PROTOCOL OF SDS-PAGE (FOR 2 MINIGELS)

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1 Assemble the glass plates.
2 Determine the volume of the gel mold.
3 Prepare the resolving gel solution (see table 1).
4 Pour the acrylamide solution into the gap between the glass plates. Leave sufficient space for the stacking gel (the length of the teeth of comb plus 1 cm).
5 Add butanol to overlay the acrylamide solution.
6 After polymerization is complete (about 30-40 minutes @ RT), pour off the overlay.
7 Install the comb in right position.
8 Prepare the stacking gel solution (see table 1).
9 Pour it directly onto the surface of the polymerized resolving gel.
10 When the stacking gel is polymerizing, prepare the samples by heating them @ 100°C for 5-10 minutes in same volume of gel-loading buffer and 1/10-1/5 volume of 2-ME to denature the proteins.
11 After polymerization is complete (about 30 minutes), mount the gel in the electrophoresis apparatus and add Tris-Glycine electrophoresis buffer to the top and bottom of the gel between the glass plates.
12 Mark the bottom with a marker and remove the combs carefully.
13 Load the samples into the bottom of the wells. It is better to load an equal volume of loading buffer into any wells that are unused.
14 Attach the electrophoresis apparatus to an electric power supply and run at 40mA. After the dye reaches the bottom of the resolving gel (about 55 minutes), turn off the power supply.
15 Remove the glass plates from the electrophoresis apparatus and pry the plates apart.
16 Stain the gel about for 20-30 minutes with Coomassie Brilliant Blue.
17 Destain the gel to get a good background.
18 Take a picture or dry the gel.