Protocol

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One-Dimensional SDS-PAGE Protocol

Introduction

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is a method commonly used in protein characterization studies. In this technique, protein samples are loaded onto a polyacrylamide gel that has pores through which the protein migrates in response to an electrical field. SDS-PAGE can give insight into protein size, subunit composition, amount, and can also purify sample for further analysis. Here, we describe a general protocol for the preparation of an SDS-PAGE gel and the electrophoresis of protein samples.

Materials

- Acrylamide/Bis-Acrylamide (GoldBio Catalog # B-900)
- Tris-Base (GoldBio Catalog # T-400)
- TEMED (N,N,N,N - tetramethylethylenediamine)
- Separating and stacking gel solutions
- H_2O-saturated isobutyl alcohol
- 1x Tris-Cl/SDS at pH 8.8 (dilute 4x Tris-Cl/SDS at pH 8.8, from Methods section)
- Protein sample, on ice
- 2x and 1x SDS sample buffer
- Protein molecular weight standards
- 6x SDS sample buffer
- Ammonium Persulfate
- 1x SDS electrophoresis buffer
- Electrophoresis apparatus: 16-cm cell with clamps, glass plates, casting stand and buffer chambers
- 0.75 mm spacers
- 0.45 µm filters (for stock solution preparation)
- 25 ml Erlenmeyer side-arm flasks
- Vacuum pump with cold trap
- 0.75 mm Teflon comb with 1, 3, 5, 10, 15 or 20 teeth
- Screw-top microcentrifuge tubes (recommended)
- 25 or 100 µl syringe with flat-tipped needle
- Constant-current power supply

Method

Preparation of gels and solutions

1. For Separating Gel
    a. In a 25 ml side-arm flask, mix 30% acrylamide/0.8% bisacrylamide solution, 4x Tris-Cl/SDS at pH 8.8, and H_2O (see Table 1).
    b. Degas under vacuum ~5 minutes.

c. Add 10% ammonium persulfate and TEMED. Swirl gently to mix. Use immediately.

2. For Stacking Gel (3.9% Acrylamide)
   a. In a 25 ml side-arm flask, mix 0.65 ml of 30% acrylamide/0.8% bisacrylamide, 1.25 ml of 4x Tris-Cl/SDS at pH 6.8, and 3.05 ml H_2O.
   b. Degas under vacuum for 10 to 15 minutes.
   c. Add 25 µl of 10% ammonium persulfate and 5 µl TEMED. Swirl gently to mix. Use immediately.

3. For 30% Acrylamide/0.8% Bisacrylamide Solution
   a. Mix 30.0 g acrylamide and 0.8 g N,N'-methylenebisacrylamide with H_2O in a total volume of 100 ml.
   b. Filter the solution through a 0.45 µm filter and store at 4°C in the dark. The 2x crystallized grades of acrylamide and bisacrylamide are recommended.
   c. Discard after 30 days because acrylamide gradually hydrolyzes to acrylic acid and ammonia.

   CAUTION: Acrylamide monomer is neurotoxic. A mask should be worn when weighing acrylamide powder. Gloves should be worn while handling the solution, and the solution should not be pipetted by mouth.

4. For 4X Tris-Cl/SDS at pH 6.8 (0.5M Tris-Cl containing 0.4% SDS)
   a. Dissolve 6.05 g Tris base in 40 ml H_2O. Adjust to pH 6.8 with 1N HCl.
   b. Add H_2O to 100 ml total volume.
   c. Filter the solution through a 0.45 µm filter, add 0.4 g SDS and store at 4°C for up to 1 month.

5. For 4x Tris-Cl/SDS at pH 8.8 (1.5M Tris-Cl containing 0.4% SDS)
   a. Dissolve 91 g Tris base in 300 ml H_2O.
   b. Adjust to pH 8.8 with 1N HCl.
   c. Add H_2O to 500 ml total volume.
   d. Filter the solution through a 0.45 µm filter, add 2 g SDS and store at 4°C up to 1 month.

Pouring the separating gel

1. Assemble the glass-plate sandwich of the electrophoresis apparatus according to manufacturer's instructions using two clean plates and two 0.75 mm spacers.

   Note: If needed, clean the glass plates in liquid Liquinox or RBS-35. These aqueous based solutions are compatible with silver and Coomassie blue staining procedures.

2. Lock the sandwich to the casting stand.

3. Prepare the separating gel solution as directed in Section 1 Preparation of gels and solutions, degassing using a rubber-stoppered 25-ml Erlenmeyer side-arm flask connected with vacuum tubing to a vacuum pump with a cold trap. After adding the specified amount of 10% ammonium persulfate and TEMED to the degassed solution, stir gently to mix.

   Note: The desired percentage of acrylamide in the separating gel depends on the molecular size of the protein being separated. Generally, use 5% gels for SDS-denatured proteins of 60 to 200 kDa, 10% gels for SDS-denatured proteins of 16 to 70 kDa and 15% gels for SDS-denatured proteins of 12 to 45 kDa (see Table 1).

   Note: The stacking gel is the same regardless of the separating gel used.

4. Using a Pasteur pipet, apply the separating gel solution to the sandwich along an edge of one of the spacers until the height of the solution between the glass plates is ~ 11 cm.

   Note: Use the solution immediately; otherwise it will polymerize in the flask.

   Note: Sample volumes < 10 µl do not require a stacking gel. In this case, cast the resolving gel as usual, but extend the resolving gel into the comb (step 10) to form the wells. The proteins are then separated under the same conditions as used when a stacking gel is present. Although this protocol works well with single-concentration gels, a gradient gel is recommended for maximum resolution.

5. Using another Pasteur pipet, slowly cover the top of the gel with a layer (~1 cm thick) of H_2O-saturated isobutyl alcohol, by gently layering the isobutyl alcohol against the edge of one and then the other of the spacers.

   Note: Be careful not to disturb the gel surface. The overlay provides a barrier to oxygen, which inhibits polymerization and allows a flat interface to form during gel formation.

Note: The H_2O-saturated isobutyl alcohol is prepared by shaking isobutyl-alcohol and H_2O in a separatory funnel. The aqueous (lower) phase is removed. This procedure is repeated several times. The final upper phase is H_2O-saturated isobutyl alcohol.

6. Allow the gel to polymerize for 30 to 60 minutes at room temperature.

Note: A sharp optical discontinuity at the overlay/gel interface will be visible on polymerization. Failure to form a firm gel usually indicated a problem with the ammonium persulfate, TEMED, or both. Ammonium persulfate solution should be made fresh before use and 'crackle' when added to the water. If not, fresh ammonium persulfate should be acquired. Purchase TEMED in small bottles so, if necessary, a new previously unopened source can be tried.

Pouring the stacking gel

1. Pour off the layer of H_2O-saturated isobutyl alcohol and rinse with 1x Tris-CL/SDS at pH 8.8.

Note: Residual isobutyl alcohol can reduce resolution of the protein bands; therefore, it must be completely removed. The isobutyl alcohol overlay should not be left on the gel longer than 2 hours.

Table 1. Volumes (ml) of stock solutions required to prepare the Separating Gel.

Stock Solution	Final acrylamide concentration in separating gel (%)									
	5	6	7	7.5	8	9	10	12	13	15
30% acrylamide/ 0.8% bisacrylamide	2.50	3.00	3.50	3.75	4.00	4.50	5.00	6.00	6.50	7.50
4x Tris-Cl/SDS at pH 8.8	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75
H_2O	8.75	8.25	7.75	7.50	7.25	6.75	6.25	5.25	4.75	3.75
10% (w/v) ammonium persulfate	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
TEMED	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

2. Prepare the stacking gel solution as directed in Table 1.

Note: Use the solution immediately to keep it from polymerizing in the flask.

3. Using a Pasteur pipet, allow the stacking gel solution to trickle slowly into the center of the sandwich along an edge of one of the spacers until the height of the solution in the sandwich is ~1 cm from the top of the plates.

   Note: Be careful not to introduce air bubbles into the stacking gel.

4. Insert a 0.75 mm Teflon comb into the layer of stacking gel solution. If necessary, add additional stacking gel to fill the spaces in the comb completely.

   Note: Again, be careful not to trap air bubbles in the tooth edges of the comb; they will cause small circular depressions in the well after polymerization that will lead to distortion in the protein bands during separation.

5. Allow the stacking gel solution to polymerize for 30 to 45 minutes at room temperature.

   Note: A sharp optical discontinuity will be visible around the wells on polymerization. Again, failure to form a firm gel usually indicates a problem with the ammonium persulfate, TEMED or both.

Preparing the sample and loading the gel

1. Dilute a portion of the protein sample to be analyzed 1:1 (v/v) with 2x SDS sample buffer and heat 3-5 minutes at 100°C in a sealed screw-cap microcentrifuge tube. Vortex briefly. If the sample is a precipitated protein pellet, dissolve the protein in 50 to 100 µl of 1x SDS sample buffer and boil for 3-5 minutes at 100°C. Vortex briefly. Dissolve protein molecular weight standards in 1x SDS sample buffer according to supplier's instructions; use these standards as controls (Table 2).

   Note: For dilute protein solutions, consider using 5:1 protein solution/6x SDS sample buffer to increase the amount of protein loaded. Proteins can also be concentrated by precipitation in acetone, ethanol, or trichloroacetic acid (TCA), but losses will occur.

   Note: For a 0.8 cm wide well, 25 to 50 µg total protein in < 20 µl is recommended for a complex mixture when staining with Coomassie blue, and 1 to 10 µg total protein is needed for samples containing one or a few proteins. If silver staining is used 10 to 100 fold less protein can be applied (0.01 to 5 µg in < 20 µl depending on sample complexity).

Note: To achieve the highest resolution possible, the following precautions are recommended. Prior to adding the sample buffer, keep samples at 0°C. Add the SDS sample buffer (room temperature) directly to the 0°C sample (still on ice) in a screw-top microcentrifuge tube. Cap the tube to prevent evaporation, vortex and transfer directly to a 100°C water bath for 3-5 minutes. Let immunoprecipitates dissolve for 1 hour at 56°C in 1x SDS sample buffer prior to boiling. DO NOT leave the sample in SDS sample buffer at room temperature without first heating to 100°C to inactivate proteases. Endogenous proteases are very active in SDS sample buffer and will cause severe degradation of the sample proteins after even a few minutes at room temperature. To test for possible proteases, mix the sample with SDS sample buffer without heating and leave at room temperature for 1 to 3 hours. A loss of high-molecular weight bands and a general smearing of the banding pattern indicate a protease problem. Once heated, the samples can sit at room temperature for the time it takes to load samples.

2. Carefully remove the Teflon comb without tearing the edges of the polyacrylamide wells. After the comb is removed, rinse wells with 1x SDS electrophoresis buffer.

   Note: The rinse removes unpolymerized monomer; otherwise, the monomer will continue to polymerize after the comb is removed, creating uneven wells that will interfere with sample loading and subsequent separation.

3. Using a Pasteur pipet, fill the wells with 1x SDS electrophoresis buffer.

   Note: If well walls are not upright, they can be manipulated with a flat-tipped needle attached to a syringe.

4. Attach gel sandwich to upper buffer chamber following manufacturer's instructions.

5. Fill lower buffer chamber with the recommended amount of 1x SDS electrophoresis buffer.

6. Place sandwich attached to upper buffer chamber into lower buffer chamber.

7. Partially fill the upper buffer chamber with 1x SDS electrophoresis buffer so that the sample wells of the stacking gel are filled with buffer.

   Note: Monitor the upper buffer chamber for leaks and, if necessary, reassemble the unit. A slow leak in the upper buffer chamber may cause arcing around the upper electrode and damage the upper buffer chamber.

8. Using a 25 or 100 µl syringe with a flat-tipped needle, load the protein sample(s) into one or more wells by carefully applying the sample as a thin layer at the bottom of the wells. Load control wells with molecular weight standards. Add an equal volume of 1x SDS sample buffer to any empty wells to prevent spreading of adjoining lanes.

   Note: Disposable loading tips can be used with automatic pipettors to simplify loading. Preparing the samples at approximately the same concentration and loading an equal volume to each well will ensure that all lanes are the same width and that the proteins run evenly. If unequal volumes of sample buffer are added to wells, the lane with the larger volume will spread during electrophoresis and constrict the adjacent lanes, causing distortions.

   Note: The samples will layer on the bottom of the wells because the glycerol added to the sample buffer gives the solution a greater density than the electrophoresis buffer. To keep bands tight, hold the tip of the needle near the bottom of the well and load the samples slowly. The bromophenol blue in the sample buffer makes sample application easy to follow visually.

9. Fill the remainder of the upper buffer chamber with additional 1x SDS electrophoresis buffer so that the upper platinum electrode is completely covered. Do this slowly so that samples are not swept into adjacent wells.

Running the gel

1. Connect the power supply to the cell and run at 10 mA of constant current for a slab gel 0.75 mm thick, until the bromophenol blue tracking dye enters the separating gel. Then increase the current to 15 mA.

   Note: For a standard 16 cm gel sandwich, 4 mA per 0.75 mm thick gel will run ~15 hours (i.e. overnight); 15 mA per 0.75 mm gel will take 4 to 5 hours. To run two gels or a 1.5 mm thick gel, simply double the current. When running a 1.5 mm gel at 30 mA, the temperature must be controlled (10° to 20°C) with a circulating constant-temperature water bath to prevent 'smiling' (curvature in the migratory band). Temperatures < 5°C should not be used because SDS in the running buffer will precipitate.

   Note: If the level of buffer in the upper chamber decreases, a leak has occurred.

2. After the bromophenol blue tracking dye has reached the bottom of the separating gel, disconnect the power supply.

Disassemble the gel

1. Discard electrophoresis buffer and remove the upper buffer chamber with the attached gel sandwich.

2. Orient the gel so that the order of the sample wells is known, remove the sandwich from the upper buffer chamber and lay the sandwich on a sheet of absorbent paper or paper towels.

3. Carefully slide one of the spacers halfway from the edge of the sandwich along its entire length. Use the exposed spacer as a lever to pry open the glass plate, exposing the gel.

4. Carefully remove the gel from the lower plate. Cut a small triangle off one corner of the gel so the lane orientation is not lost during staining and drying. Proceed with protein detection.

Note: Gradient gels are most easily picked up without tearing from the high concentration end of the gel using gloved fingers. Single concentration gels < 10% can be picked up and placed in fixative, but are more easily removed if first immersed in fixative while left on the plate, allowing the gel to float off.

Note: The gel can be stained with Coomassie blue or silver, or proteins can be electroeluted, or electroblotted onto a polyvinylidene difluoride (PVDF) membrane for subsequent staining or sequence analysis or transferred to a membrane for immunoblotting. If the proteins are radiolabeled, they can be detected by autoradiography.

Table 2. Molecular weights of protein standards

Protein	Molecular Weight (in Da)
Cytochrome C (GoldBio Catalog # C-640)	11,700
A-Lactalbumin	14,200
Lysozyme (hen egg white) (GoldBio Catalog # L-040)	14,300
Myoglobin (sperm whale)	16,800
B-Lactoglobulin	18,400
Trypsin Inhibitor (soybean) (GoldBio Catalog # T-166)	20,100
Trypsinogen, PMSF treated	24,000
Carbonic anhydrase (bovine erythrocytes)	29,000
Glyceraldehyde-3-phosphate dehydrogenase (rabbit muscle)	36,000

Lactate dehydrogenase (porcine heart)	36,000
Aldolase	40,000
Ovalbumin	45,000
Catalase	57,000
Bovine Serum Albumin (GoldBio Catalog # A-421)	66,000
Phosphorylase *b* (rabbit muscle)	97,400
B-Galactosidase	116,000
RNA polymerase, *E. coli*	160,000
Myosin, heavy chain (rabbit muscle)	205,000

Tips

- Deaerating the gel solution before polymerization catalysts are added will speed up polymerization. Deaerating is not recommended for gradient gel protocols.
- SDS-PAGE running buffers can be reused two times without increased background signals. Buffers can be used more than two times but running time increases.
- If separated protein will be used for sequence analysis, ensure that only highly pure reagents are used.
- If proteins will undergo sequence analysis, add 0.1mM thioglycolic acid to the upper gel buffer.
- If proteins will undergo renaturation or sequencing, let the gel rest for at least 5 hours post-polymerization to allow ammonium persulfate and TEMED to react with gel components, reducing their chance of reacting with the amino-terminal end of the peptide.
- SDS can be purified by recrystallization.
- After a separating gel is poured and polymerized, it may be stored up to 48 hours at 4°C with an overlay of the same buffer used to prepare the gel. This overlay should be added slowly down the spacer edge to prevent disturbance of the gel interface. Immediately before use, pour the stacking gel or the buffers in the two gels will mix.
- If Coomassie blue is being used, protein of interest amount should be 0.2 to 1 µg in a mixture of proteins.
- In a typical gel, one can load 30-50 µg of sample in a total volume of < 20 µl in each well. In mini-gels, the wells should not contain more than 150 µg.
- Protein samples should be vortexed before and after heating.
- High salt concentrations should be avoided because they cause gel artifacts. If the salt concentration of a sample is high, then concentrate the protein with 10% (w/v) trichloroacetic acid (TCA). Collect the precipitated protein by centrifugation and wash

the pellet with cold acetone. Resuspend the pellet in appropriate buffer. If too dilute, then TCA can also be used to concentrate the samples.

- Uneven heating may cause differential migration of proteins causing smiling (outer lanes move more slowly than the center lanes). This can be prevented by filling the lower buffer chamber with buffer to the level of the sample wells while maintaining a constant temperature between 10° and 20°C and stirring the lower buffer with a magnetic stirrer. Or, the gel could be run at a lower current.
- Heating the sample to 100°C in sample buffer may cause aggregation of proteins resulting in a smear or Coomassie blue material at the top of the gel. This may be prevented by using specific protease inhibitors during isolation and/or lower heating temperatures (70° to 80°C for 3 to 5 minutes for an SDS sample buffer preparation).
- Samples boiled in sample buffer can be divided into aliquots and stored for > 4 weeks at -20°C or for at least a week at 4°C. These should be warmed at 37°C for 1 to 2 minutes before use. However, repeated freeze-thawing can lead to protein degradation.
- Ensure that enough bromophenol blue is added to be able to visualize the samples when loading into the well.
- Prepare 100 ml of Laemmli sample buffer and divide into 1 ml aliquots to increase reproducibility.
- Before loading double check wells for damage and do not overfill to prevent artifacts.
- Add 10 µl of 1x sample buffer to unused well lanes to avoid gel effects.
- Tris base should be used, not Tris HCl.
- It may be advantageous to limit amount of SDS in the gel to prevent micelle formation.
- Single % gels lacking SDS could be stored for longer periods than normal gels.
- Gel enclosed in a sealed bag containing tissue paper soaked in 0.02% sodium azide can last up to 1 month.
- SDS concentration should not exceed 200 µg/30 µl sample.
- High concentrations of SDS may interfere with Coomassie blue staining times.
- If electrophoresis is done at low temperatures, use lithium dodecyl sulfate (LiDS) instead of SDS. LiDS does not precipitate at low temperatures.

Associated Products

- Cytochrome C (GoldBio Catalog # C-640)
- Lysozyme (hen egg white) (GoldBio Catalog # L-040)
- Trypsin inhibitor (soybean) (GoldBio Catalog # T-166)
- Bovine serum albumin (GoldBio Catalog # A-421)
- Acrylamide/Bis-Acrylamide (GoldBio Catalog # B-900)

- Tris-Base (GoldBio Catalog # T-400)
- BLUEstain™ 2 Protein ladder, 5-245 kDa (GoldBio Catalog # P008)
- BLUEstain™ 2 Protein ladder, 11-245 kDa (GoldBio Catalog # P007)

References

Gallagher, S. R. (2012). One-Dimensional SDS Gel Electrophoresis of Proteins. *Current Protocols in Molecular Biology, 97*(1). Doi:10.1002/0471140864.ps1001s68.