Lab 8 SDS-PAGE

Sample Preparation

- Transfer the amount of sample you want to electrophorese to a microfuge tube.
  Suggested amounts:
  5- 35 µl / lane 10µl of a 2 mg/ml protein
  Protein concentration - 100µg/ml to 500µg/ml
  Generally 1 µg of a purified protein is enough to visualize on a Coomassie stained gel
  10µg of a lysate (protein mixture) is sufficient to visualize
  Amounts you can load in a well differs based on gel thickness and depth of comb (well)
- Add an equal volume of 2X loading buffer
- Add 0.1 volume of βME or DTT
- Boil 1-5 min in a 95°C heating block
- Assemble the electrophoresis gel cassette(s)
  Don't forget to remove any coverings if using a pre-poured (purchased) gel.
  If only running one gel don't forget the clear buffer dam on the other side of the cassette.
  Be sure the short plate is facing the center.
  Check that a tight seal is achieved around the gel, especially where the short plate ends.
- Place the gels into the electrophoresis tank.
- Pour running buffer in the center cavity and allow to overflow into the outer cavity.
- If desired place the yellow gel loading guide on top of the cassette.
- Load your samples in the wells.
  Be sure to record what sample is in what lane in your notebook.
- Place the top on the chamber being sure that red and black are lined up correctly.
- Plug in the leads to the power supply.
- Run (electrophorese) your gel at 100-150 V (120 V) for 45 - 90 min.
- Stop your electrophoresis when the front migrates to the bottom of the gel.
  Depending on the size of your protein, you may run the front off the bottom of the gel.

Notes:

- In a materials and methods section an investigator reports the general procedure used for sample preparation. It is amateurish to report the volume calculations for each and every sample. Such information has no relevance for other investigators. Your reviewers and/or editor would insist on deleting such unnecessary information.
- A proper amount of protein to load depends on the distribution of individual proteins in the sample. If the sample consists of a single, nearly pure polypeptide, 10 µg would give a huge blob. A rule of thumb for mini-gels is to load about 0.5 µg protein per expected band. Since complex mixtures contain proteins of widely varying concentrations, there is no ideal single amount to load.
- Heating simply speeds up the process of denaturation by increasing molecular motion. It isn't necessary for some samples, but is necessary for membrane samples.
- Heating to the boiling point can cause aggregation of proteins, defeating the purpose of SDS-PAGE. Insufficient heating can leave some proteins incompletely denatured. It may require trial and error to achieve the best results.
- Once denatured, the samples can sit on a benchtop at room temp until it is time to load them. If they are to be saved for another day, they should be frozen.

Gel Loading Buffer Recipes:

2X from "Short Protocols in Molecular Biology"
To 40 ml of DDW add:
1.52 g Tris base
20 ml glycerol
2.0 g SDS
1 mg bromophenol blue
pH to 6.8 with HCl
bring volume to 100 ml

Need to add βME to 1% for reducing conditions

2X

		[final]
12.5 ml	4X Tris-Cl/SDS	(0.1 M)
10 ml	glycerol	(20 %)
10 ml	20% SDS	(4 % w/v)
0.5 mg	bromophenol blue	(0.001 %)

for reducing conditions add 1 ml βME or 1.55 g DTT

bring volume up to 50 ml
aliquot to microfuge tubes and freeze @ -20°C

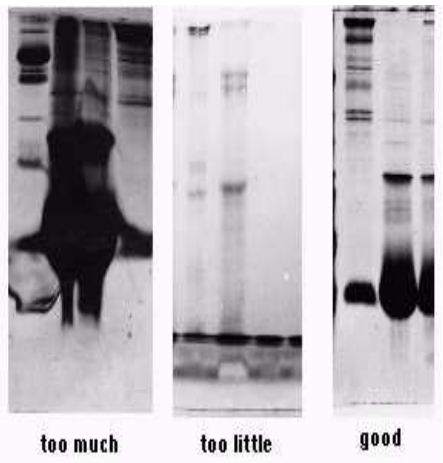
6X

	[final]
First make 4X Tris-Cl/SDS pH 8.8	
add 22.75 g Tris base to 75 ml DDW	(1.5 M)
pH to 8.8 with HCl	
bring volume to 125 ml	
filter through a 0.45 μm filter	
Add 0.5 g SDS	(0.4% w/v)

Store @ 4°C for up to 1 month

Now:
transfer 7 ml of above
add 3 ml glycerol (30 % v/v)
1 g SDS (1 % w/v)
0.93 g DTT (0.5 M)
1.2 mg brophenol blue (0.001 % w/v)

Store in 0.5 ml amounts @ -80°C



Coomassie Blue Stain

- Once your gel is finished running, disassemble the apparatus.
- Separate the short glass (plastic) plate from your gel.
- You may want to carefully separate your gel (at least partially) from the large glass plate.
  Use the flat plastic wedge between the gel and the plate.
- Carefully transfer your gel to the staining container
  Depending on the percent of your gel it may be very fragile and easily torn.
- Add the Coomassie Blue stain to your gel.
- Agitate for 1-4 h or until the gel is blue.
- Remove the stain.
  Be careful not to lose your gel as you pour off the stain.
- Rinse the gel briefly with destaining solution and pour out the destaining solution.
- Cover your gel with destaining solution.
- Agitate O/N.
  Replace the destaining solution when it turns blue.
  You may place a Kim Wipe in with your gel to absorb some of the dye.
- Continue destaining until blue bands and a clear background are obtained.

Coomassie Stain Recipe

	1 l	[ ]
Methanol	500 ml	50 %
H_2O	400 ml	40 %
Acetic acid	100 ml	10 %
Coomassie Brilliant Blue R	1-0.5 g	0.05 %

Stir well
Filter once through general filter paper

Bullerjahn Coomassie Stain Recipe
0.2% Coomassie Blue R250
50% Methanol
10% Glacial acetic acid

- Dissolve dye in 100% methanol then add = vol H_2O.
- Store in large batch (1-4 l)
- Add acetic acid to 10% to an aliquot immediately before use.

Destain Recipe

	200 ml	or		or	
50% Methanol	100 ml		40% methanol		16.5% methanol
7.5% Acetic acid	15 ml		10% acetic acid		5% acetic acid
	85 ml H_2O				

After destain the gel can be rehydrated in 5% methanol