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SDS-PAGE Gel Electrophoresis

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We are still developing and optimizing this protocol

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Abstract

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is used to separate proteins with relative molecular mass no smaller than 10 KD. Very small proteins (<10 KD) are difficult to resolve due to low ability of binding to SDS, which can be solved by gradient gels or using different eletrophoresis conditions, like Tricine-SDS-page.

Materials

MATERIALS

- Tris
- SDS **Bio-Rad Laboratories Catalog #**161-0302
- 2x Laemmli Sample Buffer **Bio-Rad Laboratories Catalog #**1610737
- Disodium phosphate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S7907
- Glycerol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**G5516
- 30% Acrylamide / Bis. solution **Bio-Rad Laboratories Catalog #**161-0158
- 10% Ammonium persulfate (APS) **Merck MilliporeSigma (Sigma-Aldrich)**
- TEMED **Merck MilliporeSigma (Sigma-Aldrich)**

Troubleshooting

Safety warnings

Acrylamide is a neurotoxin. Use gloves. Do not ingest.

SDS-PAGE Gel Preparation

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

1.1 Prepating Gel Components (4.195 lmuL)

	Deionized water	2.3 mL
	30% Acrylamide/Bis	5.0 mL
	1.5 M Tris, pH = 8.8	2.5 mL
	10% SDS	0.1 mL
	10% Ammonium persulfate (APS)	0.1 mL
	*TEMED, pH = 8.9	0.004 mL
	Total volume	10.0 mL

* After adding TEMED and APS to the SDS-PAGE separation gel solution, the gel will polymerize quickly, so add these two reagents when ready to pour.

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.

SDS-PAGE Gel Preparation

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.

SDS-PAGE Gel Preparation

4 Dry ethanol at RT to pouring the stacking gel.

SDS-PAGE Gel Preparation

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

5.1 5.0% Stacking Gel (3.0 mL)

	Deionized water	2.1 mL
	30% Acrylamide/Bis	5.0 mL
	1.0 M Tris-HCL, pH = 6.8	0.38 mL
	10% SDS	0.03 mL
	10% Ammonium pers	0.03 mL

	ulfate	
	*TEMED, pH = 8.9	0.003 mL
	Total Volume	3.0 mL

* Quickly add the TEMED when the gel is ready to be poured.

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

Sample Preparation

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

11 Centrifuge bacterial suspension at 4ºC for 3 minutes at 15000g. Discard liquid and use 500µL Buffer G (0.5 M Glycerol, 1mM Na_2HPO_4). Resuspend it.

12 Repeat step 11.

13 Centrifuge bacterial suspension at 4ºC for 3 minutes at 15000g .Combine 500µL Buffer G resuspend it. Add 100µL lysis buffer n every microcentrifuge tubes mix by gently inverting.

14 Centrifuge at 15000g for 2-3 minutes.

15 Mix 15µL of supernatant with 5µL 4x Laemmli sample buffer. Mix 500µL of precipitation with 125µL 4x Laemmli sample buffer.

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

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

Electrophoresis

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

19 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.

20 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.

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

22 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.

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

24 Connect the electrophoresis tank to the power supply.

25 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.

26 When electrophoresis is complete, turn off the power supply and disassemble the apparatus.

Protein Detection

27 If protein of interest is about 0.2 µg or more in the sample, typically use Coomassie blue staining. Otherwise, use silver staining (sliver staining), which is more sensitive and can detect as little as 5 ng protein.