SDS-PAGE Gels preparation-1

12% SDS-PAGE Gels:

1 gel: Running (Bottom) Gel

2.9ml dH_2O
4.0ml 1M Tris, pH 8.8
54ul 20% SDS
3.0ml 29:1 40% Acrylamide
54ul 10% APS
10ul TEMED

6 gels: Running (Bottom) Gel

17.2ml dH_2O
24.0ml 1M Tris, pH 8.8
324ul 20% SDS
18.0ml 29:1 40% Acrylamide
324ul 10% APS
60ul TEMED

1 gel: Stacker (Top) Gel

2.85ml dH_2O
0.57ml 1M Tris, pH 6.8
20ul 20% SDS
0.35ml 29:1 40% Acrylamide
38ul 10% APS
5ul TEMED

6 gels: Stacker (Top) Gel

17.1ml dH_2O
2.82ml 1M Tris, pH 6.8
114ul 20% SDS
2.1ml 29:1 40% Acrylamide
225ul 10% APS
30ul TEMED

1. Clean surfaces of gel plates (1.5mm) first with Windex then with 70% EtOH and wipe clean with sterile Kimwipe. Assemble glass plates accordingly; lock into green BioRad clips and subsequently into the gel assembly apparatus.
2. Mix all components of Running (Bottom) Gel ***in that order*** and promptly pipette into assembled gel plates evenly from side to side.
3. Add a small layer of water-saturated butanol in order to produce a clean, straight top of the running gel; allow Running Gel to dry (~5-30min).
4. Pour off butanol, wash once with dH_2O, blot dry (Whatman paper).
5. Mix all components of Stacking (Top) Gel ***in that order*** and promptly pipette into the assembled gel plates on top of the Running Gel, evenly from side to side. Fill plates with stacking buffer so that it will overflow upon addition of the comb.
6. Insert the comb, and prevent air bubbles from persisting. Allow to dry (~5-30min)
7. Either use immediately, or store 4C wrapped in plastic wrap.