SDS-PAGE Gels

Adapted from David Bowtell (bowtell@ariel.ucs.unimelb.edu.au)

Preparation of PAGE gels.

1. Clean glass plates with ethanol and assemble casting stand, see Biorad instruction manual

2. Mix solutions for lower gel in order shown ie. TEMED last and pour into plates leaving about 2cm at the top. There should be sufficient room to allow such that when the comb is inserted later there is about 5-8mm between the floor of the well and the top of the lower gel.

3. Carefully overlay with DDW and allow to set. Once the gel has set it can be wrapped in gladwrap and stored for several days at least in the fridge.

4. To continue remove overlay water, mix solutions for upper gel in order shown and overlay lower gel. Insert comb and allow to set for 15-20'. Generally the upper gel should not be prepared until the samples are ready as there is a pH difference between the two gels which will diffuse with time.

5. Assemble running unit, see Biorad instruction manual and aspirate bubbles and liquid from wells. Do this immediately before samples are ready as the wells will dry out quite quickly.

6. Load 10ul - 25ul (10ul for a 15 tooth mini comb) and then overlay samples with 1 x SDS running buffer. Empty wells should have 10-15ul sample buffer added and be overlayed as with the test samples so the gel runs straight. Flood the upper chamber by carefully adding 1 x SDS running buffer to unit. Avoid pouring the buffer directly onto the wells.

7. Run gels 200V for 30-45 min until dye front is at the bottom of gel. (Large BioRad gels -15 X 15cm - are run at 35mA per gel).

8. See below for Western transfers. For gels to be stained, stain for 20 min with Coomassie blue solution (see below), then destain in strong destain (50% methanol, 10% acetic acid) 20 min and leave in 10% acetic acid.

Reagents for SDS PAGE

Stock solutions:

A. Acrylamide=30 g acrylamide/0.8 g Bis to 100 ml with Super Q water and filtered through 0.2 μm filter.

B. 1.5 M Tris, pH 8.8= 36.3 g Tris in 100 ml water. pH to 8.8 and adjust to 200ml.

C. 0.5 M Tris, pH 6.8=6 g Tris in 40 ml water. pH to 6.8 and then adjust to 100 ml.

D. 10% ammonium persulfate=0.1 g in 1 ml water

E. 10% SDS

3% Stacking layer: 6.3 ml water/2.5 ml soln C/0.1 ml 10% SDS/1.2 ml soln A/10 μl TEMED/100 μl persulfate

5X Tray Buffer/liter: 15 g Tris/72 g glycine/5 g SDS. Dilute 1:5 for upper tray and 1:10 for lower tray.

Rules of thumb

1. All acrylamide solutions are to be filtered sterilized (0.2 μm).

2. All buffers and solutions are to be made up in only Super Q water (except for tray buffer which can be made up in good deionized water).

3. Deareate all acrylamide solutions 15 minutes before adding TEMED and ammonium persulfate.

4. Use 3 liters in lower buffer chamber and precool while gel is polymerizing.

5. Samples applied to the gel should have a 15-45 μl volume. Smaller volumes may cause problems with the gel.

Gel Composition

29 ml				45 ml			
Component	5%	7.5%	10%		5%	7.5%	10%
water	16.1	13.6	11.7		24.15	20.4	17.55
Soln. B	7.5	7.5	7.5		11.25	11.25	11.25
SDS	0.3	0.3	0.3		0.45	0.45	0.45
Soln A	5	7.5	10		7.5	11.25	15
TEMED (μl)	15	15	15		20	20	20
persulfate (μl)	150	150	150		200	200	200

Sample Buffers:

	Reducing Buffer (1:1)	Reducing Buffer (1/3 X)	Non-reducing Buffer
Tris buffer	(0.5 M Tris pH 6.8) 2.5 ml	1 M Tris pH 6.8 2.5 ml	0.5 M Tris pH 6.8 1 ml
glycerol	2 ml	4 ml	4 ml
20% SDS	2 ml	SDS solid 0.8 g	10% SDS 1 ml
2-ME	1 ml	2 ml	
0.05% BPB	0.8 ml	0.1% BPB 0.8 ml	0.05% BPB 0.2 ml
water	1.7 ml	to 10 ml	4.7 ml

Dehydrating Solution

30% Methanol-2.5% glycerol

Linear Acrylamide

make up a 3% solution in distilled water (stir overnight). Use 1.2 ml/12 mls gradient solution. Gallard-Schlessinger Chemical Mfg. Co.

Carle Place, NY 11514
Product # 29788
516-333-5600, TWX 510-222-5059, Telex 967792

Gradient Gels

Recipe for 30 ml Gels: GEL PERCENTAGE

Component	5%	6%	7.5%`	10%	12%	15%
water	6.8	6.33	5.74	4.4	3.8	2.54
50% glycerol	0.1	0.11	0.11	0.12	0.2	0.3
1.5 M Tris, pH 8.8	3	3	3	3	3	3
10% SDS	0.12	0.12	0.12	0.12	0.12	0.12
Acrylamide	2	2.4	3	4	4.8	6
TEMED (μl)	5	5	5	5	5	5
10% persulfate (μl)	35	35	35	35	35	35

Stacking Gel

6 ml water/2.5 ml 0.5 M Tris pH 6.8/0.1 ml 10% SDS/1.17 ml Acrylamide/15 μl TEMED/200 μl 10% persulfate.

Other Recipes (Bowtell)

30% acryl/bis-acrylamide: Bis: acrylamide 1:36 513.5 ml 342.3ml; acrylamide 150 g 100g; bis-acrylamide4.1 g 2.73g; DDW to 513.5 ml 342.3ml; Filter and store at 4 C. Use Biorad acrylamide, bis-acrylamide

SDS sample buffer 1x (clear) pH 6.8 250 ml total; Trizma base1.9 g; Glycerol 31.42g; SDS 5.75 g; DDW to 250 ml

2X SDS SB: As above and then to final volume of 125ml; pH with concentrated HCl. NOTE. Add the HCl slowly once the pH reaches ~7.2 as it will fall rapidly thereafter and if you overshoot you must start again.

Add DTT or 2-mercaptoethanol immediately prior to using: to 1 ml SDS SB add 50ul 1M DTT (from frozen stock) or 40 ul 2-ME solution.

Loading sample buffer with bromophenol blue

20 ml 1 x SDS sample buffer

2 ml bromophenol blue (1mg/ml) 0.19% in DDW. Aliquot 1ml and store 4¡C.

10x SDS running buffer pH ~8.3 (2 Lt total): Trizma base60.6 g; Glycine 288.0 g; SDS 20 g; DDW to 2 Lt. NOTE: Do not pH

Lower gel buffer (LGB); pH 8.8 (500 ml total); Trizma base91 g; SDS 2 g; DDW 450 ml, pH using concentrated HCl; Add DDW to give 500 ml total volume

Upper gel buffer (stacking gel)pH 6.8 (250 ml total); Trizma base15 g; SDS 1 g; DDW 200 ml; pH using concentrated HCl; Add DDW to give 250 ml total volume

Coomassie blue (2 Lt): 2 g Coomassie brilliant blue powder (BioRad); 1 Lt methanol; 200 mlacetic acid; 800 mlDDW; Stir for a minimum 2 hrs; Filter through Whatman filter discs

Prestained molecular weight markers: Reconstitute lyophilized standards GIBCO BRL #6041 LA in 500ul 1 mM DDT; Aliquot 7.5 ul per tube and freeze. To use add 13 ul sample buffer, boil 3 min and load 10 ul/well/ per gel.

Molecular weight markers (1:20 dilution): 50ul Biorad broad/low molecular weight markers (Low MW #161 0304 Broad MW #161 0317); 950ul loading sample buffer; Aliquot 10 ul each eppendorf, freeze, load 10 ul/well/gel

Remazole blue molecular weight markers.

1. Dilute 10 mg remazole brilliant blue (Sigma #R8001) in 1.0ml 10% SDS (can be frozen as a stock).

2. Dilute 100 ul BioRad broad range MW marker in 100 ul DDW (see above)

3. Dialyse O/N in 1lt coupling buffer (0.2M Na2HPO4, pH 9.2)

4. Dialyse for several hrs next day in 1 lt fresh coupling buffer.

5. Add 50 ul remazole solution to 200 ul dialysed MW markers and heat to 70 C for 30 min.

6. Dilute to 1.0 ml with SDS sample buffer and store as 25 ul aliquots at -20 C.