SDS-PAGE

Reactants:

- TEMED

Solutions:

- Acrylamide at 30% / bisacrylamide at 0.8%
    - 28.38 g of Acrylamide
    - 1.62 g of Bis-acrylamide
    - Dissolve in 40 mL of distilled water in constant agitation and take to a final volume of 100 mL.
    - Store at 4 °C protected from the light.

- Tris-HCl/SDS 4X pH 8.8 (1.5 M Tris-HCl, 0.4% SDS)
    - 9.08 g of TRIZMA® base
    - 0.2 g of SDS
    - Dissolve in 20 mL of distilled water.
    - Adjust pH at 8.8 with HCl and take to a final volume of 50 mL
    - Store at 4 °C

- Tris-HCl/SDS 4X pH 6.8 (0.5 M Tris-Cl, 0.4% SDS)
    - 3.025 g of TRIZMA® base
    - 0.2 g of SDS
    - Dissolve in 20 mL of distilled water.
    - Adjust pH at 6.8 with HCl and take to a final volume of 50 mL
    - Store at 4 °C

- Ammonium Persulfate at 10%
    - 100 mg of PSA (Ammonium Persulfate)
    - Dissolve in 1 mL of distilled water
    - Store at -20 ºC for long time storage, it can last 2 weeks
    - Store at 4 ºC for one time use only

- Sample Buffer 2X
    - SDS-sample buffer 2X
        - 1.52 g of TRIZMA® base
        - 2 g of SDS
        - Dissolve in 50 mL of distilled water.
        - Adjust pH at 6.8 with HCl.
        - Add 20 mL of glycerol.
        - Take it to a final volume of 100 mL with distilled water.

Work usage: add 400 uL of SDS-sample buffer 2X, 50 uL of b-mercaptoethanol and 50 uL of bromophenol blue (10 mg / mL).

SDS-PAGE

- Running buffer 5X (Stock)
    - 72 g of glycine
    - 15.1 g of TRIZMA® base
    - 5 g of SDS
    - Dissolve in 400 mL of distilled water and take to 1 L.
    - Store at 4 ºC

For work solution: prepare 1X solution from stock.

- Staining Solution
    - 0.25 g of Coomassie Blue in 30 mL of methanol
    - Add 10 mL of glacial acetic acid
    - 60 mL of distilled water
    - Filter with Whatman paper
    - Store at room temperature protected from light

Use: pour solution in a container and leave on agitation overnight to stain the SDS gel.

- Destaining Solution
    - 300 mL of methanol
    - 100 mL of glacial acetic acid
    - 600 mL of distilled water
    - Store at room temperature

Use: pour solution in a container and leave on agitation until the SDS gel is transparent or the electrophoresis bands are visible.

Protocol

NOTE: The quantities detailed below correspond to a BIO RAD Mini-PROTEAN Tetra Cell Catalog #165-8001 SDS-PAGE Chamber.

1. Clean all the electrophoresis chamber components with 70% ethanol and gauze.
2. Assemble chamber and make sure that it's not leaking by pouring some water into the gel glasses.
3. Prepare polyacrylamide gels:
    - Separation Gel (14%)

Reactants	For 2 minigels
Distilled Water	1.54 mL
4x Tris-HCl/SDS pH 8.8	3.75 mL
Acrylamide at 30% / bisacrylamide at 0.8%	4.66 mL
* Add 50 uL of PSA and 10 uL of TEMED	

4. Pour the solution between the glasses using a 1 mL micropipette leaving 1.5 cm of space to add the stacking gel. In order to help smooth the gel's surface, add isopropanol and let sit for 30 minutes or until a sharp line between the gel and the isopropanol can be seen.
    - Stacking gel (5%)

Reactants	**For 2 minigels**
Distilled Water	1.496 mL
4x Tris-HCl/SDS pH 6.8	0.65 mL
Acrylamide at 30%/bisacrylamide at 0.8%	0.406 mL
* Add 25 uL of PSA and 5 uL of TEMED	

5. Pour the solution on top of the separation gel between the glasses using a 1 mL micropipette.
6. Insert the comb carefully, avoiding bubbles and let sit for 30 minutes.
7. Once both gels are polymerized, prepare the electrophoresis chamber and fill up with electrophoresis damper 1X to cover up the gels and until the line marking 2 gels.
8. Dilute samples in sample buffer 2X with the following quantities:
    - 5 uL of protein extract
    - 5 uL of NaCl 0.15 M
    - 10 uL of sample buffer 2X
9. To denature proteins, heat up prepared samples at 65 ºC for 10 minutes.
10. Load gel(s).
11. In the first well, add 10 uL of molecular weight marker, no denature or sample buffer are needed.
12. Once the samples are loaded, start running the gel until samples reach the separator gel. Increase electric charge until samples reach the bottom of the gel.
13. Turn off chamber and disassemble.
14. Take the gel off the glass and place it in a container with staining solution. Leave the container in agitation for 1 hour.
15. After 1 h take away staining solution and pour destaining solution.
16. Leave on agitation and wait until the gel is transparent of the samples can be clearly seen. The destaining solution needs to be changed every once in a while in order to eliminate excess dye.
17. After this, analyze the gel.