SDS PAGE and Western blot

1. Wipe down the spacer plates (spacers attached) and short plates (BioRad) with D.water, 70%ethanol to remove any adherent material, dry and clamp them together.

2. Solutions used:

a. 1.5 M Tris-HCl, pH 8.8
b. 0.5 M Tris-HCl, pH 6.8
c. 30% acrylamide/bisacrylamide
d. N,N,N',N'-tetramethylethylene diamine (TEMED).
    This solution can be bought commercially and stored in fridge.
e. 10% SDS
    Add 5.0 g SDS to a 100 ml bottle
    Add 50 ml deionized water
f. 10% w/v ammonium persulfate, make fresh every month and store in fridge.

g. Running buffer: Take 100 ml of stock (10X Tris glycine running buffer) and 900 ml of Distilled water and make up to one liter.

e. Sample buffer:
    100 mM Tris, pH 6.8,
    2% SDS,
    5% ß- mercaptoethanol,
    15% glycerol,

3. Separating gel (add the following recipe)

Percentage	14%	12%		10%		7.5%	
Total	40 ml	**10 ml**	**5 ml**	**10 ml**	**5 ml**	**10 ml**	**5 ml**
D.Water	10.33 ml	3.35 ml	1.68 ml	4.0 ml	2.0 ml	4.85 ml	2.43 ml
Tris buffer (1.5M, pH 8.8)	10 ml	2.5 ml	1.25 ml	2.5 ml	1.25 ml	2.5 ml	1.25 ml
Acrylamide : Bis acrylamide	18.67 ml	4.0 ml	2.0 ml	3.33 ml	1.67 ml	2.5 ml	1.25 ml
10% SDS	400 µl	100 µl	50 µl	100 µl	50 µl	100 µl	50 µl
10% APS	200 µl	50 µl	25 µl	50 µl	25 µl	50 µl	25 µl
TEMED	40 µl	15 µl	15 µl	15 µl	15 µl	15 µl	15 µl

3. First add the add D.Water, Tris buffer, Acrylamide: Bis acrylamide solution and 10% SDS.

4. Add 10% ammonium persulfate and gently mix the solution.

5. Add 15 µl of TEMED (stored in refrigerator), and gently invert to mix the gel components thoroughly (avoid introducing air bubbles as this can inhibit polymerization).

6. Immediately pour the mix in between the plates. Fill the space up so there will be enough room to form a stacking gel of 0.5 to 1 cm.

7. Overlay with 70% ethanol to a depth of a few millimeters.

8. Allow the gel to polymerize for 20 minutes.

9. After the running gel has polymerized, rinse the ethanol from the surface with D.water. Drain excess water.

10. Prepare the stacking gel. This is composed of 4% acrylamide

Stacking gel (add the following recipe)

Percentage	**4%**	
Total	**10 ml**	**5 ml**
D.Water	3.35 ml	1.68 ml
Tris buffer (0.5M, pH 6.8)	2.5 ml	1.25 ml
Acrylamide : Bis acrylamide	4.0 ml	2.0 ml
10%SDS	100 µl	50 µl
10% APS	50 µl	25 µl
TEMED	15 µl	15 µl

11. Add the stocking gel mix. Insert appropriate combs.

12. Polymerize stacking gel for 30 minutes.

13. By the time prepare the protein. If the protein is already in solution, add an equal volume of 2X sample buffer and boil in a boiling water bath for 5 min. Boil the markers if necessary (according the instructions by the company).

14. Carefully remove comb (add some drops of running buffer in between well and combs to do the job easy)

15. Rinse wells thoroughly with running buffer and assemble the gel in the electrophoresis rig.

16. Pour running buffer in the top and bottom chambers,

18. Load the samples in appropriate wells and add more running buffer in the top chamber

20. Run the gel. Use a constant Amps power supply (80 mA). It will take approximately 1-2 hours.

21. Coomassie staining your gel

Coomassie stain

a. Dissolve 2g Coomassie Blue R, 250 in 250ml water

b. Add 75ml of glacial acetic acid.

c. Add 500ml of ethanol

d. make up to 1000ml with water

22. Immerse gel for 1 hr in Coomassie.stain solution.

23. Destain gel

Distaining solution

a. Methanol -50%
b. Acetic acid - 10%
c. Distilled water -40%

24. Immerse the stained gel into the destain solution Change the destain solution when it becomes very blue.