Protocol for Polyacrylamide Gel Electrophoresis (SDS PAGE)
Procotol code: SDSPAGE

Recipes

Recipe 1. Resolving and Stacking gels.

Reagent	Resolving Solution	Stacking Solution
Distilled Water	0.35 mL	1.575 mL
Polyacrylamide 29:1 (40%)	1.8 mL	0.3 mL
Tris (2M) - SDS (0.2%) Buffer	3 mL	1.125 mL
Glycerol (70%)	0.85 mL	-
APS (10%) *	26 uL	24 uL
TEMED *	2.6 uL	2.4 uL

*Add fast, just before the next step

Recipe 2. Sample dying buffer 5X.

Reagent	Concentration (5X)
Tris-HCl pH 6.8	250 mM
SDS 8% (p/v)	8% (p/v)
Glycerol 40% (v/v)	40% (v/v)
DTT	200 mM
Coomassie blue	0.1%

Recipe 3. Running buffers

- Cathode buffer: 0.1 M Tris, 0.1 M Tricine, 0.1% SDS.
- Anode buffer: 0.2 M Tris, pH=8.9

Recipe 4. Staining and destaining solutions

- Coomassie blue dying dilution: 10% (v/v) acetic acid, 50% (v/v) ethanol 95°, 0.25% (p/v) blue Coomassie.
- Destaining buffer I: 10% (v/v) acetic acid, 50% (v/v) ethanol 95°.
- Destaining buffer II: 10% (v/v) acetic acid, 7% (v/v) ethanol 95°

Procedure

Part 1. Glass plates assembly

1. Assemble the long and short glass plates.
2. Place them in the casting frame. Assure that the short glass plate is on top and that both of them are aligned.
3. Set it on the casting stand.

Part 2. Resolving gel preparation

1. Prepare the resolving gel by combining the reagents in the orden appeared.
2. Mix well and pour the solution into the glass plate till the mark, using the micropipette.
3. Add a layer of isopropanol to avoid air bubbles.
4. Wait for 15-20 minutes until the gel polymerize.

Part 3. Stacking gel preparation

1. When the stacking gel polymerized, drain the isopropanol with a paper towel.
2. Prepare the stacking gel by combining the reagents in the orden appeared.
3. Before adding the APS and TEMED, make sure that the comb have the dimensions needed and it's ready by the side.
4. Pour the stacking solution till the glass plate is full and place the comb.
5. Wait for 10 minutes until the gel polymerize.

Part 4. Sample preparation

1. Name the centrifugation tubes as the running map.
2. Add 3 uL of sample buffer in each tube.
3. Add 10 uL of the sample/protein of interest.
4. Heat them at 95°C for 10 minutes.
5. Make a short centrifugation of the samples.

Part 5. Running of gel

1. Put the gel into the gel box. Make sure that the short place is faced inside and there's no leaks. Remember to use plastic plates if necessary.
2. Place the gel box into the electrophoresis system and pour the cathode buffer inside.
3. Fill the electrophoresis system (outside the gel box) with the anode buffer till the mark.
4. Remove the comb vertically and carefully.
5. Wash the loading wells with cathode buffer and a micropipette of 100 uL.
6. Load the samples into the loading wells, leaving the first well free for the molecular weight marker. This one is loaded after all the other samples.
7. Place the lid in the electrophoresis system and connect it to the power supply.
8. Run the system for 30 minutes at 90 V and the, 1 hour and a half at 120 V.

Part 6. Gel staining and destaining

1. After running, switch off the power supply and take out the gel.

2. Place it in the box (named) with staining solution for 30 minutes. During this time, keep the box in slow agitation.
3. Discard the staining solution and add the destaining solution (I) for 1 hour and a half.
4. Discard the destaining solution (I) and add the destaining solution (II) for 24 hour.
5. Take a photo of the resulting gel and discard it.