Materials

Protein extracts from Experiment 1
PAGE apparatus

Reagents

TEMED
Acrylamide/Bis-acrylamide [Monomer solution]
Protein standards
70% EtOH (tech-grade)

4X Running Gel Buffer

1.5 M Tris-Cl, pH 8.8

- Store up to 3 months at 4˚C in the dark.

4X Stacking Gel Buffer

0.5 M Tris-Cl, pH 6.8

- Store up to 3 months at 4˚C in the dark.

10% SDS in water

Store up to 6 months at RT.

10% Ammonium Persulfate (Initiator)

0.1 g ammonium persulfate in 1.0 mL ddH_2O

- Use FRESH. Do NOT store.

2X SDS/PAGE Treatment Buffer

2.5 mL 4X stacking gel buffer
4.0 mL 10% SDS
2.0 mL glycerol
2.0 mg bromophenol blue
0.31 g dithiothreitol (DTT; FW 154.2) [*can be substituted with 6% v/v β-mercaptoethanol)

- Dilute with ddH_2O to 10 mL
- Store 0.5-mL aliquots at -20^{o}C for up to 6 months.

1X Tank Buffer

3.28 g Tris (FW 121.1)
14.41 g glycine
1 g SDS

- Dilute with ddH_2O to 1 L
- Store at RT or up to 1 month.

Staining Solution

0.5 g Coomasie Brilliant Blue R250
400 mL methanol

- Stir until dissolved
- Add 100 mL acetic acid
- Dilute with ddH_2O to 1 L
- Do not filter. Store at RT for up to 6 months.

Destaining Solution

10 % (v/v) acetic acid
30 % (v/v) MetOH

Procedure

Casting the running and stacking gels

1. Assemble the casting frame with the plates in the casting frame as indicated in the Mini PROTEAN manual. Make sure that the plates are properly aligned to prevent leakage.
2. In a conical tube, mix the following reagents to make the RUNNING GEL. Leave the stacking gel for later. The following is enough for 2 minigels. REMEMBER TO ADD 10% APS AND TEMED LAST.

Component	Running (Resolving) Gel	Stacking Gel
ddH_2O	Up to 12 mL	Up to 6 mL
4x Buffer	1x	1x
10% SDS	0.17%	0.17%
acylamide:bis(37.5:1)	10%	4%
TEMED	20 µL	10 µL
10% APS	100 µL	50 µL

*** *For 15-80 kDa proteins*

3. Swirl the conical tube quickly a few times and load the gel IMMEDIATELY after addition of APS and TEMED. Use the blue pipette tip for this. Avoid incorporating bubbles as oxygen inhibits polymerization.
4. Pour 70% EtOH to even the layer across the top of the running gel.
5. After polymerization (20 minutes to 1 hour), gently pour off the 70% EtOH by tilting the casting stand.
6. Prepare the stacking gel as above.
7. Rinse the remaining 70% EtOH of the running gel with stacking gel solution
8. Pipette the stacking solution to the brim of the plate.
9. Gently place the comb. Avoid trapping bubbles. Allow the gel to polymerize for about 20 minutes.

Preparing the samples

10. Combine equal parts of protein sample and 2X treatment buffer (to make 20 uL) in tubes.
11. Boil samples including appropriate protein standards for 5 minutes.
12. Place samples and standards on ice until ready for use. Samples may be stored for 6 months at -20°C.

Loading the samples

13. Assemble the PAGE apparatus as instructed in the manual. Remove the comb in ONE SWIFT MOTION before placing the gel in the system.
14. Fill the container with tank buffer.
15. Load 20-30 uL of treated samples.
16. Insert the electrical leads into a suitable power supply with the proper polarity.
17. Apply the power. Set electrophoresis at a constant 40 mA and run for about 1 hour or until the bands have traveled ¾ of the gel length.

Coomasie Gel Staining

*** Perform staining at RT. Use covered plastic trays to minimize exposure to methanol and acetic acid. Do the procedure under the fume hood.

18. Place the gel in Staining Solution. Ensure that the gel floats free in the tray. Shake slowly on shaking platform (~225 rpm) for approximately 4 hours to overnight.

    *This can be hastened by microwaving the gel in the staining solution for ~1 min and letting it incubate on the shaking platform for 30 min.

19. Replace the staining solution with Destaining Solution, and incubate this on an orbital shaker overnight.

    *This can be hastened by microwaving the gel in destaining solution for ~1min and letting it incubate on the shaking platform for 30 min – 1 hr. Remember to replace the destaining solution whenever its color becomes too dark.

21. Take a picture of the gel.

22. It is suggested to cut appropriate-sized pieces of transparency paper to store the PAGE gels in. Seal the edges using tape.

22. For long-time storage, store in Destaining Solution. Add 1% glycerol to minimize cracking before drying the gel.