2D GEL ELECTROPHORESIS SOLUTIONS

AGAROSE SEALING SOLUTION

125g Agarose
25ml Electrophoresis buffer

Add a speck of bromophenyl blue - only enough to give the solution colour. Microwave to dissolve, make 1ml aliquots and leave at room temp to solidify. Store at room temp.

ELECTROPHORESIS BUFFER

60.57g Tris base
288.27g Glycine
20g SDS
dd. H₂O up to 20 litres

DISPLACING SOLUTION

50ml Tris-CI (1.5M ph8.8)
100ml Glycerol (87% v/v)
50ml dd.H₂O

Add a speck of bromophenyl blue - only enough to give the solution colour. Make up fresh and store at 4°C until ready to use.

SDS POLYACRYLAMIDE GEL

193ml Acrylamide (40%)
103ml N-N- methyl bis. (2%)
190ml Tris HCl (1.5M ph8.8)
266ml dd. H₂O
7.71ml SDS (10%)

Make solution up fresh and store at 4°C until ready to use. Immediately prior to use add the following solutions:
7.71ml APS (10%) 1.35ml TEMED (10%)
These dilutions should also be made up fresh (800mg APS in 8ml dd.H₂O, 150 microlitres TEMED in 1.5ml dd.H₂O).
De-gas final solution immediately prior to use.

LYSIS BUFFER (CURRENT)

(8M urea, 4% CHAPS, 40mM Tris)
19.2g Urea
1.6g CHAPS
0.19g Tris base
dd. H₂O up to 40ml

Store at -20°C.
REHYDRATION BUFFER

(8m urea, 2% CHAPS)
12g Urea
0.5g CHAPS
dd. H₂O up to 25ml

Add a speck of bromophenyl blue - only enough to give the solution colour. Store at -20°C. Prior to use add 4mg DTT per ml and 5μl per ml of specific IPG buffer.

EQUILIBRATION BUFFER

(50mM Tris HCl, 6M urea, 30% glycerol, 2% SDS)
6.7ml Tris HCl (1.5M pH8.8)
72.7g Urea
69ml Glycerol (87% v/v)
4g SDS
dd. H₂O up to 200ml

Add a speck of bromophenyl blue - only enough to give the solution colour. 10ml aliquots. Store at -20°C.

LYSIS BUFFER

(From Oxford GlycoSystems Manual Sample Buffer 1)
0.03g SDS
0.31g DTT
0.044g Tris HCl
0.027g Tris base
18 meg Ohm water

CHAPS can be obtained from Amersham Pharmacia Biotech, Plus One grade, 17-1314-01.

Agarose is from SeaKem: high temperature melting one (95°C) rather than the low temp one.

(GENERALIZED) 2DE METHOD

for Buffers, see Method: 2DE Solutions

DAY 1

1. Resuspend pellet in lysis buffer. Protein assay is required on a representative cell pellet to calculate amount of lysis buffer to add.
2. Vortex/freeze/thaw sample x 3 (using LN2 or methanol/dry ice then holding in hand. Take care not to warm sample for any longer than necessary).
3. Sonicate for 5 minutes (Lab 300).
4. Add appropriate amount of DNase, RNase, and MgSO4 (see CELL PELLET LYSIS FOR 2DE).
5. Incubate on ice until able to take next sample from the viscous solution.
6. Transfer appropriate amount into another eppendorf.
7. Add 5μl of IPG buffer (of correct pH range) and 4mg DTT to 1ml of rehydration buffer.
8. Add rehydration buffer to each sample. The amount of rehydration buffer to add to sample depends on how much protein is required to be loaded onto sample. We started off adding 465µl rehydration buffer to each 60μl sample, but more recently we have added 353ul to 46ul of sample, (the ratio stays the same).
10. Microcentrifuge (13K rpm, 3 mins, 4°C). Remove supernatant (abandoning pellet that contains insoluble material).
13. Transfer sample into strip holder.
14. Remove protective backing and slide strip into holder (gel side down, acidic end at pointed end. Take care to use correct pH range strip).
15. Pipette on Drystrip Cover Fluid and cover with lid.
16. Choose appropriate protocol on IPGPhor apparatus, place strip holders in between markings and set for required number of strips.

**DAY 2**

1. Prepare electrophoresis tank approx. 2 hours before required: remove combs, add 20L HPLC water, replace combs, switch on tank (let air bubbles clear), switch on cooler (to obtain 4.5°C), add Running Buffer reagents to centre section, close lidtank, and allow to circulate to dissolve. When chemicals dissolved, lift combs to allow solution and water to mix. For Running buffer formula, see Method: 2DE SOLUTIONS.
2. Thaw equilibration buffers (2 tubes per gel) (for formula, see Method: 2DE Solutions) and add 100mg DTT to tube 1 (EB1), and 250mg iodoacetamide (CalBiochem 407710) to tube 2 (EB2).
3. Pour EB1 into strip tray. Add strip gel side down. Incubate 15 minutes on shaker.
4. Put hole in lid and melt agarose (in 95°C heat block) to seal the 1st dimension strip. Replace EB1 with EB2 in strip tray and incubate for 15 minutes.
5. Rinse polyacrylamide gels with water (outside plates) then electrophoresis buffer (between plates) and remove any loose pieces of acrylamide.
6. Briefly immerse strip in electrophoresis buffer before placing on top of acrylamide gel, acidic end at hinge of plate.
7. When strip is in contact with gel, seal in place with agarose, avoiding air bubbles.
8. Once cooled load into tank hinge side down, strip at left.
9. Check buffer level is between inside edge of spacer and edge of plate, connect up cables to tank, switch on power supply and run at eg 80V for approx. 18 hours (Use this time as a guide only - stop running just before blue dye front runs off gel).

**DAY 3**

1. If silver staining gels, start preparing solutions at least 1 hour before required. (Note that staining machine booklet gives amounts for 250ml of each solution whereas 350ml is required. Please see our staining solutions sheet for adjusted volumes).
2. Stop the current, disconnect the cables and remove first gel.
3. Remove strip and cut a corner from top of gel at acidic end (hinge), and a smaller corner from bottom to help with orientation.
4. If silver staining, transfer to staining machine, arrange magnets around drainage hole, select programme 2 and follow rest of these instructions. If using coomassie, transfer straight into stain.
5. Since the first step of the programme is fixation, the other gels can be transferred to dishes of fixative and left on the shaker until staining is performed (remembering to omit the first fix step to save time. Stopping the programme before the preserving solution step, and transferring the gel to a tray containing this solution instead can also save time).
6. Clean stainer with alcohol. Run a wash step to rinse water through tubes. It is preferable to scan gel as soon as possible but no damage should be done within a few days. Store gels in preserving solution.
IPG strips

Source: Amersham Pharmacia Biotech ('Immobiline Dry Strips'); Cat nos: pH 4-7 7cm=17-6001-10; 3-10NL 7cm=17-6001-12; 4--7 18cm=17-1233-01.

IPGPhor cleaning solution

Source Amersham Pharm. (No cat no. but all reagents are on the same page in the catalogue under the 2DE section).

Silver staining kit, protein

Source Amersham Pharm (Plus one) Cat no. 17-1150-01.