Protocol 108_Running SDS-PAGE

Stock Solutions

SDS Running Buffer (10X)

10 g	SDS
144 g	glycine
30.3 g	Tris Base

- Make to 1 L with H_2O
- Store at 4° C (stored in the cold room in designated bin)

Coomasie Blue staining solution

0.05 g	Coomassie Brilliant Blue
50 ml	methanol
10 ml	acetic acid

- Dissolve Coomassie Blue in methanol before adding acetic acid and water
- Make to 100 mL with H_2O
- Store at room temperature (stored at the bay designated for SDS-PAGE)

Coomassie Blue destain solution

50 ml	methanol
10 ml	acetic acid

- Make to 100 mL with H_2O
- Store at room temperature (stored at the bay designated for SDS-PAGE)

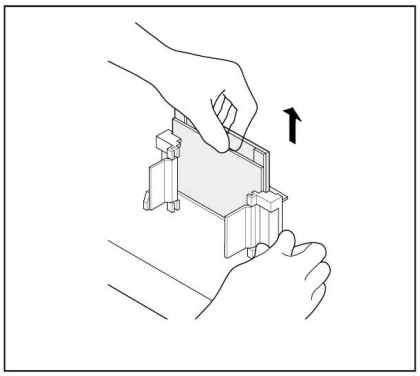
Precision Plus Protein Standard (Biorad Cat # 161-0373 or 161-0374)

- Store at 4°C

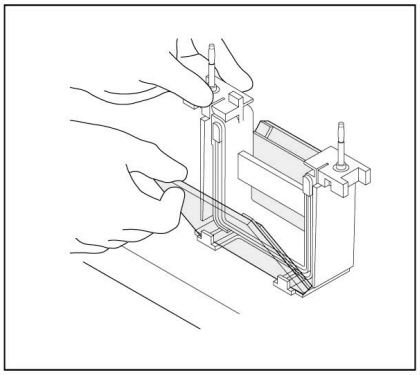
McCoy and Macpherson Lab
2013

Mini-PROTEAN 3 Electrophoresis Module Assembly

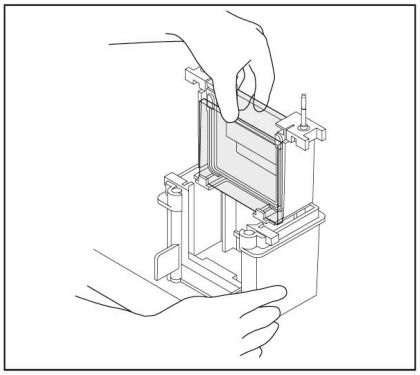
1. Remove the Gel Cassette Sandwich from the Casting Frame.



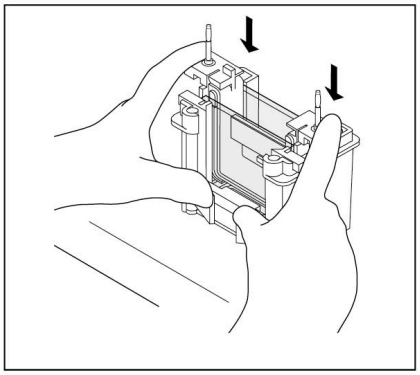
2. Place the Gel Cassette Sandwich into the Electrode Assembly with the Short Plate facing inward.



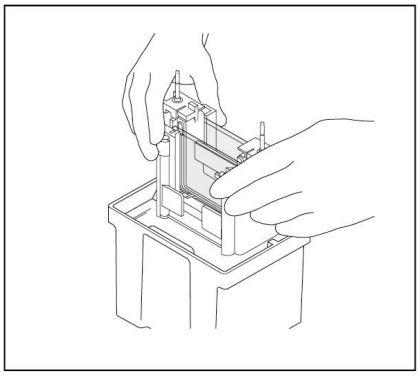
3. Slide Gel Cassette Sandwiches and Electrode Assembly into the clamping frame.



4. Press down the Electrode Assembly while closing the two cam levers of the Clamping Frame.



5. Lower the Inner Chamber into the Mini Tank.



Running the Gel

1. Clamp in your gel according to the instructions on the above.
2. Fill both chamber and minitank with 1X (dilute 10X buffer with water) gel running buffer.
   *internal chamber should be filled all the way to the top (to the top of the long glass)
   ** minitank to about 4 cm from the bottom
3. Pipet your sample and molecular weight standards into the gel adjusting the volume according to the amount of protein in your sample.
4. Be sure to include a lane with molecular weight standards (5 μl of Precision Plus Protein Standard per each gel).
5. Now attach your power leads and run the gel until the blue dye front reaches the bottom (120V for 1.5 hrs).
6. Remove the gel for the power supply and process further.
7. Visualize your proteins using Coomassie Brilliant Blue, Silver stain, or any of the other protein stains.

Coomassie Brilliant Blue Staining

1. Remove the module from minitank and disassemble it. Using green spatula carefully lift the shorter glass in such a way that gel is sitting on bigger glass.
2. Carefully remove and discard the stacking gel.
3. Clip the corners of the gels (1 corner clipped – gel#1, two corners clipped – gel#2, etc.).
4. Dislodge the separating gel from the glass using provided green spatula.
5. Submerge the gels in a shallow container filled with the Coomassie blue staining solution and gently rock overnight at room temperature on a rocking platform.
6. Destain the gels in Coomassie Destain solution for 1-2 hrs.