

Protein Gel Electrophoresis

Run a BioRad minigel apparatus at a constant voltage of 200V.
Running it at constant voltage prevents bands from smiling.

For the BioRad mini-trans blot apparatus, run it at a constant voltage of
~70V (250 mA) for 1 hour, with the ice chiller, and constant stirring.

Stock Solutions for Polyacrylamide Gels

40% (w/v) Acrylamide (37.5:1)

38.961 % Acrylamide
1.039 % bis-acrylamide

per 100 ml

38.961 grams
1.039 grams

10X Electrode Buffer

1.92 M Glycine
250 mM Tris-base
1% SDS

per 1L

144.13 grams
30.28 grams
10 grams

Stacking Buffer

0.14 M Tris pH 6.8
0.11 % TEMED
0.11 % SDS

per 500 ml

8.45 grams
0.55 ml
2.75 ml of 20 %

10x Electroblotting Buffer

1.92 M Glycine
250 mM Tris-base
1 % SDS
*** Before use, make 1X with 20 % Methanol*

per 1L

144.13 grams
30.28 grams
10 grams

Resolving Buffer

1.5 M Tris pH 9
0.4 % TEMED
0.4 % SDS

per 500 ml

90.86 grams
2 ml
10 ml of 20 %

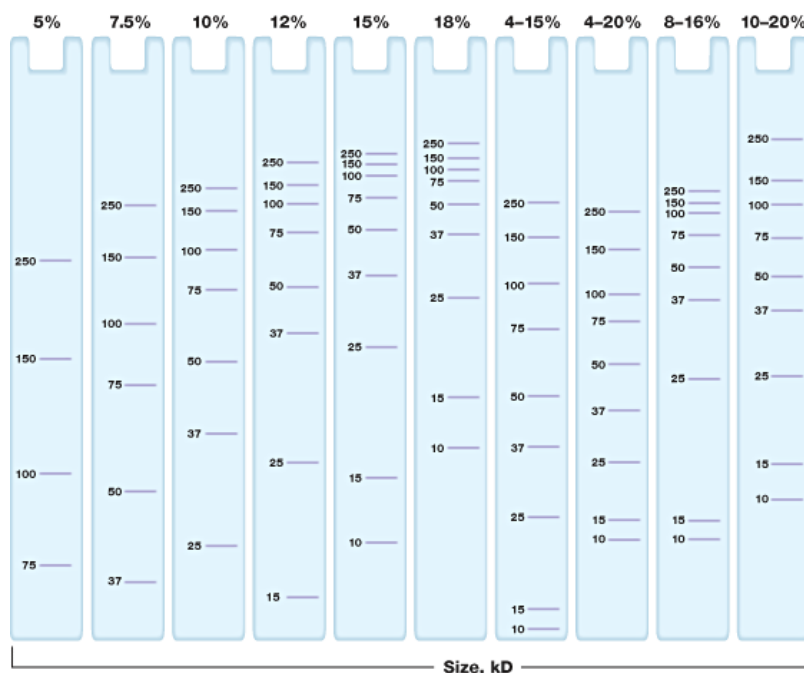
2X Sample Buffer

4 % SDS
20 % Glycerol
200 mM DTT
10 % 2-mercaptoethanol
0.2 % Bromphenol blue
0.125 M Tris-HCl pH 6.8

4x Sample Buffer (Prepare only 10ml)

8% SDS (From 20% stock) prepare 10ml stock of 20% in water
40% glycerol
20 % 2-mercaptoethanol
0.4 % Bromphenol blue
250 mM Tris-HCl pH 6.8

Layer saturated n-butanol on top of running gel to get a straight edge.



Approximate migration of proteins on various percentage gels.

SDS-PAGE Formulations for large gels

Component	3.9 % Stacking	5 %	7.5 %	10 %	12.5 %	15 %	20 %
40% Acrylamide	1 ml	5.13	7.70	10.27	12.83	15.40	20.53
Resolving Buffer	-----	10	10	10	10	10	10
Stacking Buffer	8.9	-----	-----	-----	-----	-----	-----
Distilled Water	-----	24.47	21.90	19.33	16.77	14.20	9.07
10 % APS Add after degassing	50 µl	.4	.4	.4	.4	.4	.4
Total Volume (mL)	10	40	40	40	40	40	40

SDS-PAGE Formulations for 2 mini gels

Component	3.9 % Stacking	5 %	7.5 %	10 %	12.5 %	15 %	20 %
40% Acrylamide	0.5 ml	1.28	1.93	2.57	3.21	3.85	5.13
Resolving Buffer	-----	2.5	2.5	2.5	2.5	2.5	2.5
Stacking Buffer	4.474	-----	-----	-----	-----	-----	-----
Distilled Water	-----	6.12	5.47	4.83	4.19	3.55	2.27
10 % APS Add after degassing	25 µl	0.1	0.1	0.1	0.1	0.1	0.1
Total Volume (mL)	5	10	10	10	10	10	10

If using a 30% Acrylamide Stock (37.5:1) then use the formulations below.

30% (w/v) Acrylamide (37.5:1)

Purchased from MRC Stores

Or

29.221 % Acrylamide
0.779 % bis-acrylamide

per 100 ml

1 L

29.221 grams
0.779 grams

SDS-PAGE Formulations for large gels

Component	3.9 % Stacking	5 %	7.5 %	10 %	12.5 %	15 %	20 %
30% Acrylamide	1.33 ml	6.84	10.27	13.69	17.11	20.53	27.38
Resolving Buffer	-----	10	10	10	10	10	10
Stacking Buffer	8.62	-----	-----	-----	-----	-----	-----
Distilled Water	-----	22.76	19.33	15.91	12.49	9.07	2.22
10 % APS Add after degassing	50 µl	.4	.4	.4	.4	.4	.4
Total Volume (mL)	10	40	40	40	40	40	40

SDS-PAGE Formulations for 2 mini gels

Component	3.9 % Stacking	5 %	7.5 %	10 %	12.5 %	15 %	20 %
30% Acrylamide	0.667 ml	1.71	2.57	3.42	4.28	5.13	6.84
Resolving Buffer	-----	2.5	2.5	2.5	2.5	2.5	2.5
Stacking Buffer	4.308	-----	-----	-----	-----	-----	-----
Distilled Water	-----	5.69	4.83	3.98	3.12	2.27	0.56
10 % APS Add after degassing	25 µl	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl
Total Volume (mL)	5	10	10	10	10	10	10

Always degas all solutions before use and remember to add the 10% Ammonium Persulfate only AFTER degassing and just before pouring the gels.