Protocol 107_Pouring protein gels (SDS-PAGE)

Stock Solutions

30% Acrylamide Mix (Biorad Cat # 161-0156)

- Store at 4° C (stored in the fridge near tissue culture hood in 3N42)

1.5 M Tris-Cl pH 8.8

27.23 g Tris base (tris (hydroxy methyl) aminomethane) ($C_4H_{11}NO_3$; mW: 121.14)
80 mL H_2O

- Adjust to pH 8.8 with HCl
- Make to 150 mL with H_2O
- Store at 4° C (stored in the cold room in designated bin)

1.0 M Tris-Cl pH 6.8

12.1 g Tris base (tris (hydroxy methyl) aminomethane) ($C_4H_{11}NO_3$; mW: 121.14)
80 mL H_2O

- Adjust to pH 6.8 with HCl
- Make to 100 mL with H_2O
- Store at 4° C (stored in the cold room in designated bin)

10% SDS

10 g SDS (sodium dodecyl sulfate) ($C_{12}H_{25}NaO_4S$; mW: 288.38)
90 mL H_2O

- Stir gently to dissolve
- Make to 100 mL
- Store at room temperature (stored at the bay designated for SDS-PAGE)

10% ammonium persulfate

0.1 g ammonium persulfate ($N_2H_8S_2O_8$; mW: 228.2)
1 mL H_2O

- Weigh out ammonium persulfate in eppendorf tube
- Add 1 mL of H_2O and mix by inversion
- Make up fresh every time

TEMED (Omnipur Cat # 8920)

- Store at 4° C (stored in the fridge near tissue culture hood in 3N42)

Tris-Saturated Butanol

50 ml butan-2-ol or *n*-butanol
5 ml 1.0 M Tris-Cl pH 6.8

- Combine in the bottle and shake
- Use the top phase to overlay the gels
- Store at room temperature (stored at the bay designated for SDS-PAGE)

Solutions for Tris/Glycine SDS-Polyacrylamide Gel Electrophoresis

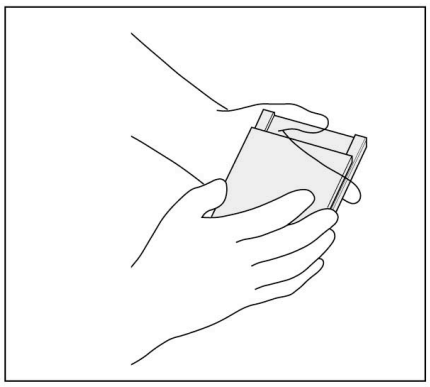
Volume (ml) of Components Required to Cast Gels of Indicated Volumes and Concentrations

Gel Volume	5 ml	10 ml	15 ml	20 ml	25 ml	30 ml	40 ml	50 ml
6% gel								
H_2O	2.6	5.3	7.9	10.6	13.2	15.9	21.2	26.5
30% acrylamide mix	1.0	2.0	3.0	4.0	5.0	6.0	8.0	10.0
Tris-Cl (1.5 M, pH 8.8)	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
SDS (10%)	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
10% APS	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED	0.004	0.008	0.012	0.016	0.02	0.024	0.032	0.04
8% gel								
H_2O	2.3	4.6	6.9	9.3	11.5	13.9	18.5	23.2
30% acrylamide mix	1.3	2.7	4.0	5.3	6.7	8.0	10.7	13.3
Tris-Cl (1.5 M, pH 8.8)	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
SDS (10%)	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
10% APS	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% gel								
H_2O	1.9	4.0	5.9	7.9	9.9	11.9	15.9	19.8
30% acrylamide mix	1.7	3.3	5.0	6.7	8.3	10.0	13.3	16.7
Tris-Cl (1.5 M, pH 8.8)	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
SDS (10%)	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
10% APS	0.05	0.1	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% gel								
H_2O	1.6	3.3	4.9	6.6	8.2	9.9	13.2	16.5
30% acrylamide mix	2.0	4.0	6.0	8.0	10.0	12.0	16.0	20.0
Tris-Cl (1.5 M, pH 8.8)	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
SDS (10%)	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
10% APS	0.05	0.1	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% gel								
H_2O	1.1	2.3	3.4	4.6	5.7	6.9	9.2	11.5
30% acrylamide mix	2.5	5.0	7.5	10.0	12.5	15.0	20.0	25.0
Tris-Cl (1.5 M, pH 8.8)	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
SDS (10%)	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
10% APS	0.05	0.1	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
Stack	**1 ml**	**2 ml**	**3 ml**	**4 ml**	**5 ml**	**6 ml**	**8 ml**	**10 ml**
H_2O	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
Tris-Cl (1.0 M, pH 6.8)	0.13	0.25	0.38	0.5	0.63	0.75	1.0	1.25
SDS (10%)	0.01	0.02	0.03	0.04	0.05	0.06	0.08	0.1
10% APS	0.01	0.02	0.03	0.04	0.05	0.06	0.08	0.1

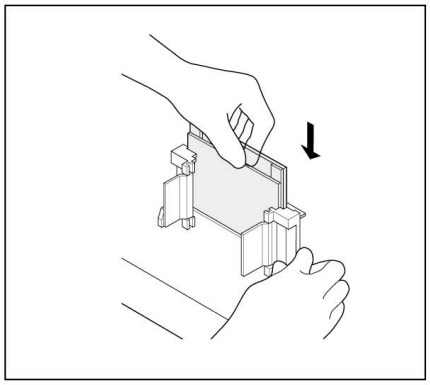
TEMED	0.001	0.002	0.003	0.004	0.005	0.006	0.008	0.01

Gel Cassette Preparation[1]

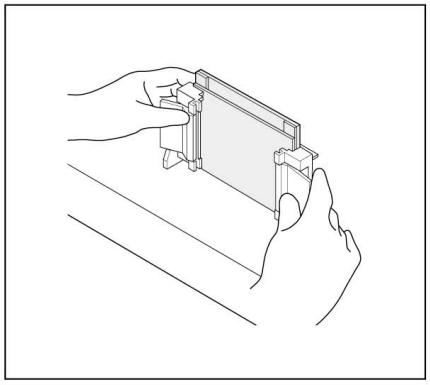
1. Place a Short Plate on top of the Spacer Plate.



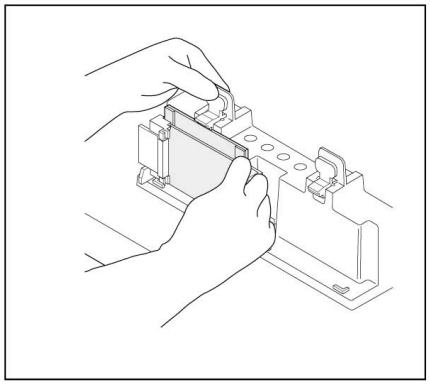
2. Slide the two plates into the Casting Frame, keeping the Short Plate facing front. Insure both plates are flush at the bottom on a level surface.



3. Lock the pressure cams to secure the glass plates.



4. Engage the spring loaded lever and place the gel cassette assembly on the gray casting stand gasket. Insure the horizontal ribs on the back of the Casting Frame are flush against the face of the Casting Stand and the glass plates are perpendicular to the level surface. The lever pushes the Spacer Plate down against the gray rubber gaskets.



Pouring Gels

1. Choose a percentage acrylamide based on the molecular weight range of proteins you wish to separate (for heavy and light chains of antibodies use 12% gel):

% Gel	M.W. Range
7	50 kDa - 500 kDa
10	20 kDa - 300 kDa
12	10 kDa - 200 kDa
15	3 kDa - 100 kDa

2. Now mix the ingredients needed for the chosen percentage and pour the solution quickly into your gel casting form - be sure to leave some room for the stacking gel (about 1 cm below the bottom of the comb for the stacking gel).
   * LEAVE OUT the ammonium persulfate and TEMED until you are ready to pour separating gels
3. Layer the top of the gel with TRIS-saturated butanol or, very carefully, with water
4. Wait for about 30 minutes for the gel to polymerize completely.
5. While waiting mix the reagents for the stacking gel
   * LEAVE OUT the ammonium persulfate and TEMED until you are ready to pour stacking gels
6. When the running gel is polymerized, pour out the butanol completely, rinse with water (from a spray bottle) and carefully dry the top of the gel with kimwipe (or your stacking gel may separate from running gel). If using water, just pour it out and carefully wipe the top of the glass with the kimwipe.
7. Mix in the polymerizing reagents and pour the stacking gel on top of the running gel. Insert green combs trying not to get bubbles underneath and allow another 30 min - 1 hour for complete polymerization.

Your gels are ready!