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Protocol

SDS-Polyacrylamide Gel Electrophoresis of Proteins

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This protocol was adapted from "Commonly Used Techniques in Molecular Cloning," Appendix 8, in *Molecular Cloning*, Volume 3, 3rd edition (eds. Sambrook and Russell). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA, 2001.

INTRODUCTION

This protocol describes the separation of proteins by SDS-polyacrylamide gel electrophoresis. SDS is used with a reducing agent and heat to dissociate the proteins. SDS-polypeptide complexes form and migrate through the gels according to the size of the polypeptide. By using markers of known molecular weight, the molecular weight of the polypeptide chain(s) can be estimated.

MATERIALS

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)

Isobutanol (overlay for gels containing ~10% acrylamide)

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

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

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

Exclusive use of one brand of SDS is recommended to ensure reproducible results.

1X SDS gel-loading buffer

TEMED (electrophoresis grade)

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

1X Tris-glycine buffer

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., Invitrogen)

Use only one type of apparatus in a given lab if possible. This makes it easier to compare the results obtained by different investigators and allows parts to be reused.

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

METHOD

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 an Erlenmeyer flask or disposable 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 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 1X 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.

REFERENCES

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

Caution

Acrylamide:bisacrylamide

Acrylamide:bisacrylamide, see Acrylamide; Bisacrylamide

Caution

Ammonium persulfate

Ammonium persulfate $(NH_4)_2S_2O_8$ is extremely destructive to tissue of the mucous membranes and upper respiratory tract, eyes, and skin. Inhalation may be fatal. Wear appropriate gloves, safety glasses, and protective clothing. Always use in a chemical fume hood. Wash thoroughly after handling.

Caution

General warning

This material contains hazardous components. Please see recipe for full details.

Caution

Isobutanol

Isobutanol (Isobutyl alcohol) is extremely flammable and may be harmful by inhalation or ingestion. Wear appropriate gloves and safety glasses. Keep away from heat, sparks, and open flame.

Caution

SDS (Sodium dodecyl sulfate)

SDS (sodium dodecyl sulfate) is toxic, an irritant, and poses a risk of severe damage to the eyes. It may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety goggles. Do not breathe the dust.

Caution

TEMED

TEMED *N,N,N′,N′*-Tetramethylethylenediamine is extremely destructive to tissues of the mucous membranes and upper respiratory tract, eyes, and skin. Inhalation may be fatal. Prolonged contact can cause severe irritation or burns. Wear appropriate gloves, safety glasses, and other protective clothing. Use only in a chemical fume hood. Wash thoroughly after handling. Flammable: Vapor may travel a considerable distance to source of ignition and flash back. Keep away from heat, sparks, and open flame.

Recipe

2X SDS gel-loading buffer

100 mM Tris-Cl (pH 6.8)

4% (w/v) SDS (electrophoresis grade)

0.2% (w/v) bromophenol blue

20% (v/v) glycerol

200 mM dithiothreitol (DTT)

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

Store the SDS gel-loading buffer without thiol reagents at room temperature. Add the thiol reagents just before the buffer is used.

Recipe

Ammonium persulfate

To prepare a 10% (w/v) solution: 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.

Recipe

SDS (10%) stock solution

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 mo at room temperature.

Recipe

Tris-Cl

Tris base

HCl

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.

pH	HCl
7.4	70 mL
7.6	60 mL
8.0	42 mL

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.

If the 1 M solution has a yellow color, discard it and obtain Tris of better quality. The pH of Tris solutions is temperature-dependent and decreases ~ 0.03 pH units for each 1°C increase in temperature. For example, a 0.05 M solution has pH values of 9.5, 8.9, and 8.6 at 5°C, 25°C, and 37°C, respectively.

Recipe

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

Table

Solutions for preparing 5% stacking gels for Tris-glycine SDS-polyacrylamide gel electrophoresis

	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

Modified from Harlow and Lane (1988).

Table

Solutions for preparing resolving gels for Tris-glycine SDS-polyacrylamide gel electrophoresis

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

Modified from Harlow and Lane (1988).