ELETTROFOR
Scientific Instruments

P Dimensional®

P-Dimensional electrophoresis unit

P Dimensional®

Index

1. Introduction:

Two dimensional electrophoresis (2-DE) is at the base of Proteomics, the science for which the aim is to separate, quantify and identify the complete set of proteins of a cell or a tissue. Thanks to this technique, proteins are separated by two different physical properties. The first dimension, isoelectric focusing (IEF), separates proteins by their isoelectric points (pI). The second dimension, SDS-PAGE, further separates the proteins by their molecular weights (MW). These two dimensions are oriented at right angles to each other. The maps obtained from proteins migration are acquired as gray level images and processed to allow the comparison of the experimental outcome for different samples.

It is known that this technique presents some drawbacks in the separation of complex protein mixture, due to the presence of proteins and/or polypeptides that have similar MW and pI, but different relative abundances. This implicates both a small likelihood of being able to identify a large part of the sample tested and difficulties in manually managing the single spots not adequately isolated.

2. Description of the P-Dimensional Electrophoresis

The purpose of the P-Dimensional electrophoresis (2-PE) is principally that of overcoming the previously mentioned drawbacks of 2-DE in order to make possible a significant separation resolution increase of different proteins and protein isoforms in particular. The patent pending 2-PE is still based on the coupling of IEF in the first dimension and SDS-PAGE in the second dimension, but it takes advantage of a SDS-PAGE step with a radial electric field instead of a Cartesian one (Figure 1).

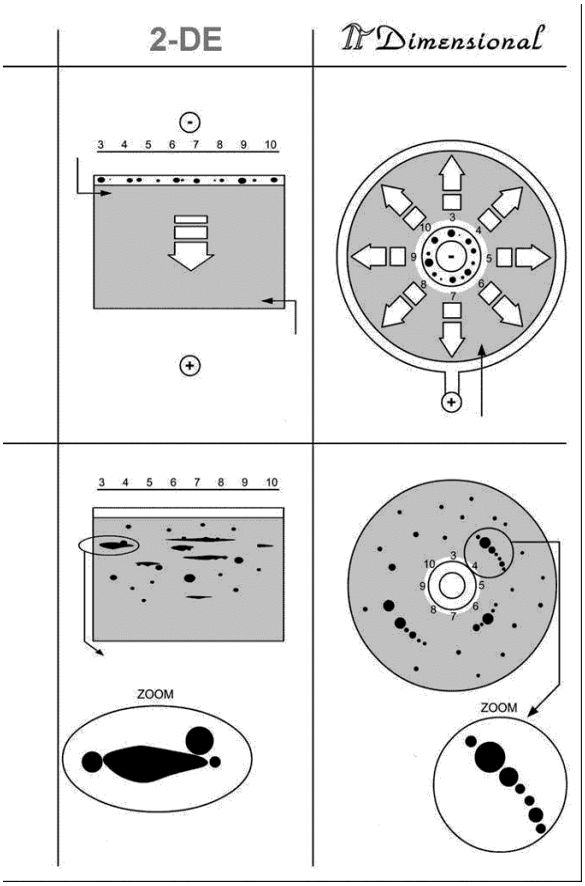

Figure 1: Schematic comparison of 2-DE and 2-PE.

The P-Dimensional radial electrophoretic chamber (figure 2) has been developed to perform the SDS-PAGE in circular crown shaped gels. This system can be used for both mono and two dimensional electrophoresis applications. Up to three gel separations can be performed simultaneously.

Figure 2: The assembled chamber to perform P-Dimensional electrophoresis.

It was shown (Millioni et al., 2010; Millioni et al., 2012) that during the second dimension run the zone movement in a radial direction substantially improves the resolution of closely spaced zones.

This increment is directly proportional to the length of the separation path of the second dimension. The loading of two or more (depending on their length) IPG strips on a single SDS-PAGE gel greatly improves the reproducibility with respect to 2-DE and makes the subsequent analysis faster and more reliable, due to easier spot matching. In fact, in this "multi-strip on one gel" approach, the conditions of electrophoresis and the procedure of staining/destaining are identical, with a consequent improvement of matching efficiency and a higher similarity of map backgrounds. This last benefit is really important since the accuracy in quantification for differentially expressed proteins will increase in absence of uneven backgrounds. 2-PE, thanks to the particular shape of the gels, makes it possible to run at the same time twice the number of IPG strips compared to 2-DE. A possible IPG loading scheme with 2-PE is reported in Figure 3.

Finally, the P-Dimensional electrophoretic chamber is able to lodge up to three stacked gels, so as to allow for simultaneous multiple runs thus making 2-PE a high-throughput method.

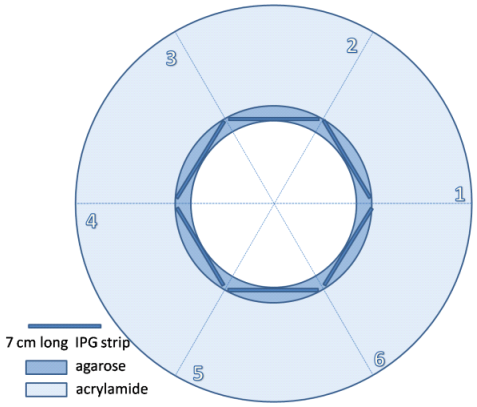

Figure 3: A purpose of P-Dimensional electrophoresis is to increase the map reproducibility. For example, it is possible to put on the same gel up to six 7-cm-long IPGs, each one occupying 1/6 of the internal circumference.

3. Preparing 2° dimension gels: P-gel casting, strip equilibration and loading

The P-gel caster device includes the following components:

- Multi-Casting Radial Chamber with a front and a backside panel
- Sixteen thumbscrews
- Six plastic discs
- Two blank inserts
- Separation sheets

P-gel caster holds up to three radial gels simultaneously suitable for the Elettrofor P-Dimensional

electrophoresis device. These radial gels are for use in the Elettrofor P-Dimensional electrophoresis device. The casting depends on the number of gels to be prepared. Two space savers (blank inserts) are included if less than three gels are made, to fill space within the caster and reduce the consumption of the acrylamide solution.

The supports at the base of the gel caster (figure 4) can be rotated: the gel caster should be placed in a horizontal position for the casting and in vertical position for the addition and polymerization of acrylamide.

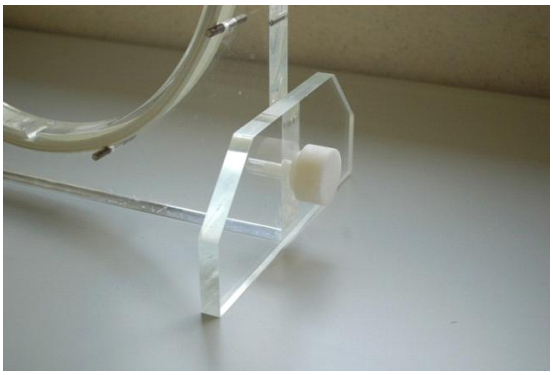

Figure 4

- Set up the gel caster on a drain board so that any liquid that overflows or spills out of the unit during acrylamide polymerization can be easily contained. The P-gel caster accommodates up to three gel cassettes with separator sheets (0.5 mm) between them. If you are planning to cast less than three gels, you will need to use the blank inserts with separator sheets between them to occupy the extra space.

- Prepare gel caster, glass plates and spacers by washing with a mild detergent and rinsing thoroughly with DI water. Attention: make sure that gel caster and the cassettes are clean and dry. The front and backside gasket of the casting chamber can be lightly greased to assure a leak free casting.
- At the base of the gel caster there is a canal that allow the passage of acrylamide during the casting. The entrance of this canal should be placed toward the front panel (figure 5).

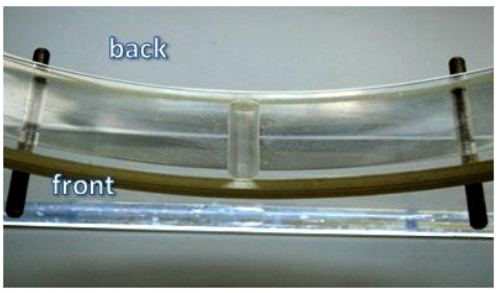

Figure 5

- Assemble the gel caster as described in figure 6.

Figure 6: 1) Place the caster in horizontal position, with the front panel facing down; 2-3) fix the backside panel using the thumbscrews, 4-5) rotate the caster and place it in horizontal position with the backside panel facing down.

- Prepare the P-gel. The P-gel includes:
- two circular crown glass plates
- an internal gasket (figure 7.1 or 7.2)
- two plastic disks (figure 7.3)

Circular crown glass plates have the same outer diameter (35 cm) while they differ for the inner one: 15 cm for the bottom, 13 cm for the upper. The glass plates have 1,5 mm thick glass spacers to create the empty space for the polyacrilamide solution entry. During the polymerization of the gel, a central circular gasket is placed between the glass plates, in order to leave, when this seal is removed, an acrylamide-free space where strips can be placed. Place the plastic disks (figure 7.3) at the centre of the glass plates to fill the empty spaces with the aim to reduce the acrylamide solution consumption.

Figure 7

There are three types of gaskets, to be chosen depending on the use which might be made of the gel. These gaskets are made of rubber, which does not create links with acrylamide.
The hexagonal gasket (figure 7.1) is used when the first dimension is performed in 7 cm long IPG strips. The circular gasket (figure 7.2, diameter= 15,8 cm) is used when the first dimension is performed in more than 7 cm long IPG strips.

- Fill the gel caster starting with a separator sheet against the backside panel. The separator sheets make it easier to remove the cassettes from the unit after polymerization.
- Add the gel cassette as shown in figure 8.

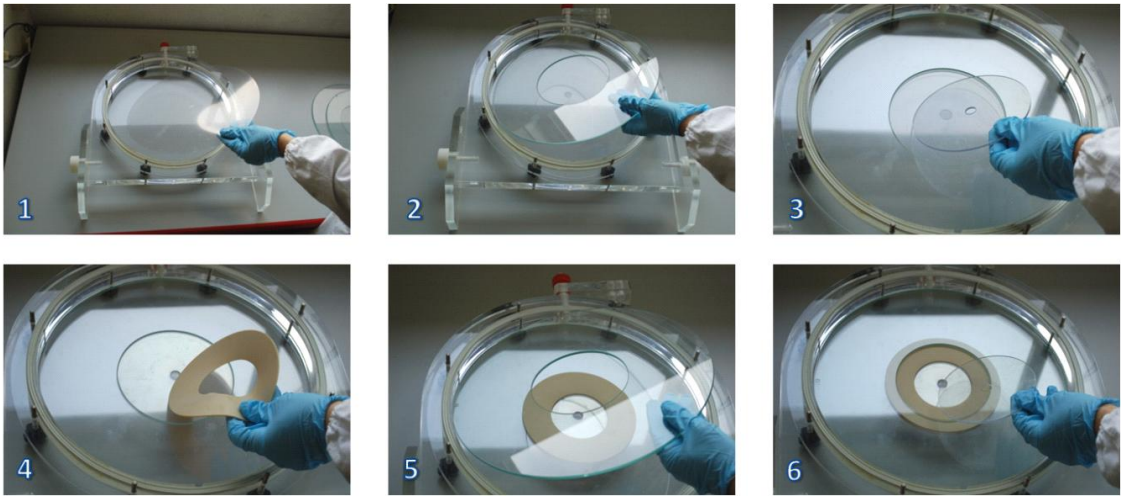

Figure 8: 1) insert a plastic sheet against the backside panel; 2-3) insert the first glass plate and its corresponding plastic disk; 4) insert the gasket; 5-6) insert the second glass plate and its corresponding plastic disk.

- Fill the caster by alternating separator sheets with gel cassettes.

- If less than three gels are made, use blank inserts to fill space within the caster to

reduce the consumption of the acrylamide solution (Figure 9.1-3), then close the caster (Figure 9.3-6).

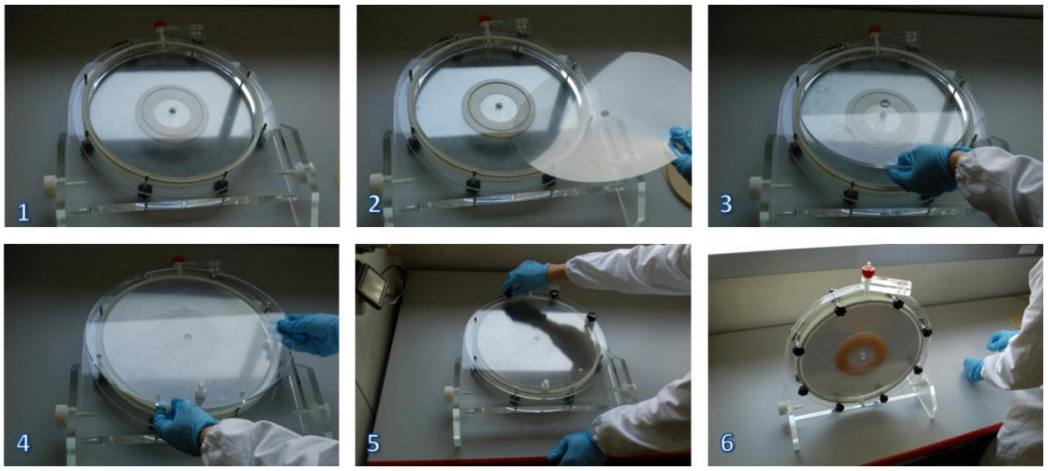

Figure 9

- Use the thicker filler sheets to bring the level of the stack of cassettes and spacers even with the edge of the caster.
- Be sure the front and backside sealing gaskets are compressed evenly by the panels creating a tight seal with the caster. Do not over tighten the screws.
- A funnel connected to a hose can be used to slowly pour the buffer from the bottom to the top of the caster, reducing the formation of bubbles (figure 10).

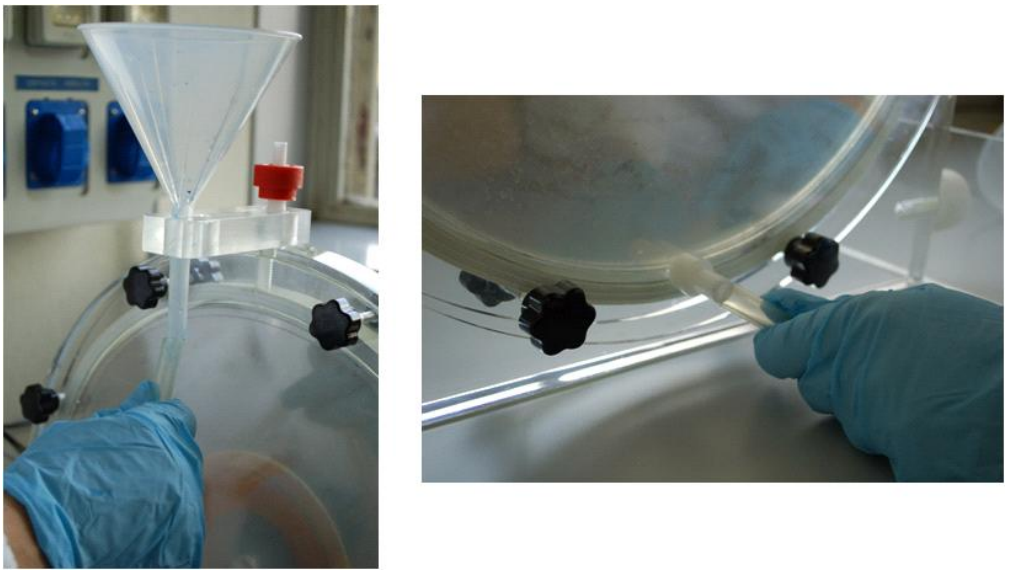

Figure 10: Connect the funnel to the caster using a hose.

- Place the funnel in its holder.
- Plug the ends of the hose to the funnel and to the barbed fitting in the faceplate.
- Pour the polyacrilamide solution.
- Allow the gel to polymerize.

WARNING! Acrylamide is a neurotoxin: always wear protective gloves when working with acrylamide solutions or surfaces that come into contact with acrylamide solutions.

NOTE: Contamination is a major issue in 2-D electrophoresis. All equipment should be carefully cleaned with a high quality detergent and rinsed twice with MilliQ water immediately before use. Always wear clean powder free gloves when performing any manipulation.

4. IPG strip equilibration steps

The first dimension separation of 2-PE should be performed on Immobilized pH Gradient Gels (IPG strips) by using the EWS-1 (Elettrofor). The proteins in a focused IPG strip are uncharged because they are at their isoelectric point, so they will not migrate into the SDS-PAGE gel. To prepare the proteins for movement into the SDS-PAGE gel, the strip must be submitted to a treatment named "equilibration".

- Prepare the SDS equilibration buffer (see paragraph 9). SDS denatures proteins and forms negatively charged protein-SDScomplexes. This buffer includes urea and glycerol in order to diminish electroendosmotic effects which are held responsible for reduced protein transfer from the first to the second dimension (Görg et al. 1988).
- Select the equilibration tray corresponding to the IPG strip length chosen for the experiment. Equilibration of the strips must be carried out in a tray (figure 11) different from that used for strip rehydratation and IEF to avoid the SDS contamination, which may prevent proper IEF. These trays include a lid that protects the strips from dust and other contaminants during the equilibration period (about 30 min).

Figure 11: Trays of different length for IPG stray rehydratation and equilibration.

- Incubate the IPG strips for 10–15 min in the SDS equilibration buffer containing 1% DTT. This step is followed by a further 10–15 min equilibration in the same solution containing 4% w/v iodoacetamide instead of DTT. The latter step is used to alkylate any free DTT, as otherwise it migrates through the second-dimension gel resulting in an artifact known as point-streaking that can be observed as thin vertical lines in silver-stained gels. Moreover, iodoacetamide alkylates the sulfhydryl groups of the proteins to prevent their potential reoxidation during the SDS PAGE run with subsequent vertical spot streaking (Görg A et al., 1987). Alternatively, tributylphosphine may be used instead of DTT and iodoacetamide (Herbert B et al., 1998).

- The equilibrated IPG gel strips are slightly rinsed and blotted to remove excess equilibration buffer and then applied onto the second dimension SDS gel.

NOTE: The second-dimension gel cassette must be ready for use prior to IPG strip equilibration

5. IPG strip loading:

During the polymerization of the gel, a central gasket is placed between the glass plates, in order to leave, when this seal is removed, an acrylamide-free space where strips can be placed. The procedure for loading the equilibrated IPG strips in radial gels varies depending on the length of the strip.

7 cm long IPG strips: removal of the hexagonal gasket leaves a space between the glass plates where up to six 7 cm long IPG strips can be loaded. The strip are placed with the plastic backing side on the bottom glass plate and then pushed between the glass plates using a thin spatula until it touches the SDS gel edge. Strips are then sealed in place by adding hot agarose.

> 7 cm long IPG strips: removal of the circular gasket leaves a space between the glass plates where > 7 cm long IPG strips can be loaded. The P-Strips have been properly developed for this application. Respect to traditional IPG strips, the P-Strip have a width of 1.4 mm instead of 3 mm. The plastic backing is flexible enough to allow the strip to be bended sideways and positioned along the SDS gel inner circumference, with the IPG gel side towards the SDS gel edge (figure 12). Strips are then sealed in place by adding hot agarose.

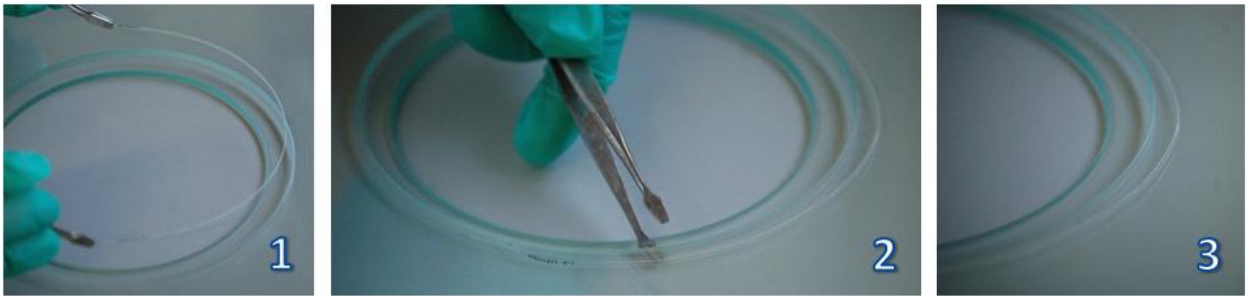

Figure 12

6. Preparing the P-Dimensional electrophoresis unit

- Radial electrophoresis is performed by using a horizontal apparatus, where the lines of force of the electric field are determined by circular and concentric electrodes. The correct position of the radial gel inside the electrophoretic chamber is obtained using the centering disk (figure 13).

Figure 13

- The outer and larger radial chamber includes the cathode electrode along its circumference, the heat exchanger on its basis and a pin at its centre (figure 14). The heat exchanger requires an external water bath for temperature control.

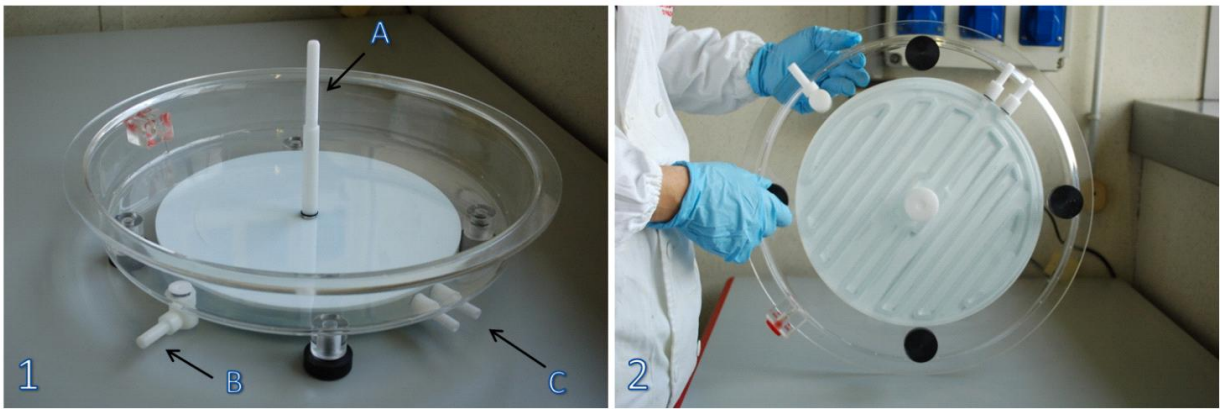

Figure 14: Panel 1: the outer chamber. A) the central pin; B) the tap to drain the buffer; C) connectors of the heat exchanger to the external water bath for the temperature control. Panel 2: the heat exchanger. When connected to a circulator bath, coolant passes through a serpentine and allow a rapid heat exchange between the coolant and the buffer in the electrophoresis tank.

- Load the P-gels following the step 1-5 of figure 15.

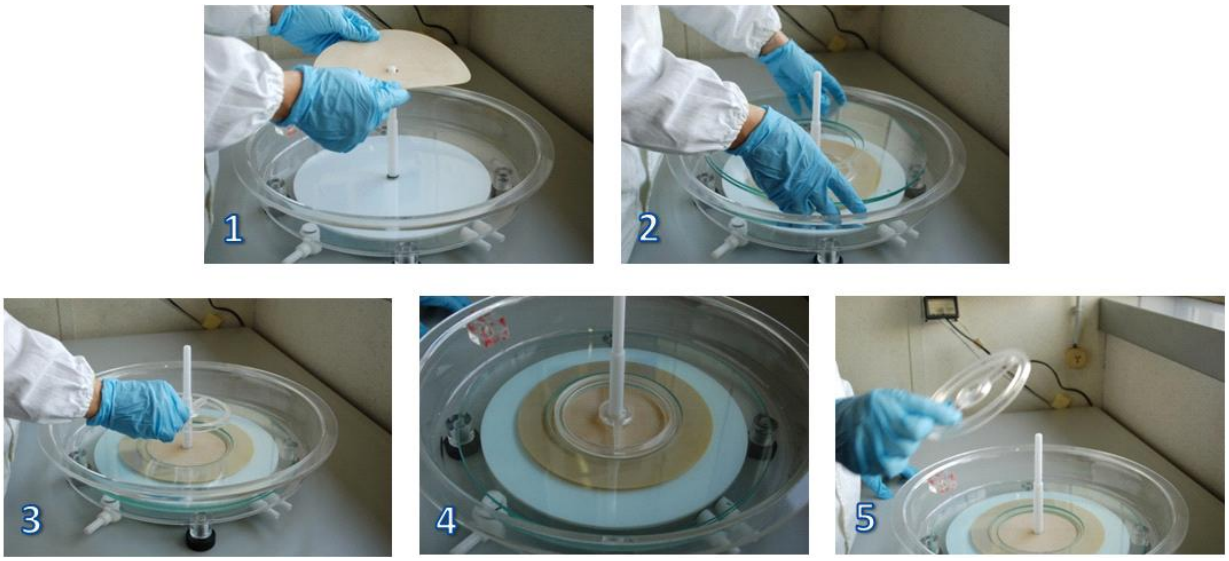

Figure 15: 1) place a circular gasket at the base of the pin, 2) put the gel cassette on the circular gasket, 3-4-5) use the centering disk to make sure that the gel is placed exactly in the center of the electric field.

- It is possible to stack up to three cassettes, alternated with each other by a circular gasket, as shown in figure 16.

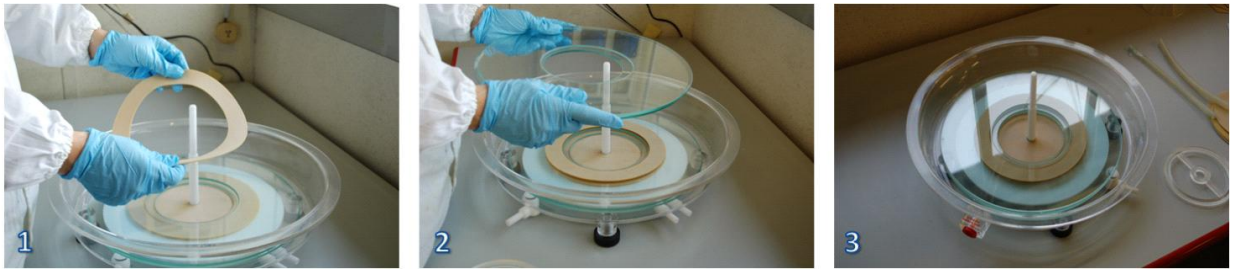

Figure 16: 1-3) put a circular gasket between each pair of gel cassettes.

- After entering the gels, the central smaller chamber (that includes the anode electrode and a tubular circular gasket) can be placed over the upper glass plate of the last placed gel cassette. The chamber cover is secured by a bolt that is screwed to the central pin. These simple operations are shown figure 17.

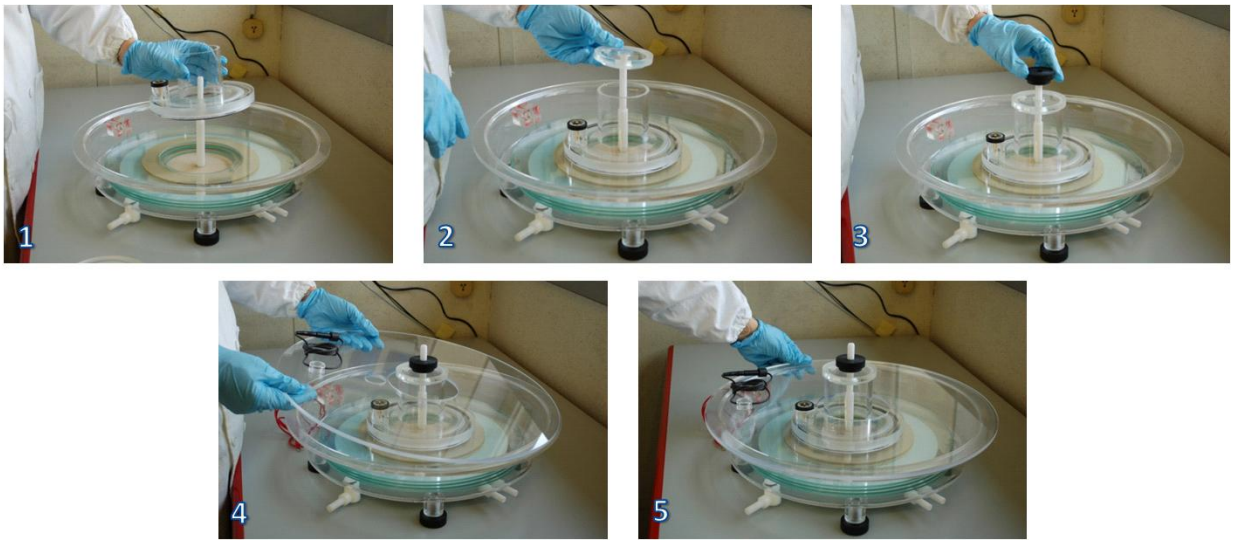

Figure 17: 1) put the inner chamber over the last inserted gel cassette; 2-3) put the lid over the inner chamber and lock it. Take care to do not over tighten the screw; 4-5) put the lid over the outer chamber.

- Pour the electrophoretic buffer into the inner chamber through the hole on the lid. A long thin funnel (or a funnel connected to a hose) can be useful to slowly pour the buffer from the bottom to the top of the chamber, thus reducing the formation of bubbles.

- Make sure that the buffer is in contact with the edge of each gel.

- Pour the electrophoretic buffer into the outer chamber. Make sure that the buffer volume is sufficient to overwhelm the gel cassettes.

- Close the outer lid, connect the cooling ports to the external recirculating water bath. Active cooling (20°C) is highly recommended.

- Connect the electrodes to the power supply and start the run.

- The characteristics of the applied electrical field influence the separation. The choice of voltage to apply depends on the time of application determined by the operator and generally will be around a value between 60 and 120 V.

NOTE: Protein transfer from the first to the second dimension should be performed slowly (field strength < 5 V/cm) in order to avoid streaking and minimize loss of proteins.

NOTE: Ammonium persulfate must be made fresh as it decomposes in water.

- Thanks to the collaboration of the bioinformatics company Decodon GmbH, the Delta2D software package allows for the conversion of radial dimensional images into Cartesian ones, a useful step since the Cartesian visualization is the more user-friendly data view for image elaboration. Thanks to this program, it is possible to obtain perfect Cartesian maps from the radial ones, retaining the increased resolution (an example is reported in figure 18).

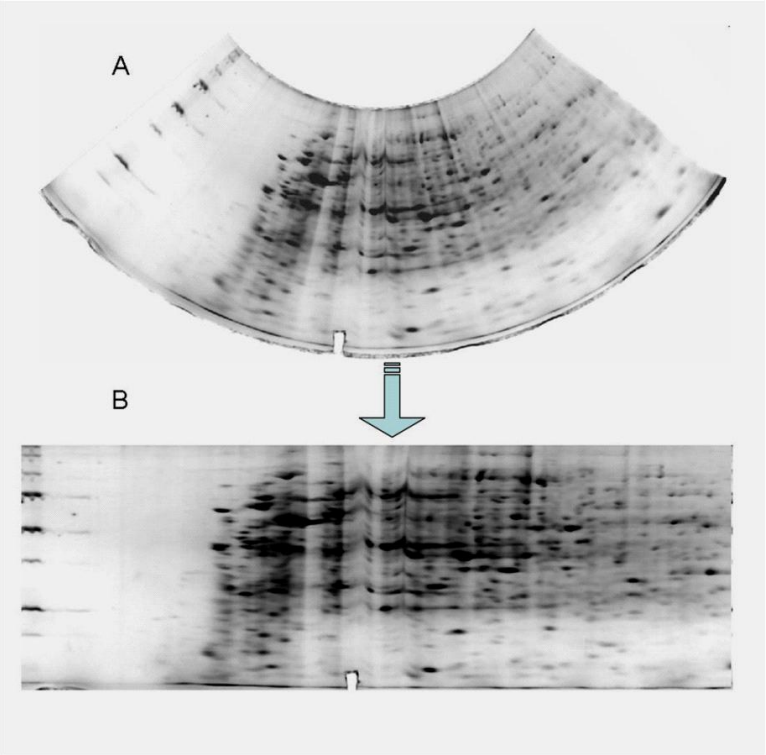

Figure 18

7. Mono-dimensional protein separation

Another application of the radial electrophoretic chamber is monodimensional electrophoresis. Compared to the above explained instruction of the unit for two dimensional electrophoresis application, the only difference is the modality of sample loading. Radial SDS-PAGE allows to load up to 60 samples at the same time (Millioni et al., 2010).

To add a stacking gel to the inner circle space, for direct contact with the running gel, another circular gasket (diameter= 15 cm), smaller than the gasket used for the gel casting (diameter= 15,8 cm) is tucked between the plates. This gasket has a slot which allows the stacking gel solution to enter the cavity made by the two glass plates, the gasket and the running gel. For 1-D electrophoresis, the Whatman filter papers containing the protein samples are inserted between the glass plates and sealed in place with the stacking gel solution.

8. Troubleshooting

Problems	**Possible causes and suggested solutions**
Gel caster leaks	Apply a light film of grease to the gaskets; do not over tighten the screws of the front and backside panel.
Incomplete gel polymerization	Use fresh APS; check the pH of the acrilamide solutions, it should be around 7; degas the acrilamide solutions: oxygen is a polymerization inhibitor; increase (+50%) both APS and TEMED; check the acrilamide solution temperature, it should be at least 20 °C; prolong the polymerization time: due to the large size of radial gel, an overnight polymerization is recommended.
Only the upper side of the caster is empty and radial gels are only partially casted	After pouring the acrilamide solution, wait for one minute to allow the solution to settle, then top with other solution.
No current	Check the electrode connections; ensure that in the inner chamber there is a sufficient quantity of

	buffer to contact the electrode.
Vertical point streaks in the gel	Add IAA during the strip equilibration process, as explained in paragraph number 4; make sure that the IPG strip is properly placed, with the IPG gel surface uniformly adherent on the second dimension gel.
Spot patterns are distorted (streaking, smearing etc)	Decrease the electrophoretic power, current and voltage; make sure that there are no bubbles between the first and the second dimension before stating the run; reduce the temperature setting; begin the electrophoresis immediately after the IPG strip equilibration to reduce the protein diffusion; many contaminants (e.g. salts, nucleic acids, lipids etc) in the sample can cause spot distortion: modify the sample preparation to reduce these contaminants.
Vertical gap in the 2-D pattern	A bubble was trapped between the strip and the radial gel.
Spots are boarder than usual	Check the equilibration time of the IPG strip. A too long time can lead to diffusion, while a too short time can lead to poor transfer; begin the electrophoresis immediately after the IPG strip equilibration to reduce the protein diffusion. Note: Take into account that there is a slight modification of the spot width and thickness during radial migration that is due to the electric field geometry.
Poor protein transfer from the first to the second dimension	Reduce the electrophoretic power, current and voltage during the entry phase; the IPG strip equilibration time is too short; for more than 7 cm long IPG strips, ensure that the strip gel side (and not the plastic backing) is facing the second dimension gel.
The second dimension electrophoresis proceeds slowly and with high current	Ensure that the buffer of the inner and outer chamber are well separated.

9. RECIPES

SDS equilibration buffer (50 mM Tris-Cl pH 8.8, 6 M urea, 30% glycerol, 2% SDS, bromophenol blue, 200 ml; store in aliquots at –20 °C).

Final concentration	**Amount**
1.5 M Tris-Cl, pH 8.8 50 mM	6.7 ml
Urea (FW 60.06) 6 M	72.07 g
Glycerol 30% (v/v)	69 ml
SDS (FW 288.38) 2% (w/v)	4.0 g
% Bromophenol blue stock solution 0.002% (w/v)	0,4 ml
Double distilled H2O	to 200 ml

Prior to use, DTT (100 mg for 10 ml of SDS equilibration buffer) or Iodoacetamide (250 mg for 10 ml of SDS equilibration buffer) is added.

1× **Laemmli SDS electrophoresis buffer**.

(25 mM Tris base, 192 mM glycine, 0.1% SDS, 10 l; the pH of this solution should not be adjusted)

Final concentration	**Amount**
Tris base (FW 121.1) 25 mM	30.3 g
Glycine (FW 75.07) 192 mM	144.0 g
SDS (FW 288.38) 0.1% (w/v)	10.0 g
Double-distilled water	to 10 l

Agarose sealing solution.

(25 mM Tris base, 192 mM glycine, 0.1% SDS, 0.5% agarose, 0.002% bromophenol blue, 100 ml)

Final concentration	**Amount**
Laemmli electrophoresis buffer (see Table 1.)	100 ml
Agarose 0.5%	0.5 g
1% Bromophenol blue stock solution 0.002% (w/v)	0.2 ml

Store at room temperature. Before use, heat in a microwave oven or on a heating stirrer until the agarose is completely dissolved. Do not allow the solution to boil over.

Acrylamide solution (500 ml)

Final %T	**10%**	**12.5%**	**15%**
Stock solutions	volume (ml)		
Acrylamide/Bis-acrylamide, 30% solution	167	208	250
1.5 M Tris-Cl, pH 8.8	125	125	125
Water	198	156	114
10% SDS	5	5	5
10% APS	5	5	5
10% TEMED	1	1	1

Add APS and TEMED to the polyacrilamide solution just before pouring the solution in the caster. This solution must be prepared just before use.

10. Ordering information

PRODUCT CODE	DESCRIPTION
P-gel caster	
PRO-0014	Multi-Casting Radial Chamber with a front and a backside panel complete with plastic discs (6/pkg), blank inserts (2/pkg), separation sheets, thumbscrews (16/pkg).
PRO-PLD	Plastic discs
PRO-BLI	Blank inserts
PRO-SES	Separation sheets
Glass plates & gaskets	
PRO-CCP3515	Circular crown plate with outer and inner diameters of 35 and 15 cm
PRO-CCP3513	Circular crown plate with outer and inner diameters of 35 and 13cm
PRO-LFC3515	Low fluorescence circular crown plate with outer and inner diameters of 35 and 15 cm
PRO-LFC3513	Low fluorescence circular crown plate with outer and inner diameters of 35 and 13cm
PRO-HEG	Hexagonal gasket
PRO-CIG	Circular gasket (diameter = 15,8 cm)
PRO-CGS	Circular gasket with the slot (diameter = 15 cm)
P-Dimensional electrophoresis unit & accessories	
PRO-OUC	Outer chamber
PRO-INC	Inner chamber
PRO-CED	Centering disk
PRO-CIB	Circulator bath
7005	EFD-300 Power supply (300V;400mA; 80W)
PRO-0020	Delta 2D Software
PRO-0016	Scanner PRO-6000
PRO-GST	Gel staining tray set (3 pcs)

PRODUCT CODE	DESCRIPTION
	Reagents
PRO-ACR	Acrylamide/Bis-acrylamide,40% solution. (Mix Ratio 19:1) 4x500ml
PRO-TEM	TEMED (N,N,N',N', -tetramethylethylenediamine) 10 ml
PRO-DTT	DTT (dithiothreitol) MB grade 5g.
PRO-IAA5	IAA (iodoacetamide) 5g.
PRO-APS	APS (ammonium persulfate) 50g.
PRO-TRI	Tris MB grade 1 Kg.
PRO-GLY	Glycine analytical grade 1 Kg.
PRO-SDS	SDS (sodium dodecylsulphate) MB grade 500g
PRO-MWM	Molecular Weight Markers Proteome markers 1 kit (5vials)
PRO-AGA	Agarose for DNA electrophoresis 500g
PRO-GLE	Glycerol MB grade 1L
PRO-BRB	Bromophenol Blue 25g
PRO-0060	IEF equilibration tray for 8cm lenght strip – 14 places
PRO-0062	IEF equilibration tray for 24cm lenght strip – 6 places
PRO-0064	IEF equilibration tray for 45cm lenght strip – 6 places

11. Reference

Görg A, Postel W, Weser J, Günther S, Strahler SR, Hanash SM, Somerlot L (1987) Elimination of point streaking on silver stained two-dimensional gels by addition of iodoacetamide to the equilibration buffer. Electrophoresis 8: 122-124.

Herbert BR, Molloy MP, Gooley AA, Walsh BJ, Bryson WG, Williams KL (1998) Improved protein solubilization in 2-D electrophoresis using tributyl phosphine as reducing agent. Electrophoresis 19: 845-851.

Millioni R, Miuzzo M, Antonioli P, Sbrignadello S, Iori E, Dosselli R, Puricelli L, Kolbe M, Tessari P, Righetti PG (2010) SDS-PAGE and two-dimensional maps in a radial gel format. Electrophoresis. 2010 Jan;31(3):465-70.

Millioni R, Polati R, Menini M, Puricelli L, Miuzzo M, Tessari P, Novelli E, Righetti PG, Cecconi D (2012) Polar electrophoresis: shape of two-dimensional maps is as important as size. PLoS One. 2012;7(1):e30911. Epub 2012 Jan 23.