

Bee T



SDS Page Gel Electrophoresis

1. Preparation of the Gel

(1) Combine all reagents except the TEMED for the 15% separating gel. (10ml)

15% Separating Gel Components (4.195 mL)

- 2.3 mL deionized water
- 5.0mL 30% acrylamide/Bis
- 2.5 mL 1.5 M Tris, pH 8.8
- 0.1mL 10% SDS
- 0.1mL 10% ammonium persulfate

When ready to pour the gel, quickly add the TEMED

0.004mL TEMED, pH 8.9

(Warning: Acrylamide is a neurotoxin. Use gloves, do not ingest.) -----10.0ml Total

(2) Mix using a Pasteur pipette, and transfer the separating gel solution between the glass plates in the casting chamber to about 3/4 inch below the short plate.

(3) Add a small layer of absolute ethyl alcohol on top of the gel prior to polymerization to straighten the level of the gel and remove unwanted air bubbles that may be present. Once the gel has polymerized, the ethyl alcohol can be removed by absorption with Kimwipes or filter paper. (4) Dry ethanol at RT to pouring the stacking gel.

(5) Combine all reagents except the TEMED for the 5% stacking gel.

5.0% Stacking Gel (3.0 mL)

- 2.1 mL deionized water
- 5.0mL 30% acrylamide/Bis
- 0.38 mL 1.0 M Tris-HCl, pH 6.8
- 0.03mL 10% SDS
- 0.03mL 10% ammonium persulfate

When ready to pour the gel, quickly add the TEMED

0.003mL TEMED, pH 8.9

(Warning: Acrylamide is a neurotoxin. Use gloves, do not ingest.) -----3.0ml Total

(6) Mix using a Pasteur pipette, and transfer the stacking gel solution between the glass plates in the casting chamber.

(7) Insert the well forming comb into the opening between the glass plates.

(8) Both the separating and stacking gels should polymerize within six minutes.

(9) Once the stacking gel has polymerized, the comb can be gently removed. The



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polymerized gel between the short plate and spacer plate forms the "gel cassette".

2. Sample Preparation

(1) Place some water in a 600 mL beaker and leave on a hot plate to boil. (This can take 15 minutes or more.)

(2) Centrifuge bacterial suspension at 4 ° C for 3 minutes at 15000g. Discard liquid and use 500µL Buffer G (0.5M Glycerol, 1mM Na₂HPO₄) Resuspend it.

(3) Repeat step (2)

(4) Centrifuge bacterial suspension at 4 ° C for 3 minutes at 15000g. Combine 500µL Buffer G resuspend it. Add 100µL lysis buffer in every microcentrifuge tubes mix by gently inverting.

(5) Centrifuge at 15000g for 2-3min.

(6) Mix 15µL Supernatant with 5µL 4x Laemmli sample buffer. Mix 500µL Precipitation with 125µL 4x Laemmli sample buffer.

(7) In separate tubes, aliquot 10 mL of MW marker. (MW markers are already prepared in Laemmli sample buffer.)

(8) Boil the samples for 10 minutes to fully denature the proteins. Leave the samples at room temperature until ready to load onto the gel.

3. Electrophoresis

(1) Remove the gel cassette from the casting stand and place it in the electrode assembly with the short plate on the inside.

(2) Slide the electrode assembly (with the gel cassette) into the clamping frame. Press down on the electrode assembly while clamping the frame to secure the electrode assembly. This step is important to minimize potential leakage during the electrophoresis experiment.

(3) Pour some 1X electrophoresis buffer into the opening of the casting frame between the gel cassettes. Add enough 1X electrophoresis buffer to fill the wells of the gel. Use a gel loading tip to pipette some buffer into each well to ensure cleanliness.

(4) When all wells are sufficiently cleaned, slowly pipette 20µL of denatured sample or MW marker into each well.

(5) When the gel has been loaded, lower the clamping frame into the electrophoresis tank.

Fill the region outside of the frame with 1X electrophoresis buffer.

(6) Cover the tank with the lid aligning the electrodes (black or red) appropriately.

(7) Connect the electrophoresis tank to the power supply.

(8) Allow the samples to run at 80V until the dye front reaches the bottom of the 5.0% Stacking Gel (3.0 mL). change the electrophoresis Voltage 120V, This can take as long as around 2 hour.

(9) When electrophoresis is complete, turn off the power supply and disassemble the apparatus.

