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SDS SLAB GEL ELECTROPHORESIS

This describes Laemmli gradient gels and can be easily modified for nongradient gels.

1. Use throughly clean glass plates with plain notch for forming sample slots or beveled notch for applying tube gels for 2D electrophoresis.

2. Heat 1.5% agar in D H_2O on boiling water bath.

3. Arrange spacers on gel with binder clamps and add agar as sealant. Leave ~1 mm space between bottom and side spacers and add extra agar to form a triangular shaped pool about 0.5 cm inside corners—this should prevent leaks here.

4. Prepare lower and upper gel solutions. Add all ingredients but ammonium persulfate (AP) and TEMED. Prepare gradient forming apparatus.

5. Mark the glass at x-3 to x-4 cm above the lower spacer, where x is the total height from notch to lower spacer. Mark this at both sides of gel to show height of lower gel to allow leveling. This leaves 3 to 4 cm for the upper gel, of which about 1.5 cm is used by slots. Add 10% AP (stored frozen aliquots) to the lighter lower gel solution and deaerate. Do likewise for heavier solution. Add TEMED, mix, and add to gradient former. Add the light solution first and pump until tubing is just filled. Then add heavy solution, and commence stirring and pumping to slabs. For gradient formation by pump, one line will lead from light to heavy and two lines from the stirred heavy solution to the slabs. For nongradient gels, pour directly from erlenmeyer flask, avoiding bubbles.

6. When acrylamide solution reaches proper height, stop and add ~ 1/8'' layer of isobutanol. After this, slabs cannot be moved until polymerized. Polymerization is finished when a refractive boundary appears beneath the isobutanol-acrylamide interface and spreads to the sides.

7. After waiting at least 15 minutes after polymerization appears completed on the sides, pour off the isobutanol and unpolymerized material and quickly wash with 0.1% SDS. Immediately add 10% AP to the upper gel solution, deaerate, and pour a portion onto lower gel for 2 minutes. Decant, add TEMED to remaining upper gel solution, pipette on top avoiding bubbles, and add comb. Allow to stand until a sample in a pasteur pipette is polymerized.

8. 15 min to 1 h later, remove comb. Straighten slots with a 2'' 20 ga. needle. Remove clamps and bottom spacer and mount on slab apparatus. Add reservoir buffer to upper chamber to just cover sample slots and to bottom chamber and squirt away all bubbles underneath slab.

9. Reservoir buffer and samples should be prepared during waits for polymerization.

10. Sample preparation—to samples containing 1-10μg protein per band, or

radioactivity, add 20-40 µl sample buffer (total volume should be 50 µl or less for 1.6mm thick slabs and 26 or 30 slot combs). For soluble proteins, mix and place in boiled water bath at least 5 minutes. Insoluble proteins as precipitates tend to coagulate and remain resistant to solubilization if incubated immediately at 100°. For antibody precipitates, incubate ~1h at 56° in sample buffer before boiling. For affinity gel-bound proteins, add sample buffer and allow the dense solution to sink into the gel for 10 minutes, then place in boiling water bath, vortex, and boil longer. Include molecular weight markers.

11. Add samples to the slots using a 50 µl Hamilton syringe. Place sample buffer in empty slots. Fill the remainder of the upper chamber with buffer.

Electrophorese (+ pole to bottom), beginning at 40V until bromophenol blue dye passes into lower gel and then the voltage may be raised to 150V. The total run for an average 13.5cm high gel is 700 V hours. Runs may also be conducted O/N at a voltage calculated to give 700 V hours by the next morning.

12. When dye is ~1cm from bottom of gel, remove slab from apparatus, prise apart plates at bottom, leaving gel adhering to one, mark lower left corner (viewed from front of apparatus) with punch, and invert the plate lose above a baking dish or tupperware container containing the appropri-te solution and allow gel to peel off and fall in.

A. Fluorography: proceed as described for PPO impregnation or other techniques.

B. Autoradiography: it is advantageous to soak the gel in 1% acetic acid for 30 minutes to remove substances causing gels to stick to film; soaking in glycerol solutions is also sometimes recommended but I have not tried this.

C. Staining: Stain with Coomassie blue according to Vesterberg (BBA 243: 345).

13. Drying: (Note: Stained gels are best photographed before drying). Transfer to oversize piece of Whatman 3MM paper by pouring off both solutions leaving gel in a backwards orientation, press on wet Whatman paper, invert tray, and allow gel to peel from tray. Cover with Saran Wrap, dry (1-3h depending on vacuum, thickness of slab, etc., check thickness and temperature of gel to determine if dry, but do not lift rubber gasket until done). Then label Whatman paper with notebook page and date (with radioactive ink for fluor- or- autoradiography).

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SOLUTIONS FOR LAEMMLI STACKING SDS POLYACRYLAMIDE GELS

(See 'SDS Slab Gel Electrophoresis' for procedures)

A. 30% acrylamide + 0.8% methylenebisacrylamide (MBA) Bis
   4 g MBA
   150 g acrylamide (Sigma, BDH, Eastman, GS)
   Bring to total volume of 500 ml with D H_2O,
   filter through pre-rinsed Whatman No. 1.
   Caution: Avoid breathing or touching acrylamide or MBA.
   Protect from light, store 4°.

B. 30% acrylamide + 1.6% MBA
   30 g acrylamide
   1.6 g MBA
   100 ml with D H_2O
   Filter through pre-rinsed Whatman No. 1 Protect from light, store 4°.

C. Lower Tris (4x) (1.5 M tris-HCl, pH 8.8, 0.4% SDS)
   90.85 g tris base
   350 ml D H_2O
   Add conc. HCl to bring to pH 8.8
   20 ml 10% SDS
   Bring to 500 ml with D H_2O, millipore, store sterile r.t.

D. Upper Tris (4x) (0.5 M Tris-HCl, pH 6.8, 0.4% SDS)
   30.3 g tris base
   350 ml D H_2O
   Add conc. HCl to bring to pH 6.8 (avoid overshooting pH)
   20 ml 10% SDS
   Bring to 500 ml with D H_2O, millipore, store sterile r.t.

E. Tris-Glycine Reservoir Buffer
   (it is easiest to prepare this fresh each time).
   3 g tris base
   14.4 g glycine
   10 ml 10% SDS
   990 ml D H_2O
   This is sufficient for one medium slab gel apparatus.

F. Sample Buffer
   10 ml glycerol
   30 ml 10% SDS
   5 ml upper tris (4x)
   10 mg bromophenol blue
   55 ml D H_2O
   For nonreducing gels, add 50 mM iodoacetamide and use fresh.
   For reducing gels, add 5% 2-mercaptoethanol.
   Store at 4°, place in 37° bath before use to dissolve SDS.

G. 10% w/w ammounium persulfate (AP)
500 mg ammonium persulfate
4.5 ml D H_2O
Store 200 µl aliquots at -80°.

H. Lower Slab Gel Gradient Formulas

	5%	7%	8%	10%	12%	15%
30% acryl 0.8% MBA	5	7	8	10	12	15
lower tris (4x)	7.5	7.5	7.5	7.5	7.5	7.5
D H_2O	17.5	15.5	14.5	12.5	10.5	6
glycerol	—	—	—	—	—	1.5
10% AP	30 µl	25 µl	25 µl	20 µl	15 µl	80 µl
TEMED	20 µl	20 µl	20 µl	20 µl	20 µl	20 µl

Note: These formulas are for forming gradients using 5, 7, 8, 10% or 12% in one chamber and 15% in the other. The glycerol is to make the 15% denser. The amounts of AP and TEMED have been found empirically to give polymerization in 30-60 min, but this depends on variables such as oxygen concentration, which inhibits polymerization, and batches of chemicals. Normally, the higher the acrylamide concentration, the lower the AP. However, the AP in the 15% acrylamide was raised because this solution is stirred and hence aerated during gradient formation.

For each slab of 1.6 mm x 138 x 125 mm, 28-30 ml of solution is sufficient.

I. Upper Gel

30% acryl 1.6% MBA	5.2 ml
upper tris (4x)	10 ml
D H_2	24.8 ml
10% AP	100 µl
TEMED	40 µl

Note: The 1.6% MBA actually yields larger pores and greater mechanical stability.