	20.0
1.5 M Tris (pH 8.8)	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
10% SDS	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
10% APS (25%)	0.05	0.1 (40 μ l)	0.15	0.2	0.25	0.3	0.4	0.5
TEMED	0.002	0.004	0.006	0.008	0.01	0.012	0.016	0.02
15%								
H ₂ O	1.2	2.3	3.5	4.6	5.7	6.9	9.2	11.4
30% Acrylamide mix	2.5	5.0	7.5	10.0	12.5	25.0	20.0	25.0
1.5 M Tris (pH 8.8)	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
10% SDS	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
10% APS (25%)	0.05	0.1 (40 μ l)	0.15	0.2	0.25	0.3	0.4	0.5
TEMED	0.002	0.004	0.006	0.008	0.01	0.012	0.016	0.02

Stacking Solution

	1 ml	2 ml	3 ml	4 ml	5 ml	6 ml	8 ml	10 ml
STACK								
H ₂ O	0.68	1.4	2.1	2.7	3.4	4.1	5.5	6.8
30% Acrylamide mix	0.17	0.33	0.5	0.67	0.83	1.0	1.3	1.7
1.0 M Tris (pH 6.8)	0.13	0.25	0.38	0.5	0.63	0.75	1.0	1.25
10% SDS	0.01	0.02	0.03	0.04	0.05	0.06	0.08	0.1
10% APS (25%)	0.01	0.02	0.03	0.04	0.05 (20 μ l)	0.06	0.08	0.1
TEMED	0.001	0.002	0.003	0.004	0.005	0.006	0.008	0.01

^aCommonly 29.2% acrylamide and 0.8% N,N'-methylene-bis-acrylamide; ^bSodium dodecyl sulfate; ^cAmmonium persulfate; ^dN,N,N',N'-Tetramethylethylenediamine.

= vol for 2 mini
or gels