



Polyacrylamide Gel Electrophoresis (PAGE)

General consideration

Polyacrylamide is a cross-linked polymer of Acrylamide. The length of the polymer chains is dictated by the concentration of Acrylamide used, which is typically between 3.5 and 20%. **Polyacrylamide** gels are significantly more annoying to prepare than agarose gels. **Because oxygen** inhibits the polymerization process, they must be poured between glass plates. **Acrylamide is a potent neurotoxin and should be handled with care!** Wear disposable gloves when handling solutions of acrylamide, and a mask when weighing out powder. Polyacrylamide is considered to be non-toxic, but **Polyacrylamide** gels should also be handled with gloves due to the possible presence of free Acrylamide.

Polyacrylamide gels have a rather small range of separation, but very high resolving power. In the case of DNA polyacrylamide is used for separating fragments of less than about 500 bp. However, under appropriate conditions, fragments of DNA differing in length by a single base pair are easily resolved. In contrast to agarose, polyacrylamide gels are used extensively for separating and

Acrylamide

Acrylamide gels are used for:

1. The separation of small fragments (<1 kb)
2. Getting the best resolution (for example, separation of sequencing reactions)
the proportion of Acrylamide and bisacrylamide in the mix will vary depending on the type of molecules to be separated:
 1. for nucleic acid sequencing, a 19:1 mix will be used
 2. for classical runs, a mix 29: 1 will be used
 3. for protein separation, a 37.5:1 mix will be used(a 19:1 mix means that the mix is prepared with 19 g Acrylamide for 1 g bisacrylamide).

How to work:

1. Pour the Separating Gel

Set up your gel apparatus, prepare separating gel monomer. Add TEMED just prior to pouring gel (I "pour" the gels using a Pasteur pipette and a rubber bulb). Allow to polymerize before adding stacking gel by overlaying gently with water or n-butanol. With higher % gels, one can immediately pour the stacking gel on the unpolymerized separating gel. Be careful not to mix the two layers.

Separating Gels, in 0.375 M Tris, pH 8.8

2. Pour the Stacking Gel

After the separating gel has polymerized, decant the overlay, prepare the stacking monomer, add the TEMED, and pour. Insert the comb and allow to polymerize completely before running.

Stacking Gels, 4.0% gel, 0.125 M Tris, pH 6.8

For best results:

1. Make ammonium persulfate solution fresh daily.
2. Degas solutions before adding TEMED for 15 min at room temperature.
3. Running the gel

I usually run my gels at constant current, 25-50 mA, depending on gel size. Here's the recipe for 5X SDS-PAGE running buffer. Dilute to 1X before use.

SOLUTIONS

30% acrylamide mix	29% (w/v) acrylamide
	1% (w/v) N,N'-methylenebisacrylamide Store at 4° C in the dark [30 days max].
5x Electrophoresis buffer	Tris base: 15.1 g
	Glycine: 94 g
	SDS: 5 g
	H ₂ O upto 1 liter
4x Resolving-gel buffer	1.5M Tris-Cl (pH 8.8)
8x Stacking-gel buffer	1M Tris-Cl (pH 6.8)
1x SDS gel-loading buffer	50mM Tris-Cl (pH 6.8)
	100mM dithiothreitol
	2% SDS
	0.1% bromophenol blue
	10% glycerol

RESOLVING GEL MIXTURE

Resolving Gel (30ml)	6%	8%	10%	12%	15%
H ₂ O	16.2	14.2	12.2	10.2	7.2
30% acrylamide mix	6.0	8.0	10	12	15
4x resolving-gel buffer	7.5	7.5	7.5	7.5	7.5
20% SDS	0.15	0.15	0.15	0.15	0.15
20% APS	0.15	0.15	0.15	0.15	0.15
TEMED	0.024	0.018	0.012	0.012	0.012

STACKING GEL MIXTURE

Stacking Gel (10ml)		
	H ₂ O	6.9 ml
	30% acrylamide mix	1.7 ml
	8x stacking-gel buffer	1.25 ml
	20% SDS	0.05 ml
	20% APS	0.05 ml
	TEMED	0.01 ml

SOLUTIONS FOR STAINING AND DISTAINING

SOLUTIONS FOR STAINING AND DISTAINING	methanol	90 ml
	H ₂ O	90 ml
	acetic acid	20 ml
	Coomassie Brilliant Blue R250	0.25 g
Staining solution	Dissolve well and filtrate through a filter paper.	
Destaining solution	isopropanol	12.5%
	glacial acetic acid	10%
	Addition of small pieces of used X-ray film with the gel will accelerate the destaining process.	

SOLUTION FOR DRYING

20% methanol, 3% glycerol in dist. water

SDS Polyacrylamide Gel Electrophoresis

WARNING: Unpolymerized acrylamide is extremely toxic. Wear gloves and never pipette by mouth.

Staining for Proteins

Stain about 30 minutes at room temperature.

0.1% Coomassie Blue R250

40 % Methanol

10% Acetic acid

Destain with several changes of destaining solution.

40 mL Methanol

75 mL Acetic acid

To 1 Litre

Literature

Rothe, G. M., 1994. *Electrophoresis of Enzymes - Laboratory Methods*. Springer Verlag, Berlin, p 231.

Protein Standards

At least four proteins of known molecular weight should be chosen to construct a standard curve. They should cover a range of values such that the molecular weight of your unknown samples will fall well within that range. Some useful proteins to use as standards are:

Protein Molecular Weight & Subunit Weight:

Phosphorylase A : 92,500 & 92,500

BSA: 67,000 & 67,000

Glutamate dehydrogenase: 220,000 & 53,000

Avian albumin: 45,000 & 45,000

Aldolase: 158,000 & 40,000

Chymotrypsinogen A: 25,000 & 25,000