SDS and native polyacrylamide gel electrophoresis of proteins

Supplies and Reagents

Acrylamide solutions (see Table 1 and Table 2 for recipes)

Premixed stock solutions are commercially available (e.g., Invitrogen)

Ammonium persulfate stock solution (10% w/v)

Dissolve 1 g ammonium persulfate in 10 mL of H_2O and store at 4°C.

Ammonium persulfate decays slowly in solution, so replace the stock solution every 2-3 weeks. Ammonium persulfate is used as a catalyst for the copolymerization of acrylamide and bisacrylamide gels. The polymerization reaction is driven by free radicals generated by an oxido-reduction reaction in which a diamine (e.g., TEMED) is used as the adjunct catalyst.

Isobutanol (overlay for gels containing ~10% acrylamide)

SDS (0.1%) (overlay for gels containing ~8% acrylamide)

Protein standard molecular-weight markers (e.g., Invitrogen, or xxx)

Protein samples to be resolved (e.g., purified protein or cell lysates)

SDS stock solution (10% w/v, electrophoresis grade) for resolving and stacking gels

Dissolve 10 g of SDS in 80 mL of H_2O, and then add H_2O to 100 mL.

This stock solution is stable for 6 months at room temperature.

1× SDS gel-loading buffer

100 mM Tris-Cl (pH 6.8)

4% (w/v) SDS (sodium dodecyl sulfate; electrophoresis grade)

0.2% (w/v) bromophenol blue

20% (v/v) glycerol

200 mM DTT (dithiothreitol)

Store the SDS gel-loading buffer without DTT at room temperature. Add DTT from a 1 M stock just before the buffer is used.

200 mM β-mercaptoethanol can be used instead of DTT.

TEMED (electrophoresis grade)

Tris-Cl (1.5 M, pH 8.8) and (1.0 M, pH 6.8)

To prepare a 1 M solution, dissolve 121.1 g of Tris base in 800 mL of H_2O. Adjust the pH to the desired value by adding concentrated HCl. Allow the solution to cool to room temperature before making final adjustments to the pH. Adjust the volume of the solution to 1 L with H_2O. Dispense into aliquots and sterilize by autoclaving.

1×Tris-glycine buffer

Prepare a 5x stock solution in 1 liter of H_2O.

15.1 g Tris base

94 g glycine (electrophoresis grade)

50 ml of 10% SDS (electrophoresis grade)

The 1×working solution is 25 mM Tris-Cl/250 mM glycine/0.1% SDS. Use Tris-glycine buffers for SDS-polyacrylamide gels.

Equipment

Erlenmeyer flask or disposable plastic tube

Hamilton microliter syringe or micropipettor equipped with gel-loading tips

Hypodermic needle (blunt) attached to a syringe

Pasteur pipette

Power supply (capable of supplying up to 500 V and 200 mA)

Squirt bottle for H_2O

Vertical electrophoresis apparatus (e.g., BioRad)

Water bath or heating block, preset to 100°C or, for extremely hydrophobic proteins, 45-55°C.

Protocol of Pouring SDS-Polyacrylamide Gels

1. Assemble the glass plates according to the manufacturer's instructions.

2. Determine the volume of the gel mold (this information is usually provided by the manufacturer). In a flask or plastic tube, prepare the resolving gel using the appropriate volume of solution containing the desired concentration of acrylamide using the values given in Table 1. Polymerization will begin as soon as the TEMED has been added. Without delay, swirl the mixture rapidly and proceed to the next step.

The concentration of ammonium persulfate recommended here and in Step 5 is higher than that used by some investigators. This eliminates the need to rid the acrylamide solution of dissolved oxygen by degassing.

3. Pour the acrylamide solution into the gap between the glass plates. Leave sufficient space for the stacking gel (the length of the teeth of the comb plus 1 cm). Use a Pasteur pipette to overlay the acrylamide solution carefully with water, or 0.1% SDS (for gels containing ~8% acrylamide) or isobutanol (for gels containing ~10% acrylamide). Place the gel in a vertical position at room temperature.

The overlay prevents oxygen from diffusing into the gel and inhibiting polymerization. Isobutanol dissolves the plastic of some minigel apparatuses.

4. After polymerization is complete (30 minutes), pour off the overlay and wash the top of the gel several times with deionized H_2O to remove any unpolymerized acrylamide. Drain as much fluid as possible from the top of the gel, and then remove any remaining H_2O with the edge of a paper towel.

5. In a disposable plastic tube, prepare the stacking gel using the appropriate volume of solution containing the desired concentration of acrylamide using the values given in Table 2.

Polymerization will begin as soon as the TEMED has been added. Without delay, swirl the mixture rapidly and proceed to the next step.

6. Pour the stacking gel solution directly onto the surface of the polymerized resolving gel. Immediately insert a clean Teflon comb into the stacking gel solution. Avoid trapping air bubbles. Add more stacking gel solution to fill the spaces of the comb completely. Place the gel in a vertical position at room temperature.

Teflon combs should be cleaned with H_2O and dried with ethanol just before use.

Preparation of Samples and Running the Gel

7. While the stacking gel is polymerizing, prepare the samples in the appropriate volume of SDS gel-loading buffer and heat them to 100°C for 3 minutes to denature the proteins.

Be sure to denature a sample containing marker proteins of known molecular weights. Mixtures of appropriately sized polypeptides are available from commercial sources.

Extremely hydrophobic proteins, such as those containing multiple transmembrane domains, may precipitate or multimerize when boiled for 3 minutes at 100°C. To avoid these pitfalls, heat the samples for 1 hour at a lower temperature (45-55°C) to denature.

8. After polymerization is complete (30 minutes), remove the Teflon comb carefully. Use a squirt bottle to wash the wells immediately with H_2O to remove any unpolymerized acrylamide. If necessary, straighten the teeth of the stacking gel with a blunt hypodermic needle attached to a syringe. Mount the gel in the electrophoresis apparatus. Add Tris-

glycine electrophoresis buffer to the top and bottom reservoirs. Remove any bubbles that become trapped at the bottom of the gel between the glass plates with a bent hypodermic needle attached to a syringe.

Do not pre-run the gel before loading the samples, since this will destroy the discontinuity of the buffer systems.

9. Load up to 15 µl of each of the samples in a predetermined order into the bottom of the wells. Use a Hamilton microliter syringe or a micropipettor, equipped with gel-loading tips, that is washed with buffer from the bottom reservoir after each sample is loaded. Load an equal volume of 1X SDS gel-loading buffer into any wells that are unused.

10. Attach the electrophoresis apparatus to an electric power supply (the positive electrode should be connected to the bottom buffer reservoir). Apply a voltage of 8 V/cm to the gel. After the dye front has moved into the resolving gel, increase the voltage to 15 V/cm and run the gel until the bromophenol blue reaches the bottom of the resolving gel (~4 hr). Then, turn off the power supply.

11. Remove the glass plates from the electrophoresis apparatus and place them on a paper towel. Use an extra gel spacer to carefully pry the plates apart. Mark the orientation of the gel by cutting a corner from the bottom of the gel that is closest to the left-most well (slot 1).

Do not cut the corner from gels that are to be used for immunoblotting.

12. At this stage, the gel can be fixed, stained with Coomassie Brilliant Blue or silver salts, fluorographed or autoradiographed, or used to establish an immunoblot.

Native PAGE

The straightforward approach to native PAGE is to leave out the SDS and reducing agent (DTT) from the standard SDS-PAGE mentioned above. The gel and electrophoresis solutions are prepared without SDS.

REFERENCES

Harlow E. and Lane D. 1988. *Antibodies: A laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Table 1

Solutions for preparing resolving gels for Tris-glycine SDS-PAGE; For native-PAGE just leave out SDS in any solution.

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

Table 2

Solutions for preparing 5% stacking gels for Tris-glycine SDS-PAGE, Native–PAGE do not contain SDS.

	Volume (ml) of Components Required to Cast Gels of Indicated Volumes							
Components Gel Volume⇒	**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
ammonium persulfate (10%)	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