

SDS-PAGE

AIM: To confirm the purity of protein by separating them on the basis of molecular weight.

Stock solutions

1. Acrylamide stock

Dissolve 29.2g of Acrylamide and 0.8g of Bis-Acrylamide into 100ml dH₂O. Then filter it with Whatman no.1 paper. Store it at room temperature in a dark bottle as it is a light-sensitive solution.

2. Running buffer (10 X)

Dissolve 30g of tris base, 144g of glycine and 10g of SDS in 1000ml dH₂O. Make 1 X buffer out of it.

3. Sample buffer- (5ml)

Tris HCl (0.25M, pH- 6.8)- 1.25ml

Glycerol (50%)-2.5ml

Bromophenol blue (0.25%) - 0.0125g

SDS (10%)- 0.5g

Beta- mercaptoethanol (0.5M) - 1ml

15% resolving gel and 5% stacking gel

Resolving gel recipe: (15%)- 5mL

Components	Quantity(mL)
Water	1.1
Acrylamide(30 %)	2.5
Tris(1.5M, pH 8.8)	1.3
SDS(10 %)	0.05
Ammonium persulphate(10 %)	0.05
TEMED	0.002

Stacking gel recipe: (5%) - 1mL

Components	Quantity(mL)
------------	--------------

Water	0.68
Acrylamide mix(30%)	0.17
Tris(1M,pH6.8)	0.13
SDS(10%)	0.01
Ammonium persulphate(10%)	0.01
TEMED	0.001

Procedure

1. Preparation of resolving gel

- Assemble gel sandwich cassette on the stand.
- Prepare a cocktail of the composition as mentioned above of resolving gel.
- Then pipette resolving gel solution in gel sandwich template in the appropriate amount.
- Then add 1cm of isopropanol on top for uniforming the margin.
- Allow it to polymerise for 30-60min.
- After the gel solidifies, remove liquid isopropanol

2. Preparation of stacking gel

- Prepare a cocktail of the composition mentioned above for stacking gel.
- Then pipette the solution on the separating gel until it reaches the top of the front plate.
- Carefully insert the comb into the gel sandwich without trapping air bubbles.
- Allow it to polymerise for about 30min.
- After it gets polymerised, remove the comb
- Take out the polymerised gel with gel cassettes.
- Fix the gel setup into the electrophoresis apparatus.

Note -

- While fixing the gel setup in the electrophoresis apparatus, make sure that the bigger plate faces outside and the small plate of gel cassette.
- Remember never to let the gel dry. Hence, wrap the gel with a gel cassette in the tissue and store it in a 4°C refrigerator dipped in distilled water.

3. Preparation and loading of sample

- Mix protein sample and 5x Sample buffer in 4:1 ratio respectively in the Eppendorf tube.
- Heat it at 95 degrees Celsius for 5 min.
- Then bring it to room temperature to cool for some time (5mins)
- Load the 2 ul of protein marker (protein ladder) in the 1st well.
- Load the sample protein in the wells of the stacking gel.
- Add the running buffer to the top and bottom of the reservoir.

4. Running a gel

- Attach the electrode plug to the appropriate electrode and turn the power supply to 50V until the sample enters the separating/resolving gel and then increase the voltage to 120V.
- The dye front should migrate to the bottom of the gel in 60-90min for 1mm gel
- Turn off the supply
- Remove electrodes and gel setup from the electrophoresis apparatus.

Coomassie staining in the gel

Staining the protein in the SDS-PAGE for primary analysis of protein separation.

1. Staining solution recipe

Coomassie blue (R-250)- 0.6g

Methanol- 90ml

Glacial acetic acid- 20ml

dH₂O- 90ml

2. Gel destainer / destaining solution

Methanol- 200ml

Glacial acetic acid- 40ml

dH₂O- 160ml

Procedure:

1. Pick up the gel from the gel cassette and transfer it into a small container containing water.
2. Then transfer the gel to a staining solution.
3. Agitate for 45mins-1hr in the staining solution on a slow rotary shaker at room temperature.
4. Pour out the stain and rinse the gel with a few changes of water.
5. Agitate for 10min in the dH₂O on a slow rotary shaker at room temperature.
6. Pour out the water and add the destaining solution to it.
7. Agitate overnight in the destaining solution on a slow rotary shaker at room temperature.
8. Pour out the destaining solution. The blue color protein bands will be visible.
9. Add water to the container having the gel to avoid it from drying

Note- • Cover the container with a lid during staining and destaining to prevent evaporation.