

SDS-Polyacrylamide Gel Electrophoresis

Proteins can be separated on Polyacrylamide gels on the basis of size. Choose a polyacrylamide gel concentration that will allow a good separation of the band(s) of interest using the table below.

% Acrylamide	Best Resolution Range (kDa)
5	25-200
10	15-70
15	12-45

- Prepare a gel according to the recipe below. Mix the components in an Erlenmeyer flask in the fume hood with constant stirring under vacuum on for 15 min to remove air bubbles. Handle unpolymerized acrylamide powder with caution as it is a potent neurotoxin and its effects are cumulative.

	Resolving Gel						Stacking Gel						
	7.5%		10%		12%		15%		20%		4%		
# of Gel	1	2	1	2	1	2	1	2	1	2	1	2	4
H ₂ O (mL)	2.5	5	2	4	1.75	3.5	1.25	2.5	0.25	0.5	3.1	6.2	12.4
0.5 M Tris-HCl(pH 6.8) -0.4% SDS (mL)	-	-	-	-	-	-	-	-	-	-	1.25	2.5	5
1.5 M Tris-Base(pH 8.8) -0.4% SDS (mL)	1.25	2.5	1.25	2.5	1.25	2.5	1.25	2.5	1.25	2.5	-	-	-
30% Acrylamide -0.8% Bis (mL)	1.25	2.5	1.75	3.5	2	4	2.5	5	3.5	7	0.65	1.3	2.6
10% Am Persulfate (μL)	30	50	30	50	30	50	30	50	30	50	30	50	100
TEMED (μL)	3	5	3	5	3	5	3	5	3	5	3	5	10

- Pour running buffer into the anode and cathode chambers.

10x Running Buffer

		1x Final Conc
Glycine	144 g	192 mM
Tris-base	30 g	25 mM
SDS	10 g	0.1% (w/v)

q.s. to 1 L with distilled H₂O and dilute to 1x before use.

- Preparation of samples:
 For human cell extracts: add 100 μL of 4x sample buffer into 300 μL of cell extract (A₂₈₀=3.0).
 For bacterial cell extracts: dissolve the final pellet in equal amount of 2x sample buffer.
 For insect cells infected with baculovirus: use a lysate from 3000 cells per lane in 10-well comb with diluted sample buffer. Heat the samples at 95°C for 10 min before loading on the gel. Please refer to the formula for sample buffer.

Sample Buffer

	2x	3x	4x	5x
Tris-HCl (pH 6.8)	50 mM	75 mM	100 mM	125 mM
SDS	4%	6%	8%	10%
Bromophenol blue	0.004%	0.006%	0.008%	0.01%
Glycerol	20%	30%	40%	50%
2-Mercaptoethanol	10%	15%	20%	25%

- Load a lane of 10 μ L of pre-stained molecular weight standards; 30 μ L of sample/well or 300-500 μ L/space.
- Run the gel according to the electrophoresis unit's manufacturer's instructions.
- Transfer proteins at 4°C from the SDS-PAGE gel to either a nitrocellulose or a PVDF membrane. (Note: PVDF membranes need to be activated with methanol before use. Smaller proteins require less transfer time than larger proteins.)

5x Transfer Buffer

		1x Final Conc
Tris-HCl	7.68 g	10 mM
Tris-Base	9.25 g	15 mM
Glycine	72.08 g	192 mM
SDS	2.5 g	0.05% (v/v)

q.s. to 1 L with distilled H₂O and dilute to 1x before use.