

Preparing SDS-PAGE Gels

1. Grab glass plates
 - Scrub side w/ ridges by dousing w/ soap and using scrub brush
 - Scrub both sides of flat glass
 - Douse all sides of all plates w/ 70% ethanol
 - Dry w/ kimwhipe
2. Grab green tray holders from drawer
 - Setup plates. Make sure they're flush at bottom. When you put them in contraption pick up and down a few times to make sure it's level at bottom. Feel with fingertips.
 - Push clamps back to hold plates together and place into larger contraption.
3. Mix up gel in 50 ml conical tube
 - Follow chart on wall (volumes are in mL for 2 gels)
 - Accrylamide is in door of fridge. TEMED is next to ethidium bromide. APS in freezer in reagents #2 box.

 - Vortex to mix together.
 - **Note: Do not add APS or TEMED until right before you're ready to pour b/c it'll start polymerizing.
4. Pour into gap.
 - Use 5 mL pipette to press up against the gap b/w the 2 plates. Add 4.7 ml to each plate.
 - To smooth out bubbles at the top, use transfer pipette to apply top layer of water-saturated isobutanol dropwise.
 - You will see 3 layers when the resolving gel has solidified.
5. Start w/ the stacking gel while that's solidifying (Again, don't add APS or TEMED until ready to pour).
6. Before adding stacking gel, pour off excess isobutanol.
 - Turn contraption over into sink. Use ddH₂O to rinse b/w plates. Use filter paper to absorb excess water.
7. Fill stacking layer
 - Fill up to top to prevent bubbles when you add combs (15-well combs almost always used).
 - Take kimwipes & wipe off excess stuff that floated out.
8. You can tell it's ready when the gel begins to pull away from the well-plate. If storing, wrap in water-saturated paper towel and saran wrap. Label with name, date, and gel percentage. Can be stored in the fridge for ≤ 1 week.