SDS-PAGE

by Michael Koelle

1. To use commercial BRL boxes: Clean the plates with soap and hot water, wipe dry, scrub with ethanol, wipe dry, and clamp them together with spacers in between. The notched spacers interlock to form a tight seal. I use the thin (0.8 mm) spacers for routine analytical gels, and 1.5 mm spacers to run more sample. I usually use a comb that makes 20 wells.

2. Solutions:

I. 1.5 M Tris-HCl, pH 8.8

II. 1 M Tris-HCl, pH 6.8

III. 30% acrylamide/bisacrylamide (37.5:1). This solution can be bought commercially from Biorad. Store in fridge.

IV. 20% SDS (some people filter)

V. 10% w/v ammonium persulfate, make fresh every month and store in fridge. Note: old bottles of dry AP can go bad.

VI. N,N,N',N'-tetramethylethylene diamine (TEMED). Buy it and store in fridge.

VII. 0.1% SDS. Keep a squirt bottle of it.

VIII. Running buffer: Keep a jug of this. 24 g Tris base, 115.2 g glycine, 20 ml 20% SDS, H2O to 4 liters.

IX. Sample buffer: 100 mM Tris, pH 6.8, 2% SDS, 5% β-mercaptoethanol, 15% glycerol, enough bromophenol blue to make it dark looking, and optional: 4 M urea. Store in a tightly sealed container to keep the BME from going off. You may still need to add more BME if your solution is old, since it is highly volatile. Some people keep sample buffer -BME and add it right before use.

3. Resolving gel: mix for the correct percentage. A 7% gel resolves down to ~40 kd, and 100 kd runs in the middle of the gel. 10% resolves down to ~20 kd, and 50 kd runs in the middle. Here are recipes for 30 mls, which is enough for two 0.8 mm thick gels, or one 1.5 mm gel.
5% 6% 7% 8% 9% 10%
5 6 7 8 9 10 ml 30% acrylamide/bis
7.5 7.5 7.5 7.5 7.5 7.5 ml 1.5 M Tris 8.8
17 16 15 14 13 12 ml H2O

Then add 150 µl 20% SDS
400 µl 10% AP
20 µl TEMED

Mix gently and pour between plates up to ~1.5 inches below the top, depending on your apparatus. (A rule of thumb: want the stacking gel to be about as deep below the wells as the wells are deep). Immediately and very gently squirt a 0.1% SDS overlay over the acrylamide solution (keeps oxygen away so that the top of the resolving gel polymerizes well). If you squirt this on gently enough you won't disturb the acrylamide solution, and a sharp line of refractive index change will be seen between the gel and the overlay. (Note: a more widely used alternative overlay is water saturated isobutanol. This probably works somewhat better, but it's smelly so I only use it when pouring really thick (3 mm) gels).

4. After the gel polymerizes (10 min) pour off the overlay, and rinse out the well with dH2O. Then pour all the water out, and dry the well by shoving in a paper towel or kimwipes (carefull not to touch the gel!) Insert the comb.

5. Stacking gel : enough for two 0.8 mm thick gels or one 1.5 mm gel

627 µl 30% acryl:bis
780 µl 1M Tris pH 6.8
5 ml H20
31 µl 20% SDS
62 µl 10% AP
3.8 µl TEMED

Pour in between plates, or squirt in with a pasteur pipette. Carefull not to get airbubbles trapped around the teeth of the comb.

6. After polymerization (10-15 min), remove the clamps and bottom spacer.

7. Pour running buffer in the top and bottom chambers, and clamp into a gel box. Get rid of bubbles between the plates under the gel (avoid these best by pouring buffer in the botton tank first, then inserting the gel at an angle so that air bubbles float off the upper
8. Sample preparation. Mix protein with 1 volume or more of sample buffer, and boil in a boiling water bath for 2-5 min. Can freeze the sample at this point and use it later; just boil again briefly before loading. Also want to use some markers; Biorad unstained standards are good; boil 1 µl in 10 µl sample buffer to prepare. Prestained markers are nice for westerns; you can see them transfer on to the blot. However, they give inaccurate molecular weights.

9. Samples of _E. coli_ extracts: resuspend the pellet from 1 OD ml of culture in 50 µl sample buffer (1 OD ml= 1 ml of culture at OD600=1). On a BRL gel box with the 0.8 mm thick 20 well comb, load 20 µl of this (this is about 80 µg of protein). This sample is a bit viscous, and is best loaded with a Hamilton syringe: I use the type that holds 50 µl and has a 22 g needle cemented permanently on. If you are running a Western on _E. coli_ expressing a high abundance (fusion) protein, load only 0.0125 OD ml. For _Drosophila_ tissue culture cells, suspend 3X10^6 cells per 90 µl cracking buffer, and load about 20 µl per lane (0.8 mm thick gel with the BRL gel box and 20 well comb).

10. Running. Use a constant Amps power supply. For the BRL gel box and 0.8 mm thick gels, start the gel at 11 mAmps, and after 1.75 hours (dye should now be into the resolving gel) turn it up to 16 mAmps and run a further 3.15 hours. (Total run takes ~5 hours.) Using a programmable power supply like the Biorad model 1000/500 you can do a run like this automatically (without having to be in lab to switch up the Amps in the middle or shut them off at the end). Alternatively, for overnight runs, run the gel at 11 mAmps and set the timer to turn the gel off after 6.5 hours. Note that the resistance of the gel increases during the run, so to maintain constant Amps the power supply will increase the voltage.

11. If you're running a western, go to the western protocol at this point to blot your gel.

12. Coomassie staining your gel.

The stain is:

1 g Coomassie brilliant blue R-250

50 ml glacial acetic acid

500 ml MeOH
450 ml H2O

(Note: This is a somewhat weak stain recipe: it destains faster and gives a prettier gel, but may be less sensitive than more concentrated mixtures.) Stain the gel 20 min in a tray on a rotating platform. Longer staining helps see faint bands, but then you have to destain longer. Pour the used stain down the sink, rinse gel in a little water to get excess stain off. Destain by soaking gel in destain solution in a tray on a rotator. Throw a couple of bunched up large kimwipes into the tray to absorb the coomassie as it is extracted from the gel.

Destain is:

10% glacial acetic acid

5-50% MeOH

With 5% MeOH you can destain overnight. With 20% MeOH can destain a 0.8 mm thick gel in ~1.5 hours at room temp. For faster destaining, use more methanol, or do it in a warm room. Brief pulsing of the gel in destain in the microwave also speeds it up.

12.5 To visualize very heavy bands without coomasie (eg for prep gels of bacterial fusion proteins to be injected for immunization):

soak gel 5 min in ice cold 0.25 M KCl. This precipitates SDS, so the bands show up white against a black background.

13. To preserve the gel. Can dry it down on Whatman 3mm paper. For a much prettier gel, you can dry your gel between acetate sheets clamped in a frame. You can buy setups for this (from Fisher or Promega). Place one half of the frame down, puddle water all over it, add one wet acetate sheet, add the wet gel, add the next wet sheet, the other half of the frame, and then put black clips all around the edges. Make sure not to get any air bubbles between the acetate sheets- these can crack your gel. Supposedly the acetic acid in destain can make your gel crack, so you should soak the gel in H2O for a few minutes to get rid of the acetic acid before drying. High percentage gels (>12%) will crack for sure unless you soak them in 3% glycerol for 15-30 minutes first.

14. Hoeffer minigels are great: they run in 1 hour, and you can keep them prepoured in the fridge. The Biorad minigel apparatus is not as good.