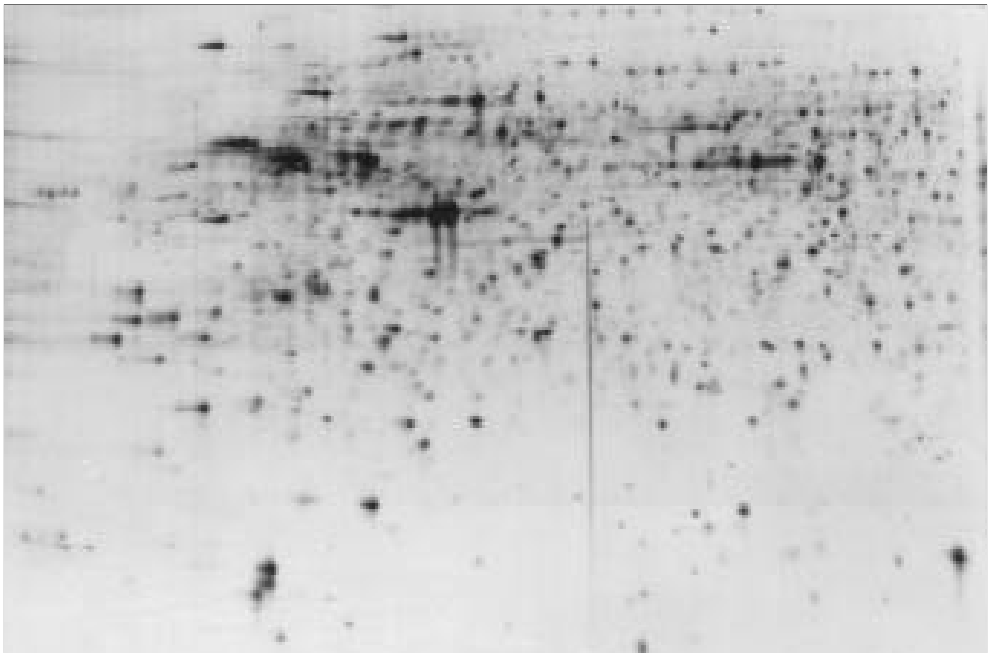
Immobiline® DryStrip Kit for 2-D Electrophoresis with Immobiline® DryStrip and ExcelGel™ SDS



Instructions

18-1038-63
Edition AC

Amersham Biosciences

Important user information

Reading this entire manual is recommended for full understanding of the use of this product.

The exclamation mark within an equilateral triangle is intended to alert the user to the presence of important operating and maintenance instructions in the literature accompanying the instrument.

Should you have any comments on this manual, we will be pleased to receive them at:

Amersham Biosciences
S-751 82 Uppsala
Sweden

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Contents

1. Introduction

Immobiline DryStrip kit is an integral part of the horizontal system for 2-D electrophoresis originally devised by Görg et al (1-3). The essential features of this method are:

Technique	Equipment
1: IEF on Immobiline DryStrip	Immobiline DryStrip kit Multiphor II Electrophoresis Unit
2: SDS electrophoresis on ExcelGel SDS gradient 8-18 ExcelGel SDS XL 12-14	Multiphor II Electrophoresis Unit

This Instruction manual presents all necessary information for the entire method for horizontal 2-D electrophoresis. By appropriate adaptations, the SDS electrophoresis can also be performed using vertical electrophoresis equipment (4).

1.1 Characteristics of the horizontal 2-D method

Isoelectric focusing with an immobilized pH gradient (IPG), makes true isoelectric focusing possible and significantly improves the reproducibility of the spot distribution along the pH gradient axis of 2-D maps. IPG also makes it possible to focus basic proteins in the gel and to obtain distinct protein spots.

Immobiline DryStrip kit facilitates sample application and running and equilibration of Immobiline DryStrip for the first dimension in 2-D (two dimensional) electrophoresis. The kit includes the accessories necessary to run up to 12 Immobiline DryStrip strips simultaneously on Multiphor II. The high sample capacity allows the application of up to 100 µl on each Immobiline DryStrip.

2. Kit contents and description of parts

2.1 Kit contents

The kit includes the accessories necessary to run Immobiline DryStrip on Multiphor II and instructions.

Designation	Code No.
Cathode electrode	18-1018-67
Anode electrode	18-1018-66
Sample cup bar	18-1004-33
Tray	18-1004-31
Sample cups (pkg/10 x 6)	18-1004-35
Immobiline strip aligner (pkg/4)	18-1004-34
IEF electrode strips (pkg/100)	18-1004-40
Sample application pieces (pkg/200)	80-1129-46
Instruction manual	18-1038-63

2.2 Description of parts

The tray consists of a thin glass plate with a glued glass-filled polyester frame. The frame and glue are resistant to commonly used solvents. The frame also acts as the electrode holder and the metal bars affixed to the frame conduct the current to the electrodes. The black and red leads connect the tray to Multiphor II unit. The moveable electrodes are made of polysulfone and have a platinum wire which rests against the electrode strip.

The sample cup bar, also of polysulfone, supports the sample cups (styrene-acrylnitrile) and makes it possible to adjust the height of the sample cups. The cups take up to 100 µl of sample.

Note: Do not leave the tray in water or other cleaning agents overnight. Parts like connectors, cables, and voltage conducting parts may not resist long exposure to such agents.

3. Other chemicals and equipment required

3.1 Gel media for the 2-D method

First dimension:

Immobiline DryStrip, precast polyacrylamide gels,(T=4%, C=3%) are cast on plastic support film and contain an immobilised pH gradient.

Immobiline DryStrip gels are available in different sizes (110 x 3 mm and 180 x 3 mm) and pH ranges (4-7 and 3-10). The shapes of the pH gradients are described in Table 1.

Table 1

pH gradient	length (mm) of the strip 110	180
4-7 L	linear	linear
3-10 L	linear	linear
3-10 L		non-linear*

* The pH gradient is flattened in the pH range 5-7 to achieve a high resolving area in this part of the gradient. This means that this pH gradient is steeper in the pH ranges 3-5 and 7-10 than the linear pH 3-10 gradient. The linear 3-10 gradient gives improved resolution at pH values above 7.

The strips are delivered in a dehydrated form. After rehydration, the gel thickness of the strips is 0.5 mm.

Second dimension:

ExcelGel SDS polyacrylamide gradient gels are 0.5 mm thin precast polyacrylamide pore gradient gels cast on plastic support film.

The gels are available in different sizes and pore gradients. See Table 2.

Table 2

	Gradient	Size (mm)
ExcelGel SDS, gradient 8-18	8-18	245 x 110
ExcelGel XL SDS 12-14	12-14	245 x 180

Each of the gels consists of a stacking gel zone followed by a polyacrylamide gradient (Table 2). During the run the gel is supplied with buffer ions from precast ExcelGel SDS buffer strips.

The buffer system in the strips, in combination with the gel buffer, forms a discontinuous buffer system.

3.2 Recommended chemicals and consumables

Designation	Code No.	No. per pack
Immobiline DryStrip for 1st dimension		
pH 4-7 L, 11 cm	18-1016-60	12/pkg
pH 3-10 L, 11 cm	18-1016-61	12/pkg
pH 4-7 L, 18 cm	17-1233-01	12/pkg
pH 3-10 L, 18 cm	17-1234-01	12/pkg
pH 3-10 NL,18 cm	17-1235-01	12/pkg
Precast gel media for 2nd dimension		
ExcelGel SDS, gradient 8-18	80-1255-53	6/pkg
ExcelGel XL SDS 12-14	17-1236-01	3/pkg
ExcelGel SDS buffer strips	17-1342-01	for 6 gels
PlusOne Chemicals		
Repel-Silane ES	17-1332-01	500 ml
Urea	17-1319-01	
Triton X-100	17-1315-01	
DTT (Dithiothreitol)	17-1318-01	0.5 g
	17-1318-02	5 g
2-Mercaptoethanol	17-1317-01	
Bromophenol Blue	17-1329-01	
Tris	17-1321-01	
SDS (Sodium Dodecyl Sulfate)	17-1313-01	
Glycerol	17-1325-01	
DryStrip Cover Fluid	17-1335-01	
Silver Staining Kit, Protein	17-1150-01	
Other Chemicals		
Pharmalyte 3-10	17-0456-01	
Orange G	Sigma	
PMSF (Phenylmethanesulfonyl fluoride)		
Glacial Acetic Acid		
PhastGel Blue R	17-0518-01	
Ethanol		
Accessory items		
Sample Cups	18-1004-35	10 x 6/pkg*
Immobiline Strip Aligner	18-1004-34	4/pkg*
IEF Electrode Strips	18-1004-40	100/pkg*
Sample Application Pieces	80-1129-46	200/pkg*
Cellophane Preserving Sheets	80-1129-38	
Test Tubes (>110 or >180 mm in length)		
Parafilm and Aluminium Foil		
Filter paper		
Petri dishes 90 mm Φ		

* Items included in DryStrip Kit Code No. 18-1004-30

3.3 Recommended equipment accessory

Designation	Code No.
Immobiline DryStrip kit	18-1004-30
Reswelling cassette for dry gels	18-1013-74
Multiphor II Electrophoresis Unit	18-1018-06
Electrophoresis Power Supply EPS 3500 XL*	19-3500-01
MultiTemp III thermostatic circulator, 110 V	18-1102-77
or	
MultiTemp III thermostatic circulator, 220 V	18-1102-78
Hoefer Automated Gel Stainer, 230 V	80-6330-04
Hoefer Automated Gel Stainer, 115 V	80-6330-23
Staining tray 1	18-1018-08
Staining tray 2	18-1018-09
Cellophane preserving sheets	80-1129-38

** AN IMPORTANT NOTE*

An alternative power supply may only be used if it does not shut off when the current drops below 0.1 mA. If you plan to use a power supply other than EPS 3500 XL, please check the manufacturers specifications. Immobiline DryStrip has a very high resistance and run therefore at high voltages with very low currents. Many power supplies are designed to shut off when the current drops below a certain value. A power supply other than EPS 3500 XL will more than likely shut off during the overnight run of the first dimension.
If you must use another power supply, 0.8% Pharmalyte 3-10 may be added to the rehydration solution to increase the conductivity of the strips. This should help but still does not guarantee success.

4. A survey of the 2-D method

Note: Times do not include staining of 2nd dimension. Add 1-2 hours for Coomassie staining and 3-4 hours for Silver staining.

4.1 Miscellaneous Notes and Tips

- Always wear gloves when handling Immobiline DryStrip and ExcelGel SDS, as well as equipment that comes in contact with them. This will help avoid protein contamination which can result in artefactual spots in the silver stained 2-D patterns. The artefacts originate from keratins and will appear in the pH range 5-8 and in the molecular weight range 50-65 kDa.
- Clean all assemblies (i.e. Reswelling Cassette, Tray, Sample Cups, etc) that come in contact with the gels or your sample with a detergent designed for glassware and rinse well with distilled water.
- Solutions containing urea may be warmed briefly. Do not heat any solutions containing urea above 30-40°C as isocyanate, a urea degradation product, will carbamylate the proteins in your sample, thus changing their isoelectric points.
- Immobiline DryStrip may be labelled by writing directly on the plastic support.
- Homogeneous SDS-PAGE gels may be cast for the second dimension and different pH gradients may be used for the first dimension. Contact your local Amersham Biosciences representative or Amersham Biosciences technical support for further information.
- After rehydration Immobiline DryStrip can be stored in tight plastic bags in refrigerator for 3–4 weeks or 2–3 months in freezer before use. This means that you can start rehydration a Thursday and put the rehydrated strips in refrigerator/freezer on Friday in order to start the first dimension on Monday. The rehydration step can not be performed during a weekend*, because the rehydration solution is not stable at room temperature nor in refrigerator where the urea can crystallize.
- Rehydrated Immobiline DryStrip must be handled in a fast way because evaporation can cause urea to crystallize on the strips reducing the amount of protein transferred to the second dimension gel.

* Neither in room temperature nor in a refigerator.

5. Sample preparation

Due to the different origins of samples which can be subjected to 2-D electrophoresis, it is not possible to give a standardized universal protocol for sample preparation. We recommend consulting recent reviews covering the subject (5,6). Any of the procedures described in the literature can be used, followed by dilution with sample solution prior to application.

Protein Load

How much sample to load? There is no clear cut answer, but some general guidelines will help. The total amount of protein loaded is not the only parameter that must be considered; the complexity of the sample (is it a purified mixture or is it a cell lysate?) and the relative proportions of the proteins are also important (i.e., is the sample 50% BSA, while the protein of interest is less than 1% of the total). If the protein of interest is in very small quantities, it may be necessary to overload the gel relative to some proteins to get enough material to see the protein(s) of interest.

While 2-D gels can be Coomassie stained, the method generally requires loading too much protein to be of use. Coomassie staining is sometimes preferred for a first run because of the somewhat simpler methodology compared to silver staining. Coomassie staining can also be useful if the protein of interest is in abundant supply.

For silver staining gels, much less protein need be loaded.

If the proteins have been radioactively labelled, the quantity loaded will depend as much on the specific activity of your sample as on the protein concentration.

Use these suggestions as guidelines, see Table 3. Running samples as a test on a one dimensional gel (SDS-PAGE or IEF) can provide a good hint as to how much to load on a two dimensional gel.

Table 3. Recommended sample load

	Silver (µg)	**Coomassie (µg)**
110 mm strips + ExcelGel SDS, gradient 8-18		
pH 4-7 L	2-15	40-300
pH 3-10 L	1-5	20-100
180 mm strips + ExcelGel XL SDS 12-14		
pH 4-7 L	20-50	400-1000
pH 3-10 NL + L	10-30	200-600

Salts and Detergents

While the Immobiline system is more tolerant to salt than traditional IEF, it is best to keep salt concentrations as low as possible. Salt concentrations should be kept below 50 mM.

In cases where the sample proteins have been solubilized with SDS, the final concentrations of SDS (once sample buffer has been added) should be less than 0.25%.

6. 2-D Electrophoresis Protocol

The 2-D method is described in a step-by-step protocol using Immobiline DryStrip Kit in the first dimension and ExcelGel precast SDS-gradient gels in the second dimension.

6.1 Day 1

6.1.1 Rehydration of Immobiline DryStrip

- Prepare 25 ml Rehydration Solution: See 10 Solutions

  While the Orange G (or Bromophenol Blue) is optional, it is highly recommended as it helps with strip alignment and serves as an indicator that current is moving through Immobiline DryStrip.

  This solution may be warmed briefly to dissolve the Urea.

  Do not heat urea solutions above 30-40°C for long periods as urea will break down into isocyanate which will cause protein carbamylation.

  This solution should be made fresh the day you use it.

- Wash the reswelling cassette well with detergent and rinse well with distilled water (dH_2O). Dry thoroughly.

  The reswelling cassette consists of a U-frame (glass plate with 0.5 mm spacer) and a plain glass plate.

  Use a detergent designed for glassware.

  For details of cassette assembly see Multiphor II Manual (Code No 80-1103-43).

- Treat the new U-frame with Repel-Silane.

  Place the U-frame face up on the bench in a fume hood. Pipette 1-2 ml of Repel-Silane onto the glass and distribute evenly with a paper tissue. Let dry for 1-2 minutes and rinse with dH_2O. Shake excess water from the frame and air dry.

  The Repel-Silane prevents the swelling gel from sticking too hard to the U-frame.

- Remove the appropriate number of dry strips from their foil case (Fig. 1).

  Up to 12 dry strips may be run in the first dimension.

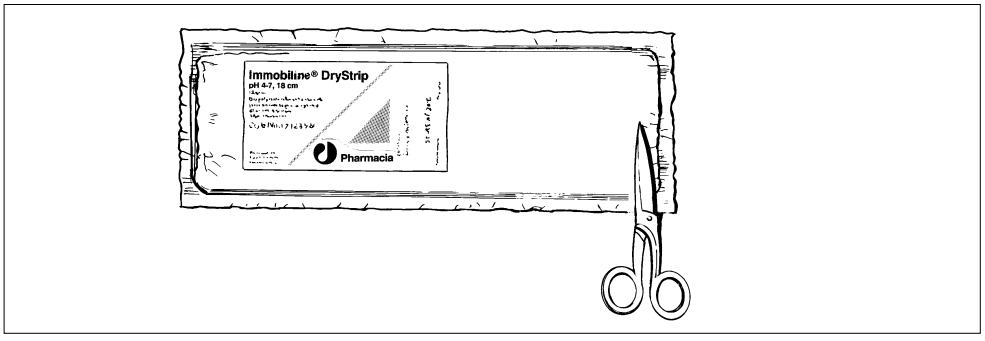


Fig. 1.

- Pull apart the dry strip from its protective cover (Fig. 2).

  The dried gel is very thin and often difficult to see. The protective cover is made of a thinner plastic than the dry strip.

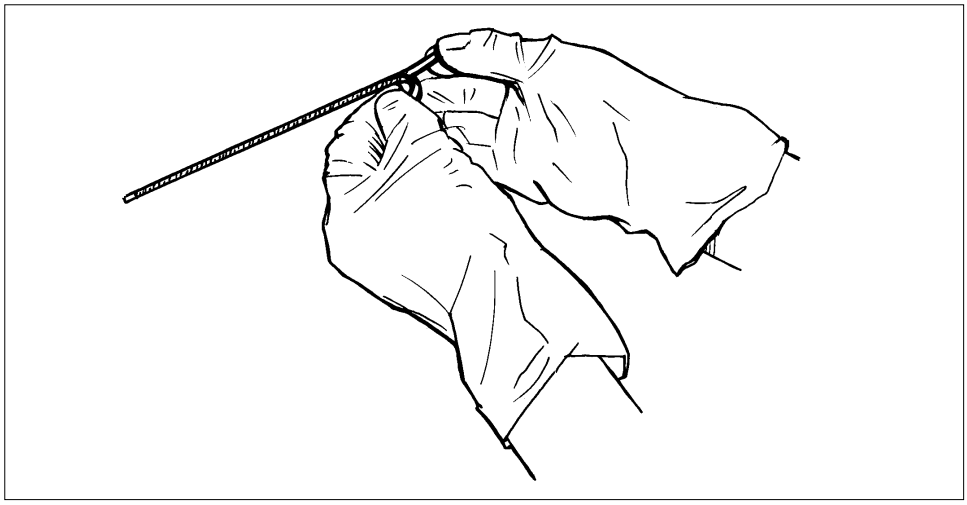


Fig. 2.

- Wet the plain glass plate with a few drops of water and distribute with a paper tissue.

  The water is necessary to hold the strips in place while assembling the reswelling cassette.
- Place the dry strips on the wetted surface of the plain glass plate gel side UP (Fig. 3).

  The gel surface will be facing the U-frame that has been treated with Repel-Silane.

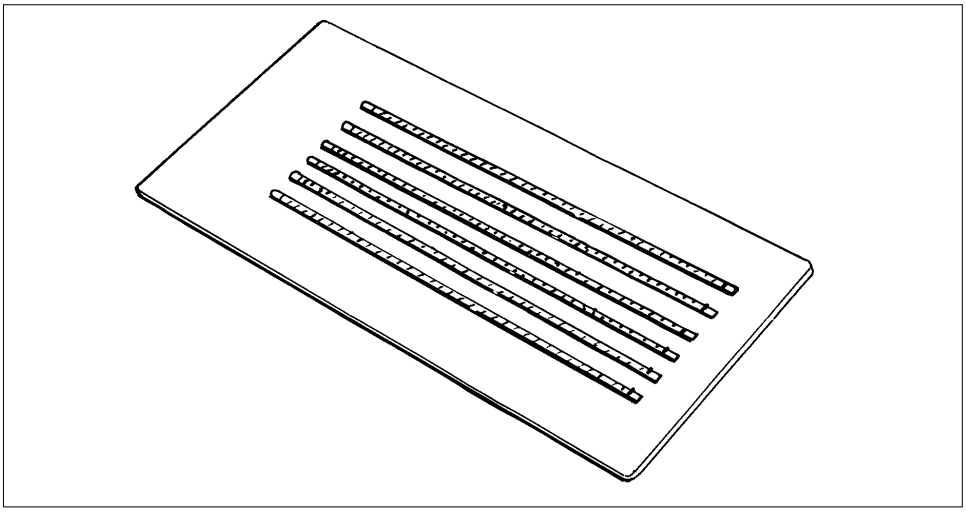


Fig. 3.

- Place the U-frame on top of the plate containing the strips and clamp the cassette together using FlexiClamps (Fig. 4).

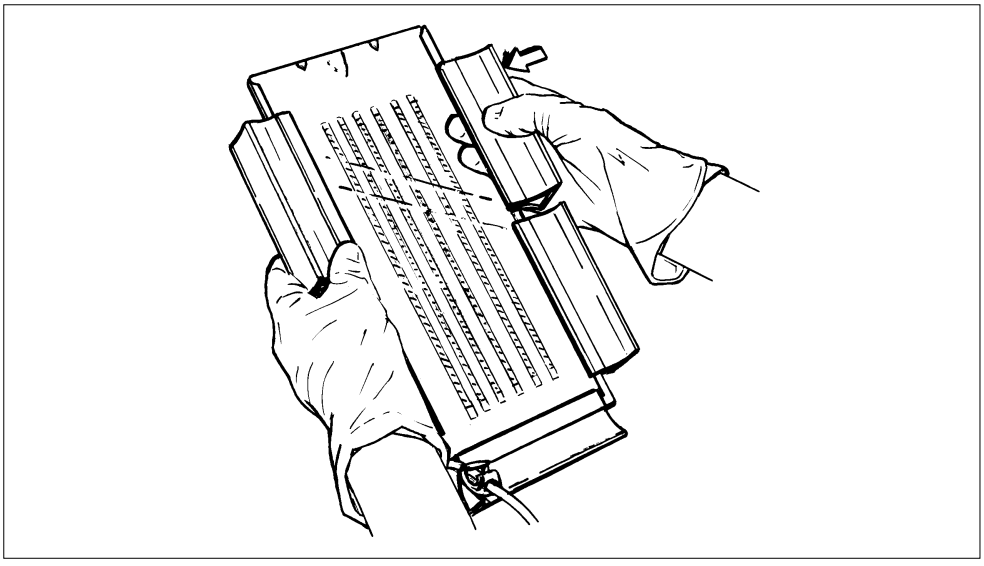


Fig. 4.

- Fill the cassette to the top with rehydration solution, close the pinchcook on the tubing and cover the top with Parafilm* (Fig. 5).

  It may be helpful to lay the cassette on its side at a 30–45 degree angle to help prevent leakage.

  It is normal for urea crystals to form around the edge of the frame even if it is not leaking.
- Allow the strips to rehydrate overnight.

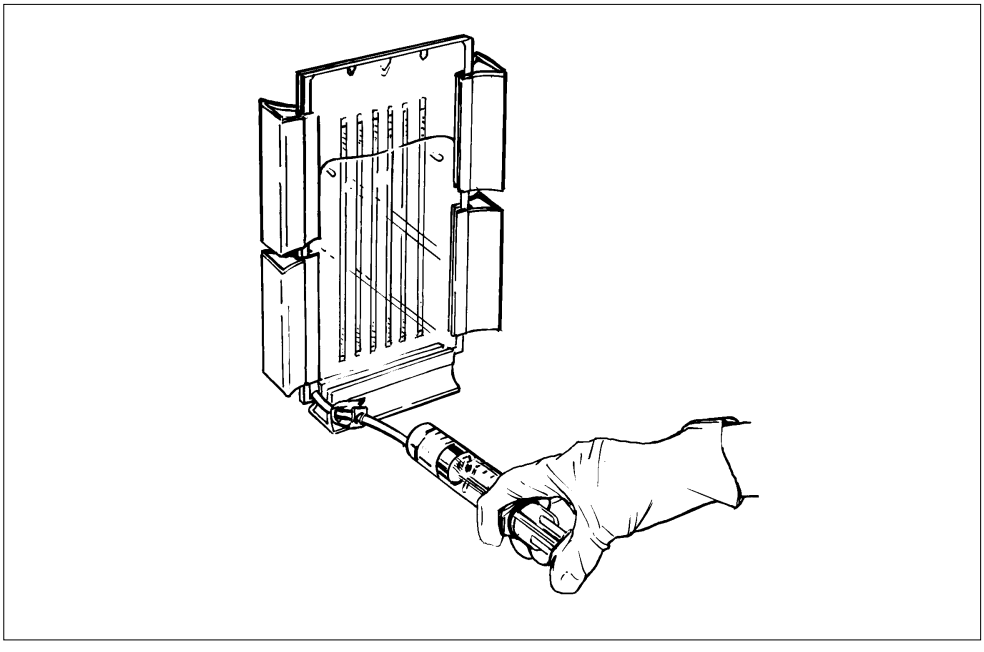


Fig. 5.

* Trademark of American National Can Greenwich CT 06836.

6.2. Day 2

6.2.1 Sample preparation

Preparation

If your samples/cells have not already been solubilized the Lysis buffer A can be used, see 10. Solutions.

If your protein has already been solubilized in a lysis buffer or is already in solution, you will not need to make this solution.

Prepare Sample Solution B, see 10. Solutions.

Take a volume of sample with the appropriate amount of protein as suggested on page 10. Mix your sample in at least a 1:4 ratio with sample solution. If the sample has high salt or SDS concentrations, more than a 1:4 ratio of sample solution may be used.

6.2.2 Preparation for the first dimension run

Set the temperature on MultiTemp to 20°C

- Make sure that the red bridging cable in Multiphor II is connected.
- Place the cooling plate in Multiphor II Electrophoresis unit and make sure the unit is level.
- Pipette approximately 3-4 ml of DryStrip Cover Fluid onto the cooling plate and position Immobiline DryStrip tray on the cooling plate (Fig. 6).

  Make sure that the red (anodic) electrode connection of the tray is positioned at the top of the plate near the cooling tubes.

  Make sure that there are no large bubbles between the tray and the cooling plate; small bubbles are OK.

  (Silicon oil may substitute at this step).

  The DryStrip Cover Fluid at this point serves as an insulating fluid to ensure good contact between the cooling plate and the tray.

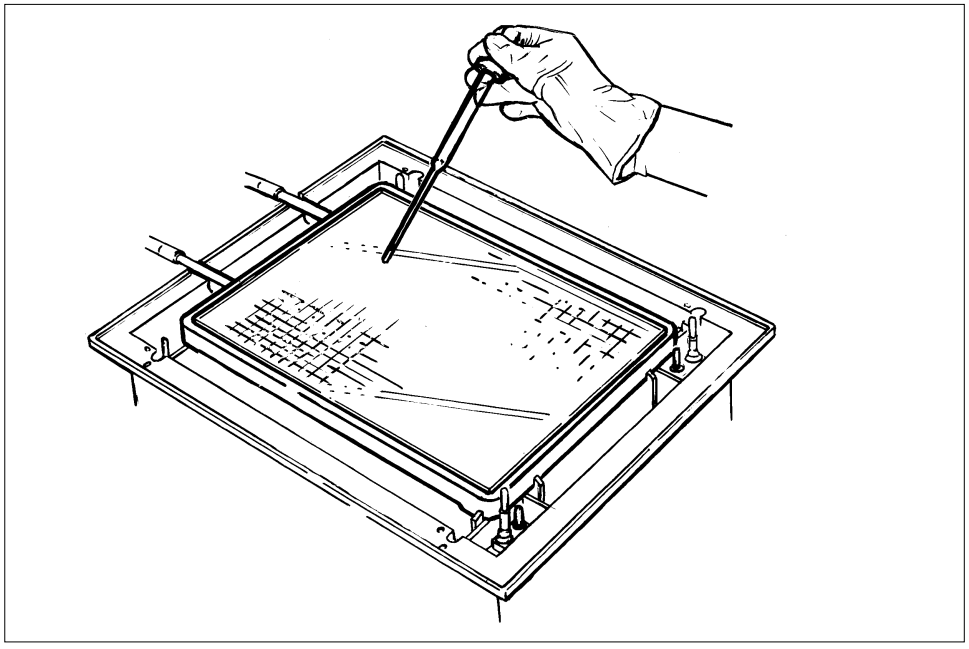


Fig. 6.

- Connect the red and black electrode leads on the tray to Multiphor II Unit (Fig. 7).

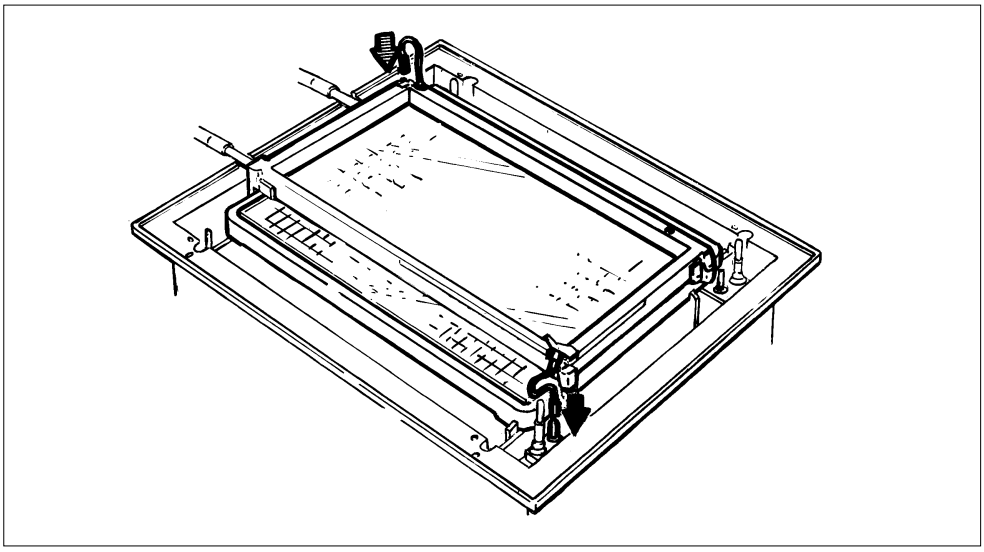


Fig. 7.

- Pour about 10 ml of DryStrip Cover Fluid into the tray.

  DryStrip Cover Fluid protects the strips from evaporation and the samples from oxidation.
- Place Immobiline strip aligner, 12 grooves side up, into the tray on top of the oil (Fig. 8).

  The presence of air bubbles in the spaces under the strips in Immobiline strip aligner will not affect the experiment.

  Avoid getting oil on top of the aligner at this point.

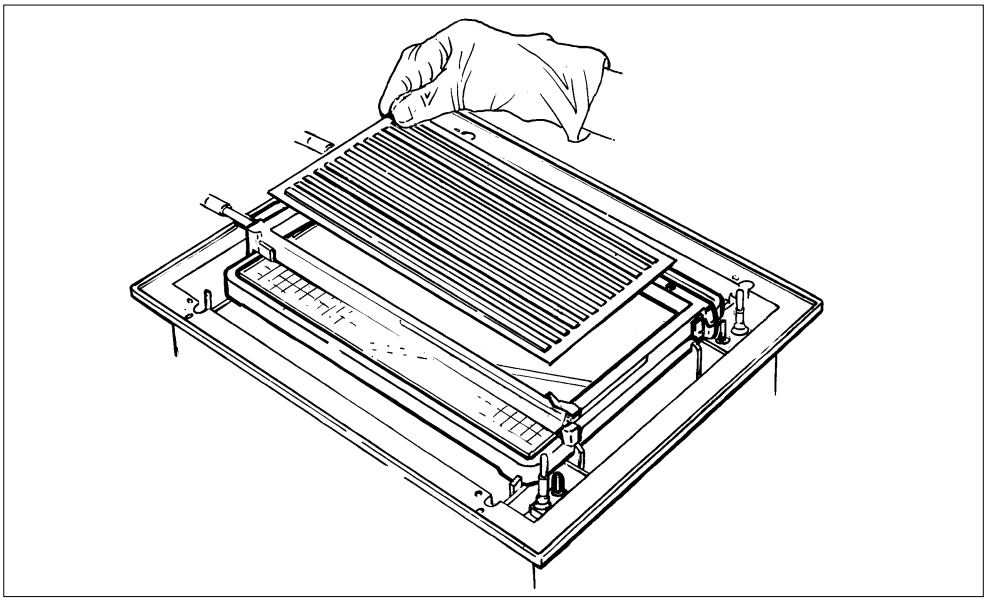


Fig. 8.

- Cut two IEF Electrode Strips to a length of 110 mm.

  These should always be cut to the same length regardless of the number of strips you are running.
- Place IEF Electrode Strips onto a clean flat surface (a glass plate works well) and soak each one with 0.5ml dH_2O. Remove excess water by blotting with tissue paper (Fig. 9).

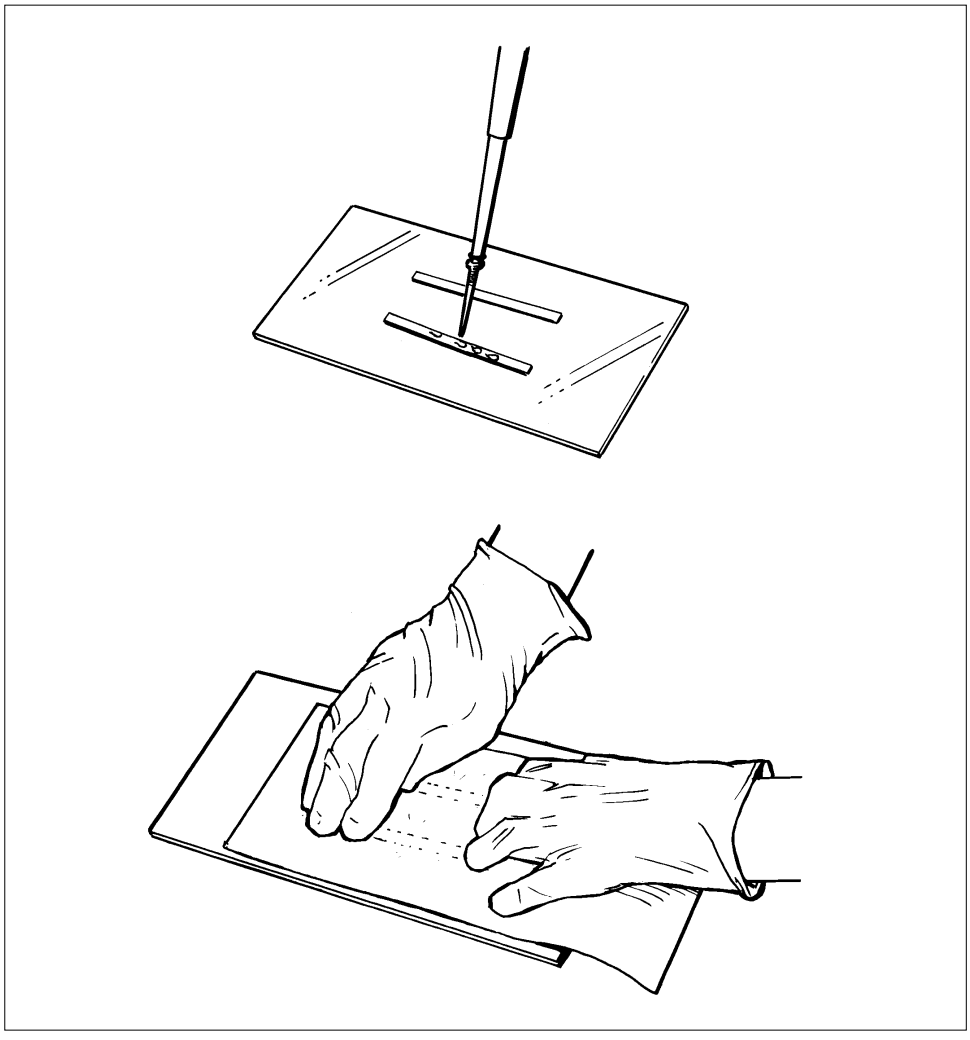


Fig. 9.

It is very important to blot IEF Electrode Strips well. Any excess water may cause streaking. IEF Electrode Strips should just be damp and evenly soaked.

- Make sure that the electrodes, sample cup bar and sample cups are ready. All the above steps should be completed before going on.

6.2.3 Applying Immobiline DryStrip in the strip aligner

- Pour the rehydration solution out of the reswelling cassette and remove the clamps.

- Open the cassette by prying apart the plates (Fig. 10).
  The strips normally stick to the U-frame treated with Repel-Silane ES.

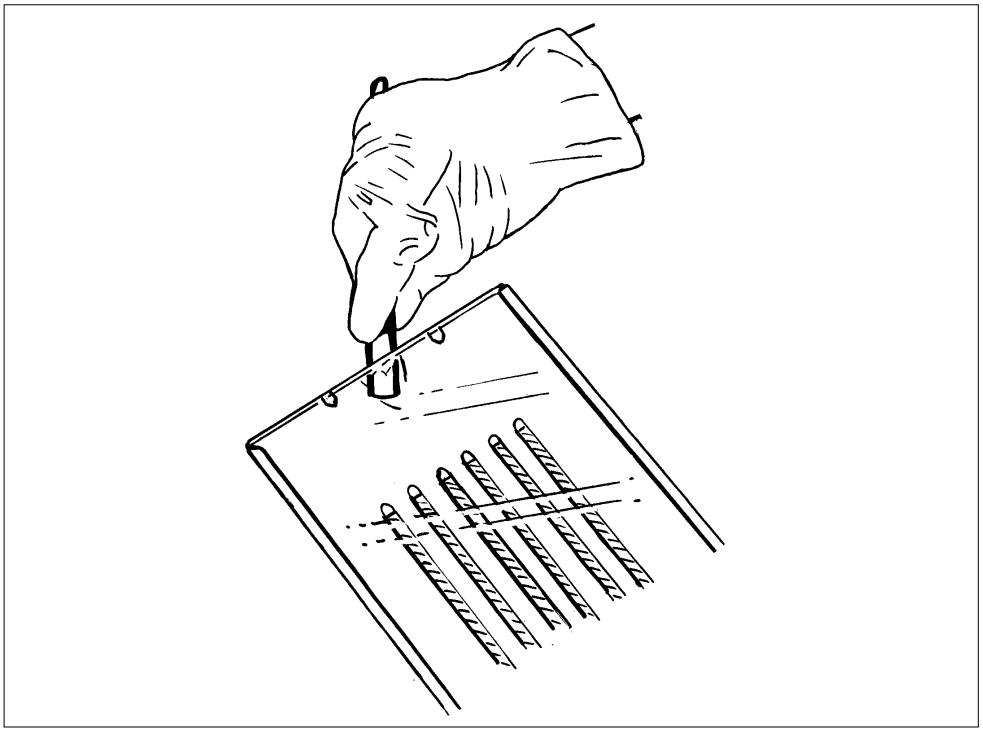


Fig. 10.

- Use a pair of clean forceps to place the strips on the plain glass plate gel side up (Fig. 11).

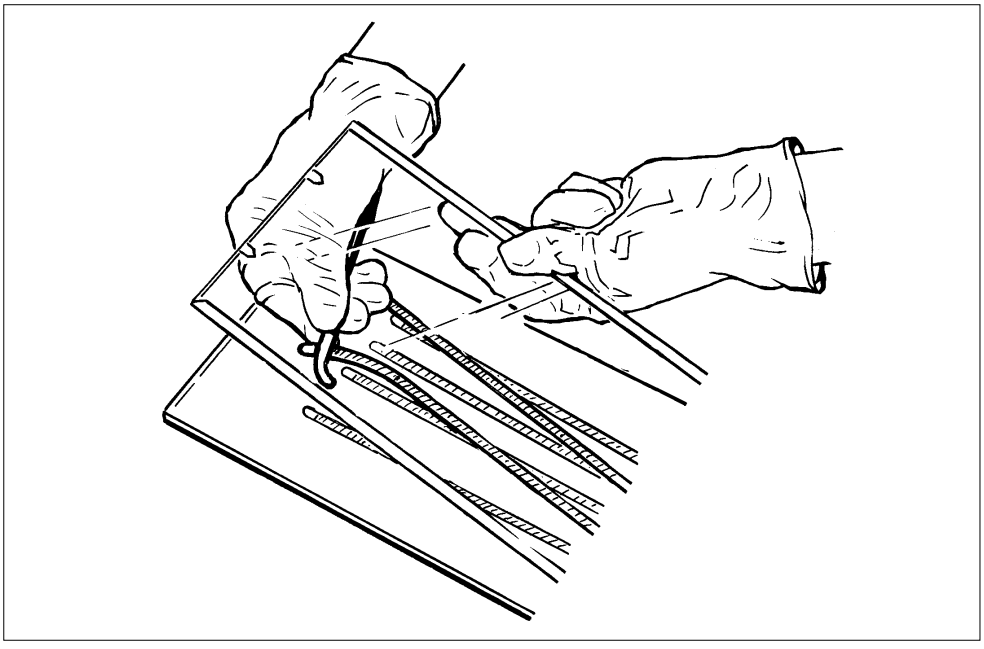


Fig. 11.

- Place a tissue paper or a humid filter paper on top of the strips, blot very gently and then remove the tissue paper (Fig. 12).

  It is normal for the tissue paper to stick to the strips. You may need to "peel" the tissue paper off.

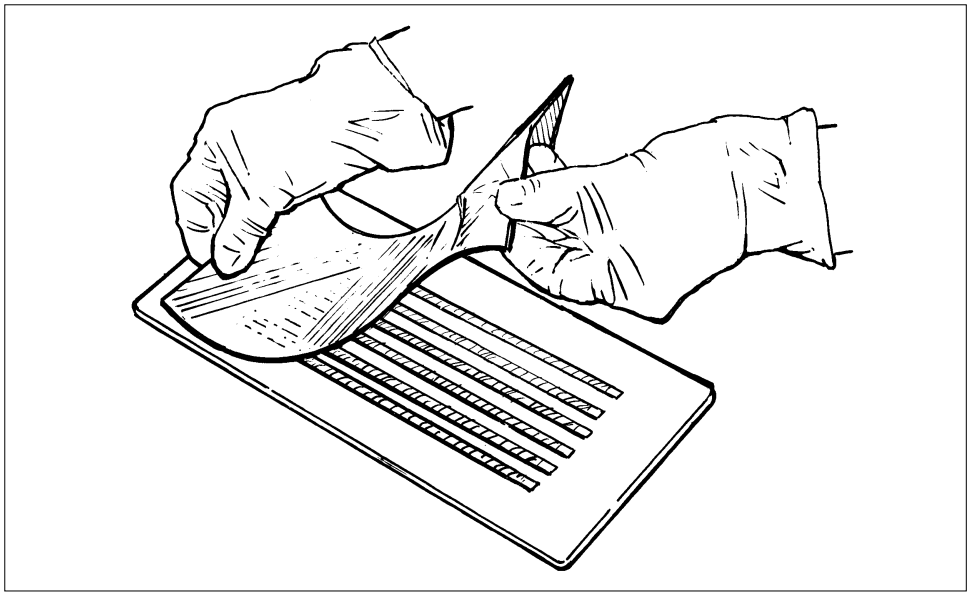


Fig. 12.

- Transfer the strips to adjacent grooves of the aligner in the tray (Fig. 13).

  Place the strips with the rounded (acidic) end at the top of the tray near the cooling tubes and red electrode (anode). The squared end should be at the bottom of the tray near the black electrode (cathode).

  Align the strips such that the anodic gel edges are lined up.

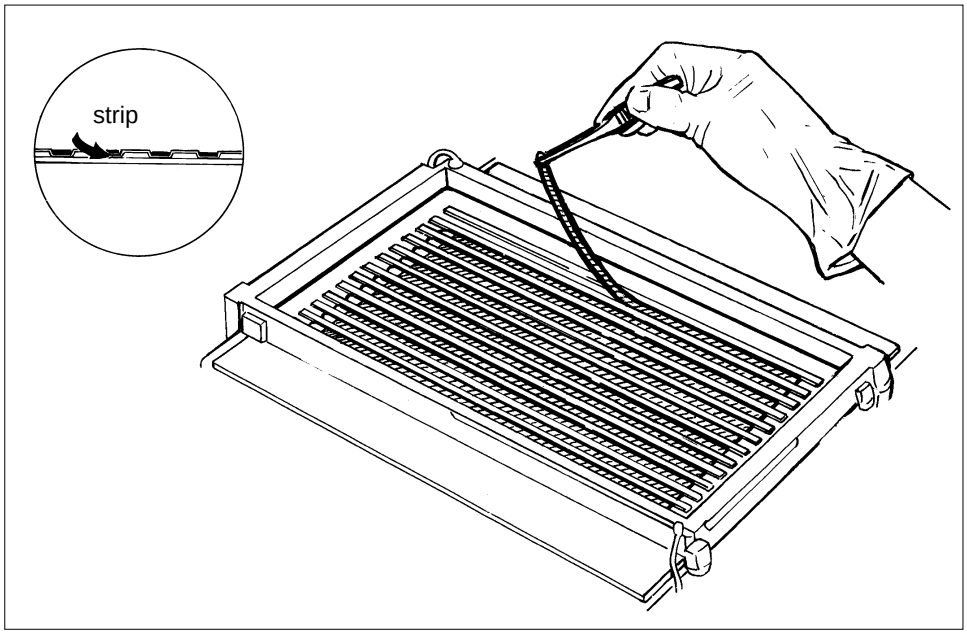


Fig. 13.

- Place the moistened IEF Electrode Strips on top of the aligned strips at the Cathode and Anode (Fig. 14).

  The IEF Electrode Strips should be at least partially on top of the gel surface.

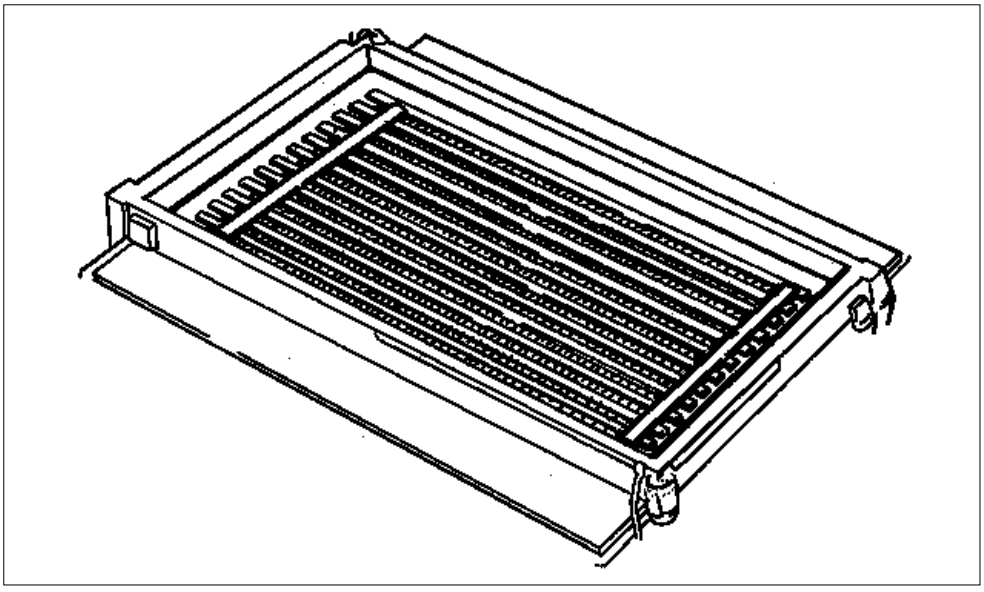


Fig. 14.

- Position the electrodes and press them down on top of IEF Electrode Strips (Fig. 15).

  Each electrode has a side marked red (anode) or black (cathode). Make sure the marked side corresponds to the side of the tray giving electrical contact.

  Once the electrodes are in place, make sure the strips have remained aligned in their grooves.

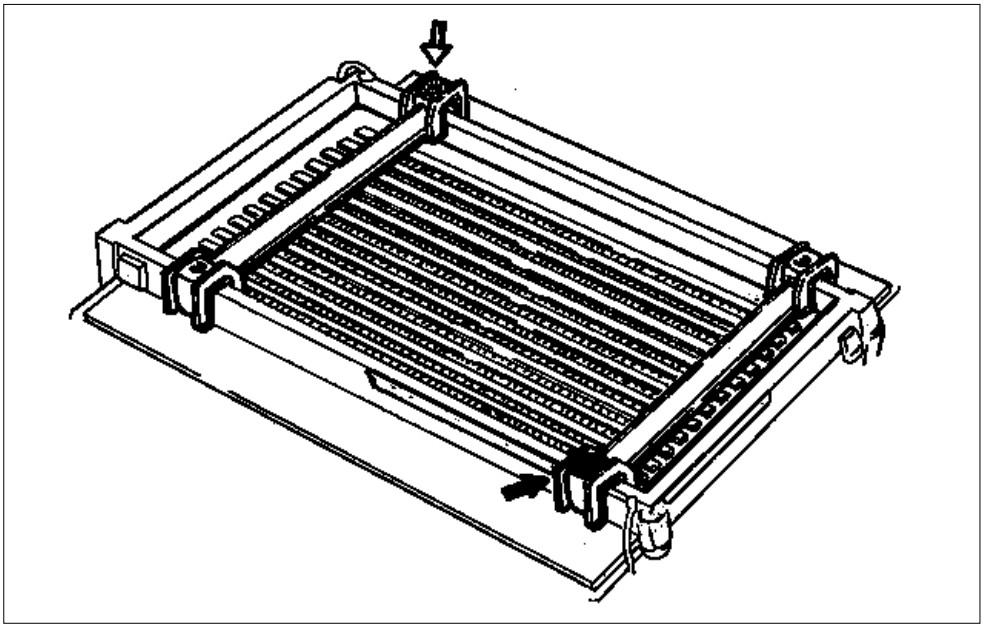


Fig. 15.

- Put the sample cups on the sample cup bar. Place the cups high enough on the bar to avoid touching the gel surface. Put the sample cup bar in position so that the sample cups are a few millimeters away from the cathodic or anodic electrode. The sample cups must face the electrode (Fig. 16).

  There is a “spacer” on one side of the sample cup bar. Slide the sample cup bar towards the anode/cathode until this spacer just touches the anodic/cathodic electrode.

  If the proteins of interest have acidic pI’s, or the concentration of SDS is high, we recommend loading the samples at the cathode.

  We recommend anodic application for the pH gradients 3-10 L, 180 mm and 3-10, 110 mm.

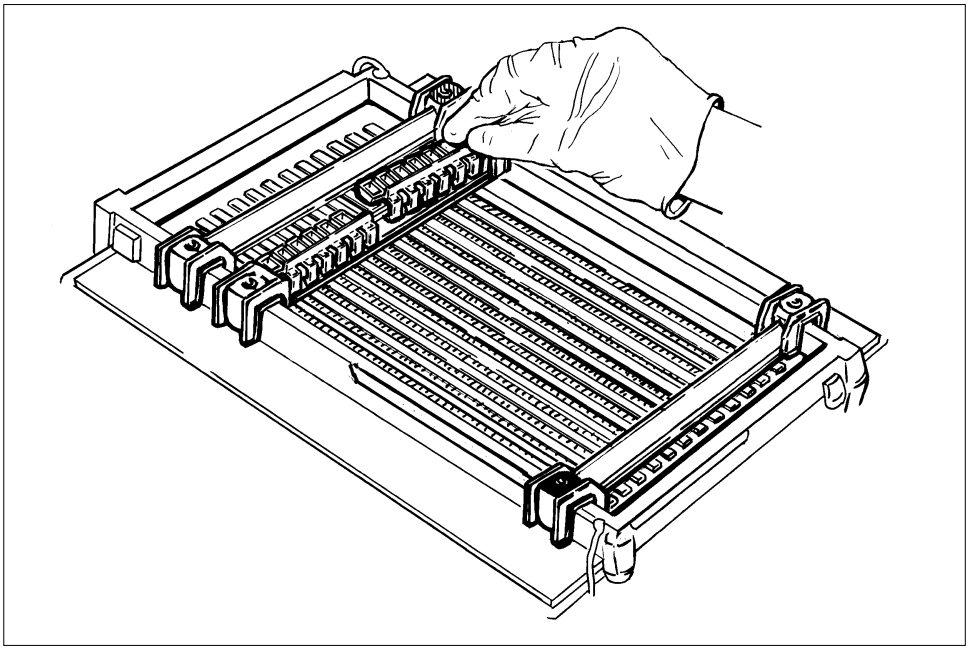


Fig. 16.

- Move the sample cups into position, one sample cup above each Immobiline DryStrip, and finally press the sample cups down to ensure good contact with each strip. (Fig. 17).

  This is perhaps the most critical part of the setup. See figures for correct position of sample cups.

  Check that the strips are in their right and straight position in the strip aligner.

- Once the sample cups are properly positioned, pour 70-80 ml DryStrip Cover Fluid into the tray to completely cover the strips. If the oil leaks into the sample cups suck the oil up, adjust the leakage and check for leakage again. Add approximately an additional 150 ml of DryStrip Cover Fluid to completely cover the sample cups. (Fig. 18)

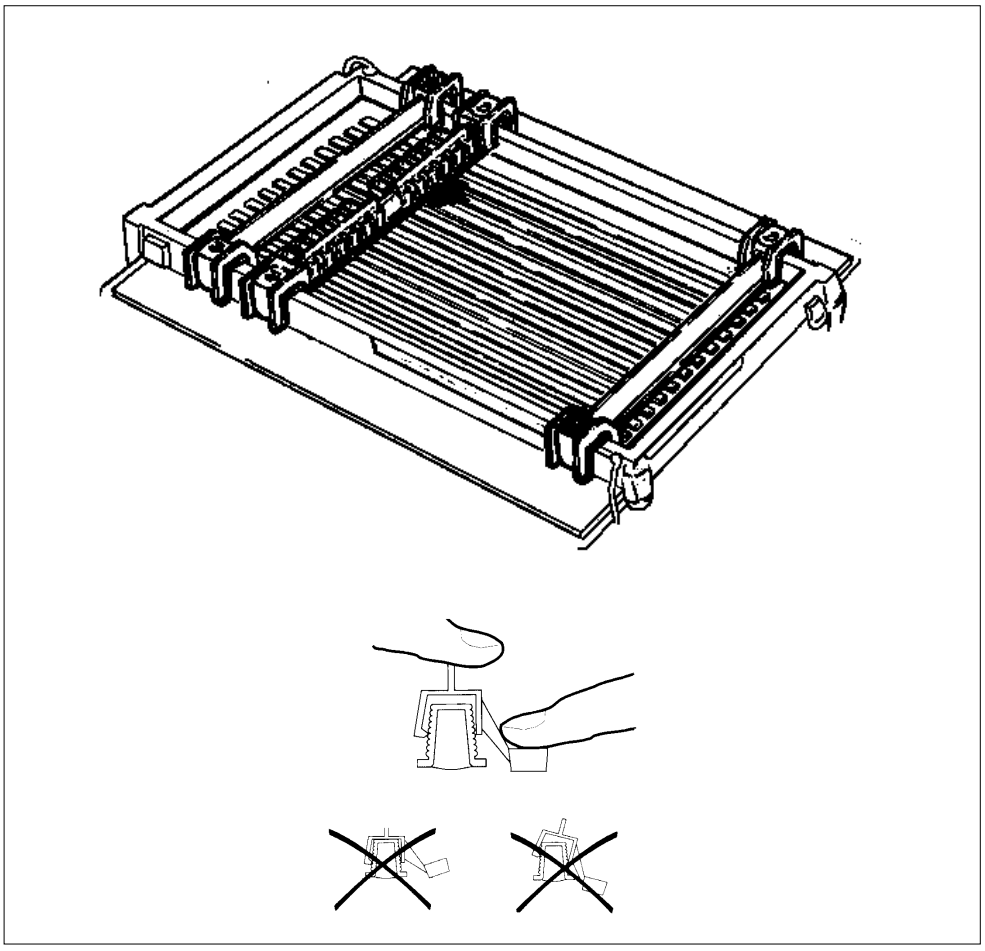


Fig. 17.

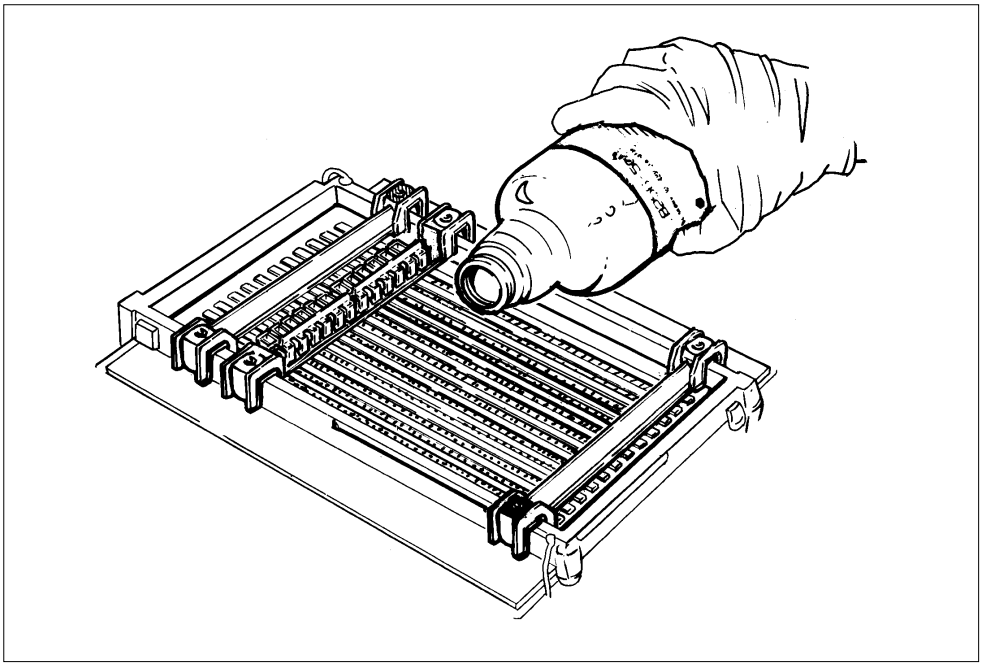


Fig. 18.

- Pipette your samples into the cups by underlaying. The sample should sink to the bottom of the cup. Watch again for leakage (Fig. 19).

  The samples should have already been prepared according to the instructions at the beginning of Day 2 activities.

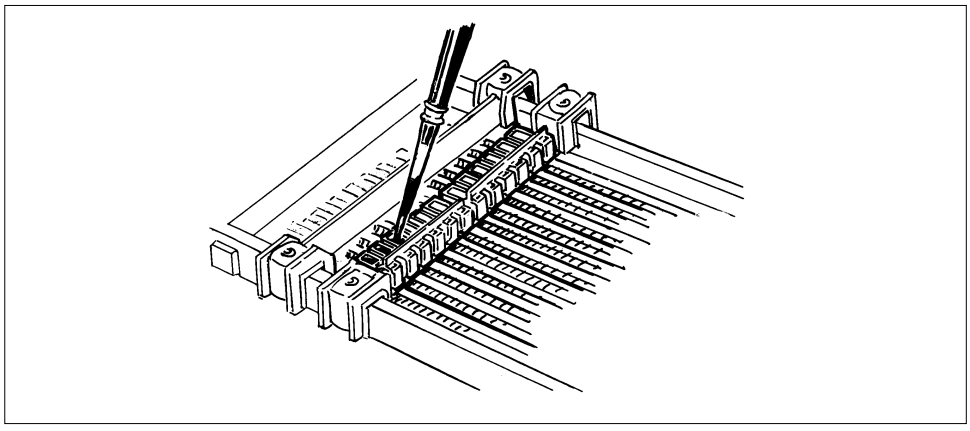


Fig. 19

- Make sure the electrodes on the tray are connected and place the lid on Multiphor II unit. Connect the leads on the lid to the power supply.
- Fig. 20 shows the complete arrangement of the equipment.

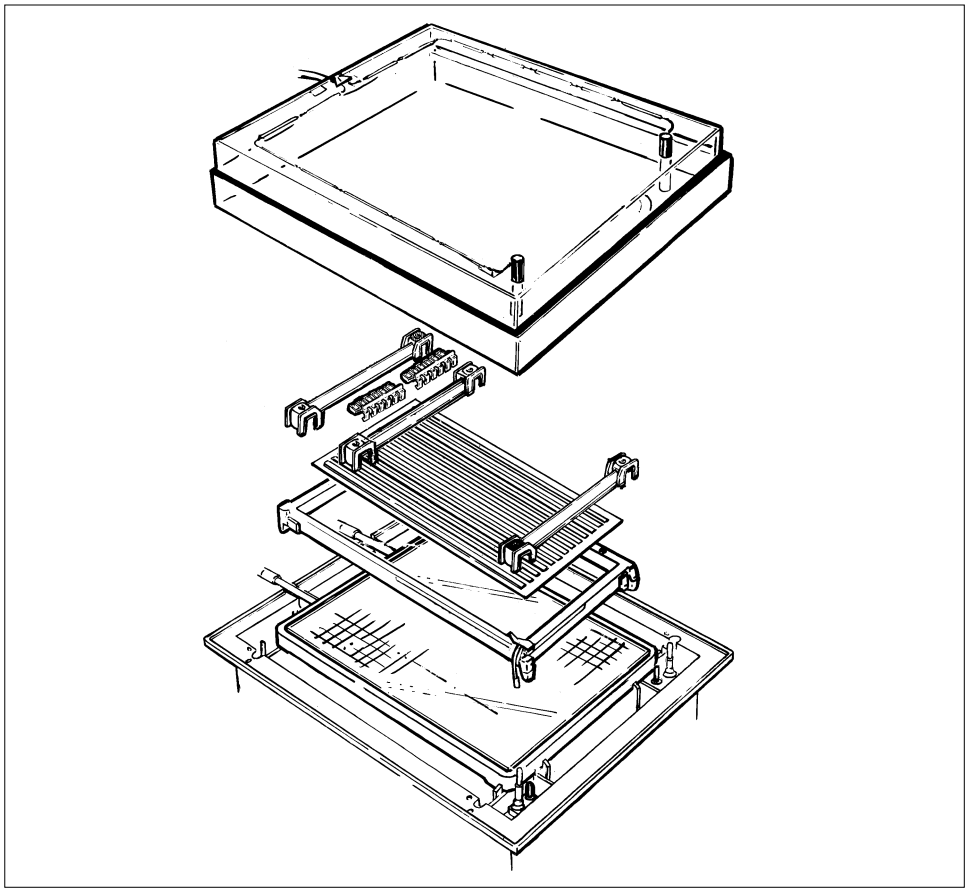


Fig. 20.

- Start the power supply and run according to the parameters discussed on the following pages.
  If you put Orange G in the rehydration buffer, you should see it starting to migrate from the cathode to the anode within the first 5-10 minutes of the run.

6.2.4 First dimension run

Recommended running conditions for 1 or up to 12 Immobiline DryStrip
Option 1: Using Electrophoresis Power Supply EPS 3500 XL
Programme Mode: Gradient programming

- Note that the program mode “Gradient ” (/) is indicated. This means that the voltage will be ramping up to the maximum set in the phase, starting from zero in the first phase and in phases to follow from the end point of the phase before.

Program for Immobiline DryStrip pH 3-10 L, 110 mm

Phase	Voltage	mA	W	Time (hr)	Vh
1	300	1	5	0.01	1
2	300	1	5	4.5	1350
3	2000	1	5	5	5750
4	2000	1	5	6.5	13000
Total:				16	20100

The total focusing time is 16 hours (20100 Vh, Volt-Hours).
To arrange a more convenient stop time, Phase 2 and 4 can be adjusted to give a longer run, using the same number of Vh as recommended. This is applicable to all pH intervals.
For example using Immobiline DryStrip pH 3-10 L, 110 mm, Phase 2 can be 300 V, 1 mA, 5 W, 6.5 hr, 1950 Vh and Phase 4 2000 V, 1 mA, 5 W, 6 hr, 12000 Vh. The total run time would then be 17.5 hours, 19700 Vh.
Changes in the program should be done without changing Phase 3 or the voltage of any Phase, or the total number of Vh.

Program for Immobiline DryStrip pH 3-10 L, 180 mm

Phase	Voltage	mA	W	Time (hr)	Vh
1	500	1	5	0.01	1
2	500	1	5	3	1500
3	3500	1	5	5	10000
4	3500	1	5	12.5	43750
Total				20.5	55250*

Program for Immobiline DryStrip pH 3-10 NL 180 mm

Phase	Voltage	mA	W	Time (hr)	Vh
1	500	1	5	0.01	1
2	500	1	5	5	2500
3	3500	1	5	5	10000
4	3500	1	5	9.5	32400
Total				19.5	44900*

* The total number of Volt-hours for these pH gradients should be 50-60 kVh or 40-50 kVh respectively. The optimal total number of Volt-hours depends on the kind of sample, sample load (µg) and sample volume.

Program for Immobiline DryStrip pH 4-7 L, 110 mm

Phase	Voltage	mA	W	Time (hr)	Vh
1	300	1	5	0.01	1
2	300	1	5	6	1800
3	3500	1	5	5	9500
4	3500	1	5	5.5	19250
Total:				16.5	30550*

Program for Immobiline DryStrip pH 4-7 L, 180 mm

Phase	Voltage	mA	W	Time (hr)	Vh
1	500	1	5	0.01	1
2	500	1	5	1	500
3	3500	1	5	5	10000
4	3500	1	5	10	35000
Total				16	45500*

* The optimal number of Vh depends on the kind of sample, sample load (µg) and sample volume.

Option 2: Using a Manual Power Supply

- When using a manual power supply, the power supply should run at constant voltage with the parameters set as below.

Running conditions for Immobiline DryStrip pH 3-10 L, 110 mm

Phase	Voltage	mA	W	Time	Vh
1	300	1	5	1	300
2	1400	1	5	14-15	20000*

Running conditions for Immobiline DryStrip pH 3-10 L, 180 mm

Phase	Voltage	mA	W	Time	Vh
1	500	1	5	1	500
2	3500	1	5	15-16	55000*

Running conditions for Immobiline DryStrip pH 3-10 NL, 180 mm

Phase	Voltage	mA	W	Time	Vh
1	500	1	5	4	2000
2	3500	1	5	12,5	43750*

Running conditions for Immobiline DryStrip pH 4-7 L, 110 mm

Phase	Voltage	mA	W	Time (hr)	Vh
1	300	1	5	3	900
2	2200	1	5	13	28600*

Running conditions for Immobiline DryStrip pH 4-7 L, 180 mm

Phase	Voltage	mA	W	Time (hr)	Vh
1	500	1	5	4	2000
2	3500	1	5	12.5	43750*

* The optimal number of Vh depends on the kind of sample, sample load (µg) and sample volume.

6.3. Day 3

6.3.1 Equilibration of Immobiline DryStrip

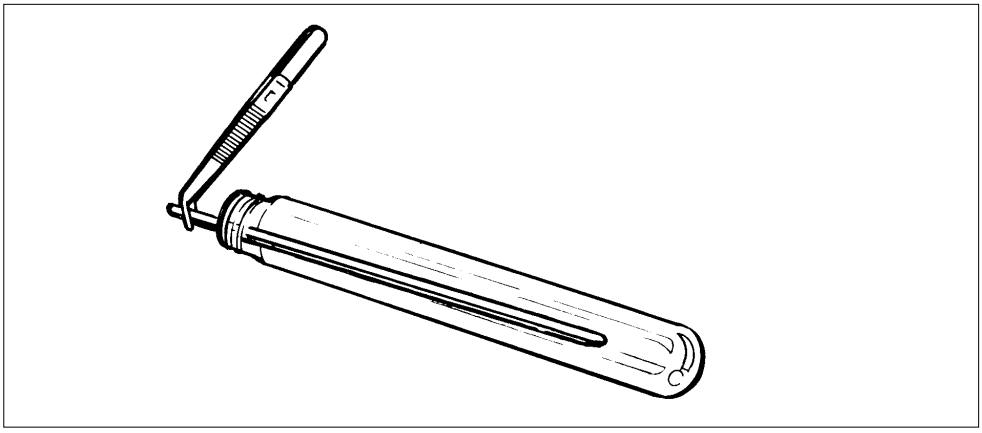
- Prepare equilibration solutions.

  The equilibration is a two step process (7, 8). Prepare 20 ml of each of equilibration solutions, see 10 Solutions.
- When the IEF run is completed, disconnect the power supply and remove the Multiphor II cover.
- Remove the electrodes, sample cup bar and IEF Electrode Strips from the tray.
- Using a pair of forceps, remove the strips that will be immediately run in the second dimension.

  1 or 2 strips can be run per ExcelGel in the second dimension.

  If more than two strips have been run in the first dimension, the unused strips can be wrapped in plastic foil and stored at –80°C for later use. The strips should be stored prior to equilibration. When the strips are to be run in the second dimension, they should be equilibrated as indicated.
- Place the strips into individual test tubes, 20 ml, and add 15 ml of equilibration solution No.1 to each tube (Fig. 21). For the longer strips it is convenient to use a petri dish. Place the strip with the support film towards the wall of the test tube or petri dish.

  Seal the test tube with Parafilm and lay it on its side on a rocker. If a petri dish is used, put this on a rocker.



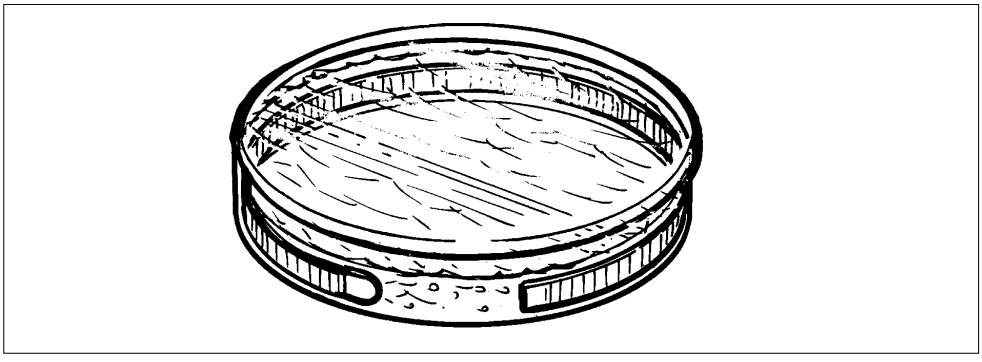


Fig. 21.

- Equilibrate solution No.1 for 10 minutes.
- After 10 minutes, pour out solution No.1 and add equilibration solution No. 2 as above. Equilibrate for 10 minutes.
- After the second equilibration, place the strips on a piece of filter paper that has been moistened with dH_2O (Fig. 22).

  The strip should be turned up on one of it's edges to help it drain.

  The strips can be left in this position for up to 10 minutes without noticeably affecting spot sharpness.

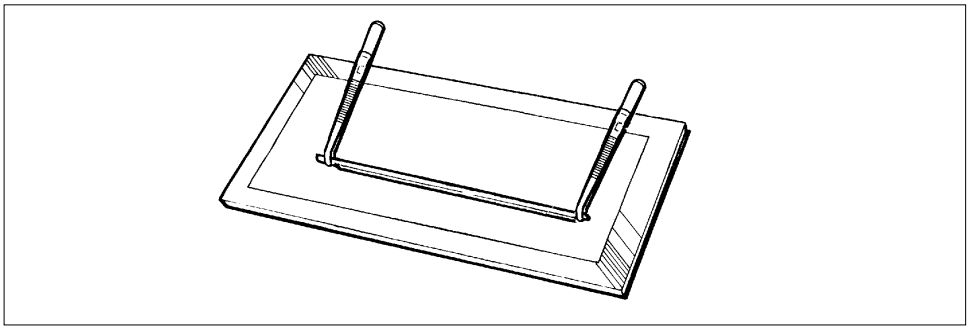


Fig. 22.

6.3.2 Second dimension run

- While the strips are equilibrating and draining, begin the assembly of the ExcelGel for the second dimension.
- Set the temperature on MultiTemp to 15°C.

  If there are problems with condensation at this time, the MultiTemp can be left at room temperature until the start of the electrophoresis.
- Remove the ExcelGel from its foil package by cutting around the edges (Fig. 23).

  The ExcelGel is cast on a plastic support film. It also has a protective plastic cover on top of it which indicates the direction the gel should be run.

  The lower right hand corner of the ExcelGel support film has a notch cut out of it. This notch is on the 18% or 14% (i.e. Anodic) side of the gel.

  Note also that the gel itself does not generally cover the entire support film.

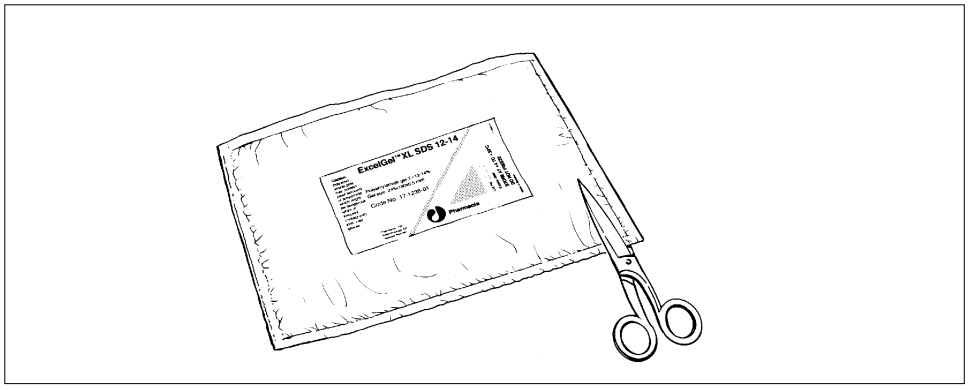


Fig. 23.

- Place approximately 2.5-3.0 ml of DryStrip Cover Fluid on the cooling plate of Multiphor II (Fig. 24).

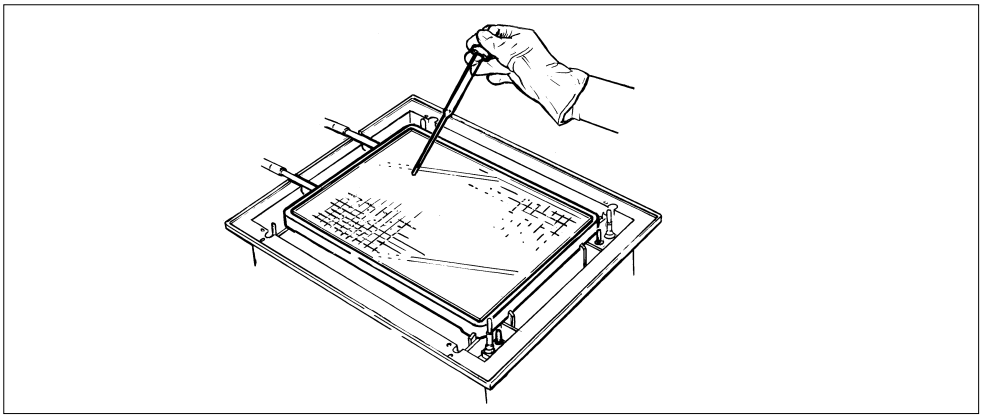


Fig. 24.

- Remove the protective cover from the top of ExcelGel XL SDS 12-14 or SDS, gradient 8-18.
- Place the gel on the cooling plate. Avoid getting bubbles trapped between the gel and the cooling plate (Fig. 25).

  Avoid getting DryStrip Cover Fluid or oil on the gel surface as this may cause the buffer strips to slide during the electrophoresis.

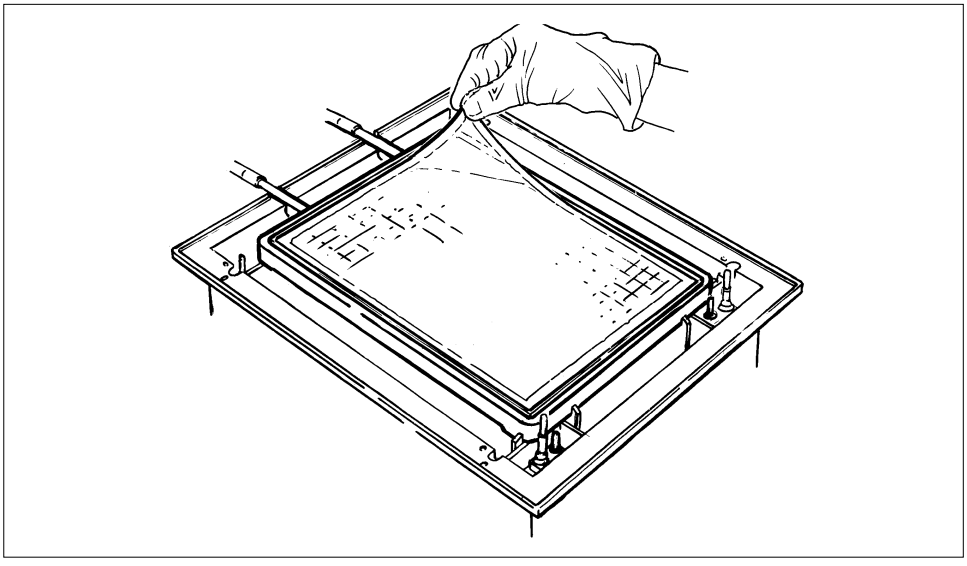


Fig. 25.

- Peel back the foil on the Anodic (+) SDS Buffer Strip (Fig. 26).

  Vinyl gloves tend to stick less to the buffer strips than other types of plastic gloves.

  If you find your gloved fingers stick to the buffer strips, wet your fingers lightly with distilled water or a 5-10% SDS solution.

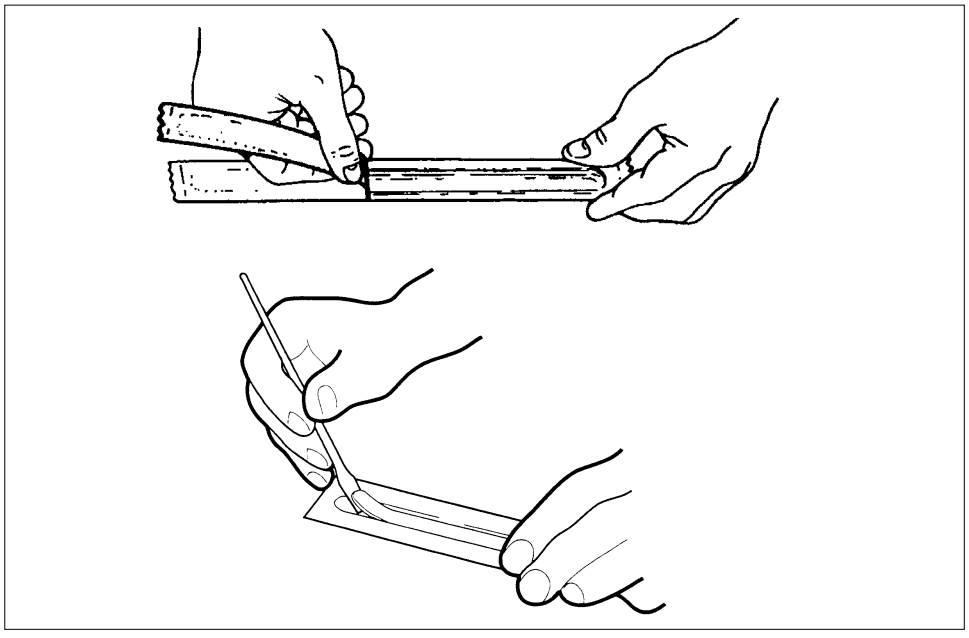


Fig. 26.

- Place the anodic buffer strip on the anodic side of the gel (this side of the gel has the notch cut from it). Avoid air bubbles between the gel and the buffer strips (Fig. 27).

  The smooth (narrower) side of the buffer strip should face down and contact the gel surface.

  Should the buffer strip break, it can be pieced together on the gel.

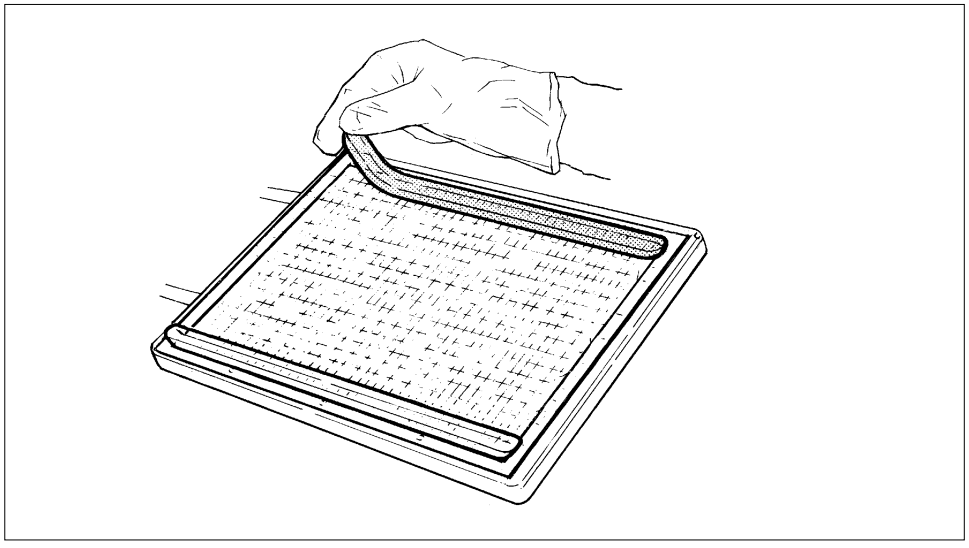


Fig. 27.

- Repeat the procedure with the Cathode (-) buffer strip.
- Once the strips (from above) have drained for at least 3 minutes, place the strips, gel side down, on the ExcelGel as indicated in Figure 28. If you wish to run SDS Markers in the Second Dimension apply an extra application piece for them.

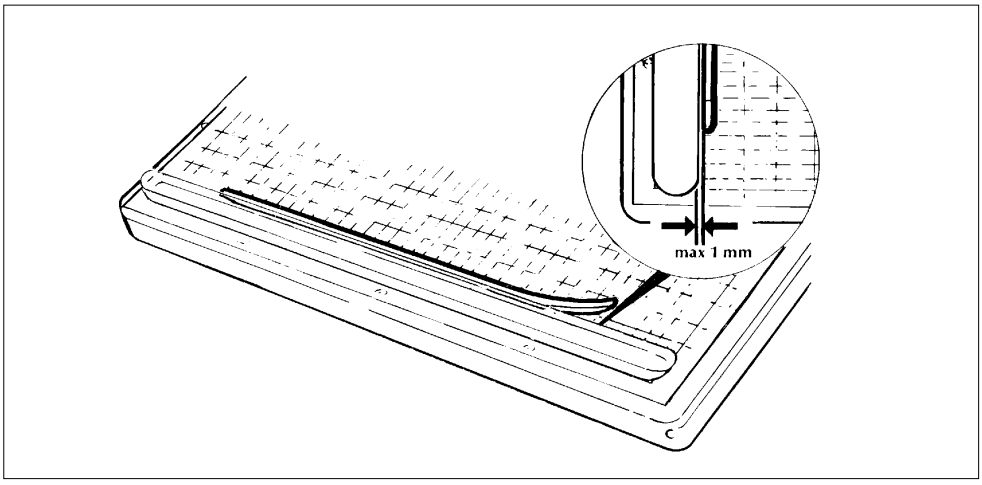


Fig. 28.

- Place sample application pieces at each end of the strips (Fig. 29).

  The sample application pieces are used to absorb buffer that is "pumped" out of the strips during the electrophoresis.

  These sample application pieces should be tucked underneath the "tab" at each end of the strip(s). The pieces should be in contact with the gel.

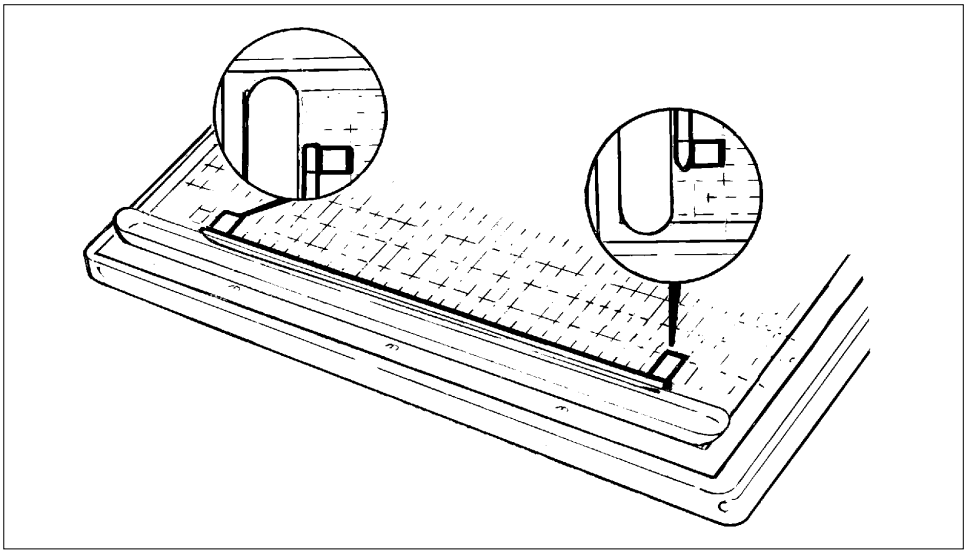


Fig. 29.

- Pipette SDS markers onto the extra sample application piece.

  Apply the markers in a volume of 15-20 µl. For less volume, cut the sample application piece proportionately.

  The markers should contain 200-1000 ng of each component for Coomassie staining and about 10-50 ng of each component when silver staining is used.
- Stroke the top of the strips gently with the forceps to remove any trapped air bubbles (Fig. 30).

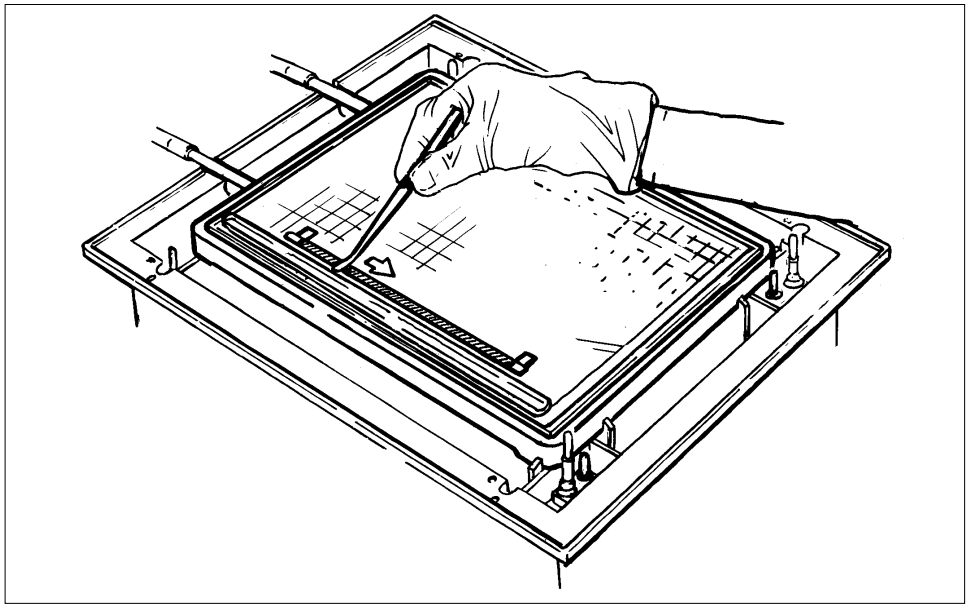


Fig. 30.

- Place the electrode holder in the upper position and align the electrodes with the buffer strips (Fig. 31).

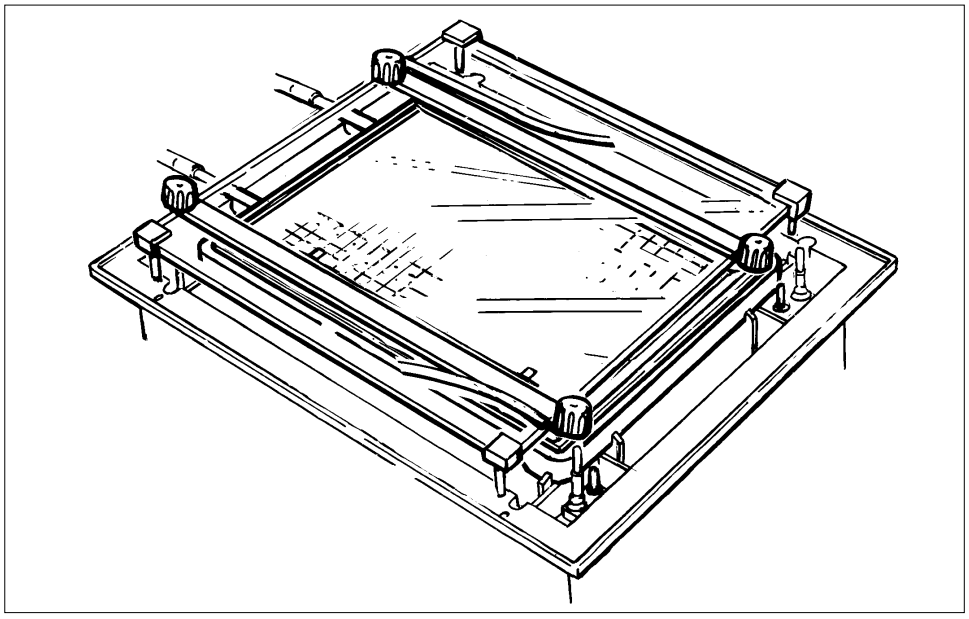


Fig. 31.

- Plug in the electrodes and lower the electrode holder carefully on to the buffer strips.
- Place the lid on the Multiphor II and run as indicated below.

Running Conditions for

ExcelGel XL SDS 12-14. Using EPS 3500 XL, program step mode.

Step	Voltage	mA	W	Time (min)
1	1000	20	40	45[1]
2	1000	40	40	5[2]
3	1000	40	40	160[3]

ExcelGel SDS, gradient 8-18%. Using EPS 3500 XL, program step mode

Step	Voltage	mA	W	Time (min)
1	600	20	30	25-30[1]
2	600	50	30	3-5[2]
3	600	50	30	70[3]

[1] When the bromophenol blue dye front has moved 4-6 mm for ExcelGel XL SDS 12-14 and 1-2 mm for ExcelGel SDS, gradient 8-18 from Immobiline DryStrip, remove the strip and the application pieces.

[2] When the front has moved a further 2 mm, move the cathodic buffer strip forward to cover the area of removed Immobiline DryStrip by 1-2 mm. Adjust the position of the cathodic electrode.

[3] When the Bromophenol Blue front has just reached the anodic buffer strip, electrophoresis is continued for 5 min and should then be stopped. Remove the buffer strips.

7. Detection

7.1 Staining

For automated silver and coomassie staining of polyacrylamide gels see Protocol Guide, Hoefer Automated Gel Stainer (80-6343-34).

All current detection methods used for isoelectric focusing and SDS electrophoresis can be applied. For Silver staining we recommend to use PlusOne Silver Staining Kit, Protein (Code No. 17-1150-01).

Note: For ExcelGel XL SDS 12-14 400–500 ml solutions are needed per gel.

7.2 Electrophoretic transfer

The support film can be removed with Film Remover (Code No. 18-1013-79). The separated proteins can then be electrophoretically transferred to a blotting membrane for specific probing.

8. Evaluation

ImageMaster Software is a powerful and agile software program for protein quantification and data analysisd.

ImageMaster exists in two program versions:

ImageMaster version 1.20 for evaluating both 1-D and 2-D gels (Code No. 18-1108-29), and ImageMaster 1D for 1-D evaluation (Code No. 18-1108-30). The 1-D functions are the same for both versions.

Together with the new Sharp Scanner JX-330 (Code No. 18-1108-95), ImageMaster forms a fast, high resolution personal computer-based evaluation system for both 1-D and 2-D electrophoresis gels and other images. ImageMaster DeskTop Scanner (DTS) (Code No. 18-1033-00) and UltroScan™ XL Laser Densitometer (Code No. 18-1013-70). With the ImageMaster Software it is also possible to import certain TIFF file formats of images scanned by other scanners and some Video cameras. The connection of the Sharp Scanner or ImagesMaster DTS to a PC is achieved via a GPIB-interface. The connection of the UltroScan to a PC is via RS 232.

The software allows configuration for the densitometer in use and all programming is done from the PC-keyboard or via the mouse.

The 2-D function analyses complex protein samples separated by 2-D electrophoresis. Protein spots are automatically detected and quantified. ImageMaster Software reveals concentration changes in spot patterns and presents them as histograms. This function makes feasible analysis of all data generated with 2-D gels. Up to 6 gels, each containing up to 10,000 spots can be compared and analysed simultaneously.

9. References

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10. Solutions

Note: All chemicals should be of the highest purity, PlusOne grade recommended. Double distilled water should be used.

A. Lysis solution

Urea	13.5 g
Triton X-100	0.5 ml
2-Mercaptoethanol or	0.5 ml
500 mg DTT	
Pharmalyte 3-10	0.5 ml
PMSF	
(Phenylmethansulfonyl fluoride) or	35 mg
Pefabloc (Merck)	5 mg

Make up to 25 ml with distilled water.

B. Sample solution

Urea	13.5 g
2-Mercaptoethanol or	0.5 ml
250 mg DTT	
Pharmalyte 3-10	0.5 ml
Triton X-100	0.13 ml

Add a few grains of Bromophenol Blue (BPB). Make up to 25 ml with distilled water.
Can be stored in small portions at -20°C for 2 months.

C. Rehydration solution

Urea (8 M)	12.0 g
Triton X-100 (0.5%)	0.13 ml
Pharmalyte 3-10	0.13 ml
DTT	50 mg

Make up to 25 ml with distilled water

Make fresh solution every day.

Optionally 0.5 mg Orange G (or a few grains of Bromophenol Blue for cathodic application) can be added, which makes the strips faintly yellow (or blue) and may be helpful for strip alignment.

The following changes in the above rehydration solution can be made for rehydration of 110 mm long strips:

Exclude Pharmalyte and add 0.10 ml of 1.0 mol/l Acetic acid

D. Equilibration

Tris-HCl stock solution:

Tris	61 g
1.0 mol/l HCl	460-490 ml

Dissolve Tris in 300 ml distilled water.

Adjust to pH 6.8 with 1.0 mol/l HCl.

Make up to 1.0 l with distilled water.

Equilibration solution:

Tris-HCl stock solution:	20 ml
Urea	72 g
Glycerol	60 ml
SDS	2 g

Add 67 ml distilled water to give a final volume of 200 ml.

Add 25-100* mg DTT/10 ml equilibration solution for the first equilibration step and 0.45 g iodoacetamide plus a few grains of BPB/10 ml solution for second step.

* Depending on the amount of sample