APPLICATION: For laboratory use only in the electrophoretic separation of proteins and nucleic acids.

Capillary Gel Electrophoresis

O'Farrell first developed two-dimensional electrophoresis in 1975 to overcome the limitations of one-dimensional SDS-PAGE slab gels in separating the complex protein mixtures within cell lysates. Conventional one-dimensional slab gels are limited because individual proteins are resolved purely on the basis of their size or charge-to-mass ratio. Consequently, there is a high probability that multiple proteins will be resolved at the same point within the gel, either because they fall into the same size range or are of a similar charge-to-mass ratio. By performing 2-D electrophoresis in which proteins within cell lysates are first separated on the basis of their isoelectric point (pI) by isoelectric focusing (IEF) and then by their molecular weight with SDS-PAGE, the probability of multiple proteins resolving at the same point within the gel is significantly reduced. This means, therefore, that complex cell lysates, which once only resolved into 30 or so proteins bands on a one-dimensional gel, can now be separated into thousands of proteins on a second-dimension SDS-PAGE gel, simply as a result of including this first-dimension IEF step.

First Dimension Capillary Gel Isoelectric Focusing

IEF is based on the principle that, at any given pH, the net charge of a protein, determined by the charged groups on its surface, influences its movement within an electric field. The charged groups within each protein each have a pK value corresponding to the pH at which half of these groups are protonated. Accordingly, the charged groups are deemed to be fully protonated if the pH is below the pK value, or deprotonated if the pH is above the pK value. This change in protonation influences the protein's movement within an electric field, so that at a low pH below its pK value the protein will have a net positive charge and migrate towards a higher pH and the cathode, while the converse would be true for the same protein at a pH above it pK value. In IEF each protein is resolved within a pH gradient by its pI - *i.e.* the pH at which it has no net charge and, therefore, ceases to move within an electric field.

In capillary gel IEF a pH gradient is established by applying a voltage to a complex mixture of ampholytes: low molecular weight amphoteric molecules, which, like proteins, migrate through the gel until they reach their respective pI. Capillary gels are prepared using a syringe to inoculate the ampholyte mixture into glass capillaries sealed at one end with tape or parafilm. The samples are then prepared in a high pH cathode solution containing sodium hydroxide and urea. The same cathode solution is added to the upper chamber of the CAP-GRM containing the capillary gels, while a low pH anode solution is added to the gel tank. The capillary gels are prerun to generate the pH gradient, when the samples are loaded onto the gel and the separation or focusing step performed.

During the separation or focusing step, there is a gradual decline in current - as each protein migrates through the pH gradient - which ceases once the proteins reach the pH zones equivalent to their respective pI values. The voltage gradient is orientated so that if a protein enters a pH below its pK value it will acquire a positive charge and migrate towards the cathode to a region of higher pH and, ultimately, its pI. Consequently, the focusing step allows proteins to move back into tight bands determined by their respective pI values. This continues until protein movement ceases.

Second Dimension Slab Gel Electrophoresis

After the IEF the gel is removed from the glass capillary and overlaid along the top of an SDS-PAGE slab gel, when each protein is resolved by its molecular weight and then analysed as spots which are assigned *x,y* coordinates. The sensitivity of this technique is such that protein isoforms indistinguishable by 1-D electrophoresis can be detected.

1ST DIMENSION ELECTROPHORESIS USING THE CAPILLARY GEL RUNNING MODULE

Outline 2-D Capillary Gel Electrophoresis Protocol

The protocol listed below is based on method first reported by O'Farrel in 1975 and further developed by Anderson, and Adams and Gallagher in 1991.

Capillary Tube Preparation

1. Clean the outside of the tubes in a mild laboratory detergent. A piece of wire with a cotton wool plug placed over the end can be used to clean the insides of the tubes.

2. **DO NOT** use abrasive creams or scourers. **NEVER** allow organic solvents or chromic acid to come into contact with the plastic components.

3. Handle clean tubes with gloved hands (remove any fingerprints with acetone).

IMPORTANT: Lubricate the Capillary Tubes and Internal Running Module slots with distilled water or running buffer before inserting the tubes.

Capillary Gel Preparation and Pouring

Tube gels can be poured either by sealing one end of the tube and injecting with acrylamide solution or by placing the tubes in a beaker containing acrylamide solution and then allowing the tubes to fill by capillary action.

Sealing Method

1. Seal one end of each glass capillary with Parafilm® and place upright in the CAP-GRM. Insert the glass capillary from the bottom upwards to prevent the Parafilm® getting dislodged.

2. Prepare the following gel solution, using the ampholyte corresponding to the desired pH gradient. There is sufficient volume for twenty V10-CAPROD or ten V20-CAPROD capillary gels.

   Ampholyte Gel Solution
   7.8ml Distilled Water
   1.2ml SERVALYT™ 3-10 Standard, 40%
   2.52ml 40% (w/v) 37.5:1 Acrylamide/Bis solution
   15µl TEMED
   150µl 10% (v/v) Ammonium Persulphate
   13.11g Urea (to prepare 9M solution)
   2.43ml 20% Triton® X-100

3. Fill a 10ml syringe, attached to a 24ga needle, with gel solution. Insert the needle into the glass capillary until it is 1cm from the bottom. Fill the capillary with gel solution, slowly withdrawing the needle as the fluid level rises. Stop once the gel solution rises to within 1cm of the top of the capillary.

4. Overlay the top of the gel solution with water-saturated isobutanol. The 1cm gap will serve as a loading chamber for the sample. Allow the gel solution to set for at least 30'. Insert the blanking ports into the grommets, if using fewer than 10 capillary gels in the CAP-GRM.

5. Fill the tank with low pH anode solution (0.1% phosphoric acid).

6. Insert the CAP-GRM into the tank, having first removed the Parafilm® from the bottom of each glass capillary. Fill the inner chamber with high pH cathode solution (20mM NaOH), ensuring that the top of each capillary is immersed completely.

7. Add 5µl of Sample Overlay Solution (see below) to the top of each gel, before replacing the lid and performing the prerun (pre-focusing) step for 30' at 200V.

   Sample Overlay Solution
   4.8g Urea
   0.5ml SERVALYT™ 3-10 Standard, 40%
   Distilled water to 40ml

8. The current will gradually decline as the pH gradient forms. After 30' stop the power supply and load 2 to 10µl of sample, previously prepared in sample buffer (see below), with a syringe into the top of each gel. The sample should sink to the bottom of the well.

   Sample Buffer
   5.4g Urea
   1ml SERVALYT™ 3-10 Standard, 40%
   2ml 20% Triton® X-100
   0.1g Dithiothreitol (DTT)
   Distilled water to 10ml

9. Perform the Separation step at 400V for the time period suggested in the Technical Specification, depending on the length of the capillary gel. Meanwhile, prepare a 12.5% polyacrylamide resolving gel (see Preparation of SDS-PAGE gels for further details). A gradient gel can be used to resolve proteins of highly contrasting molecular weights.

10. Perform the 'End of Run' stage as suggested in the Technical Specification. Turn off the power supply and remove the CAP-GRM. Using 24ga needle, gently squirt water into the top of the glass capillary so that the gel is extruded onto the gel extraction platform. Equilibrate the gel in 0.125M Tris-Cl, 2% SDS, 10% glycerol, 4.9mM DTT and Bromophenol Blue, pH 6.8.

11. Remove the gel from the buffer solution and align the gel extraction platform with the top of the SDS-PAGE. Allow the gel to slide gently onto the top of the SDS-PAGE gel, making sure that it is in contact with the resolving gel over its entire length.

12. Load the desired protein markers for analysis. Pipette molten 0.5% agarose in 0.125M Tris-Cl (to pH 6.8) and 0.1% SDS over the tube gel, allowing it to set.

13. The gel is now ready for second-dimension SDS-PAGE.

Capillary Method

1. If capillary action is the pouring method of choice, prepare double the amount of acrylamide solution to that in the recipe above. Pipette ~70% of the acrylamide solution into a flat-bottomed beaker and stand the capillary tubes upright in the acrylamide solution. Allow the tubes to equilibrate with the acrylamide solution. Check the height of the acrylamide in the tubes. If the tubes are full so that there is less than a1cm non-filled space at the top, remove some of the acrylamide solution from the beaker until the height is 1 cm from the top. If there is a greater than1cm space at the top, add more acrylamide solution, so that the solution rises in the tubes until there is a 1cm space at the top. Fill the 1cm space, which will act as the sample well, with water saturated isobutanol.

2. Leave to polymerise which will normally take 1 – 2 hours.

3. After polymerisation, remove the water-saturated isobutanol.

4. The tube gels can now be removed from the beaker and inserted into the slots in the Internal Running Module with the 1cm sample well facing upwards. Alternatively, they can be stored wrapped in a damp paper towel and Clingfilm at 4^{o}C. The tubes may contain a residual of acrylamide on the outside and may need cleaning with distilled water before insertion.

1st Dimension (IEF) Phase

Buffer and run conditions will vary according to the type of ampholyte used. The following conditions are given as guidelines only and apply when 4-8 Resolyte is the ampholyte used. Other ampholytes will require different buffer solutions. Please consult your laboratory manuals.

1. Prepare ~ 500ml of 10mM H_3PO_4 Anode Buffer (1 litre for TV400-CAPGRM) and use this to fill the bottom chamber of the unit so that the bottoms of the capillary tubes are submerged. If less than 10 capillary tubes are to be run, block up the unused tube slots in the internal running module with the blanking ports provided. Place the capillary gel-running module into the unit and fill the upper buffer reservoir with ~100ml of 20mM NaOH Cathode Buffer (200ml for TV400-CAPGRM) so that the tops of the capillary tubes are submerged. See Technical Specification for a summary of running conditions.

2. For the pre-run, load the gels with 10µl of 1% ampholyte solution and run for 30 minutes at 200V. The pre-run stage is recommended as it helps set up the pH gradient.

4. Replace the safety lid firmly making sure that the electrical connectors form a good contact.

5. Connect the electrophoresis apparatus to the power pack and connect the power pack to the mains supply. Turn all settings to zero before turning on the mains supply.

6. Run 8cm tubes within the TV100-CAPGRM and TV200-CAPGRM units at 400V for 3 hours and then 500V for 1 hour. 17cm tubes need to be run at 400V for 6-18 hours and then 500V for 1 hour in the TV400-CAPGRM.

7. At the end of the run, turn the power supply settings to zero, turn off the mains supply and disconnect the power leads.

8. Turn off the water supply if the unit is being cooled. Remove the safety lid by gripping the handles and pressing on the locating lugs with your thumbs.

9. Remove the Internal Module and remove the tubes from their slots. The gels can be extracted from the capillary tubes by: **a)** inserting a piece of wire with a small plug of cotton wool on the end and using this as a piston to push the gel out; **b)** inserting a Gilson tip into the end of the gel and gently squeezing the gel out with air or water. Whichever of these two methods is used, the gels should be handled with care, as they are fragile.

2-D Size Determination Phase.

1. To prepare the tube gel(s) for the 2-D, size-determining phase, equilibrate them by soaking for 30 minutes in the running buffer to be used for the 2-D phase.

2. Remove the gel(s) from the running buffer pre-soak, and place each lengthways onto the top of a pre-poured slab gel. The slab gel should be cast using a blank or 2-D comb. Hold the tube gel in place by pouring over it a low % agarose gel containing the tracker dye.

3. Electrophorese as usual for Slab Gels until the tracker dye has advanced the required distance down the gel.

4. The samples can be visualized using any of the standard staining methods or can be blotted.

At the End of the Run

- Tubes can be cleaned using a mild detergent and rinsing in distilled water. A clean sheet of foam rubber placed at the bottom of the sink serves as a tube support and minimises the risk of tube damage.

- Empty the tank buffer chambers with a vacuum line and trap or carefully decant the buffer away from the electrical connectors. Rinse the chambers with distilled water then dry the electrode connectors with tissue. Ensure that the connectors are clean and dry before usage or storage.

Vertical - Polyacrylamide Gel Electrophoresis (PAGE)

Gel Plate Preparation

1. Clean the plates, spacers and combs in mild laboratory detergent. **DO NOT** use abrasive creams or scourers. If a particularly clean finish is required (e.g. for silver-stained gels) glass plates can be soaked in chromic acid overnight, rinse with water then wipe successively with ethanol, acetone and ethanol again. **NEVER** allow organic solvents or chromic acid come into contact with the plastic components.

2. The notched glass plate can be siliconised in a fume hood with dimethyldichlorosilane if required to assist in plate separation after the run.

3. Handle clean plates with gloved hands (remove any fingerprints with acetone).

Gel Plate Assembly

1. On a clean level bench, position the two side spacers flush with the edges of the rectangular glass plate and then overlay the notched plate.

2. The gel plates can be sealed either with tape, or by clamping greased spacers between the plates with bulldog clips, or by using a casting base or 2-gel or 10-gel multicaster.

3. For tape sealing hold or clamp the plates firmly and seal the edges of the gel cassette with gel sealing tape. The tape should be applied smoothly with no wrinkles. Reinforce the corners by overlapping extra pieces of tape onto the glass. Grease or fingerprints will prevent a good seal being formed.

4. If greasing is the preferred method, smear a little silicone grease or Vaseline over the spacers before assembly and use the long spacer to seal the bottom of the gel and clamp with bulldog clips. Note that the side spacers will seem too long if this sealing method is chosen. The side spacers should be cut to size - make sure the cut is a clean right angle.

Casting using the notched gel-running module

1. Loosen the clamping plates on the gel-running module by untightening the screws. Place the gel-running module on its side on a level bench surface, and slide the gel cassettes, comprising the glass plates and spacers, into the gel-running module until they meet notched overhangs. The notched glass plates should face the centre of the gel-running module.

2. Put the gel-running module in an upright position on the bench surface. If using plain glass plates without bonded spacers use the spacer aligner to push the spacers into position so that they are perfectly aligned with the bottom of the glass plates (there is no need to do this with plain glass plates with bonded spacers). Tighten the screws, using your other hand to hold the plates and spacers in position on the bench surface.

3. Once the gel cassettes are tightened into the gel-running module, invert the gel-running module to check that the plates and spacers are aligned correctly in the gel-running module. Readjust the plates and spacers if necessary.

4. Place the gel-running module onto the casting base with the silicone seals sitting in the grooves and the cam-pin levers pointing into the bench surface.

5. Slot the cam pins into position, turning them in a clockwise direction so that the gel-running module and gel cassettes are drawn onto the silicone seals. The cam pins should then point upwards at 90° to the bench surface.

6. Check for leaks by adding 2 ml distilled water into each gel cassette. The glass plates should be firmly embedded within the silicone seals, ensuring that the gel cassettes remain leak-free. Discard the water and pour the acrylamide gels.

Gel Pouring

1. For reproducibility and uniform polyacrylamide crosslinking, **we recommend using 'Electran' grade materials and degassing gel solutions before use.** Acrylamide solutions should be stored in a cool, dark environment, such as a refrigerator, and allowed to reach room temperature before pouring. Avoid exposure to heat and sunlight.

2. Polymerisation conditions should be adjusted to effect polymerisation within about **5 - 10 minutes.** Test a small volume in a vial before pouring the gel. As a rough guide 100ml of degassed 6% acrylamide gel will set in about 5 minutes at room temperature when gently mixed with 450µl of freshly prepared 10% (w/v) ammonium persulphate plus 200µl TEMED. The setting time increases to about 10 minutes if the TEMED volume is reduced to 100µl, and to approximately 15 minutes with 75µl. The amount of catalyst may need to be reduced under warm conditions. Do not pour under direct sunlight.

3. Gel pouring can be carried out directly in a gel-casting unit or by clamping a taped gel into the tank unit.

4. Run the acrylamide separating gel mix slowly down the inside edge of the gel cassette. **Avoid aeration.** If a stacking gel is to be used, carefully overlay the separating gel to a depth of 3 - 5mm with 1 x separating gel buffer or water-saturated butanol (If no stacking gel is to be used, insert the required comb into the solution at the top of the gel cassette before polymerisation).

5. Following polymerisation of the separating gel, pour off the overlay layer (rinse off the butanol with electrophoresis gel buffer) and pour a stacking gel. Insert the comb ensuring bubbles are not trapped. Once the stacking gel has polymerised the gel may be used immediately.

6. If tape has been used, remove the gel tape from the bottom of the gel and from any region that could affect the seal between the glass and the silicone gasket. Clean both the silicone gasket, located on the upper buffer chamber, and the outside of the gel plates. If the gasket becomes unseated from its groove simply press it back into place.

7. Secure the gel plates into position in the gel running unit with the short or notched plate innermost. **DO NOT OVER TIGHTEN** the screws as this will cause the glass plates to crack. When running only one gel, a blank glass plate is required on the other side of the unit to retain the top buffer level.

8. Place the gel running unit into the bottom buffer chamber.

9. Add the appropriate volume of running buffer to the upper and lower chambers (see Table 1). **IMPORTANT: DO NOT** fill above the Maximum fill lines or above the top of the rectangular gel plate if using glass plates smaller than 10x10cm.

Falls Sie Fragen zu einem Produkt haben oder allgemeine Informationen benötigen, wenden Sie sich bitte an:

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