SDS-PAGE (Laemmli)

Casting and running protein gels according to Laemmli using BioRad's Miniprotean II.

The protocol

1. Take two spacers, a comb, one small and one large glass plate, a casting block and the casting stand.
2. Clean the glass plates with ethanol.
3. Assemble the "sandwich", take case that the lower edges of blass plates and spacers are well aligned. Otherwise the whole thing will leak when you pour the gel.
4. Prepare 10 % (w/v) ammoniumpersulfate (APS) solution fresh
5. Prepare the separating gel mixture (at least 10 ml per gel when using the 1.5 mm spacers):

	30 % acrylamide	Buffer I	MilliQ
10 % gel	3.3 ml	2.5 ml	4.0 ml
12 % gel	4.0 ml	2.5 ml	3.3 ml
15 % gel	5.0 ml	2.5 ml	2.3 ml
18 % gel	6.0 ml	2.5 ml	1.3 ml

6. Add 100 μl of 10 % SDS, 80 μl of 10 % APS and 10 μl of TEMED
7. Pour the gel leaving ca. 2 cm free from the top of the lower glass plate. Carefully pipette water or water-satyrated iso-butanol on top to create smooth top surface.
8. When the separating gel has polymerised, prepare the stacking gel mixture (3.75 % acrylamide):

2.4 ml	MilliQ
1.0 ml	Buffer II
0.5 ml	30 % acrylamide
40 μl	10 % SDS

9. Pour the water from the top of the separating gel completely.
10. Add APS and TEMED to stacking gel mix, pour it to the top and insert the comb. Be careful not to introduce air bubbles to the gel. Let it polymerise.
11. Attach the gel sandwich to the electrode assembly. Grease the gasket to prevent leakage. If you are running only one gel, turn the gasket from the other side around and put only the acryl block on that side.
12. Pour running buffer inside the gel assembly. The buffer should cover be in contact with the gel. Let stand for few minutes while preparing the samples to check for leakage. If there is no leakage, fill the outer chamber (tank) with buffer to cover the bottom of the gels.

13. Prepare the samples by mixing one volume of sample and one volume of sample buffer. Heat at 95°C for few minutes and load on the gel.

14. run with 150V for ca. 1 h 20 min.

Solutions needed

Buffer I (4x)	1.5 M Tris-HCl pH 8.8
Buffer II (4x)	0.5 M Tris-HCl pH 6.8
30 % Acrylamide	29.2 % acrylamide 0.8 % bis-acrylamide
Sample buffer (2x)	0.125 M Tris-HCl pH 6.8 20 % glycerol 4 % SDS 2 % β-mercaptoethanol (β-ME) 0.02 % bromphenolblue
Running buffer (1x)	25mM Tris 192mM glycine 0.1 % SDS

Notes

Acrylamide is very toxic and should be handled with care. Wear always gloves and clean any spills etc. immediately. If you have to weight it out, do it in the fume hude as the powder is very light. I would recommend to buy ready made solutions whenever possible to avoid the need to handle the powder.
Prepare the sample buffer without β-ME and add it after thawing new batch from -20°C. For non-reduced samples leave it out. In practice to make 100 ml mix all the components except β-ME and fill the volume to 98 ml. Freeze the solution in 9.8 ml aliquotes. Add 200 μl of β-ME before use.
Running buffer can be prepared as 5- or 10-fold stock solution. To make 5 l of 5-fold stock, you will need 75.6 g of Tris base, 360 g of glycine and 25 g of SDS. Do not adjust the pH, it should be correct as is.