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SDS-PAGE Protocol

REAGENTS

Resolving gel solution（1.5M Tris-HCL, 0.4%SDS.pH 8.8）

181.7g Tris
40mL 10%SDS 或 4g SDS
Use HCL to adjust pH to 8.8
Add ddH2O to 1L, store at RT.

Stacking gel solution（0.5M Tris-HCL, 0.4%SDS, pH6.8）

60.6g Tris
40mL 10%SDS or 4g SDS
Use HCL to adjust pH to 6.8
Add ddH2O to 1L, store at RT.

30%Arc-Bis

300g Acrylamide
8g Bis-acrylamide
add ddH2O to 1L, store at 4℃.

10×Running (electrophoresis) Buffer

Tris 30.3g
Glycine 144g
SDS 10% 100 mL or 10g SDS
add ddH2O to 1L, store at RT.

10×Transfer Buffer

Tris 30.3g
Glycine 144g
add ddH2O to 1L, store at RT. When use, dilute 100mL stock in 700 mL of ddH2O, add 200mL of methanol to bring up to 1L.

5X Loading Buffer (10 mL)

150 mg Tris
1 g SDS
5 mL Glycerin
25 mg Bromophenol blue
1mL β-mercaptoethanol
Use HCL to adjust pH to 6.8
Add ddH_20 to 10 mL.

INTRODUCTION

SDS-PAGE, is short for sodium dodecyl sulfate polyacrylamide gel electrophoresis. In SDS-PAGE, the detergent SDS and a heating step determine that the electrophoretic mobility of a single kind of protein is only affected by its molecular weight in the porous acrylamide gel.

SDS PAGE Preparation:

The SDS PAGE gel in a single electrophoresis run can be divided into stacking gel and separating gel. The acrylamide percentage in SDS-PAGE gel depends on the size of the target protein in the sample.

Acrylamide %	M.W. Range
6%	350 kDa - 500 kDa
8%	150 kDa - 350 kDa
9%	75 kDa - 150 kDa
10%	35 kDa - 75 kDa
12%	10 kDa - 35 kDa

- **For stacking gel:**

ddH2O	1.8 mL
0.5M Tris-HCL, 0.4%SDS, pH6.8	0.75 mL
Acrylamide/Bis-acrylamide (30%/0.8% w/v)	0.4 mL
10% (w/v) ammonium persulfate (AP)	15 μL
TEMED	5 μL

- **For separating gel:**

Acrylamide percentage	6%	8%	9%	10%	12%
ddH2O/mL	2.7	2.4	2.2	2	1.7
1.5M Tris-HCL, 0.4%SDS. pH 8.8)/mL	1.25	1.25	1.25	1.25	1.25
Acrylamide/Bis-acrylamide (30%/0.8% w/v)/mL	1	1.25	1.46	1.66	2
10% (w/v) ammonium persulfate (AP)/μL	50	50	50	50	50
TEMED/μL	5	5	5	5	5

Note: AP and TEMED must be added right before each use.

MAKE THE SEPARATING GEL:

Set the casting frames (clamp two glass plates in the casting frames) on the casting stands.

Prepare the gel solution (as described above) in a separate small beaker.

Swirl the solution gently but thoroughly.

Pipet appropriate amount of separating gel solution (listed above) into the gap between the glass plates.

To make the top of the separating gel be horizontal, fill in water (either isopropanol) into the gap until an overflow.

Wait for 20-30min to let it polymerize.

MAKE THE STACKING GEL:

1. Discard the water and you can see separating gel left.

Pipet in stacking gel until an overflow. Insert the well-forming comb without trapping air under the teeth. Wait for 20-30min to let it polymerize.

2. Make sure a complete gelation of the stacking gel and take out the comb. Take the glass plates out of the casting frame and set them in the cell buffer dam. Pour the running buffer (electrophoresis buffer) into the inner chamber and keep pouring after overflow until the buffer surface reaches the required level in the outer chamber.

3. Prepare the samples: Mix your samples with 5× loading buffer. Heat them in 95℃ for 5min, let cool down on ice. Centrifuge samples briefly, and then vortex, finally centrifuge them briefly again before loading.

4. Load prepared samples into wells and make sure not to overflow. Don't forget loading protein marker into the first lane. Then cover the top and connect the anodes.

5. Set an appropriate volt and run the electrophoresis when everything's done. In general, run the gel at 4℃ or on ice, first at 90V for 20 min, then shift to 120V for another 60 min.

6. As for the total running time, stop SDS-PAGE running when the downmost sign of the protein marker almost reaches the foot line of the glass plate.

Note: Various factors affect the properties of the resulting gel.

-Higher concentration of ammonium persulfate and TEMED will lead to a faster gelation, on the other hand, a lower stability and elasticity.

-The optical temperature for gel gelation is 23°C-25°C. Low temperature will lead to turbid, porous and inelastic gels.

-The pH is better to be neutral and the gelation time should be limited in 20-30 min.

APPENDIX: SAMPLE PREPARATION

1. Prepare the IntactProtein™ lysis buffer by adding 2 μL of Reagent A into 1 mL of Reagent B immediately before use. Mix thoroughly by vortexing and place on ice.

Tips: Calculate the volume of the lysis buffer you need as per Step 3; discard the unused buffer after use.

2. Discard the cell culture medium and wash the cells twice with ice-cold PBS.

3. Place the culture dish/plate on ice or ice water and add 1 mL of the premixed lysis buffer per $5x10^6$ cells (e.g. add 300 μL of lysis buffer to a 35 mm dish containing 1 $x10^6$ cells). Keep the dish/plate on ice for an additional 5 min and swirl occasionally to allow the lysis buffer to completely cover the cells.

4. After 5 min of lysis, scrape the cells off the dish/plate using a clean plastic scraper and collect the lysate into a centrifuge tube.

5. Vortex the lysates thoroughly (3 x 10 sec) and place the lysates on ice or ice water for another 10 min to complete the lysis.

6. Heat the lysates on a 95°C heat block for 5 min.

7. Cool the lysates on ice or ice water for 3 min.

8. Centrifuge the lysates at 13, 000g for 5 min at 4°C.

9. Measure the protein concentration using a NanoDrop spectrophotometer or SDScompatible protein assay.

10. Store the lysates at -20°C for future use or use immediately for further analysis.