SDS-PAGE Protocol

SDS-PAGE Solutions

40% Acrylamide (37.5:1)

Acrylamide	116.8 g
N,N'-Methylene bisacrylamide	3.2 g
DDI H_2O	to 300 ml

Filter and store in a dark bottle at 4°C. (We buy this premade)

30% Ammonium Persulfate

Ammonium Persulfate	1.5 g
DDI H_2O	5 ml

Store at 4°C. Replace every month.

RG Buffer--1.5 M Tris•Cl, pH 8.8

DDI H_2O	300 ml
Tris–free base	90.75 g
Conc. HCl	8 ml

Adjust to pH 8.8 with conc. HCl, and bring final volume to 500 ml with DDI H_2O.

SG Buffer--1.0 M Tris•Cl, pH 6.8

DDI H_2O	300 ml
Tris–free base	60.54 g
Conc. HCl	36 ml

Adjust to pH 6.8 with conc. HCl, and bring final volume to 500 ml with DDI H_2O.

4x SDS-PAGE Sample Buffer

125 mM Tris•HCl, pH 6.8	1 M	5 ml
20% Glycerol		8 ml
4% SDS	20%	8 ml
10% ß-Mercaptoethanol		4 ml
0.5 mg/ml Bromophenol Blue		20 mg
DDI H_2O		15 ml
Total		40 ml

10x SDS-PAGE Running Buffer

30.3 g	Tris base
144.0 g	Glycine
10.0 g	SDS

Dissolve and bring total volume to 1,000 ml with deionized water. Do not adjust pH with acid or base (pH is normally 8.3 as prepared).

Coomassie Stain Solution

Ethanol	150 ml
Glacial Acetic Acid	50 ml
DDI H_2O	300 ml
Coomasie Brilliant Blue–R-250	1 g

Dissolve Coomasie Brilliant Blue–R-250 in EtOH first.

Destain Solution

Ethanol	1200 ml
Glacial Acetic Acid	400 ml
DDI H_2O	2.4 l

Mini-Protean SDS-PAGE Protocol

Casting the Gel

1] Assemble glass plates and spacers in gel casting apparatus–see BioRad instruction manual.

2] Mix the components for the resolving gel as described in the Mini-Protean II protocol.

3] Pour the resolving gel mixture into the gel plates to a level 2 cm below the top of the shorter plate.

4] Pace a layer of DDI H_2O over the top of the resolving gel to prevent meniscus formation in the resolving gel.

5] Allow resolving gel to stand 30 min at room temperature.

6] Drain the DDI H_2O from top of the resolving gel. Rinse with DDI H_2O, drain, and wick any remaining DDI H_2O away with a Kimwipe.

7] Mix components for stacking gel.

8] Pour stacking gel solution into gel plates (on top of running gel), so that gel plates are filled. Insert comb to the top of the spacers.

9] Allow gel to stand for at least 1 hr at room temperature, or overnigt at 4°C (wrapped in saran wrap).

Preparing Samples

Note: 10 well combs will hold up to 30 μl of prepared sample. 15 well combs will hold up to 20 μl of prepared sample.

Cell Samples

1] Harvest 100 μl of cells at O.D. > 0.6. Decant the supernatant media.

2] Resuspend cells in 20 μl of 2x sample buffer.

3] Incubate tubes in boiling water for 5 min.

4] Centrifuge at 12,000 x g for 30 s.

Solution Samples

1] Place a volume of protein solution (or 1 μl of standard) into a μfuge tube, such that there

is 5-10 μg of protein in the solution.

2] And an equal volume of 2x sample buffer (or 10 μl for standards).

3] Incubate tubes in boiling water for 5 min.

4] Centrifuge at 12,000 x g for 30 s.

Running the Gel

1] Remove comb and assemble cast gel into Mini-Protean II apparatus.

2] Add freshly prepared 1x running buffer (300 ml) to both chambers of the apparatus.

3] Load the prepared samples into the wells of the gel.

4] Run the gel at 100 V until the dye front migrates into the running gel (~15 min), and increase to 200 V until the dye front reaches the bottom of the gel (~45 min).

Staining & Destaining the Gel

1] Remove the run gel from the aparatus and remove the spacers and glass plates. Place the gel into a small tray. *Note:* Never us a metal spatula to separate the glass plates.

2] Add ~20 ml staining solution and stain for > 30 min with gentle shaking.

3] Pour off and save the stain.

4] Add ~5 ml destain solution and destain for ~1 min with gentle shaking.

5] Pour off and discard the destain solution. Add ~ 30 ml of destain solution.

6] Destain with gentle shaking until the gel is visibly destained (> 2 hr).

5] Pour off and discard the destain solution.

6] Rinse with DDI H_2O. Add ~30 ml DDI H_2O and rinse for 5 min with gentle shaking.

7] Dry the gel on the gel dryer at 60°C for 1 hr with a sheet of Whatman filter paper below the gel and a piece of Seran wrap over the gel.

% Acrylamide in running gel	Separation size range (kDa)
Single percentage:	
5%	100–250
7.5%	40–200
10%	30–150
12%	20–120
15%	10–100
18%	6–50
Gradient:	
4–15%	20–250
4–20%	10–200
10–20%	10–100
8–16%	6–70

From the BioRad Readygel Manual.

Adapted from Laemmli, U.K. (1970) Nature 227, 680-685. (see page 681).

Mini-Protean II
SDS-PAGE Gels

Resolving Gel	**8 %**		**10%**	
	1 gel	2 gels	1 gel	2 gels
DDI H_2O	1.8 ml	3.6 ml	1.6 ml	3.2 ml
1.5 M Tris-HCl, pH 8.8 (RG Bfr.)	1.3 ml	2.6 ml	1.3 ml	2.6 ml
40% Acrylamide Stock	800 μl	1.6 ml	1.0 ml	2.0 ml
20 % SDS	100 μl	200 μl	100 μl	200 μl
30% Ammonium Persulfate	10 μl	20 μl	10 μl	20 μl
TEMED	4 μl	8 μl	4 μl	8 μl

	12 %		**15%**	
	1 gel	2 gels	1 gel	2 gels
DDI H_2O	1.4 ml	2.8 ml	1.1 μl	2.2 ml
1.5 M Tris-HCl, pH 8.8 (RG Bft.)	1.3 ml	2.6 ml	1.3 ml	2.6 ml
40% Acrylamide Stock	1.2 ml	2.4 ml	1.5 ml	3.0 ml
20 % SDS	100 μl	200 μl	100 μl	200 μl
10% Ammonium Persulfate	10 μl	20 μl	10 μl	20 μl
TEMED	4 μl	8 μl	4 μl	8 μl

Stacking Gel (4 %)

	2 gels
DDI H_2O	3.9 ml
1.0 M Tris-HCl, pH 6.8 (SG Bfr.)	500 μl
40% Acrylamide Stock	500 μl
20 % SDS	100 μl
30% Ammonium Persulfate	16 μl
TEMED	8 μl

Note: Always add Ammonium Persulfate and TEMED immediately before casting gel.

Number of gels refers to 1 mm thick gels. Use the 2 gel recipe for 1.5 mm thick gels.