

Protocol for pouring mini-gels for BioRad Tetra System

1. Assemble stack of two clean glass plates, making sure to form a tight seal at the bottom with the rubber gasket to prevent acrylamide solution from running out.
2. Combine components to pour the resolving gel in a conical tube. Add APS as the penultimate component. When ready to pour the gel, add TEMED and mix thoroughly and quickly, as polymerization will begin.
3. Use a serological pipet to dispense the appropriate volume of resolving gel between the plates.
4. Using a glass Pasteur pipet, dispense enough water-saturated butanol on top of the resolving gel mixture to cover the top of the acrylamide solution. This will minimize the acrylamide meniscus and help flatten what will become the interface between the resolving gel and the stacking gel. [To make water-saturated butanol, add some water to butanol in a small culture bottle, and shake it up. Butanol will phase separate out to the top layer. Thus, pipet only from the top layer.]
5. Reserve the falcon tube with leftover acrylamide solution so that you can see when polymerization is complete in the tube, and therefore also between the glass plates.
6. While the resolving gel is polymerizing, mix up components for the stacking gel, but do not add APS and TEMED.
7. Once polymerization is complete, rinse the butanol out with MilliQ water, and use a Kimwipe to dry the inside of the plates at the top to remove any bulk water. [Some people just dump the butanol without rinsing.]
8. Add APS and TEMED to stacking gel mixture, and pipet into space between plates. Add enough that you get complete wells when you add the comb, but it is clearly not necessary to fill to the top of the plates because the comb will displace some volume.
9. Again, reserve the tube with leftover acrylamide mixture to see when it gels.
10. If you are pouring gels in advance and not planning to use for a few hours or even days, store the plate/gel sandwiches with comb still present in a plastic bag or Tupperware container with enough running buffer that the plates are submerged. Gels can be stored in this way for up to 5-7 days.

NOTE: APS needs to be fresh to work well. Store it frozen at -20°C in aliquots to keep it fresh.

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