SDS Protein Gel (Price Lab)

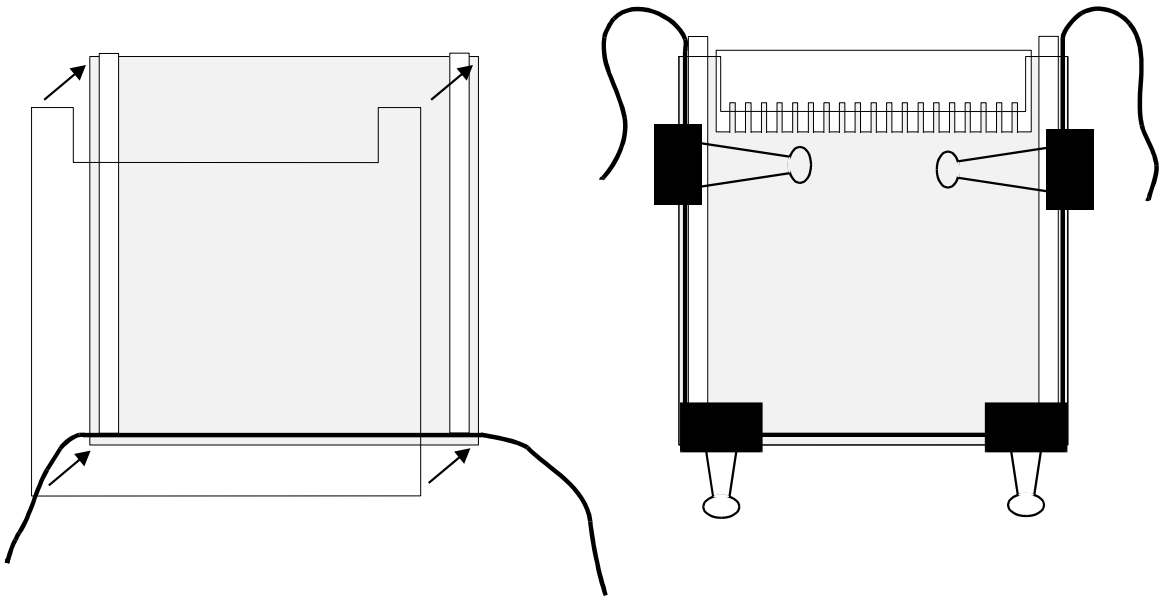
Materials:

Lower Tris (1.5 M Tris-HCl, pH 8.8)
30% Acrylamide/0.8% Bis
Upper Tris (0.5 M Tris-HCl, pH 6.8)
20% Acrylamide/1% Bis
10% SDS
10% Ammonium Persulfate
TEMED
1 M DTT (stored at -80°C in aliquots)
1% agarose in Tris/glycine (or thin silicone tubing)
10 x Tris/glycine buffer (30 g Tris + 144 g glycine per liter)

Setting up the gel:

1. Wash plates, spacers, and comb very well (Formula 409 cleaner), and let them air dry in a clean plastic finger rack.

2. Assemble the gel by placing the two side spacers onto the square plate so that 1-2 mm of glass is left exposed on the outside edges. Put silicone tubing across the bottom edge of the plate, and set the top glass plate (with cut out on top) onto the bottom plate. Clamp the bottom corners (temporarily) with small clamps. Pull the tubing up along the sides of the spacers, and clamp the sides with large spacers. Remove the small, temporary clamps and clamp the bottom of the gel with two large clamps (positioned at the outside edges of the plates, and snug against the bottom). Stand the gel upright so that it sits on the two bottom clamps and adjust the gel to level.



3. To a small side arm flask add the Lower Tris, 30% acrylamide, and water. Degas the solution under vacuum (rubber stopper and aspirator) by swirling the liquid and tapping smartly on the bench until bubbles cease to appear.

4. Add 10% SDS, 10% APS and TEMED. Pour the solution down the inside of the glass plates and avoid air bubbles. Leave about 2.5 cm for a stacking gel. Layer water saturated butanol carefully on top and allow gel to polymerize. Remove butanol after polymerization and rinse the top of the gel with water.

5. Make stacking gel in the same manner as steps 3 & 4, but use Upper Tris and 20% acrylamide. Make sure that no air bubbles are present when comb is inserted into the gel.

6. Make one liter of running buffer (1x Tris/glycine + 0.1% SDS) and pour half into the upper buffer chamber and half into the lower buffer chamber. Remove bubbles from under the gel using a bent Pasteur pipet or a 10 ml syringe with a bent, dull needle.

7. Samples should be in 1% SDS, 10 mM DTT, 4% Ficoll, 10 mM Tris (pH 7.5). Heat samples to 80-90°C for two minutes before loading on the gel.

8. Attach (+) electrode to lower tray and (-) electrode to upper tray. [*SDS proteins run toward the (+) electrode*.] Run at constant current: 7 mA overnight or 25 mA for 4-5 hours.

Running gel

Straight percentage

12.5%	**30 ml**
30% Acrylamide/0.8% Bis	12.5 ml
Lower Tris (1.5 M Tris, pH 8.8)	7.5 ml
Water	9.5 ml
10% SDS	300 µl
10% APS	150 µl
TEMED	15 µl

6-15% gradient

6% (upper component)	**13.5 ml**
30% Acrylamide/0.8% Bis	2.7 ml
Lower Tris (1.5 M Tris, pH 8.8)	3.4 ml
Water	7.2 ml
10% SDS	135 µl
10% APS	67.5 µl
TEMED	6.8 µl

15% (lower component)	**13.5 ml**
30% Acrylamide/0.8% Bis	6.8 ml
Lower Tris (1.5 M Tris, pH 8.8)	3.4 ml
2.2 M Sucrose	3.2 ml
10% SDS	135 µl
10% APS	67.5 µl
TEMED	6.8 µl

Stacking gel

4%	**10 ml**
20% Acrylamide/1% Bis	2.0 ml
Upper Tris (0.5 M Tris, pH 6.8)	2.5 ml
Water	5.3 ml
10% SDS	100 µl
10% APS	50 µl
TEMED	10 µl

Sample buffer	**10 ml**	**5x concentration**
Ficoll	2 g	20%
SDS	1 g	10%
1 M Tris (pH 7.5)	500 µl	50 mM

•Add bromophenol blue to the desired level.
•Just before use, 1 M DTT must be added to the 5x sample buffer to achieve 50 mM.

PolyBlot Electrotransfer System

The polyblot manual for protein transfer to other membranes is located in the file cabinet of the technician desk, under the P listing. Information on how to set up the apparatus, voltage setting, running time, buffers recipes and other specifications are in this manual.

PolyBlot Buffers

Buffer 1 (Anode buffer 1)
0.3M Tris, 20% Methanol, pH 10.4

Buffer 2 (Anode buffer 2)
25mM Tris, 20% Methanol, pH 10.4

Buffer 3 (Cathode buffer)
25mM Tris, 40mM 6-Aminohexanoic Acid, 20% Methanol, pH 9.4

Note*: 6-Amino Caproic Acid is a common name for the 6-Aminohexanoic Acid