Protocol

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis of Proteins

Clara L. Kielkopf, William Bauer, and Ina L. Urbatsch

Most analytical electrophoreses of proteins are achieved by separation in polyacrylamide gels under conditions that ensure dissociation of proteins into individual polypeptide subunits and minimize aggregation. Most commonly, the anionic detergent sodium dodecyl sulfate (SDS) is used in combination with a reducing agent (β-mercaptoethanol or dithiothreitol) and with heating to dissociate proteins before loading onto the gel. SDS binding denatures the polypeptides and imparts a negative charge that masks their intrinsic charge. The amount of SDS bound is generally sequence-independent and proportional to molecular weight; at saturation, approximately one SDS molecule is bound per two amino acids, or ~1.4 g of SDS per gram of polypeptide. Therefore, the migration of SDS–polypeptide complexes in an electric field is proportional to the relative size of the polypeptide chain, and its molecular weight can be estimated by comparison to protein markers of known molecular weight. However, hydrophobicity, highly charged sequences, and certain posttranslational modifications such as glycosylation or phosphorylation may also influence migration. Thus, the apparent molecular weight of modified proteins does not always accurately reflect the mass of the polypeptide chain. This protocol describes preparation and running of SDS-PAGE gels, followed by staining to detect proteins using Coomassie Brilliant Blue. Finally, the stained SDS-PAGE gel may be scanned to an image or preserved by drying.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.

Reagents

Acrylamide (30%) and *N*,*N*′-methylene-bis-acrylamide mixture (29:1 [w/w] ratio)

See Table 1 for resolving gel recipes and Table 2 for stacking gel recipes.

Several manufacturers sell electrophoresis-grade acrylamide that is free of contaminating metal ions. A stock solution containing 29% (w/v) acrylamide and 1% (w/v) N,N′-methylene-bis-acrylamide may be prepared in deionized warm H_2O to assist dissolution of bis-acrylamide. Alternatively, premixed solutions are convenient and safer to use than the powdered reagents and may be purchased from several suppliers (including National Diagnostics, Fisher BioReagents, and Sigma-Aldrich, among others). These premixed solutions may have a higher total concentration of acrylamide (e.g., 40%) and/or a different ratio of acrylamide:bis-acrylamide (19:1, 29:1, and 37.5:1 are common ratios). Increasing the amount of bis-acrylamide cross-linker decreases the pore size of the gel; therefore, the guidelines for total acrylamide concentrations given in Table 3 may need to be adjusted if a different ratio of cross-linker is used.

During storage, slow deamination of acrylamide and bis-acrylamide to acrylic acid and bis-acrylic acid is catalyzed by light and alkaline pH. Check that the pH of the solution is 7.0 or less, and store the solution in dark bottles at 4°C. Fresh solutions should be prepared every few months.

From the Molecular Cloning collection, edited by Michael R. Green and Joseph Sambrook.

Cite this protocol as *Cold Spring Harb Protoc*; doi:10.1101/pdb.prot102228

TABLE 1. Solutions for preparing resolving gels for Tris-glycine SDS-PAGE

	Volume of components required to cast gels of indicated volumes and concentrations							
Components	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
Acrylamide mix (30%)	1.0	2.0	3.0	4.0	5.0	6.0	8.0	10.0
Tris (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
Ammonium persulfate (10%)	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
Acrylamide mix (30%)	1.3	2.7	4.0	5.3	6.7	8.0	10.7	13.3
Tris (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
Ammonium persulfate (10%)	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
Acrylamide mix (30%)	1.7	3.3	5.0	6.7	8.3	10.0	13.3	16.7
Tris (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
Ammonium persulfate (10%)	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
Acrylamide mix (30%)	2.0	4.0	6.0	8.0	10.0	12.0	16.0	20.0
Tris (1.5 M, pH 8.8)	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
10% SDS	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
Ammonium persulfate (10%)	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
Acrylamide mix (30%)	2.5	5.0	7.5	10.0	12.5	15.0	20.0	25.0
Tris (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
Ammonium persulfate (10%)	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).

TABLE 2. Solutions for preparing 5% stacking gels for Tris-glycine SDS-PAGE

	Volume of components required to cast gels of indicated volumes							
Components	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
Acrylamide mix (30%)	0.17	0.33	0.5	0.67	0.83	1.0	1.3	1.7
Tris (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 3. Effective range of separation of SDS–polyacrylamide gels

Acrylamide concentration (%)	Linear range of separation (kDa)
15	10–43
12	12–60
10	20–80
7.5	36–94
5.0	57–212

The molar ratio of bis-acrylamide:acrylamide is 1:29.

Ammonium persulfate (APS) (10%, w/v)

A small amount of a 10% (w/v) stock solution should be prepared in deionized H_2O and stored at 4°C. APS slowly decomposes, and fresh solutions should be prepared frequently (every 1–2 wk).

Coomassie Brilliant Blue R-250 stain solution

Dissolve 0.05 g of Coomassie Brilliant Blue R-250 per 100 mL of Destain solution. Filter through a Whatman No. 1 filter to remove any particulate matter.

Coomassie Brilliant Blue R-250 dye as well as "ready-to-use" staining solutions are available from many suppliers (Sigma-Aldrich, Bio-Rad, Life Technologies, Promega, and GE Healthcare). Nontoxic staining solutions have also been developed (e.g., Bio-Safe Coomassie, Bio-Rad; BluePrint Fast-PAGE Stain, Life Technologies).

Destain solution

Prepare Destain solution (glacial acetic acid:methanol:H_2O, 10:50:40 [v/v/v]). The acetic acid should be added last to the methanol:H_2O mixture for safety.

Ethanol (20%) and 3% glycerol

Fixing solution for SDS-PAGE gels <R>

Methanol (20%) containing 3% glycerol (optional; see Step 15)

Protein markers

Individual or mixtures of defined molecular weight proteins (high- or low-molecular-weight range, or full-range markers) are available from many commercial sources (e.g., Sigma-Aldrich, Bio-Rad, Life Technologies, Promega, and GE Healthcare). Prestained marker mixes allow visualization of their separation during the run, but the mobility and apparent concentrations of individual proteins may be somewhat altered from the staining.

Protein sample of unknown purity

SDS gel-loading buffer (5×) <R>

TEMED (*N*,*N*,*N*′,*N*′-tetramethylethylenediamine)

Electrophoresis-grade TEMED should be used and is sold by several manufacturers (National Diagnostics, Fisher BioReagents, Sigma-Aldrich, EMD Chemicals, etc.).

TEMED works only as a free base; polymerization is inhibited at low pH.

Tris base (1.5 M, pH 8.8) (for preparation of a resolving gel) or Tris base (1.0 M, pH 6.8) (for preparation of a stacking gel)

It is essential that the Tris buffers used for the preparation of resolving and stacking gels be prepared starting with Tris base. If Tris-HCl or Trizma is used to prepare buffers, the concentration of salt will be too high and polypeptides will migrate anomalously through the gel, yielding extremely diffuse bands. After the Tris base has been dissolved in deionized H_2O, adjust the pH of the solution with HCl.

Tris-glycine electrophoresis buffer (10×, pH 8.3) (reservoir buffer) <R>

Prepare Tris-glycine electrophoresis buffer (1×, pH 8.3) (reservoir buffer).

Equipment

Cellulose acetate sheets and plastic frame (e.g., AP Biotech, Owl Scientific)

Desktop scanner

Erlenmeyer flask or disposable tube

Hamilton microliter syringe or gel-loading tips

Heating plate (optional)

Microwave oven (optional)

Cite this protocol as *Cold Spring Harb Protoc*; doi:10.1101/pdb.prot102228

Plastic wrap

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

Rocking platform

Vacuum gel dryer

Gel dryers are available from several commercial sources (e.g., Life Technologies, Promega). It is best to purchase the dryer from the manufacturer of the SDS-PAGE to ensure that the size of the dryer will be tailored to that of the gels and will accommodate several SDS–polyacrylamide gels simultaneously.

Vertical electrophoresis apparatus

The use of discontinuous buffer systems in SDS-PAGE requires the vertical gel format. Although the basic design of the electrophoresis tanks and plates has changed little since Studier (1973) introduced the system, many small improvements have since been incorporated. Standard-size as well as minigel systems for separation and blotting are now sold by many manufacturers (e.g., Bio-Rad, Life Technologies). Which system to purchase is a matter of personal choice, but it is sensible for a laboratory to use only one brand. This uniformity makes it easier to compare results obtained by different investigators; it also allows parts of broken apparatuses to be scavenged and reused.

Whatman 3MM paper

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 an appropriate volume of solution containing the desired concentration of acrylamide/bis-acrylamide for the resolving gel, using the values given in Table 1. Mix the components in the order shown. Rapid polymerization will begin as soon as the TEMED has been added. Without delay, swirl the mixture rapidly and proceed to the next step.

   Ammonium persulfate provides free radicals to initiate polymerization of acrylamide and bis-acrylamide.

   TEMED accelerates the polymerization of acrylamide and bis-acrylamide by catalyzing the formation of free radicals from ammonium persulfate.

   Avoid air bubbles during mixing of the solutions because oxygen inhibits polymerization of acrylamide solutions.
3. Pour the acrylamide solution into the gap between the glass plates of the electrophoresis apparatus, leaving sufficient space for the stacking gel (the length of the teeth of the comb plus 1 cm). Use a Pasteur pipette to carefully overlay the acrylamide solution with 0.1% SDS (for gels containing ~8% acrylamide) or isopropanol (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, and removes any bubbles from the surface.
4. After polymerization is complete (30 min), pour off the overlay and wash the top of the gel several times with deionized H_2O to remove 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 appropriate volume of the stacking gel mixture using the values given in Table 2. Mix the components in the order shown. 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 or pipette the stacking gel mix to fill the space above the resolving gel. Immediately insert a clean comb into the stacking gel solution, taking care to avoid trapping air bubbles. Add more stacking gel solution as needed to fill the spaces of the comb completely. Place the gel in a vertical position at room temperature.

Preparation of Samples and Running the Gel

7. As the stacking gel is polymerizing, add an appropriate volume of SDS gel-loading buffer to the samples, and boil or heat the samples for 2 min in an 85°C heat block to denature the proteins. Be sure to denature a sample containing marker proteins of known molecular weights.

   Extremely hydrophobic proteins, such as those containing multiple transmembrane domains, may aggregate or oligomerize when boiled. To avoid this pitfall, denature these samples for 10–60 min at 45°C–55°C.

8. After polymerization is complete (30 min), carefully remove the comb. Use a squirt bottle to wash the wells immediately with deionized H_2O to remove 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, and, if needed, remove any bubbles that have become trapped at the bottom of the gel between the glass plates. This may be accomplished with a U-bent hypodermic needle attached to a syringe.

   Do not prerun the gel before loading the samples because this will defeat the purpose of the discontinuous buffer system.

9. Load each of the samples in a predetermined order into the bottoms of the wells. This is best done with a Hamilton microliter syringe that is washed with buffer from the bottom reservoir after each sample is loaded or with a micropipettor equipped with gel-loading tips. The sample volume loaded will depend on the comb and gel thickness; for example, 20 µL of sample is commonly used with a 3.35-mm well width comb and 1-mm gel thickness.

   Loading an equal volume of 1× SDS gel-loading buffer into any unused wells will reduce differences in the migration of samples near the edge of the gel.

10. Attach the electrophoresis apparatus to an electric power supply (the red 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. Minigels (~8 cm in length) are usually completed within an hour; larger gels will require longer electrophoresis time.

    Slow migration through the stacking gel enhances resolution. Many manufacturers recommend higher than 15 V/cm to accelerate migration through the resolving gel; however, overheating of the gel can distort the bands and even cause plates to fracture.

11. Remove the gel sandwich from the apparatus and place on a paper towel. Use an extra gel spacer or wedge to carefully pry the plates apart. If samples are loaded in a symmetric manner, the orientation of the gel can be marked by clipping a corner from the bottom of the gel that is closest to the leftmost well (Slot 1).

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

    At this stage, the gel can be fixed, stained with Coomassie Brilliant Blue as described in the following steps, stained with silver salts, fluorographed or autoradiographed, or used for an immunoblot.

Staining SDS–Polyacrylamide Gels with Coomassie Brilliant Blue

Coomassie Brilliant Blue R-250 is an aminotriarylmethane dye that forms strong but noncovalent complexes with proteins, most probably by a combination of van der Waals forces and electrostatic interactions with NH_3^+ groups. The amount of dye bound is approximately proportional to the amount of protein. Polypeptides resolved on SDS–polyacrylamide gels can be simultaneously fixed and stained with Coomassie Brilliant Blue R-250. The gel is soaked for several hours in a solution of the dye in Destain solution of methanol:acetic acid, and excess stain is then allowed to diffuse from the gel during a prolonged incubation with the same solvent lacking the dye ("destaining"). See Protocol: ***Variations of Staining Sodium Dodecyl Sulfate–Polyacrylamide Gels with Coomassie Brilliant Blue*** *(Kielkopf et al. 2021) for several variations of the standard procedure given here. If the gel is to be dried as described in Steps 15–21, we recommend the "End of the Day" Coomassie Blue staining method given in that protocol.*

12. Immerse the gel in at least five volumes of Coomassie Brilliant Blue stain solution, and place it on a slowly rotating platform for at least 4 h at room temperature.

13. Remove the stain, and rinse the gel briefly with water. Destain the gel by soaking it in methanol: acetic acid solution without dye on a slowly rocking platform for 4–8 h, changing the Destain solution three to four times.

    Prolonged destaining results in a clearer background but also causes protein bands to fade. Destaining for 2–4 h usually allows as little as 0.1 µg of protein to be detected in a single band.

    A more rapid rate of destaining can be achieved by placing KimWipes paper tissues or sponge fragments in the Destain solution. These will absorb the stain as it leaches from the gel. Tie the tissues in a simple knot and place two to four of them around the gel, but avoid laying them directly on the gel because this will cause uneven destaining.

14. After destaining, store the gels in H_2O in a sealed plastic container.

    Gels may be stored indefinitely without any diminution in the intensity of staining; however, polyacrylamide gels stored in H_2O after fixation will swell. To avoid distortion, store fixed gels in H_2O containing 20% glycerol. Stained gels should not be stored in destaining solution because the protein bands will fade.

    To obtain a permanent record of the gel, either scan the stained gel on a desktop scanner between transparencies, photograph the stained gel, or dry it by following Steps 15–21.

Drying SDS–Polyacrylamide Gels

SDS–polyacrylamide gels containing radiolabeled proteins (e.g., ^{35}S-, ^{32}P-, or ^{3}H-labeled) require drying before autoradiography, and drying is also useful to preserve a primary record after staining. The major problems encountered when a gel is dried are (1) shrinkage and distortion and (2) cracking of the gel. The first of these problems can be minimized if the gel is attached to a sheet of Whatman 3MM paper before dehydration. However, there is no guaranteed solution to the second problem, the likelihood of which becomes greater for thicker gels and at higher polyacrylamide concentrations. Gel cracking will occur if it is removed from the drying apparatus before dehydration is complete. Therefore, it is essential to keep the drying apparatus in good condition, to use a reliably steady vacuum source that has few fluctuations in pressure, and to use the thinnest gel possible for the purpose. For nonradioactive gels, it may be preferable to slowly air-dry the gel between cellulose acetate sheets clamped into a simple plastic frame (available from AP Biotech, Owl Scientific, or other suppliers) to allow for transillumination and clear visualization of the dried gel. We recommend soaking the gel in 20% ethanol/3% glycerol before drying by this method. Regardless of the drying method chosen, because of the unavoidable risk of breakage, it is advisable to record an image of the stained gel, either by scanning or photography, before drying.

15. If the proteins loaded on the gel have not been stained (e.g., for proteins that will be visualized by autoradiography or luminescence instead), then fix the gel before drying. Fix the gel in 5–10 volumes of fixing solution at room temperature. Bromophenol blue from the loading dye will turn yellow as the acidic fixing solution permeates the gel. Continue fixation for 5 min after the blue color from the loading dye has disappeared (~15–30 min total).

    If cracking of polyacrylamide gels during drying is a constant problem, soak the fixed gel overnight in 20% methanol/3% glycerol before proceeding to Step 16. However, longer drying times may be required if glycerol is present.

    *For Coomassie staining of gels that will be dried, the "End of the Day" method given in Protocol: **Variations of Staining Sodium Dodecyl Sulfate–Polyacrylamide Gels with Coomassie Brilliant Blue** (Kielkopf et al. 2021) is recommended.*

    Silver-stained gels may be briefly equilibrated in fixing solution (15–30 min) before drying.

16. On a piece of plastic wrap slightly larger than the gel, arrange the gel with its cut corner (Slot 1) on the lower right-hand side.

17. Place a piece of wet Whatman 3MM paper on the damp gel. The paper should be large enough to create a 1- to 2-cm border around the gel and small enough to fit on the gel dryer. Do not attempt to move the 3MM paper once it has contacted the gel.

18. Arrange a larger piece of dry 3MM paper on the drying surface of the gel dryer, and place the sandwich of paper/gel/plastic wrap on top of this. The plastic wrap should be uppermost.

19. Close the lid of the gel dryer and apply vacuum so that the lid makes a tight seal around the gels. If the dryer is equipped with a heater, apply low heat (50°C–60°C) to speed the drying process.

20. Dry the gel for the time recommended by the manufacturer (usually 2–3 h for standard 0.75-mm gels). If heat was applied, turn off the heat for a few minutes before releasing the vacuum.
21. Remove the gel, which is now affixed to the paper, from the dryer. The dried gel may be stored indefinitely. For autoradiography, remove the plastic wrap and expose to film.

TROUBLESHOOTING

Problem: Protein does not migrate at the expected position relative to molecular weight standards.
Solutions:

- Relatively high hydrophobicity, highly charged sequences, and certain posttranslational modifications such as glycosylation or phosphorylation influence protein mobility on SDS-PAGE gels (Weber et al. 1972). Thus, apparent molecular weight can differ from the true mass of the polypeptide chain; the anomalous migration will be consistently observed.
- In addition to degradation, unintended splicing or translational errors can result in truncated proteins. Reexamine the DNA sequence for rare codons, internal start or stop codons, or other mutations that could result in incomplete translation.

Problem: Protein bands do not stain well, become diffuse, or disappear after initial staining.
Solutions:

- This is most common with smaller proteins (<12 kDa) that can diffuse from the gel during staining and destaining. Using a faster staining protocol and minimizing delay between steps may improve the appearance of these bands.
- Very small proteins (<4 kDa) may require a fixing reagent that covalently cross-links protein, such as formaldehyde or glutaraldehyde. Before staining, incubate the gel in fixing solution for at least 1 h or overnight at room temperature with gentle shaking. Wash with deionized H_2O three times for 30 sec each, then proceed with Coomassie staining as described in this protocol. Of course, recovery of fixed proteins from gels will be drastically reduced.
- Gels that have been destained too long may simply be restained.
- Not enough protein was loaded onto the gel. Coomassie stain will detect a minimum of 0.1 μg in each protein band. If Coomassie stain is not sensitive enough, the gel can be rinsed and restained with silver salts.

Problem: The dye front and bands curve up ("smiling") or down ("frowning") at the edges of the gel.
Solutions:

- "Smiling" may result from uneven temperature across the width of the gel, with the spacers acting as heat sinks near its edges. This may be remedied by using active temperature control (i.e., placing the apparatus at 4°C), adding more buffer to the outer reservoir to help dissipate heat, and/or reducing the power. Overheating can distort bands or even cause the plates to fracture.
- "Frowning" can be caused by an uneven field due to electrical discontinuities such as air bubbles trapped at the bottom of the plates, or uneven gel thickness. Incomplete polymerization at the edges of the gel may also contribute to "frowning." Be sure that no air is trapped in the gel or under the gel sandwich and that the gel has polymerized completely before starting.

Problem: There is inadequate resolution of the protein bands.
Solutions:

- Optimization of acrylamide monomer concentration, bis-acrylamide concentration, or running time may be required. In general, resolution of smaller proteins is favored with higher-percentage

gels and more extensive cross-linking, and vice versa for larger proteins. Increasing the amount of bis-acrylamide cross-linker decreases the matrix pore size and will influence the guidelines for total acrylamide concentration given in Table 3. Longer running times may improve separation of larger proteins. The use of a prestained molecular weight standard can help to visualize band separation during the run.

- In some cases, resolution of closely spaced bands can be improved by loading less sample or a lower concentration of protein so that bands are thinner.

Problem: There are diffuse, streaky, or smeared bands.
Solutions:

- It is essential that gel buffers be prepared from Tris base. If Tris-Cl is used, the ionic strength will be too high, resulting in stacking failure and extremely diffuse bands.
- High concentrations of salt in a sample can result in band distortion. Reduce the salt concentration of the sample by dialysis, precipitation, or desalting.
- The voltage may be too high. Electrophorese at 8 V/cm until the dye front has passed through the stacking gel, then increase to 10–15 V/cm.
- Aggregated material will accumulate in the well and slowly dissolve during electrophoresis, creating a streak. Hydrophobic proteins such as those containing multiple transmembrane domains may aggregate or multimerize when boiled. To avoid this, denature these samples for 10–60 min at 45°C–55°C. For other insoluble proteins, add 4–8 M urea or increase the concentration of SDS to solubilize the protein.

Problem: There is a metallic sheen on the gel after Coomassie staining.
Solution: The solvent was allowed to evaporate, causing the dye to dry on the gel or precipitate out of solution. Rinse the gel for 15 sec in methanol and immediately return to water or Destain solution.

Problem: There is high background in silver stain.
Solution: Only high-purity water and clean dishes should be used. Glassware used for silver staining may be very effectively cleaned with 1% nitric acid.

DISCUSSION

Most applications use a discontinuous polyacrylamide gel and buffer system first devised by Ornstein (1964) and Davis (1964) and modified by Laemmli (1970) to contain 0.1% SDS in all components of the system. The system is discontinuous with respect to the percent polyacrylamide used in the stacking and resolving gels and with respect to the pH and ionic composition of the buffer components. The sample and the stacking gel both contain Tris-Cl (pH 6.8), the resolving gel contains Tris-Cl (pH 8.8), and the upper and lower buffer reservoirs contain Tris-glycine (pH 8.3). At pH 6.8, glycine migrates much slower than chloride ions. Thus, when voltage is applied, chloride ions in the sample and stacking gel begin to migrate away from glycine and form the leading edge of a moving boundary, whereas glycine molecules comprise the trailing edge. Between these boundaries, a zone of low conductivity and steep voltage gradient develops, which pulls the glycine along. SDS-coated polypeptides are intermediate in their mobilities and become concentrated ("stacked") between the two successive fronts. At the interface to the resolving gel, the pH changes abruptly to 8.8, which favors the ionization of glycine. The glycine ions accelerate past the polypeptides and stack them even further. This stacking effect compresses the polypeptides into thin, sharp zones before separation and greatly increases the resolution of the samples. Glycine ions continue to travel through the resolving gel immediately behind the chloride ions. Once freed from the moving boundary, the

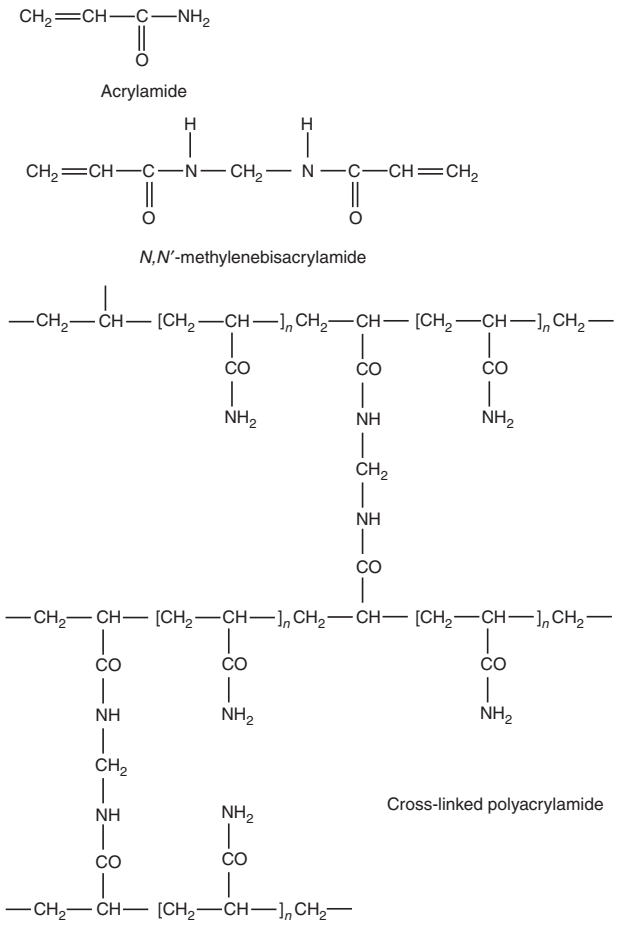

FIGURE 1. Chemical structure of polyacrylamide. Monomers of acrylamide are polymerized into long chains in a reaction initiated by free radicals. In the presence of *N*,*N*′-methylenebisacrylamide, these chains become cross-linked to form a gel. The porosity of the resulting gel is determined by the length of chains and degree of cross-linking that occurs during the polymerization reaction.

SDS–polypeptide complexes move through the resolving gel in a zone of uniform voltage and pH and are separated according to size by the sieving action of the polyacrylamide matrix.

The porosity of the polyacrylamide matrix determines the range of molecular weights that can be resolved effectively. Polyacrylamide gels are composed of linear chains of polymerized acrylamide cross-linked by a bifunctional agent *N*,*N*′-methylene-bis-acrylamide (Fig. 1). Porosity depends on the concentration of acrylamide used to prepare the gel and on the degree of cross-linking; that is, the size of these pores decreases as the acrylamide and bis-acrylamide concentrations increase. Cross-links also add rigidity and tensile strength to the gel. Most SDS–polyacrylamide gels are cast with a molar ratio of bis-acrylamide:acrylamide of 1:29, which can resolve polypeptides differing in size by as little as 3%. Table 3 shows the effective separation range for gels made with polyacrylamide concentrations ranging from 5% to 15%. "Ready-to-use" gels with a variety of polyacrylamide concentrations, including gradient gels, are offered by many vendors (Bio-Rad, Life Technologies, GenScript, and others). Note that precast gels are often prepared without SDS and can be used for either SDS-PAGE or native PAGE with nondenaturing detergent systems (Ramjeesingh et al. 1999).

Proteins separated by PAGE are typically detected by staining with Coomassie Brilliant Blue or with silver salts. Coomassie Brilliant Blue permeates the gel and binds nonspecifically and relatively rapidly to proteins, thereby allowing their visualization as discrete blue bands within the translucent polymer matrix (Wilson 1983). Silver staining of protein is based on the differential reduction of

Cite this protocol as *Cold Spring Harb Protoc*; doi:10.1101/pdb.prot102228

silver ions in a reaction similar to that used in photographic processes. Silver staining, although somewhat more laborious, is significantly more sensitive because it allows detection of proteins at concentrations nearly 100-fold lower than Coomassie Brilliant Blue (Switzer et al. 1979; Merril et al. 1984).

RECIPES

Fixing Solution for SDS-PAGE Gels

Reagent	Quantity	Final concentration
Ethanol	30 mL	30% (v/v)
Glacial acetic acid	12 mL	12% (v/v)
Formaldehyde (from 37% [w/v] formaldehyde stock)	50 µL	0.018% (v/v)
H_2O	100 mL	

SDS Gel-Loading Buffer (5×)

Reagent	Quantity (for 1 mL)	Final concentration
Tris-Cl (1 M, pH 6.8)	0.25 mL	250 mM
SDS (electrophoresis grade)	80 mg	8%
Bromophenol blue	1 mg	0.1%
Glycerol (80%, v/v)	0.5 mL	40% (v/v)
Dithiothreitol (1 M)	0.1 mL	100 mM
H_2O	0.25 mL	

SDS gel-loading buffer (5×) lacking DTT can be stored at room temperature. Add DTT from a 1 M stock just before the buffer is used.

Tris-Glycine Electrophoresis Buffer (10×, pH 8.3) (Reservoir Buffer)

Reagent	Quantity	Concentration in 10× stock	Final 1× concentration
Tris base	30.3 g	250 mM	25 mM
Glycine, electrophoresis grade	144 g	1.9 M	192 mM
SDS, electrophoresis grade	10 g	1%	0.1% (w/v)
H_2O	to 1 L		

The pH should be ~8.3.

Several manufacturers sell electrophoresis-grade SDS. Although any one of these will give reproducible results, they are not interchangeable, and we recommend exclusive use of a single brand of SDS because migration of polypeptides may change noticeably when one brand of SDS is substituted for another. If proteins are to be eluted from the gel for sequencing, electrophoresis-grade SDS should be further purified as described by Hunkapiller et al. (1983). A 20% (w/v) stock solution of SDS should be prepared in deionized H_2O and stored at room temperature.

ACKNOWLEDGMENTS

We thank Dr. Ellen Hildebrandt for review and technical editing of this protocol.

REFERENCES

Davis BJ. 1964. Disc electrophoresis. II. Method and application to human serum proteins. *Ann N Y Acad Sci* **121:** 404–427.

Harlow EL, Lane D. 1988. *Antibodies: A laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Hunkapiller MW, Lujan E, Ostrander F, Hood LE. 1983. Isolation of microgram quantities of proteins from polyacrylamide gels for amino acid sequence analysis. *Methods Enzymol* **91:** 227–236.

Kielkopf CL, Bauer W, Urbatsch IL. 2021. Variations of staining sodium dodecyl sulfate–polyacrylamide gels with Coomassie Brilliant Blue. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot102236.

Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:** 680–685.

Merril CR, Goldman D, Van Keuren ML. 1984. Gel protein stains: Silver stain. *Methods Enzymol* **104:** 441–447.

Ornstein L. 1964. Disc electrophoresis. I. Background and theory. *Ann N Y Acad Sci* **121:** 321–349.

Ramjeesingh M, Huan LJ, Garami E, Bear CE. 1999. Novel method for evaluation of the oligomeric structure of membrane proteins. *Biochem J* **342:** 119–123.

Studier FW. 1973. Analysis of bacteriophage T7 early RNAs and proteins on slab gels. *J Mol Biol* **79:** 237–248.

Switzer RC III, Merril CR, Shifrin S. 1979. A highly sensitive silver stain for detecting proteins and peptides in polyacrylamide gels. *Anal Biochem* **98:** 231–237.

Weber K, Pringle JR, Osborn M. 1972. Measurement of molecular weights by electrophoresis on SDS–acrylamide gel. *Methods Enzymol* **26:** 3–27.

Wilson CM. 1983. Staining of proteins on gels: Comparisons of dyes and procedures. *Methods Enzymol* **91:** 236–247.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis of Proteins

Clara L. Kielkopf, William Bauer and Ina L. Urbatsch

Cold Spring Harb Protoc; doi: 10.1101/pdb.prot102228