10% Acrylamide Gels for SDS-PAGE

Resolving gel master mix:
400 ml H2O
250 ml 1.5 M Tris pH 8.8
10 ml 10% SDS

Stacking gel master mix:
340 ml H2O
62.5 ml 1.0 M Tris pH 6.8
5 ml 10% SDS

Pouring resolving gel:

1. Make 6 ml of resolving gel (makes 1 gel, with a little bit leftover)
   3.96 ml of resolving gel master mix
   1.98 ml of 30% acrylamide
   60 µl of 10% APS
   2.4 µl of TEMED

2. Immediately load gel mixture into the casing with a pipette – fill to the line on the casing. Add EtOH on top of gel. Save any leftover mixture to help you determine when the gel is set. It should take about 30 minutes to polymerize at room temperature. To speed up polymerization, you can add more APS and TEMED to the mixture. You can also degas the solution under a vacuum for about 10 minutes before adding the APS and TEMED.
3. Once resolving gel is set, pour off EtOH. Rinse with DI water. The gel can be stored for later use (store at 4 °C in 1x Tris Glycine buffer) or can be used immediately.

Pouring stacking gel:

1. Using a paper towel, dry the inside of the casing as well as possible. Try not to disturb the top of the resolving gel.

2. Make 3 ml of stacking gel (makes stacker for 1 gel).
   2.51 ml of stacking gel master mix
   0.5 ml of 30% acrylamide
   30 µl of 10% APS
   3 µl of TEMED

3. Make sure you have gel comb ready.

4. Load stacking solution onto the top of the resolving gel. It takes about 2 mls of solution to fill the gel casing. Make sure there are no air bubbles. Insert gel comb carefully (sometimes the solution will squirt out!). Save any leftover mixture to help you determine when the gel is set. It should take about 10-15 minutes to polymerize at room temperature. To speed up polymerization, you can degas the solution under vacuum for about 10 minutes before adding the APS and TEMED.