SDS-PAGE for Protein Separation

Materials

SDS-PAGE precast gel
1× Running Buffer
5× Sample Buffer
Protein Marker
Protein samples
ddH_2O
Electrophoresis tank and power supply
Ice packs or ice-water bath

Procedure

SDS-PAGE

Gel Preparation

1. Install the precast gel in the electrophoresis tank.
2. Add an appropriate volume of 1× Running Buffer.

Sample Preparation

1. Bring 5× Sample Buffer to room temperature.
2. Prepare the following 10 µL sample mixture.

Reagent	**Volume**
Protein sample	6 µL
5× Sample Buffer	2 µL
ddH_2O	2 µL
Total	**10 µL**

3. Mix thoroughly.
4. Incubate at 100 °C for 10 min to denature the proteins.
5. Briefly centrifuge to collect the liquid at the bottom of the tube.

Gel Electrophoresis

1. Load the Protein Marker into the first well.
2. Load 10 µL of each sample into the remaining wells.
3. Run the gel at 80 V for 15 min, then at 140 V for 55 min.

Parameter	**Condition**
Running buffer	1× Running Buffer
Initial electrophoresis	80 V for 15 min
Main electrophoresis	140 V for 55 min
Cooling	Ice packs or an ice-water bath

4. Keep the electrophoresis system cool during the run.