SDS-PAGE ELECTROPHORESIS

COMPOSITION

● Protein ladder for SDS page

● Solubilization buffer (2X SDS-PAGE solubilizer 10 mL)
  • 0.5M Tris HCl (pH 6.8) – 3.12 mL
  • SDS – 0.5 g
  • Glycerol – 2.5mL
  • 1M DTT – 0.5 mL
  • Distilled water – 3.88 mL

● 12% resolving gel (10 mL)
  • Distilled water – 3.2 mL
  • 1.5M Tris HCl (pH 8.8) – 2.6 mL
  • 30% acrylamide with Bis Acrylamide - 4 mL
  • 10% SDS – 100 μL
  • 10% Ammonium persulfate (APS) – 100μL
  • TEMED – 8 μL

● 5% stacking gel (10 mL)
  • Distilled water – 2.8 mL
  • 1.5 M Tris HCl (pH6.8) – 0.5 mL
  • 30% Acrylamide – 0.66 mL
  • 10% SDS - 40 μL
  • 10% APS(Ammonium persulphate) - 40 μL
  • TEMED – 8 μL
• Upper running buffer for SDS PAGE (2 L)
  • Tris base – 6.5 g
  • Glycine – 28.8 g
  • SDS – 2 g
  • 0.3% Bromophenol blue – 200 μL

• Lower running buffer for SDS PAGE (4 L)
  • Tris base - 13 g
  • Glycine – 57.6 g

• SDS-PAGE staining solution
  • 10% glacial acetic acid
  • 0.1% Coomassie R250
  • 30% methanol

• SDS-PAGE destaining solution
  • 10% glacial acetic acid solution
  • 30% methanol

INSTRUMENTS
• Weighing balance
• Pipettes
• Centrifuge
• Spectrophotometer
• Bio-Rad SDS-PAGE apparatus
• SDS-PAGE power pack
• Gel Documentation System
SAMPLE PREPARATION
● 1 mL of liquid media culture cells are harvested at 5000 rpm for 5 minutes.
● The sample is mixed with the 50 uL of SDS-PAGE solubilizer and boiled for 3 min at 100 °C.

GEL PREPARATION
● 12% resolving gel is prepared and poured in the gel casting tray.
● Once the resolving gel gets solidified, the stacking gel is poured on top of it and the comb is put.
● Once the stacking gel is solidified, the comb is removed and the gel is transferred to the running tank with the buffers.
● Samples are mixed with loading dye and loaded onto the gel.
● The gel is run at 120 V and 15 mA.
● After running the gel, it is removed from the plate and stained using staining solution for 30 minutes, followed by overnight destaining with the de-staining solution.