SDS-PAGE GELS

Lower Gel Buffer:
36.4 g Tris in 100 mL dH_2O
pH to 8.8
add 0.8 g SDS

Upper Gel Buffer:
6.05 g Tris Base in 100 mL dH_2O
pH to 6.8
Add 0.4 g SDS

10X Laemelli Running Buffer:
For 1L:
30.3 g Tris Base
144.1 g Glycine
10g SDS
pH should be 8.3

# gels→	**1**	**2**	**3**	**4**
5%				
Acrylamide	0.62	1.25	1.87	2.5
Lower Gel Buffer	1.25	2.50	3.75	5.00
dH_2O	3.13	6.26	9.4	12.25
TOTAL	5	10	15	20
8%				
Acrylamide	1	2	3	4
Lower Gel Buffer	1.25	2.50	3.75	5.00
dH_2O	2.75	5.50	8.25	11.00
TOTAL	5	10	15	20
10%				
Acrylamide	1.25	2.50	3.75	5.00
Lower Gel Buffer	1.25	2.50	3.75	5.00
dH_2O	2.5	5.0	7.5	10
TOTAL	5	10	15	20
12%				
Acrylamide	1.5	3	4.5	6
Lower Gel Buffer	1.25	2.50	3.75	5.00
dH_2O	2.25	4.5	6.75	9
TOTAL	5	10	15	20
Upper Gel				
Acrylamide	0.28	0.56	0.85	1.13
Upper Gel Buffer	0.63	1.25	1.88	2.5
dH_2O	1.6	3.2	4.8	6.4
TOTAL	2.5	5.0	7.5	10.0
**This is for 0.75 mm*	*gels.*	*Double*	*for 1.5mm*	*gels.*

**For 5 mL gel solution*	*add*	*10 uL*	*TEMED*	
	and	*20 uL*	*10% APS*	

1. Assemble the glass plates according to the manufacturer's instructions.

2. In an Erlenmeyer flask, prepare the appropriate volume of solution containing the desired concentration of acrylamide for the resolving gel, using the values given in the table below. Mix the components in the order shown. Polymerization will begin as soon as the TEMED has been added. Without delay, swirl the mixture rapidly and proceed to the next step.

3. Pour the acrylamide solution into the gap between the glass plates. Leave sufficient space for the stacking gel. Using a Pasteur pipette , carefully overlay the acylamide solution with 0.1% SDS (for gels containing less than 8% acrylamide) or isobutanol (for gels containing more than 10% acrylamide). Place the gel in a vertical position at room temperature.

4. After polymerization is complete, pour off the overlay and wash the top of the gel several times with deionized water to remove any unpolymerized acrylamide. Drain as much fluid as possible.

5. Prepare the stacking gel as follows: In a disposable plastic tube, prepare the appropriate volume of solution containing the desired concentration of acrylamide, using the second table below.

6. Pour the stacking gel solution directly onto the surface of the polymerized resolving gel. Immediately insert a clean Teflon comb into the stacking gel, being careful to avoid bubbles. Add more stacking gel if there are any spaces. Place the gel in a vertical position at room temperature.

7. While the stacking gel is polymerizing, prepare the samples by heating them to 100°C for 3 minutes in 1 X SDS gel-loading buffer to denature the proteins.

8. After polymerization is complete, wash with deionized water to remove unpolymerized acrylamide. Remove the Teflon comb. Mount the gel in the electrophoresis apparatus. Add Tris-glycine electrophoresis buffer to the top and bottom reservoirs.

9. Load up to 15 uL of each of the samples in a predetermined order into the bottom of the wells. Add an equal volume of 1 X SDS gel-loading buffer to the unused wells.

10. Attach the electrophoresis apparatus to an electric power supply. Apply a voltage of 8 V/cm to the gel. Once the dye front has run into the resolving gel, increase the voltage to 15 V/cm. Run the gel until the bromophenol reaches the bottom of the resolving gel and then turn off the resolving gel.

11. Remove the glass plates from he electrophoresis apparatus and place them on a paper towel. Using a spatula, pry the plates apart. Mark the orientation of the gel by cutting a corner form the bottom of the gel that is closest to the leftmost well.

12. The gel can now be fixed, stained with Coomassie Blue, or used to establish a western blot.