

SDS-PAGE Gel Instructions

The following recipes are more than enough for two 1.5 mm Gels
The buffers/solutions do not need to be degassed if using a minigel

	<u>% Resolving Gel</u>					
	<u>6%</u>	<u>8%</u>	<u>10%</u>	<u>12%</u>	<u>14%</u>	<u>16%</u>
Soln A (ml)	2.25	3.00	3.75	4.50	5.25	6.00
Soln. B (ml)	3.75	3.75	3.75	3.75	3.75	3.75
H ₂ O (ml)	9.00	8.25	7.50	6.75	6.00	5.25
TEMED (μl)	10	5	5	5	5	5
10% APS (μl)	75*	75*	75*	75*	75*	75*

- Add the APS last. Once added it will start to polymerize

<u>4% Stacking Gel</u>	<u>10X Electrophoresis Buffer</u>	<u>1X Conc.</u>
Solution A (ml)	0.60	30.0 g Tris Base -- 25 mM
Solution C (ml)	1.50	144.0 g Glycine --192 mM
H ₂ O (ml)	3.96	10.0 g SDS --0.1%
10% APS (μl)	40.0	QS to 1 liter, pH should be 8.3
TEMED (μl)	10.0	

Working Solutions:

Solution A: 40% (w/v) acrylamide, 1.09 % (x/v) bis-acrylamide

194.8 g acrylamide
5.2 g bis-acrylamide
QS to 500 ml w/ H₂O

5X Sample buffer, 10 ml

0.6 ml 1 M Tris-HCl (pH 6.8)
5 ml 50% glycerol
0.5 ml β Mercaptoethanol
1 ml 1% Bromophenol blue
0.9 ml H₂O

Solution B: 4X Separating gel Buffer

Dissolve 91 g Tris base in 300 ml H₂O
Adjust pH to 8.8 w/NaOH
Add 2 g SDS
QS to 500 ml with H₂O and filter

Solution C: 4X Stacking Buffer

Dissolve 6.05 g Tris base in 40 ml H₂O
Adjust pH to 8.8 w/NaOH
Add 0.4 g SDS
QS to 500 ml with H₂O and filter

10% APS

0.5 g Ammonium persulfate
5.0 ml H₂O (stable frozen)

Well capacity:

At 1.0 mm gel thickness 10 wells = 32 μl and 15 well = 18 μl
At 1.5 mm gel thickness 10 wells = 48 μl and 15 well = 27 μl

Coomasie Gel Stain, 1 liter

1.0 g Coomassie Blue R-120
450 ml methanol
450 ml H₂O
100 ml Glacial Acetic Acid

Coomasie Gel Destain, 1 liter

100 ml Methanol
100 ml Glacial Acetic Acid
800 ml H₂O