

a. Gel Running:

1. Assemble gel plates in casting frame.
2. Prepare 12% resolving gel as follows:

Distilled water	1600 μ l
Acrylamide/bisacrylamide solution (30%)	4200 μ l
1.5M Tris pH 8.8	2500 μ l
10% SDS	100 μ l
50% glycerol	1700 μ l
10% APS*	100 μ l
TEMED*	10 μ l

* Add immediately before loading between the plates.

3. Load the resolving between the plates leaving sufficient space for stacking gel.
4. Add a few drops of isopropanol.
5. After resolving gel solidifies, remove isopropanol using filter paper.
6. Prepare 4.5% stacking gel as follows:

Distilled water	2950 μ l
Acrylamide/bisacrylamide solution (30%)	750 μ l
1M Tris pH 6.8	1250 μ l
10% SDS	50 μ l
10% APS*	40 μ l
TEMED*	5 μ l

* Add immediately before loading between the plates.

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7. Load stacking gel and put the comb.
8. After stacking gel solidifies, remove gel plate from casting frame and assemble the running chamber.
9. Add running buffer and remove comb.
10. Boil sample at 95°C for 5 minutes.
11. Load sample.
12. Run the gel at 125V for 80 minutes.

b. Staining:

1. Stain the gel*

* Kindly check the protocol of the Coomassie stain.

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