2D gel electrophoresis

Required solutions

Solution	Component	Amount
Cathode buffer:	TRIS	7.6g
	Glycine	36g
	SDS	2.5g
	Fill up with ddwater to 250ml	
ESS (equilibration stock solution)	SDS	2g
	Urea	36g
	EDTA	3mg
	50 mM TRIS-HCl pH 6.8	10 ml
	Glycerol	35ml of 87% solution
	Fill up with ddwater to 100ml	
APS (ammonium persulfate solution)	APS	400mg
	Disolve in 1ml of ddwater	
Acrylamide , Bis solution (T=30%, C=3%)	Acrylamide	29.1g
Use protective equipment when handling powder	Bis	0.9g
	Fill up with ddwater to 100ml	
	Filter with 45um filter	
Gel buffer pH 8.8	TRIS	18.18g
	pH to 8.8	
	add SDS	0.4g
	Fill up with ddwater to 100ml	
Glycerol 87%	Glycerol (1.2 g/ml)	104.4g
	dd water (makes 100ml)	13ml
	Filter with 45um filter	
Water saturated butanol	butanol	
	dd water	

Choose strip pH range and size

- Immobiline Dry Strips from Pharmacia are available in different pH ranges and sizes (see catalog or brochure).
- For a mini-gel use a 70mm strip.
- Two 11 cm strips can be easily ran in one high resolution gel;
- Two 13 cm strips can ran, but the acidic end will hang out of the gel;
- Most of the non-zeins are in the 4-7 pH range

Preparation of protein samples

- Proteins from extract or specific purification procedures should be resuspended in 125 ul of rehydration buffer for a 70mm strip (table 1 of brochure).
- **Add the corresponding IPG buffer (pH range) just before use (0.5% for Multiphor equipment)**. In case of pellets containing Agarose or Sepharose beads , resuspend in 140ul, mix well, spin to pellet the beads and pippet out 125ul (for 7cm) 250ul (for 13cm) for strip rehydration.

Rehydration of the Dry Strip

- Add 125ul of the protein sample to the groove in the rehydration tray;
- Peel off the cover foil from the strips with a forceps;
- Place the strips on top of the protein sample (gel side down). **Remove any air bubbles** by sliding strip carefully into the groove;
- Cover the strips with mineral oil to avoid evaporation and crystallization of urea;
- Rehydrate strip for a minimum of 12 hours (overnight);

Separation of the first dimension

- Remove the strip from the rehydration tray and place it in the flat bed. **The acidic end (pointed) of the strip should be pointing the positive electrode (RED);**
- Place a damp IEF electrode strip transversally on top of the gel strip ends;
- Place the electrode wire on top of that;
- Cover the whole flat bed with mineral oil;
- Turn on the circulating water bath @ 20 C;
- Run according with the strip pH range and size (see brochure).

- For 180mm use program **#1** (gradient to 500-3500 V in 1:30 h and another 7.5h @ 3500 V)
- For 70mm pH 4-7 use program **#2** (gradient 300-3500 V in 1:30 h and another 2h @ 3500 V)
- For 130 and 110mm use program **#3** (gradient to 500-3500 V in 1:30 h and another 6h @ 3500 V)

Separation of the second dimension in minigels

- Prepare a 12.5% or gradient gel according to protein properties and sample complexity. If silver staining, make sure solutions are clean (filtered).
- In order to run the molecular markers , make one well using a cut comb or using a paper loaded with makers just before turning voltage on;
- Incubate the strips in 1x sample Laemmli sample buffer for 15';
- Place the rehydrated strip on top of the stacking gel. Press down to make sure the strip is making contact with the stacking gel;
- Add molecular weight makers (for silver staining, 1ul of a 1/10 dilution of Benchmark is enough);
- Turn the apparatus on @ 120V.

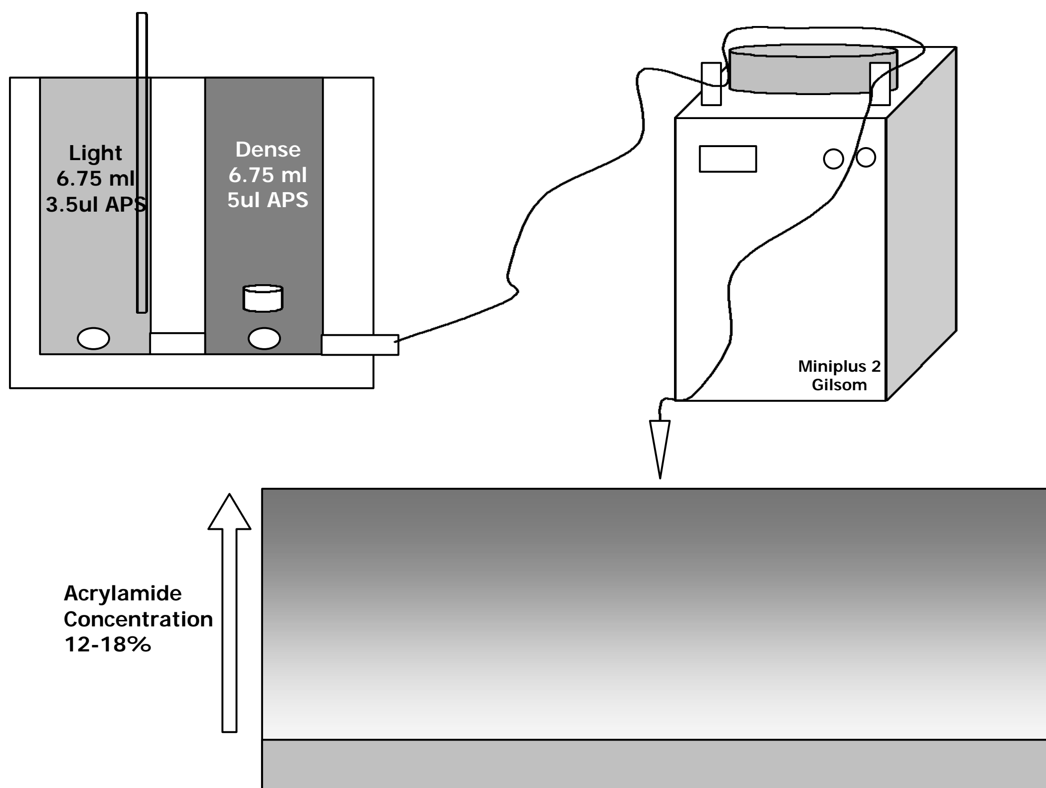
Separation of the second dimension in High resolution gels

Preparing gradient gel

- Get clean glass plates, Gel Bond Pag Film and Flexi clamps;
- Identify the hydrophobic side of the film;
- Sprinkle ddwater on top of the glass plate (w/o rubber). Lay film on that with the hydrophobic side down.
- Use a roller to attach the film to the glass;
- Dry excess water with kim wipes;
- Put the two glass pieces together and clamp them;
- Prepare acrylamide solutions according to the table:

Reagent (% acrylamide)	Super Dense (6%)	Dense (12%)	Light (18%)
Glycerol 87%	6.50 ml	4.3 ml	0.0
Acrylamide	3.00 ml	6 ml	9.0 ml
Gel buffer	3.75 ml	3.75 ml	3.75 ml
TEMED	10 ul	10 ul	10 ul
Fill up to 15ml in a falcon tube			

- Pour the stacking gel: 3ml of super dense acrylamide + 10ul APS;
- Overlay with ddwater and let it polymerize for 20';
- Prepare to pour the gradient gel. Get a stir plate and peristaltic pump.
- Close the 2 valves in the gradient maker, connect the tubes in the pump, place stir bar in the first well;
- Pour acrylamide according to the diagram. **APS is added just before start pouring.** Mix it thoroughly with the glass rod.



- Turn the pump on, open the valves and collect the first few drops with a piece of paper.
- Place the tip into the gel groove. Make sure the solution are being mixed in the gradient maker;
- Once the gel reaches the top of the glass (0.5 cm below), **remove the gradient maker and tubes and wash them immediately to avoid acrylamide polymerization**.
- Overlay the gel with water saturated butanol;
- Cover with saran wrap. Let it polymerize at room temperature overnight.

Setting up electrophoresis

- In a culture tube, equilibrate the strips in 10 ml of ESS buffer + 50 mg DTT for 15';
- Transfer them to 10 ml of ESS buffer + 250 mg of iodoacetamide + 100ul of 1% bromophenol blue. Incubate for 15';
- Set up circulating water bath for 15 C;
- Cut 10 pieces of IEF wick in half, longwise;
- Place the 2 stacks on a pyrex dish. For each stack add 20 ml of Cathode buffer. With gloves on, press them down making sure they are damp;
- Pour some oil on the flat bed . Take the gel out of the glass plates and lay on top of the oil (gel side up, stacking towards the (-) side). End of the gel in the 3 cm mark.
- Lay the wick paper on the ends of the gel with ~ 3 mm overlay;
- Press down the ends to make sure the wicks are making good contact with the gel;
- Take the strips out from the equilibration solution step, place them sideways on paper towel;
- Lay the strips on top of the 4 cm mark. Press it down with forceps;
- Spot 1ul of 1/10 dilution of BenchMark marker on the side of the strips;
- Place the electrodes on top of the wicks, plug them, cover apparatus;
- Select program #4, turn it on;
- When it reaches 70 V/h (~20min.) pause the program and remove the strips.
- Put cover back on and run it until ~1000 V/h;
- Take it out and start staining !!!!!!!!!