Preparing SDS-PAGE gels

WARNING: Unpolymerized acrylamide is a neurotoxin!

(1) **Clean the plates and combs.** For each gel, you will need one short plate, one spacer plate, and one comb. These are usually found on the gray rack by the sink. Spray a little bit of 70% ethanol on the plates, and wipe dry with Kimwipes. Wash the combs thoroughly with tap water. It is critical to remove all dust and small particles, especially any bits of left-over polyacrylamide.

(2) **Set-up the plates on the rack.** Layer the short plate on the spacer plate, with the spacers in between and slide the two plates into the green holder. Make sure that the bottom edges of the two plates are flush to avoid leakage. Lock the plates in, and place the holder on the rack, with the bottom edges of the plates pushed into the gray foam gasket to make a water-tight seal. Test the seal by pipetting or squirting a small volume of water between the plates and making sure there is no leakage. Blot dry with filter paper.

(3) **Pour the separating gel.** For each minigel (1 mm thick) you will need slightly more than 5 mL of reagent mix. Use the table below as a guide to calculate the total volumes you will need.

Pipette solutions in order. Avoid introducing bubbles, which will inhibit polymerization. Swirl the solution gently to mix thoroughly after addition of each component.

	7.5% gel	10% gel	12.5% gel	15% gel	18% gel
ddH_2O	2.81 mL	2.50 mL	2.19 mL	1.88 mL	1.50 mL
40% acrylamide/bis stock	0.94 mL	1.25 mL	1.56 mL	1.88 mL	2.25 mL
1.5 M Tris, pH 8.8	1.25 mL	1.25 mL	1.25 mL	1.25 mL	1.25 mL
10% ammonium persulfate	50 µL	50 µL	50 µL	50 µL	50 µL
TEMED	5 µL	5 µL	5 µL	5 µL	5 µL
TOTAL VOLUME	~5 mL	~5 mL	~5 mL	~5 mL	~5 mL

Once TEMED is added, the gel will begin to polymerize, so you need to work fast (but carefully). Pipette the gel mix between the plates, making sure you leave enough space at the top for the stacking gel and comb. Carefully layer water on top of the gel solution.

Once the gel has polymerized (about 10-15 mins), wash off the top of the gel with water. Carefully blot off excess water with a filter paper. Take care not to disturb or damage the top of the gel.

(4) **Pour the stacking gel.** For each gel you will need about 1.2 mL of reagent mix. Again, pipette the solutions carefully and swirl to mix after addition of each component. Pipette the gel mix between the plates up to just below the edge of the short plate. Carefully place in the comb.

ddH_2O	3.13 mL
40% acrylamide/bis stock	0.62 mL
1.5 M Tris, pH 6.8	1.25 mL
10% ammonium persulfate	50 µL
TEMED	5 µL
TOTAL VOLUME	~5 mL

Once the gel has polymerized, slowly remove the comb under running water. Wash the wells carefully to avoid distorting them.

(5) **Gel storage.** Put back the comb, and sandwich the gel between two wet pieces of paper towels. The gel can be stored horizontally at 4 °C for up to 1 week.

Things to look up / think about:

Learn about the chemical structures of acrylamide and bis-acrylamide and the mechanism of polymerization. What are the purposes of ammonium persulfate and TEMED? Why do air bubbles inhibit polymerization?
What is the purpose of bis-acrylamide? What would happen if you used a higher ratio of bis:acrylamide? A lower ratio?
Why is unpolymerized acrylamide neurotoxic? Why is polymerized acrylamide (polyacrylamide) non-toxic?
What is the purpose of the stacking gel? Why is the pH of the stacking gel different from the pH of the separating gel?
If these are supposed to be SDS-PAGE gels, where is the SDS?
What is the primary difference between a gel made of agarose and one of polyacrylamide? Why do we use agarose for DNA and polyacrylamide for proteins?