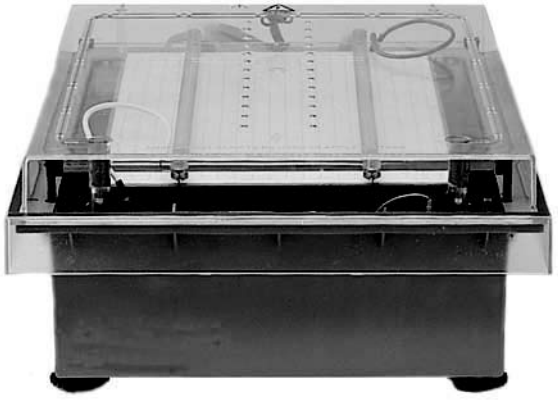
GE Healthcare
Life Sciences

# Multiphor II Electrophoresis System

User Manual



## Important user information

All users must read this entire manual to fully understand the safe use of Multiphor II Electrophoresis System.

### WARNING!

WARNING indicates a hazardous situation which, if not avoided, could result in death or serious injury. It is important not to proceed until all stated conditions are met and clearly understood.

Indicates that hazardous voltages occur inside the instrument.

### CAUTION!

CAUTION indicates a hazardous situation which, if not avoided, could result in minor or moderate injury. It is important not to proceed until all stated conditions are met and clearly understood.

### NOTICE!

NOTICE indicates instructions that must be followed to avoid damage to the product or other equipment.

### *Note*

The Note sign is used to indicate information important for trouble-free and optimal use of the product.

## CE Certifying

This product meets the requirements of applicable CE-directives. A copy of the corresponding Declaration of Conformity is available on request.

The **CE** symbol and corresponding declaration of conformity, is valid for the instrument when it is:

– used as a stand-alone unit, or

– connected to other CE-marked GE Healthcare instruments, or

– connected to other products recommended or described in this manual, and

– used in the same state as it was delivered from GE Healthcare except for alterations described in this manual.

## Recycling

This symbol indicates that the waste of electrical and electronic equipment must not be disposed as unsorted municipal waste and must be collected separately. Please contact an authorized representative of the manufacturer for information concerning the decommissioning of equipment.

# Contents

# 1 Introduction

Multiphor™ II electrophoresis system is a versatile modular system for horizontal electrophoresis, isoelectric focusing, 2-D electrophoresis and electrophoretic transfer.

For ease of use and reproducible results, an innovative range of precast gels for all major electrophoretic techniques is available with Multiphor II:

| **Technique** | **Precast Gel** |
|---|---|
| SDS and Native PAGE | ExcelGel™ SDS Gradient<br>ExcelGel SDS Homogeneous |
| IEF | CleanGel™ IEF |
| 2-D electrophoresis | Immobiline™ DryStrip<br>ExcelGel SDS gradient Homogeanus |

If laboratory cast gels are preferred an application kit and accessories can be added to the basic electrophoresis unit.

The following guide summarizes how you can expand and use Multiphor II Electrophoresis Unit with application kits and accessories.

| Application | Recommended Kit/Accessory | Code No. |
|---|---|---|
| SDS and Native PAGE<br>homogeneous and gradient ge | SDS and Native PAGE, IEF Kit<br>Gradient Maker | 18-1102-45<br>80-6196-09 |
| ExcelGel SDS | Buffer Strip Positioner | 80-6442-90 |
| IEF in polyacrylamide<br>0.5 x 125 x 260 mm | SDS and Native PAGE, IEF Kit | 18-1102-45 |
| 2-D, first dimension: Immobiline DryStrip | Immobiline DryStrip Kit | 18-1004-30 |
| Electrophoretic transfer | NovaBlot Kit<br>FilmRemover | 18-1016-86<br>18-1013-75 |

This User Manual is comprised of the following sections:

1. "Introduction" includes a general description of Multiphor II system and dedicated precast gels, a guide to the application kits and the manual structure.
2. "Description of parts" describes in detail the components of Multiphor II Electrophoresis Unit.
3. "Installation" contains a detailed description of how to install Multiphor II Electrophoresis Unit and Multiphor II NovaBlot™ Unit.
4. "Operation" contains information on the operating procedure for SDS and native polyacrylamide gel electrophoresis, isoelectric focusing, 2-D electrophoresis and electrophoretic transfer.
5. "Maintenance" gives cleaning recommendations to help you maintain your Multiphor II unit.
6. "Trouble shooting" offers suggestions for correcting problems that may occur.
7. "Multiphor II application kits and accessories" describes in detail the contents, assembly and use of each Multiphor II application kit.
8. "Ordering information."
   - Multiphor II Electrophoresis Unit, application kits, accessories and replacement parts
   - MultiTemp™ and EPS
   - Precast gels and buffer strips
   - Molecular weight and pI markers
   - Pharmalyte™ carrier ampholytes
   - Gel casting and electrophoresis chemicals

# 2 Safety information

To avoid any risk of injury, the instrument should be operated only by properly trained personnel and always in accordance with the instruction provided. Read this entire manual before using the instrument.

**WARNING!** Multiphor II is a high voltage instrument that can cause fatal electrical shock if the safety features are disabled. The safety lid must be securely closed before starting a protocol.

**WARNING!** The instrument is designed for indoor use only.

**WARNING!** Do not operate tthe system in extreme humidity (above 95% RH). Avoid condensing by equilibrating to ambient temperature, when taking the unit from a cooler to a waremer environment.

**WARNING!** Always check the wires for damage before using the unit.

**WARNING!** Always check that the electrodes are properly connected before using the lid.

**WARNING!** Always connect the lid according to the mounting instruction.

**WARNING!** Always connect the cables to the Power supply BEFORE turning the Power Supply ON.

**WARNING!** Always TURN OFF the Power Supply before removing the lid.

**WARNING!** Do NOT use concentrated acids, bases or halogenated and aromatic hydrocarbons.

**WARNING!** Only use water or coolant with high electrical resistance in the cooling plate, e.g., deionized water.

**WARNING!** NEVER EXCEED the maximum pressure 0.35 bar in the cooling plate.

**WARNING!** NEVER EXCEED the maximum allowed voltage, current or power.

**WARNING!** The cooling plate is rated for operation at up to
3.5 kV (± 1750 V with floating output with reference to ground).

**WARNING!** When using hazardous chemicals, take all suitable protective measures, such as wearing protective glasses and gloves resistant to the chemicals used. Follow local regulations and instructions for safe operation and maintenance of the system.

**WARNING!** When using hazardous chemicals, make sure that the entire system has been flushed thoroughly with bacteriostatic solution, e.g. NaOH, and distilled water before service and maintenance.

**WARNING!** Always make sure that the surfaces around the cooling plate are clean and dry before starting a run.

**WARNING!** Make sure not to spill buffer outside the buffer chambers! Clean up any spillage immediately! There is a risk of electric shock and personal injury if there is spilled buffer in the areas where the electrical connectors are located.

**WARNING!** Never use any flammable liquids in the product when it is powered!
Risk for fire!

# 3 Description of parts

The Multiphor II Electrophoresis Unit includes; buffer tank with 4 levelling feet, cooling plate with accessories, safety lid, electrode holder with movable EPH/IEF electrodes (for buffer strips and electrode strips).

**Unit contents – Code No. 18-1018-06**

| Designation | Code No |
|---|---|
| Buffer Tank | 18-1122-25 |
| Levelling Foot (4/pkg) | 18-1026-40 |
| Cooling Plate ceramic, 210 x 270 mm | 18-1103-46 |
| Grommet (2/pkg) | 80-1106-58 |
| Cooling Tubing, 8/12 mm, 4 m | 80-1106-56 |
| Tubing Connector Set 2 pcs female, 2 pcs male | 18-1104-26 |
| Hose Clamp (10/pkg) | 18-1104-27 |
| Safety Lid | 18-1122-26 |
| Electrode Holder | 80-1106-55 |
| EPH/IEF Electrode cathode | 18-1122-19 |
| EPH/IEF Electrode anode | 18-1122-20 |

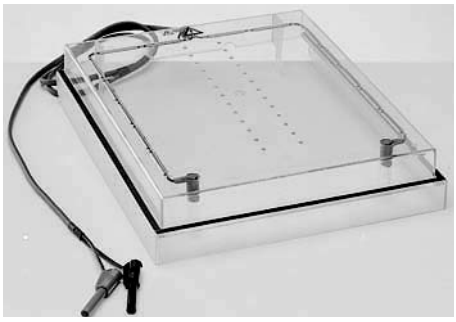
The buffer tank is made of polypropylene, which is resistant to nearly all chemicals at room temperature.

The buffer tank contains four pin contacts. Viewing the buffer tank from the front, the cathode pins are located to the left and the anode pins to the right.

The larger pins are for connection to the safety lid and complete the electrical circuit when the lid is in position.

The small left hand pin is used to connect the EPH/IEF or card-mounted cathode electrodes. The small pin on the right connects to the card-mounted anode or the EPH/IEF cathode via the red lead mounted on the unit.

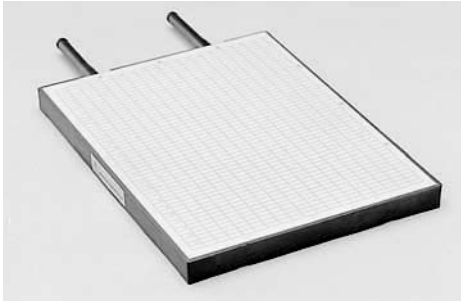
The buffer tank holds the four adjustable levelling feet, supports the cooling plate and is covered during electrophoresis with the safety lid. The buffer tank includes four buffer chambers, each with a 1 liter capacity, allowing the user to choose one of two orientations for electrophoretic runs.



The safety lid contains electrode leads, apertures for voltage measurement, and a safety interlock.

The well-recessed cathode connector (black) and anode connector (red) for connection to power supplies ensure safe operation at high voltages. The polycarbonate lid snugly fits the contours of the buffer tank. This makes it possible to reduce the atmospheric $CO_2$ content around the gel (important for IEF at basic pH intervals) and provides increased protection against condensation.

**NOTICE** Polycarbonate is not resistant to concentrated acids and bases, or to halogenated and aromatic hydrocarbons



The ceramic (aluminium oxide $Al_2O_3$) cooling plate measures 210 x 270 mm, supports the gel, and provides uniform temperature control. Aluminium oxide is an excellent heat conductor and electrical insulating material.

**WARNING!** The cooling plate is rated for operation at up to 3.5 kV (± 1750 V with floating output with reference to ground).

To facilitate the correct positioning of electrophoresis gels, the surface of the cooling plate is screened with a template measuring 190 x 250 mm.

The two grommets are connected to the inlet and outlet tubes of the cooling plate which can then be connected to a thermostatic ciculator such as MultiTemp.

**WARNING!** Only use water with high electrical resistance as coolant and NEVER EXCEED maximum pressure of 0.35 bar.

The cooling tubing, tubing connector set and hose clamps provide a flexible and safe way to connect Multiphor II to a thermostatic circulator such as MultiTemp.

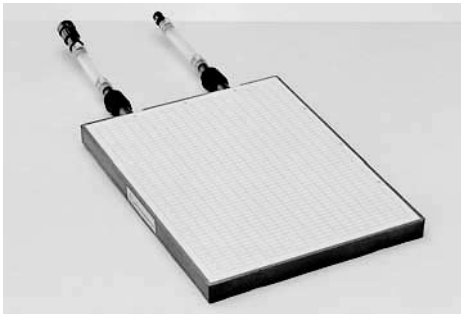
The electrode holder holds the movable EPH/IEF electrodes. The holder keeps the electrodes away from the gel surface during alignment and then provides a uniform pressure over the buffer strips or electrode strips during the separation.

The electrode holder consists of a double strength glass plate with ground edges and four corner feet made of Rynite™ FR530. The electrode holder holds one anode and one cathode electrode.
The EPH/IEF electrodes consist of moulded polysulfone bars which support the platinum wire, held taut by stainless steel springs. The cables are spring reinforced for safety.
The anode cable (red) carries the pin contact to be connected to the socket connector on the buffer tank.
The cathode cable (black) carries a female socket connector which fits to the buffer tank pin connector.
Clamping nuts located at each end of the electrode allow easy adjustment of the electrodes on the holder. The distance between the electrodes can be varied from 10 mm to 240 mm.

***NOTICE*** Polysulfone is not resistant to ketones, esters, halogenated and aromatic hydrocarbons.

# 4 Installation

For all products, check the unassembled parts against the Packing List for the respective product to ensure that all items heve been included.



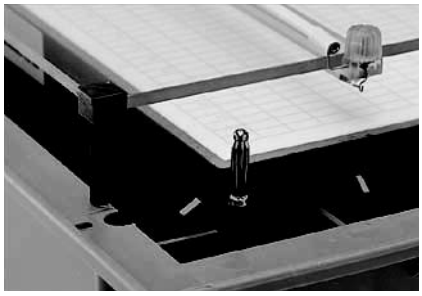
For easy connection of Multiphor II Electrophoresis Unit to the cooling device, install tubing connectors on both inlet and outlet tubings. Use one male and one female connector on the Multiphor II unit side and thermostat unit side respectively. The cooling plate tubings and thermostatic circulator tubings can then be locked separately.

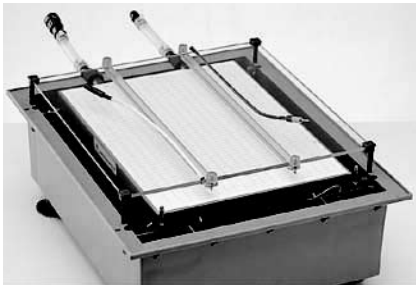
Two Multiphor II units can also be connected in series to one thermostatic circulator.

Place the rubber grommets on the cooling plate inlet and outlet tubes. Slide a short piece of tubing onto each tube and secure with hose clamps. Attach the male and female tubing connectors as described above. Repeat this process with the thermostatic circulator using longer pieces of tubing.

To lock the connectors, insert the male connector into the female and turn clockwise one-quarter turn until it clicks.

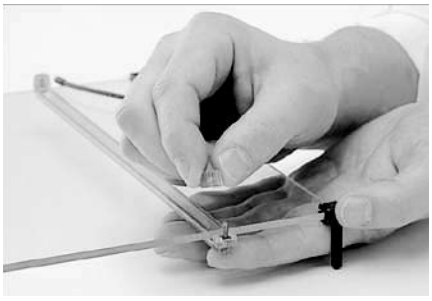
Screw one levelling foot into each corner of the buffer tank. Place the buffer tank on the lab bench where it will be used. Place the cooling plate on the unit, using the moulded guides to position it correctly. Fit the grommets into the cutouts in the back of the unit. Place a spirit level on the cooling plate and adjust the levelling feet until the unit is levelled.

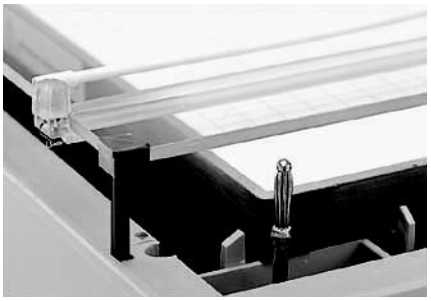




To mount the EPH/IEF electrodes on the electrode holder, unscrew the clamping nut from each electrode.

When running electrophoresis across the width of the cooling plate, mount the electrodes as illustrated. Place the electrode under the electrode holder. Replace the nut and lightly tighten until the electrode is held firmly in place. To align the electrodes with the electrode strips, place the electrode holder with the electrodes onto the false holes on the buffer tank using the four corner feet.





When running electrophoresis along the length of the cooling plate, mount the electrodes as illustrated. Place the electrodes under the electrode holder.

Replace the nut and lightly tighten until the electrode is held firmly in place. To align the electrodes with the electrode strips, place the electrode holder with the electrodes onto the false holes on the buffer tank using the four corner feet.

Install the safety lid.

# 5 Operation

**WARNING!** When using hazardous chemicals, take all suitable protective measures, such as wearing protective glasses and gloves resistant to the chemicals used. Follow local regulations and instructions for safe operation and maintenance of the system.

This section, together with the information supplied with the precast gels, gives all the necessary information to run most analytical electrophoresis techniques using our precast gels. Running procedures for electrophoretic transfer are also included.

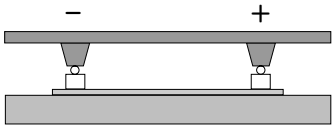
For laboratory cast gels, use the running conditions recommended in Electrophoresis in Practice, A Guide to Theory and Practice by Reiner Westermeier.

Multiphor II contains two alternative electrode configurations.

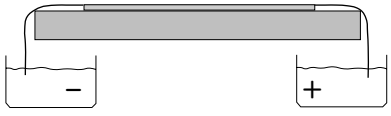
– EPH/IEF electrodes for use with buffer strips or electrode strips

– EPH electrodes for use with electrode wicks and buffer chambers. This metod is used for SDS-PAGE and native PAGE.

IEF, SDS-PAGE and native PAGE are most conveniently performed using EPH/IEF electrodes and buffer strips. The strips are applied on the gel edges with the electrodes on top.




ExcelGel




Electrode wicks

The buffer chambers are located below the cooling plate with the electrodes immersed in buffer solution. Paper wicks connect the buffer solution with the gel.

This method is used for SDS-PAGE and native PAGE.

The optional card-mounted EPH electrodes (18-1122-19 and 18-1122-20) – for electrophoresis using buffer chambers across the width of the cooling plate – are moulded from polypropylene and support the platinum wire. The anode cable (red) and cathode cable (black) carry female pin connectors for attachment to the male pins at the front of the buffer tank.

## *5.1 Electrophoresis using ExcelGel SDS and buffer strips*

This section describes the running procedure for SDS PAGE using buffer strips. The running of ExcelGel SDS, gradient 8-18 using ExcelGel SDS buffer strips is chosen as an example, but the basic method is applicable to all SDS PAGE and native PAGE gels.

ExcelGel SDS, gradient 8-18, is a 0.5 mm-thin, precast polyacrylamide gel for horizontal electrophoresis of SDS denatured proteins. To facilitate handling, the gel is cast on a plastic support. During the run, the precast SDS buffer strips supply the gel with buffer ions. For further information, see the information supplied with ExcelGel SDS gels.

### Sample preparation

Dissolve the samples in sample buffer B (for recipes, see Section 4.9 Stock solutions). Then heat the sample solution at 95 °C for 3 minutes. The sensitivity of your development technique and the volume of sample applied to the gel will determine the lower limit of your sample concentration. Generally, the sample must contain 200 to 500 ng of each component for Coomassie staining, and at least 10-25 ng of each component for silver staining. For molecular weight determination, we recommend the use of molecular weight calibration kits LMW and HMW/SDS.

### Sample application

In horizontal electrophoresis there are three methods of applying the sample: application strips, paper pieces and sample wells. Sample application strips are put on the gel surface, forming sample slots. Silicone rubber sample application strips are specially designed for easy sample application.

The following application strips are available:

SDS application strips for up to 40 µl of sample in 26 slots. IEF/SDS application strips for up to 20 µl of sample in 52 slots.

Immobiline applicator strip for up to 5 µl of sample in 52 slots. Immobiline applicator strip is designed to counteract lateral band spreading.

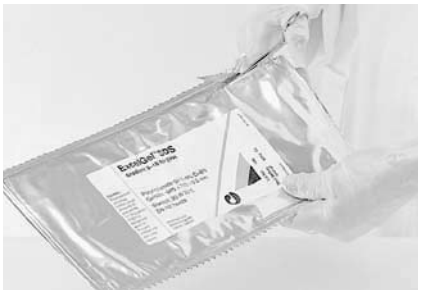
Sample application pieces hold approximately 20 µl of sample. For smaller volumes, cut the paper pieces to an appropriate size. At least 24 application pieces size 5 x 10 mm and 50 application pieces size 2.5 x 5 mm can be placed on one gel. Apply the sample about 1 cm away from the cathodic buffer strip and 1 cm away from each short side of the gel. For the best results, remove the application pieces 15 min after electrophoresis has started.

ExcelGel SDS, ExcelGel Homogeneous 7.5, 12.5, 15 and CleanGel are available with various numbers of sample wells for various volumes. Samples are applied directly into the wells immediately prior to the run.

### Electrophoresis

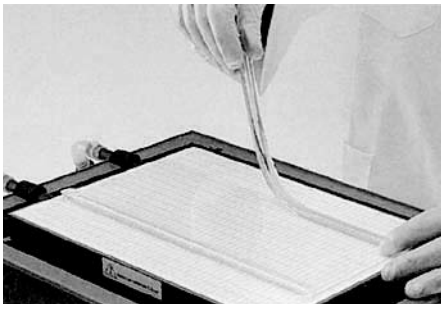
Connect Multiphor II to MultiTemp thermostatic circulator. Switch on MultiTemp 15 minutes before starting the experiment and set the temperature to 15 °C.

Always wear clean gloves when working with polyacrylamide gels and buffer strips, particularly when using sensitive staining methods.



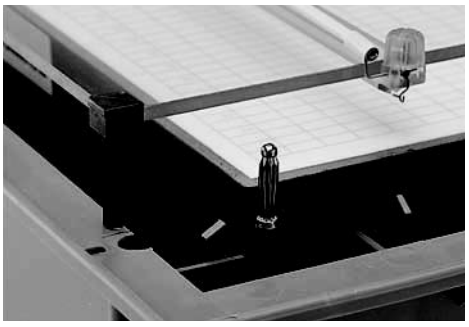
Remove one ExcelGel SDS from the package. Pipette 1 ml of insulating fluid (kerosene or light paraffin oil) onto the cooling plate. Place the gel with the stiff plastic film facing down in the middle of the cooling plate, making sure no air bubbles are trapped under the gel. Position the gel so that the polarity of the gel corresponds to that of the plate. Use the screened template on the cooling plate to centre the gel. Remove any excess solution with paper tissue. Remove the protective cover film from the gel.

Open the ExcelGel SDS strip packages and apply the cathodic and the anodic SDS buffer strips on the respective sides of the gel.



***Note:*** *The narrowest side of the buffer strip should be placed on the gel surface.*

Choose an appropriate sample application method and apply the sample.

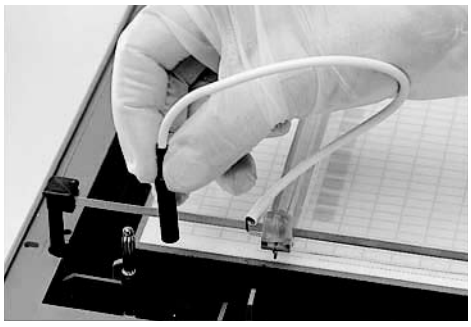


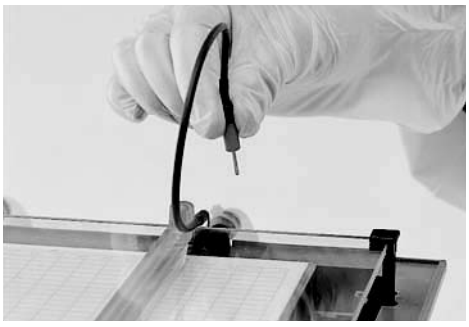
Place the electrode holder on the electrophoresis unit in the shallow depressions.

Align the EPH/IEF electrodes with the centre of the buffer strips by loosening the clamping nuts and sliding the electrodes to the appropriate position. Retighten the clamping nuts. Lift the electrode holder slightly and reposition the supporting feet over the deep holes. Lower carefully, so that the electrodes rest on the buffer strips. Connect the electrodes to the buffer tank.

During electrophoresis, the socket on the bridging cable MUST be attached to the pin connector at the front of the buffer tank as shown in the picture.

Connect the two electrodes to the buffer tank using the spring-loaded cables on the electrodes.





Connect the socket of the cathode electrode to the pin at the front of the unit and the anode pin to one of the sockets at the back.

Place the safety lid in position by matching the extensions on the back of the lid with the openings on the base unit. Using the extensions at the back as a hinge, connect the male and female banana plugs by pressing down firmly on the front of the lid.

Connect Multiphor II to the power supply. Follow the recommended electrical settings and running times given in the instructions supplied with the precast gel.

**Running conditions**

| | Voltage (V) | Current (mA) | Power (W) | Time (min) |
|---|---|---|---|---|
| Run | 600 | 50 | 30 | 75* |

* Approximate time, or until the Bromophenol Blue front reaches the anode buffer strip.

When the Bromophenol Blue front has reached the anodic buffer strip, electrophoresis is complete and should be stopped.

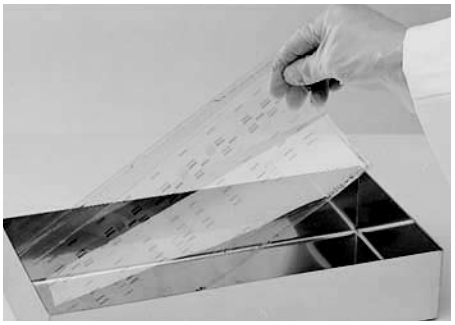
## Ending the run

Turn off the power supply. Disconnect the Multiphor II unit from the power supply. Remove the safety lid from the unit. Carefully remove the electrode holder. Gently pull the strips from the gel and continue with detection techniques as required.

**WARNING!** Always TURN OFF the power supply before opening the safety lid.

## Detection

*Coomassie staining*
On completion of the electrophoresis, immediately immerse the gel in Staining Tray 1 using the solutions and times indicated in table below.



The staining and destaining steps should be carried out on a shaking table. (See section 4.9 for stock solutions). Each step requires 250 ml of solution.

| Step No | Solution | Time (min) | Temp °C |
|---|---|---|---|
| 1 | Fixing solution C | 20 | 23 |
| 2 | Destaining solution I | 2 | 23 |
| 3 | Staining solution K | 10 | 60 |
| 4 | Destaining solution I | 20 | 23 |
| 5 | Destaining solution I | 30 | 23 |
| 6 | Preserving solution L | 10 | 23 |

The staining solution should be heated to 60 °C and poured over the gel. No further heating is necessary. Destain the gel using several changes of destaining solution (I) until the background is clear. Change the solution frequently (particularly at the beginning) in order to speed up the destaining. To preserve the gel, soak a cellophane sheet in preserving solution (L). Place it on the gel surface. Remove any air bubbles and wrap the excess cellophane around the glass plate. An additional glass plate may be placed underneath during drying to stop the cellophane from shifting or wrinkling. Leave the gel at room temperature until it is completely dry.

### Silver staining

Silver staining is performed essentially as described by. J. Heukeshoven and R. Dernick, Electrophoreses Forum 1986, 22-27. On completion of the electrophoresis, immediately immerse the gel in Staining Tray 1 using the solutions and times indicated in the table below. All steps should be carried out at room temperature in daylight, while gently shaking the solution. Use 250 ml of solution for each step. (See section 4.9 for stock solutions).

Time schedule for silver staining

| Step No. | Solution | Time (min) |
|---|---|---|
| 1 | Fixing solution C | 30 |
| 2 | Incubation solution D | 30 |
| 3 | Distilled water | 3 x 5 |
| 4 | Silver solution E | 40 |
| 5 | Developing solution F | 5–15 * |
| 6 | Stop solution G | 10 |
| 7 | Distilled water | 3 x 5 |
| 8 | Preserving solution L | 20 |

* Short development times will give a lightly stained gel. Long development times will give a dark gel.

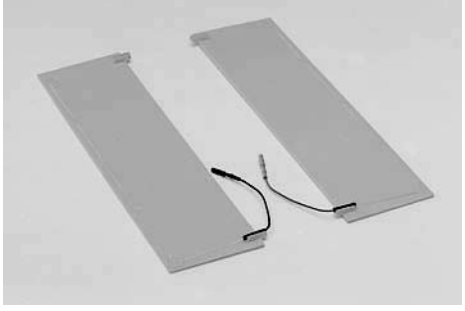
To preserve the gel, soak a cellophane sheet in preserving solution (L) and lay it on the gel surface. Remove any air bubbles and wrap the excess around the glass plate. An additional glass plate may be placed underneath during drying to stop the cellophane from shifting or wrinkling. Leave the gel at room temperature until it is completely dry.

## *5.2 Electrophoresis using buffer chambers*

This section describes the running procedure when using buffer chambers. To place the electrodes in the buffer chamber, remove the cooling plate from the buffer tank.

To reduce the effect of electrolysis products during the electrophoresis, the electrodes should be positioned as far as possible from the wicks.

Therefore, when performing electrophoresis across the large cooling plate, the electrodes should be placed in the grooves in the wall closest to the centre of the unit. The wicks lie at the outer edge of the buffer chamber. Place the cathode electrophoresis electrode in the left buffer chamber and the anode in the right buffer chamber.



Fill each chamber to the moulded line (indicating 1 liter volume) with buffer solution. (When running 120 x 250 mm gels, pour 1.2 liters of buffer into each chamber to ensure adequate buffer contact with the wicks.) Replace the cooling plate, making sure that the electrode socket connectors lie to the front and that the connecting cable is clear of the feet on the plate. Disconnect the anode bridging connector for isoelectric focusing and connect the electrodes to their respective pins.

**WARNING!** Make sure not to spill buffer outside the buffer chambers! Clean up any spillage immediately! There is a risk of electric shock and personal injury if there is spilled buffer in the areas where the electrical connectors are located**.**

When performing immunoelectrophoresis or agarose electrophoresis, center a small (84 x 94 mm) glass plate on the dry cooling plate and attach a strip of tape along the width of the cooling plate in alignment with the edges of the glass plate. These stop the small gels from shifting during application of the wicks and ensure that they are centred between the two buffer chambers during electrophoresis.

Switch on MultiTemp thermostatic circulator and set the desired temperature (normally 10 °C for PAGE or agarose electrophoresis and 15 °C for SDS-PAGE) 15 minutes before starting the experiment. To ensure efficient heat transfer from the gel during electrophoresis, a uniform layer of a non-charged insulating fluid is applied under the gel. To do this, pour a few milliliters of kerosene or light paraffin oil towards one end of the cooling plate.

Starting with one end of the gel support, gradually lower the gel to the horizontal position, constantly checking for trapped air bubbles. If air becomes trapped, raise the gel just enough to release the air and then continue to lower it onto the cooling plate. Use the template markings to centre the gel on the cooling plate. Remove any excess solution with a tissue. Repeat this procedure for each gel. If voltage probe measurements are required, the gel(s) must be positioned with the direction of the current path across the width of the cooling plate.

Prepare the electrode wicks by aligning 8-10 pieces of filter paper for each buffer chamber. Starting at one end, slowly immerse the electrode wicks in the buffer, using capillary action to reduce the amount of air trapped in the paper.

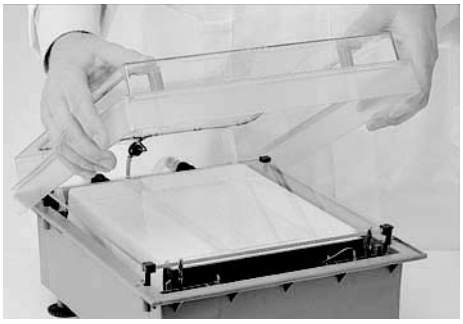
When running large (195 x 250 mm) or medium sized gels (120 x 250 mm), place the wicks so that the long edge overlaps the gel by 15 mm over the entire length. The template markings are useful for checking that the alignment is correct.

For running small gels (84x94 mm) on the large cooling plate, place the wicks with the short edge overlapping the gel by 15 mm. In this case, one set of wicks is required for each gel.

Apply the samples as required. If voltage probe measurements are not required, remove the isoelectric focusing electrodes from the electrode holder.

Position the empty electrode holder so it lies directly on the electrode wicks. This will ensure even contact between the wicks and the gel and stop any moisture from condensing on the gel surface.

**WARNING!** Make sure not to spill buffer outside the buffer chambers! Clean up any spillage immediately! There is a risk of electric shock and personal injury if there is spilled buffer in the areas where the electrical connectors are located



Replace the safety lid on the unit and connect the Multiphor II unit to the power supply. Set the power requirements, and start the experiment.

Typical power settings for agarose immunoelectrophoresis using buffer chambers are: constant voltage 20 V/cm and current and power set to maximum. Run time is 40-60 minutes. Bromophenol Blue is used as a tracking dye.

Typical power settings for SDS PAGE electrophoresis using buffer chambers.

| Separation distance (cm) | Voltage (V) | Current (mA) | Power (W) | Time (min | Temp (°C) |
|---|---|---|---|---|---|
| 8 | 600 | 50 | 30 | 100 | 15 |
| 16 | 1200 | 50 | 30 | 165 | 15 |

### Ending the run

Turn off the power supply. Disconnect the Multiphor II unit from the power supply. Take off the safety lid from the unit. Carefully remove the electrode holder. Gently pull the wicks from the surface of the gel and continue with detection techniques as required.

**WARNING!** Always TURN OFF the power supply before opening the safety lid. Although user safety is not endangered, arcing may damage the contacts.

## *5.3 Isoelectric focusing using CleanGel IEF*

### Prepare cooling and add the gel

1 Connect the Multiphor II electrophoresis unit to MultiTemp™ II thermostatic circulator and set the temperature to 10°C (15°C for IEF with urea).

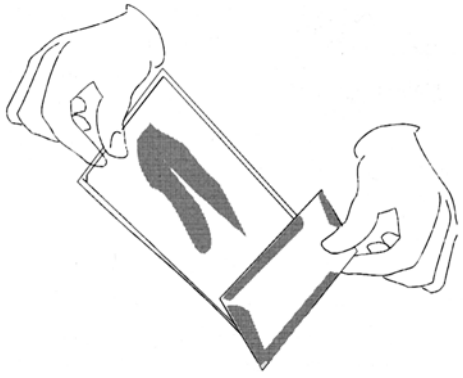
2 Switch on the thermostatic circulator 15 minutes before starting the analysis. Isoelectric focusing has to be performed at a defined constant temperature, as the pH gradient and the isoelectric points are dependent on the temperature.

3 Position the gel on the cooling plate.

***Note:*** *Wear clean gloves to avoid contamination of the gel surface, particularly when using sensitive silver stain.*

4 Pipette about 2 ml of insulating fluid (kerosene or light paraffin oil) onto the cooling plate of the Multiphor II.

5 Remove the gel from GelPool and dry the gel surface with the edge of a filter paper



Drying the gel surface with the edge of a filter paper.

***Note:*** *The gel surface should be absolutely dry, otherwise the gel will extrude water during the isoelectric focusing.*

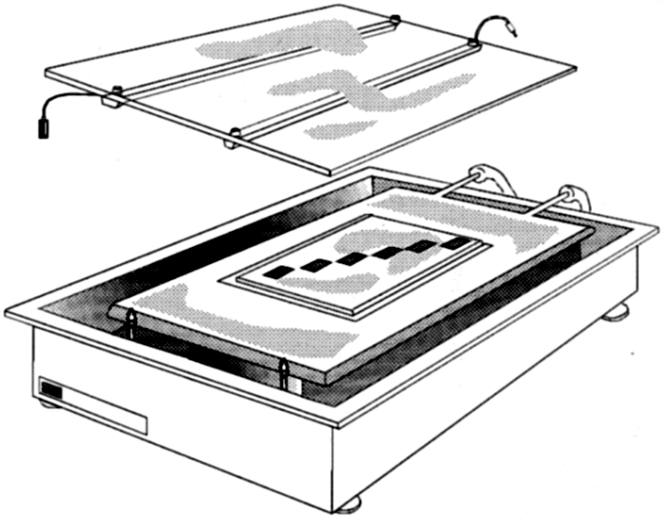
6 Position the gel in the centre of the cooling plate, use the screen print as a guide. No air bubbles should be trapped beneath the gel.

### Prefocusing

1 Clean the platinum electrode wires, before and after each electrophoresis run, with a wet tissue paper.

2 Place the electrode holder with the IEF electrodes on the electrophoresis unit and align the electrodes so that they rest on the outer edges of the gel. Electrode wicks between the gel and the electrodes are not necessary.

3 Connect the cables of the electrodes to the unit, see illustration below.



CleanGel IEF (half a gel) placed on the Multiphor II. Samples applied at various positions across the gradient.

4 Place the safety lid in position. Connect the power supply. Follow the recommended electrical settings and running conditions given in the table in Section "Running conditions"

5 Prefocus for 20 minutes without samples in order to establish the pH gradient in the gel.

### Sample application

***Note:*** *If water is excluded from the gel during the prefocusing procedure it should be removed, See illustration in previous Section "Prepare cooling and add the gel"***.**

In IEF there is, for most samples, one optimal position within the pH gradient for sample application. This should be found by applying the samples at various positions across the gradient, using sample application pieces (See illustration above).

There are three different methods for sample application. The optimal method is determined primarily by the sample and the volume to be applied.

- *IEF/SDS applicator strip (Code No. 18-1002-26)*
  Up to 52 samples can be applied with a sample volume of 5 to 20 µl in each well. The applicator strip is made of silicon and is applied directly on the gel surface
- *Sample application pieces (Code No. 80-1129-46)*
  Recommended sample volumes 15 to 20 µl, (or for smaller volumes cut the paper). The sample application pieces are made of Paratex. These sample application pieces are valuable when the samples are to be applied at different positions on the gel.
- Very small sample volumes (e.g. 2 µl) can be applied as droplets directly onto the gel surface.

When the protein concentration is between 1 to 3 mg/ml apply 10 µl /sample, by using an applicator strip or sample application pieces.

The pH gradient can be determined by using pI markers. Dissolve the Broad pI Kit pH 3 to 10 (Code No. 17-0471-01) according to the instructions supplied and apply 10 µl at two different places on the gel. For determination of the calibration curve please consult the instructions to the pI Kit.

Place the electrode and safety lid in position (Fig 6) and start the isoelectric focusing according to Table below.

### Running conditions

Recommended running conditions for one CleanGel IEF pH gradient 3 to 10.

| | Voltage (V) | Current (mA) | Power (W) | Time (min) | |
|---|---|---|---|---|---|
| **Prefocusing** | 700 | 12 | 8 | 20 | After this step, pause, again remove excess fluid and place sample application pieces onto the gel, and then apply sample onto those pieces. |
| **Sample entrance** | 500 | 8 | 8 | 20 | After this step, remove the sample application pieces. If excess fluid is observed along the cathode electrode, gently wipe it away again at this area avoiding the area where samples were applied. |
| **Isoelectric focusing** | 2000 | 14 | 14 | 90 | |
| **Band sharpening** | 2500 | 14 | 18 | 10 | For the Low pI Kit his step should omitted due to the risk of electrical burns. |

***Note:*** *If only half of a gel is used, remember to divide the current and power settings by two*

**WARNING!** For voltage above 1750 V ⎓ (dc), use a power supply that have floating output with reference to ground (± 1750 V). EPS 3501 and EPS 3501 XL are fulfilling this.

***Narrow gradients***

When running narrow pH gradients e.g. pH 5 to 7, use the same electrical settings, but prolong the isoelectric focusing time to 4 hours.

**WARNING!** Always turn OFF the power supply before opening the safety lid

**Detection**

All current detection methods used for isoelectric focusing can be used with CleanGel IEF. In case of background staining, try a prolonged washing step after fixation by 2 hours, or if needed over night, to eliminate this problem.

## *5.4 2-D electrophoresis using Immobiline DryStrip and ExcelGel SDS*

This chapter gives a brief description of the 2-D (two dimensional) electrophoresis method using Immobiline DryStrip and ExcelGel. For more detailed information on running conditions, please refer to the instructions supplied with Immobiline DryStrip Kit.

Isoelectric focusing using Immobiline DryStrip makes true isoelectric focusing possible and significantly improves the reproducibility of the spot distribution along the pH gradient axis of 2-D maps. Immobiline DryStrip also makes it possible to obtain distinct protein spots, even of basic proteins.

The Immobiline DryStrip Kit facilitates sample application, running and equilibration of Immobiline DryStrip for the first dimension of 2-D electrophoresis. The kit includes the accessories necessary to run up to 12 Immobiline DryStrip strips simultaneously on Multiphor II. Sample cup loading allows the application of up to 100 µl on each Immobiline DryStrip.

See application Note 80-1443-47 for unultiple miniformat 2-D lectrophoresis using ExcelGel 2-D, 12.5.

## *5.5 Electrophoretic transfer Introduction*

The method of horizontal semi-dry electrophoretic transfer gives fast, even and efficient transfer of proteins from a gel to an immobilizing membrane. The resulting membranes may be used for a wide range of applications including general protein staining, identification of specific antigens or antibodies (immunoblotting) and glycoprotein detection. By using different electrode solutions and running conditions, it is possible to transfer proteins from SDS PAGE, native PAGE, agarose gels and isoelectric focusing gels.

The speed and efficiency of the electrophoretic transfer using NovaBlot system is dependant on:

- Characteristics of the immobilizing membrane
- Characteristics of the transfer buffer
- Molecular weight and charge of the protein
- Gel thickness and concentration of acrylamide and bisacrylamide
- Voltage, current and transfer time

The semi-dry transfer technique uses filter papers soaked in buffer as the only buffer reservoir. Both discontinuous and continuous buffer systems can be used in the filter paper layers. Methanol in the buffer solution prevents swelling of polyacrylamide gels. However, it may have the disadvantage of denaturing or fixing the proteins in the gel, resulting in a lower transfer efficiency. Conversely, methanol may increase the protein binding capacity of the nitrocellulose membrane by strengthening the hydrophobic interactions. The transfer speed and efficiency can also be increased by increasing the protein charge, i.e. adding 0.05% SDS in the transfer buffer.

The transfer is normally finished in about one hour. If it is necessary to transfer for more than 1 hour due to unusual sample characteristics, rewetting of the cathode filter paper is recommended. No cooling is necessary since negligible heat is produced during the transfer.

### Immobilizing membranes

The immobilizing membrane is an important factor affecting the efficiency of the electrophoretic transfer. The most important requirements for an immobilizing membrane are:

- High binding capacity for the molecules of interest
- Preservation of the biologic activity of the molecules of interest
- No interference with subsequent detection methods
- Chemical and mechanical stability to assay conditions
- Provision of an accurate reflection of the original separation

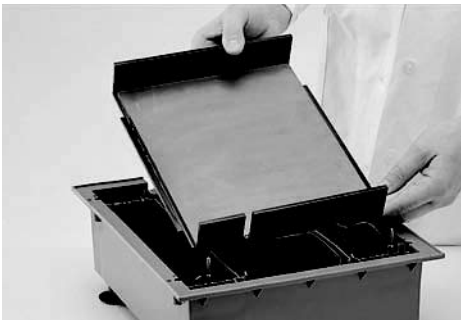
Nitrocellulose membranes are the standard medium for electrophoretic transfer of proteins and nucleic acids. This is due to their high binding capacity, versatility and easy use. Nitrocellulose membranes are available in various pore sizes, 0.45 µm is most commonly used, however low molecular proteins may be lost. By using pore sizes of 0.2 or 0.1 µm, most proteins are retained.

Nitrocellulose membranes can be probed several times. The membranes require no activation and the functional groups have an extended lifetime. Protein patterns on nitrocellulose membranes can be easily detected with most conventional stains, as well as by autoradiographic, immunoenzymatic and fluorescent methods.

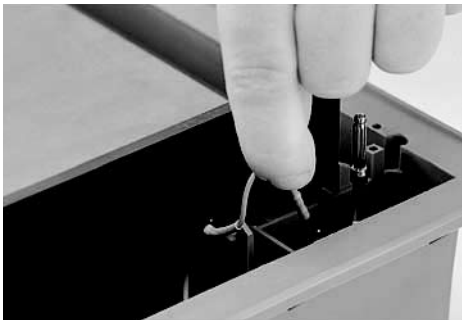
Other membranes are: nylon-based membranes and polyvinyldifluoride (PVDF) membranes.

### Transferring proteins from SDS polyacrylamide gels to nitrocellulose membrane.

The support (or backing) film must be removed from all polyacrylamide and agarose gels before electrophoretic transfer. Using FilmRemover, the film is removed quickly and cleanly. Instructions for use are supplied with FilmRemover.



1. Saturate the graphite anode plate with distilled water and remove the excess water with absorbent paper. With the electrode lead to the front of the instrument, fit the lower anode plate onto the buffer tank.

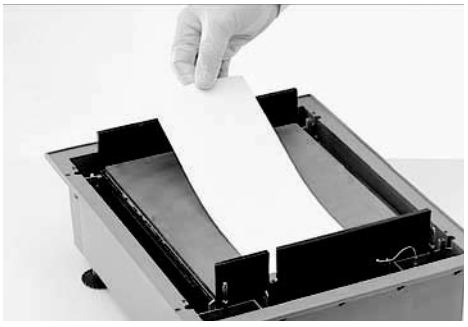


Connect the anode socket (red lead) to the anode pin on the right side of the buffer tank. The transfer sandwich can now be assembled on the anode electrode.

***Note:*** *When assembling the transfer sandwich in NovaBlot unit, always wear gloves.*

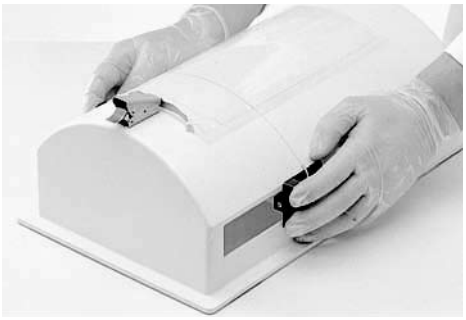
2. To ensure that the current passes only through the gel, cut the filter papers and the immobilizing and dialysis membranes to the same size as the gel to be transferred.

   When using a discontinuous buffer system, carefully soak the first layer of six filter papers in anode solution R (see Section 4.9 Stock solutions) by slowly immersing the papers under the surface of the electrode solution. Allow them to become wet by capillary action and avoid trapping air bubbles that may interfere with the transfer.



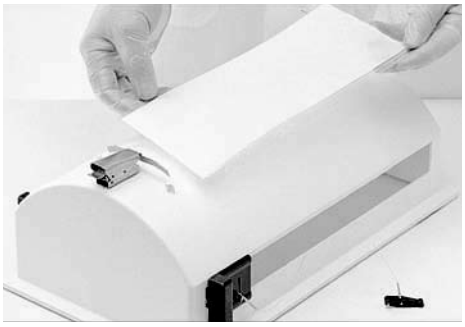
3. Carefully place the filter papers onto the anode electrode. When forming the first transfer sandwich, soak a further layer of three filter papers in anode solution S (see Section 4.9, Stock solutions), using the same method as above. Place them on top of the six filter papers on the anode electrode plate, again taking care to avoid trapping air bubbles. When using a continuous buffer system, all filter papers, cathodic and anodic are wetted in the same solution.

***Note:*** *To obtain optimal transfer of molecules from the gel, care should be taken to avoid trapping air bubbles at all stages of the assembly of the transfer sandwiches.*

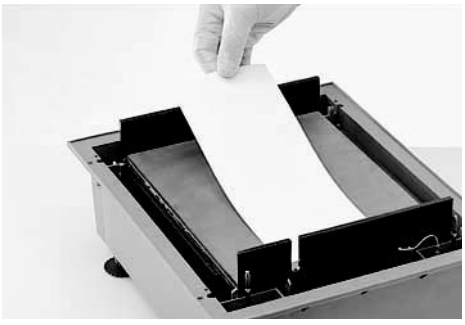


4. Cut the gel loose from the support film using FilmRemover. Do not move the gel, leave it on FilmRemover. Wet the immobilizing membrane in electrode solution S and carefully place it on top of the gel on the FilmRemover.

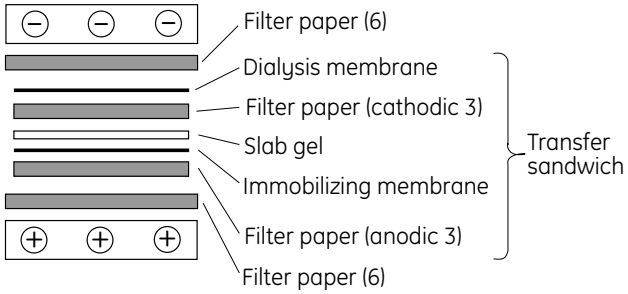
***Note:*** *Wear gloves to avoid contamination of the membrane.*



5. Loosen the support film from FilmRemover by pressing the handle and carefully lift the whole sandwich with the support film, immobilizing membrane and gel. Turn it over (support film up, immobilizing membrane down) and place it on the layer of three filter papers on the anode. Carefully remove the support film. If air bubbles become trapped under the gel, wet the surface of the gel with a few drops of electrode solution, and gently push out the bubbles.



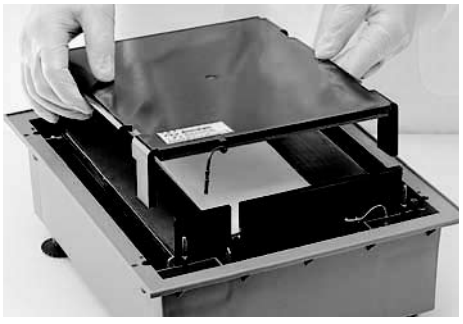
6. Immerse nine filter papers in cathode solution T. Place these filter papers on top of the gel to complete the transfer sandwich.



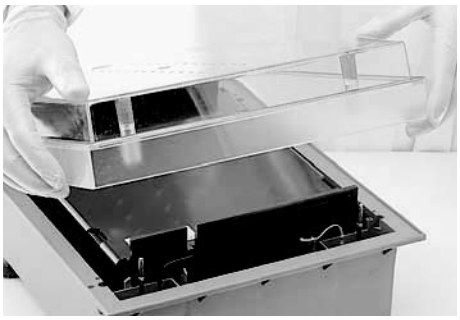

7. Several gels of the same type and size can be transferred simultaneously. Two transfer sandwiches can be put on top of each other. The cellophane dialysis membrane placed between each transfer sandwich prevents crosscontamination between transfer sandwiches.

   The maximum gel size is 200 x 250 mm. If small gels (125 x 250 mm) are to be transferred, NovaBlot will accept up to four gels for simultaneous transfer by assembling two transfer sandwich stacks side by side.

To ensure that the current passes through the gel, all components of the transfer sandwich are cut to the same size as the gels to be transferred.



8. Saturate the cathode electrode plate with distilled water and remove any excess with absorbent paper. Place the cathode on top of the transfer sandwich and connect the socket on the black cathode lead to the cathode pin in the Multiphor II base.



9. Close the Multiphor II safety lid and connect the unit to the power supply. It is recommended to run the transfer at a constant current of 0.8 mA/cm$^2$. A transfer time of approximately 1 hour is normal.

***Note:*** *The current is calculated using the surface area (total length x width) of the transfer sandwiches, and this calculation applies irrespective of the number of transfer sandwiches in the stack.*

***Note:*** *For transfer times longer than one hour turn off the power supply, remove the safety lid and carefully lift the cathode (top) electrode without disturbing the filter papers or gel. Carefully pour on additional transfer buffer to re-wetting the filter paper.*

10. When the transfer is complete, turn off the power supply and disconnect NovaBlot from the power supply. Remove the safety lid and the upper cathode electrode. Carefully disassemble the transfer sandwiches and remove the immobilizing membranes for analysis. If necessary, save and stain the gel to monitor the transfer efficiency.

Clean the electrodes with distilled water.

**WARNING!** Always turn off the power supply before opening the safety lid. Although user safety is not endangered, arcing may damage the contacts.

*Detection methods*

Following electrophoretic transfer, the membrane can be stored, stained or probed immediately.

**Further reading**

Electrophoresis in Practice: A guide to theory and practice. Westermeier, R., Ed., (1993) *VCH Verlagsgesellschaft mbH.* Weinheim. Westermeier, R.

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Handbook of immunoblotting of proteins. ed. Bjerrum, O. J. & Heegaard, N. H. H., CRC Press, Florida, USA, Volume 1 Technical descriptions, Volume II experimental and clinical applications.

Hancock, K. and Tsang, V.S.W., *Anal. Biochem. 133, 157-162 (1983)*

Towbin, H., Staehlin, T., Gordon, J., *Proc. Natl. Acad. Sci. USA, 76, 4350- 4354 (1979)*

## *5.6 Stock solutions*

B. Sample buffer
0.050 mol/l Tris-HAc pH 7.5
Dissolve 0.3 g Tris in 40 ml distilled water. Carefully adjust to pH 7.5 with HAc (approximately 0.14 ml). Make up to 50 ml with distilled water. Add 0.4 g SDS and a few grains of Bromophenol Blue. Immediately before use add 40 mg of DTT.

| C. Fixing solution | |
|---|---|
| Ethanol | 400 ml |
| Acetic acid, HAc | 100 ml |
| Make up to 1000 ml with distilled water. | |

| D. Incubation solution | |
|---|---|
| Ethanol | 75 ml |
| Sodium acetate | 17.00 g |
| Glutaraldehyde (25% w/v) | 1.25 ml |
| Sodium thiosulphate, $Na_2S_2O_3 \times 5H_2O$ | 0.50 g |
| Make up to 250 ml with distilled water. | |

| E. Silver solution | |
|---|---|
| Silver nitrate | 0.25 g |
| Formaldehyde | 50 µl |
| Make up to 250 ml with distilled water. | |

| F. Developing solution | |
|---|---|
| Sodium carbonate | 6.25 g |
| Formaldehyde | 25 µl |
| Make up to 250 ml with distilled water. | |

| G. Stop solution | |
|---|---|
| EDTA-$Na_2 \times 2H_2O$ | 3.65 g |
| Make up to 250 ml with distilled water. | |

| H. Preserving solution | |
|---|---|
| Glycerol (87% w/w) | 25 ml |
| Make up to 250 ml with distilled water. | |

| | |
|---|---|
| I. Destaining solution | |
| Ethanol | 250 ml |
| Acetic acid | 80 ml |
| Make up to 1000 ml with distilled water. | |
| K. Coomassie solution | |
| PhastGel Blue R | 1 tablet |
| Make up to 400 ml with destaining solution. | |
| Heat to 60 °C, stirring constantly, and filter before use. | |
| L. Preserving solution | |
| Glycerol (87% w/w) | 25 ml |
| Make up to 250 ml with destaining solution. | |
| N. Fixing solution | |
| Trichloroacetic acid | 100 g |
| Make up to 500 ml with distilled water. | |
| Transfer buffers using a discontinuous buffer system | |
| R. Anode solution 1, pH 10.4 | |
| Tris | 36.3 g |
| Methanol | 200 ml |
| Make up to 1000 ml with distilled water. | |
| S. Anode solution 2, pH 10.4 | |
| Tris | 3.03 g |
| Methanol | 200 ml |
| Make up to 1000 ml with distilled water. | |
| T. Cathode solution, pH 7.6 | |
| 6-Amino-n-hexanoic acid | 5.20 g |
| Methanol | 200 ml |
| Make up to 1000 ml with distilled water | |
| U. Transfer buffer using a continuous buffer system | |
| Glycine | 2.93 g |
| Tris | 5.81 g |
| SDS | 0.375 g |
| Methanol | 200 ml |
| Make up to 1000 ml with distilled water. | |

***Note:*** *In a continuous buffer system, this solution is used for both anode and cathode electrode solutions.*

## *5.7 Running conditions for precast gels*

### ExcelGel SDS Gradient 8–18

Running conditions

| Phase | Voltage (V) | Current (mA) | Power (W) | Time (min) | Temp °C |
|---|---|---|---|---|---|
| Run | 600 | 50 | 30 | 75* | 15 |

* Or until the Bromophenol Blue front reaches the anode buffer strip.

### ExcelGel XL SDS Gradient 12-14

Running conditions

| Phase | Voltage (V) | Current (mA) | Power (W) | Time (min) | Temp °C |
|---|---|---|---|---|---|
| Run | 1000 | 40 | 40 | 165* | 15 |

* Or until the Bromophenol Blue front reaches the anode buffer strip.

### ExcelGel SDS Homogeneous 7.5, 12.5 and 15

Running conditions

| ExcelGel SDS Homogeneous | Voltage (V) | Current (mA) | Power (W) | Time (min) | Temp °C |
|---|---|---|---|---|---|
| 7.5 and 12.5 | 600 | 50 | 30 | 80* | 15 |
| 15 | 600 | 30 | 30 | 140* | 15 |

* Or until the Bromophenol Blue front reaches the anode buffer strip.

### CleanGel IEF 3–10

Running conditions for one CleanGel IEF 3–10.

| Phase | Voltage (V) | Current (mA) | Power (W) | Time (min) | Temp °C |
|---|---|---|---|---|---|
| Prefocusing | 700 | 12 | 8 | 20 | 10 |
| Sample entry | 500 | 8 | 8 | 20 | 10 |
| Isoelectric focusing | 2000 | 14 | 14 | 90 | 10 |
| Band sharpening | 2500 | 14 | 18 | 10 | 10 |

If half a gel is used, halve the current and power settings.

**WARNING!** For voltage above 1750 V ⎓ (dc), use a power supply that have floating output with reference to ground (± 1750 V). EPS 3501 and EPS 3501 XL are fulfilling this.

## 2-D electrophoresis using Immobiline DryStrip and ExcelGel SDS

### First dimension

Option 1: EPS 3501 XL Power Supply, using a voltage gradient.
The parameters below may be used for up to 12 strips.

Programme for Immobiline DryStrip, pH 3–10, 110 mm. All steps are run at 10 °C.

| Phase | Voltage (V) | Current (mA) | Power (W) | Time (h) | Vh |
|---|---|---|---|---|---|
| 1 | 300 | 1 | 5 | 0.1 | 1 |
| 2 | 300 | 1 | 5 | 4.5 | 1350 |
| 3 | 2000 | 1 | 5 | 5 | 5750 |
| 4 | 2000 | 1 | 5 | 6.5 | 13000 |
| Total | | | | 16 | 20100* |

Programme for Immobiline DryStrip, pH 3–10 L, 180 mm. All steps are run at 10 °C.

| Phase | Voltage (V) | Current (mA) | Power (W) | Time (h) | Vh |
|---|---|---|---|---|---|
| 1 | 500 | 1 | 5 | 0.1 | 1 |
| 2 | 500 | 1 | 5 | 3 | 1500 |
| 3 | 3500 | 1 | 5 | 5 | 10000 |
| 4 | 3500 | 1 | 5 | 12.5 | 43750 |
| Total | | | | 20.5 | 55250* |

**WARNING!** For voltage above 1750 V ⎓ (dc), use a power supply that have floating output with reference to ground (± 1750 V). EPS 3501 and EPS 3501 XL are fulfilling this.

Programme for Immobiline DryStrip, pH 3–10 NL, 180 mm. All steps are run at 10 °C.

| Phase | Voltage (V) | Current (mA) | Power (W) | Time (h) | Vh |
|---|---|---|---|---|---|
| 1 | 500 | 1 | 5 | 0.1 | 1 |
| 2 | 500 | 1 | 5 | 5 | 2500 |
| 3 | 3500 | 1 | 5 | 5 | 10000 |
| 4 | 3500 | 1 | 5 | 9.5 | 32400 |
| Total | | | | 19.5 | 44900* |

Programme for Immobiline DryStrip, pH 4–7, 110 mm. All steps are run at 10 °C.

| Phase | Voltage (V) | Current (mA) | Power (W) | Time (h) | Vh |
|---|---|---|---|---|---|
| 1 | 300 | 1 | 5 | 0.1 | 1 |
| 2 | 300 | 1 | 5 | 6 | 1800 |
| 3 | 3500 | 1 | 5 | 5 | 9500 |
| 4 | 3500 | 1 | 5 | 5.5 | 19250 |
| Total | | | | 16.5 | 30550* |

Programme for Immobiline DryStrip pH 4–7, 180 mm. All steps are run at 10 °C.

| Phase | Voltage (V) | Current (mA) | Power (W) | Time (h) | Vh |
|---|---|---|---|---|---|
| 1 | 500 | 1 | 5 | 0.1 | 1 |
| 2 | 500 | 1 | 5 | 1 | 500 |
| 3 | 3500 | 1 | 5 | 5 | 10000 |
| 4 | 3500 | 1 | 5 | 10 | 35000 |
| Total | | | | 16 | 45500* |

* The optimal total number of Volt-hours for these pH gradients depends on the type of sample, sample load (µg) and sample volume.

**WARNING!** For voltage above 1750 V ⎓ (dc), use a power supply that have floating output with reference to ground (± 1750 V). EPS 3501 and EPS 3501 XL are fulfilling this.

Option 2: Using a Manual Power Supply
The power supply should run at constant voltage with the parameters set as below. All steps are run at 10 °C.

Running conditions for Immobiline DryStrip, pH 3–10, 110 mm.

| Phase | Voltage (V) | Current (mA) | Power (W) | Time (h) | 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 (V) | Current (mA) | Power (W) | Time (h) | 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 (V) | Current (mA) | Power (W) | Time (h) | Vh |
|---|---|---|---|---|---|
| 1 | 500 | 1 | 5 | 1 | 500 |
| 2 | 3500 | 1 | 5 | 13 | 45000 |

Running conditions for Immobiline DryStrip, pH 4–7, 110 mm.

| Phase | Voltage (V) | Current (mA) | Power (W) | Time (h) | Vh |
|---|---|---|---|---|---|
| 1 | 300 | 1 | 5 | 1 | 300 |
| 2 | 2200 | 1 | 5 | 13.5 | 29700 |

Running conditions for Immobiline DryStrip, pH 4–7, 180 mm.

| Phase | Voltage (V) | Current (mA) | Power (W) | Time (h) | Vh |
|---|---|---|---|---|---|
| 1 | 500 | 1 | 5 | 1 | 500 |
| 2 | 3000 | 1 | 5 | 14.5 | 43500 |

**WARNING!** For voltage above 1750 V ⎓ (dc), use a power supply that have floating output with reference to ground (± 1750 V). EPS 3501 and EPS 3501 XL are fulfilling this

### Second dimension

Running conditions for ExcelGel XL SDS gradient 12–14.

| Step | Voltage (V) | Current (mA) | Power (W) | Time (min) | Temp. (° C) |
|---|---|---|---|---|---|
| 1 | 1000 | 20 | 40 | 45* | 15 |
| 2 | 1000 | 40 | 40 | 5** | 15 |
| 3 | 1000 | 40 | 40 | 160*** | 15 |

Running conditions for ExcelGel SDS gradient 8–18.

| Step | Voltage (V) | Current (mA) | Power (W) | Time (min) | Temp. (° C) |
|---|---|---|---|---|---|
| 1 | 600 | 20 | 30 | 25-30* | 15 |
| 2 | 600 | 50 | 30 | 3-5** | 15 |
| 3 | 600 | 50 | 30 | 70*** | 15 |

* When the Bromophenol Blue dye front has moved 4-6 mm for ExcelGel XL SDS gradient 12–14 and 1–2 mm for ExcelGel SDS, gradient 8–18 from Immobiline DryStrip, remove the strip and the application pieces.

** 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.

*** 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.

Further information about the gels and running conditions are supplied with the products.

Running conditions for ExcelGel 2-D Homogeneus 12.5

| Phase | Voltage (V) | Current (mA) | Power (W) | Duration (h:min) |
|---|---|---|---|---|
| 1 | 600 | 20 | 30 | ~0:35[1] |
| 2 | 600 | 50 | 30 | ~1:15[2] |

# 6 Maintenance

**WARNING!** Remove liquid or dirt from the system surface using a cloth and, if necessary, a mild cleaning agent.

**WARNING!** When using hazardous chemicals, take all suitable protective measures, such as wearing protective glasses and gloves resistant to the chemicals used. Follow local regulations and instructions for safe operation and maintenance of the system.

**WARNING!** When using hazardous chemicals, make sure that the entire system has been flushed thoroughly with bacteriostatic solution, e.g. NaOH, and distilled water before service and maintenance.

A few standard measures are necessary to keep Multiphor II in full functioning order.

After isoelectric focusing, remove the electrodes from the electrode holder and rinse with distilled water to remove the strong acidic and basic solutions. Do not submerge the cable containing the pin or socket. Air dry or carefully dry with paper tissue. Check that the platinum wire is not damaged.

After electrophoresis using the buffer chambers, remove the electrodes. Rinse them in distilled water and air dry. Take care not to damage the platinum electrodes.

Rinse the buffer chambers with distilled water between buffer changes and after use. Do not immerse the socket connector. Air dry or carefully dry with a paper towel.

Following electrophoretic transfer, remove all remaining filter papers from the NovaBlot unit. Remove the anode and cathode plates and rinse them in distilled water. Do not immerse the electrode leads in water. Leave to air dry. For longer life of NovaBlot electrodes store them either by:
1. Placing 3 cm thick plastic foam between the electrodes as if for transfer
or
2. Store the electrodes on “backs” without foam sandwiched between.

## *6.1 Recycling*

This symbol indicates that the waste of electrical and electronic equipment must not be disposed as unsorted municipal waste and must be collected separately. Please contact an authorized representative of the manufacturer for information concerning the decommissioning of equipment.

# 7 Technical specifications

| | |
|---|---|
| Maximum Voltage | 3500 V ⎓ (dc), ± 1750 V floating output with reference to Ground) |
| Maximum Power | 100 W |
| Max pressure cooling plate | 0.35 bar |
| Dimensions | 16 x 31 x 40 cm |
| Weight | 4 kg |
| Environment | 4ºC to 40ºC, 20% to 95% relative humidity |
| Material of wetted parts | |
| Chemical resistance | The wetted parts are resistant to solvents commonly used in electrophoresis and solutions containing inorganic and organic acids, alkalis and alcohols. |
| Compliance with standards | The declaration of conformity is valid for the instrument only if it is:<br>• used in laboratory locations<br>• used in the same state as it was delivered from GE Healthcare except for alterations described in the User Manual<br>• connected to other CE labelled GE Healthcare modules or other products as recommended. |
| Safety standards | This product meets the requirement of the Low Voltage Directive (LVD) 2006/95/EC through the following harmonized standards:<br>• EN 61010-1<br>• IEC 61010-1<br>• CAN/CSA-C22.2 No. 61010-1<br>• UL61010-1 |

# 8 Trouble shooting

**WARNING!** Always TURN OFF the Power Supply before opening the lid.

### Trouble shooting guide to PAGE

| Symptom | Cause | Remedy |
|---|---|---|
| No current reading | Safety plug improperly inserted in power supply outlet | Check the safety plug insertion |
| | Pin and socket connection from electrode to base incomplete | Check the pin and socket connections |
| | Anode bridging contact disconnected | Connect the bridging contact |
| | Banana plug connection in safety lid not completed | Press firmly on the safety lid |
| | Electrode holder not seated properly | Lower the electrode holder so that the electrodes are in contact with the electrode strips |
| | Poor contact between the electrodes and electrode strips | Check that the electrodes are clean and intact, and sit in the centre of the isoelectric focusing strips over the entire length |
| Uneven migration of the dye front | Bad electrical contact between the gel and the wicks and electrodes | Check the contact |
| | Poor cooling | Check the cooling |
| Burning at slots or accumulation of water in the slots | Polypeptide complexes are too big to enter the gel and cause electroendosmosis | If SDS PAGE, add DTT once again and boil the sample |

### Trouble shooting guide to IEF

| Symptom | Cause | Remedy |
|---|---|---|
| Current increases with time | Electrode strips applied incorrectly in relation to electrode polarity | Check the electrode polarity and the pH of the electrode strips |
| | Cathode and anode polarities reversed | Check the pin and socket connections, the gel orientation and the pH of the applied electrode strips. |

| Symptom | Cause | Remedy |
|---|---|---|
| Sparking on the gel | Gel dried out, insufficient cooling | Check the temperature and flow of the cooling fluid. Lower the power |
| Water droplets on gel | Excessive condensation | Decrease condensation by adjusting the temperature of the cooling fluid. Wipe the electrode holder periodically to remove condensation |
| Drying out of the gel near the electrodes | Incorrect electrode solutions | Use the recommended electrode solution at the specified concentration. |
| | Excessive power setting | Check the power setting |
| Sparking along edge of gel onto cooling plate | Excess moisture on gel or under cooling plate | Remove the excess moisture |
| | Electrode strips overhanging the ends of the gel | Cut the electrode strips short of the ends of the gel |
| | Liquid expelled at sides of electrode strips due to electroendosmotic of water towards the cathode | Occasionally remove the excess flow fluid by blotting |
| Condensation over the entire surface of the glass electrode holder | Excessive power setting | Check the power setting. When only a portion of the gel is used, reduce the power setting proportionally |
| | Insufficient cooling | Check the temperature and flow of the cooling fluid |
| Local condensation on the glass electrode holder | Local overheating due to a high salt concentration in the sample | Reduce the salt content of the sample by gel filtration using PD-10 columns pre-packed with Sephadex G-25 |
| | Incorrect electrode solutions in relation to electrode polarity | Check the electrode polarity. Check the pH of the applied electrode strips |
| | Localized hot spots due to air bubbles under the gel | Use insulating fluid under the gel and check for air bubbles |
| Excessive amount of condensation along electrode strips | Cathodic drift may cause an electroendosmotic flow of water towards the cathode. Thus, cathode strip may become over saturated | Cut the electrode strips shorter than the edge of the gel. If necessary, blot the pooled liquid. |
| | Reversed polarity of electrode strips (lower pH at cathode, higher pH at anode) | Check pH of the strips and polarity of the plugs in the power supply. Reverse polarity if the strips have been incorrectly applied (should be acid at anode) |

| Symptom | Cause | Remedy |
|---|---|---|
| Skewed or wavy bands | Localized gradient disturbances due to excessive salt | Reduce the salt content of the sample by gel filtration using PD-10 columns pre-packed with Sephadex G-25. Salt content should be <50 mmol/l. Too much ammonium persulphate may also cause wavy bands |
| | Unevenly wetted electrode strips | Electrode strips must be evenly wetted and be neither too wet nor too dry |
| | Electrode strips too short | The strips should be cut just short of the edges of the gel |

### Trouble shooting guide to electrophoretic transfer

| Symptom | Cause | Remedy |
|---|---|---|
| Incomplete transfer | Gel concentration too high | Use reversible cross-linkers (e.g. DATD, BAC, DHEBA) and depolymerize gel before transfer. Lower monomer concentration. Convert molecule to smaller form by limited digestion with proteases (for proteins) or with nucleases or acid hydrolysis (for nucleic acids) |
| | Methanol present in transfer buffer | Remove methanol from transfer buffer |
| | Transfer time too short | Increase transfer time |
| | Field strength too low | Increase field strength |
| | Too low charge/mass ratio | Change transfer buffer pH further away from molecules pI. Add 0.1% SDS |
| Poor transfer | Air trapped between gel and membrane | Carefully push out all air bubbles from the layers of the transfer sandwich |
| Inefficient transfer | Too low binding efficiency (molecules migrate from the gel, but pattern is faint) | Use different immobilizing membrane (DEAE, NC, DBM, DPT). Immobilizing membrane needs to be activated. Remove interfering substances (denaturants, detergents). Raise/lower salt concentration. Raise/lower pH |

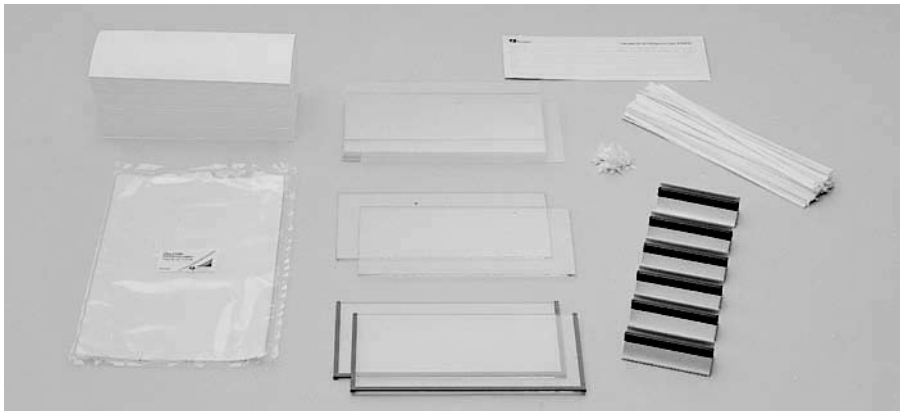
| Symptom | Cause | Remedy |
|---|---|---|
| | Field strength too high (e.g. low Mol. Wt. DNA) | Lower field strength |
| | Transfer time too long | Shorten transfer time |
| | Pore size too large | With nitrocellulose, use smaller pore filters (0.1 or 0.7 µm) |

# 9 Multiphor II application kits and accessories

This section describes the contents of the Multiphor II application kits and accessories and provides instructions for assembly and use. For experimental details including preparation of samples and stock solutions, running conditions, staining and preserving procedures see Chapter 4. Operation. Further information can be found in "Electrophoresis in Practice" – Code No. 18-1104-12.

## *9.1 SDS and Native PAGE, IEF Kit*

This kit is used for casting 0.5 mm homogeneous or gradient polyacrylamide gels. The gels are cast on a 1 mm thick glass plate (125 x 260 mm). Alternatively, casting can be done on GelBond™ PAGfilm (124 x 258 mm). Optional glass plates with U-frames allow casting of 1.0 and 2.0 mm thick gels. The kit includes sample application pieces and strips for applying the sample onto the gel surface. An optional template and tape allow preparation of a slot-former.



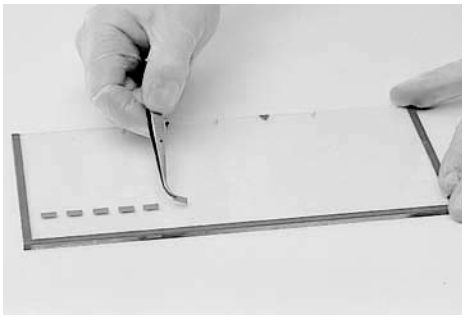
**Kit contents – Code No. 18-1102-45**

| Designation | Code No. |
|---|---|
| Glass Plate, 125 x 260, 0.5 mm U-frame (2/pkg) | 80-1106-89 |
| Glass Plate, 125 x 260x1 mm (15/pkg) | 80-1106-29 |
| Glass Plate, 125 x 260x3 mm (2/pkg) | 80-1106-99 |
| FlexiClamp (6/pkg) | 18-1013-73 |
| IEF Electrode Strip (100/pkg) | 18-1004-40 |
| Electrophoresis Wick 104 x 253 mm (500/pkg) | 80-1129-52 |
| IEF Sample Application. Pieces (200/pkg) | 80-1129-46 |
| IEF/SDS Sample Application. Strip, 52 samples 5–20 µl (5/pkg) | 18-1002-26 |
| Cellophane Sheets (50/pkg) | 80-1129-38 |

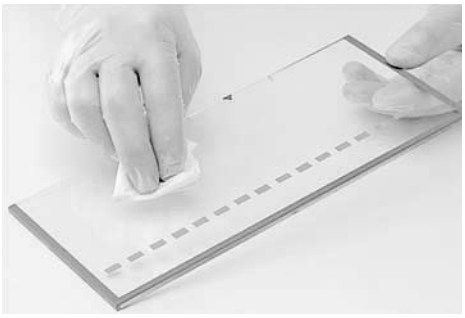
## Optional accessories

| Designation | Code No. |
|---|---|
| Roller | 80-1106-79 |
| GelBond PAGfilm, 124 x 258 mm (50/pkg) | 80-1129-36 |
| Bind-Silane, 100 ml | 17-1330-01 |
| Repel-Silane, 500 ml | 17-1332-01 |
| SDS Sample Application Strip, 26 samples, 40 µl | 18-1002-74 |
| Gradient Maker SG 100, 100 ml | 80-6196-09 |
| Glass Plate, 125 x 260 mm, 1,0 mm U-frame (2/pkg) | 80-1106-91 |
| Glass Plate, 125 x 260 mm, 2,0 mm U-frame (2/pkg) | 80-1106-92 |

The 3 mm thick glass plate is used as a support, either for the 1 mm glass plate or GelBond PAGfilm. The mould comprising the 3 mm glass plate, 1 mm glass plate or GelBond PAGfilm and glass plate with U-frame is clamped together using four FlexiClamps.



To prepare a slot-former for individual sample slots, a glass plate with Uframe, tape, 0,25 x 9 mm, and a template should be used. One or several layers of tape can be applied to the glass plate. For instance, 3 layers of 5 x 3 mm will make a sample slot for 10-20 µl of sample. Wash the glass plate with detergent, rinse with distilled water and dry with a paper tissue. Apply tape 30 mm from the open edge of the U-framed glass plate avoiding air bubbles. Check that all edges of the tape are cut perfectly even. Leave the slot former over night to ensure that the tape adheres completely.



To prevent the gel from sticking to the U-framed glass plate, coat the plate with Repel-Silane.

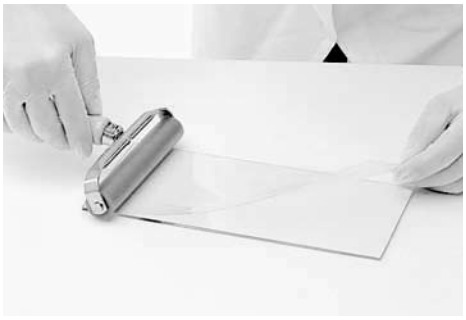
***Note:*** *For this operation use gloves and a fume hood.*

Pour about 2 ml of Repel-Silane onto the glass plate and distribute it evenly with a tissue. Leave it to dry for a few minutes. Rinse the glass plate with distilled water and remove water drops by shaking or wiping lightly with a tissue. Leave the glass plate to dry.

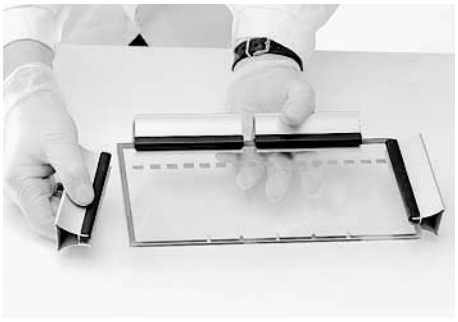
When using the 1 mm thick glass plate as the gel support, simply lay it directly on top of the 3 mm thick glass plate. If the gel is to be permanently bound to the 1 mm thick glass plate, coat the plate with Bind-Silane, before preparing the mould.

***Note:*** *For this operation use gloves and a fume hood.*

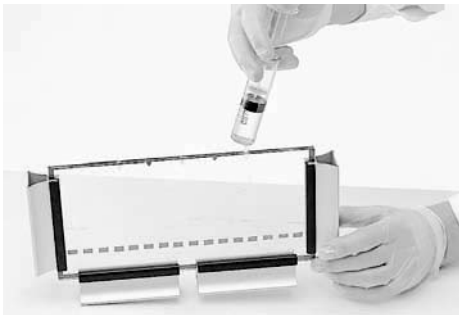
Pour about 2 ml of diluted Bind-Silane onto the glass plate and distribute it evenly with a tissue. Leave the glass plate to dry for a few minutes, rinse with distilled water and leave to dry.



When using GelBond PAGfilm, pour a few ml of water on to the 3 mm thick glass plate and lay the film over it with the hydrophilic side up (see Instructions supplied with the film). Centre the film on the glass plate. Beginning at one end, use the roller to apply even pressure over the film surface in order to eliminate air bubbles and seal the film to the plate with a minimum of water. Remove any excess water with a tissue.



Form the mould by placing the U-framed glass plate in position and clamp together using four FlexiClamps.



***Note:*** *Gloves must be worn to protect the user from contact with the toxic acrylamide solution.*

Draw the gel solution into a syringe or a graduated pipette. Fill the mould, checking that air bubbles are not trapped along the rubber U-frame or around the slots.

When casting a gradient gel, position the mould horizontally using the Levelling Set and place the Gradient Maker as illustrated. Lay the end of the tubing from the Gradient Maker against the 1 mm glass plate or GelBond PAGfilm. The slot-former will otherwise disturb the flow of the solution.

To open the 1 mm glass plate mould, remove the four FlexiClamps. Carefully insert one or two thin-bladed spatulas between the gel surface and slot former on one of the short sides. Twist gently in order to introduce air across the whole of the short side. Twist more firmly to slowly separate the U-frame from the gel surface. Remove the U-frame. Carefully remove any unpolymerized acrylamide from the edge of the gel with a paper tissue. Separate the gel support from the thick glass plate. The gel is now ready to use.

To open the mould including GelBond PAGfilm, remove the four FlexiClamps and insert the spatula between the 3 mm thick glass plate and film. Remove the glass plate and dry the back of the film. Turn the mould upside down (with the glass plate with U-frame on top) and gently peel the film with gel away from the glass.

## *9.2 Buffer Strip Positioner*

The Multiphor II Buffer Strip Positioner is a frame with slots thats sits on top of an ExcelGel SDS gel on the Multiphor II cooling plate. Theslots in the positioner facilitate placement of the buffer strips for electrophoresis and hold them securely in place. A locking cam secures the positioner on the cooling plate.

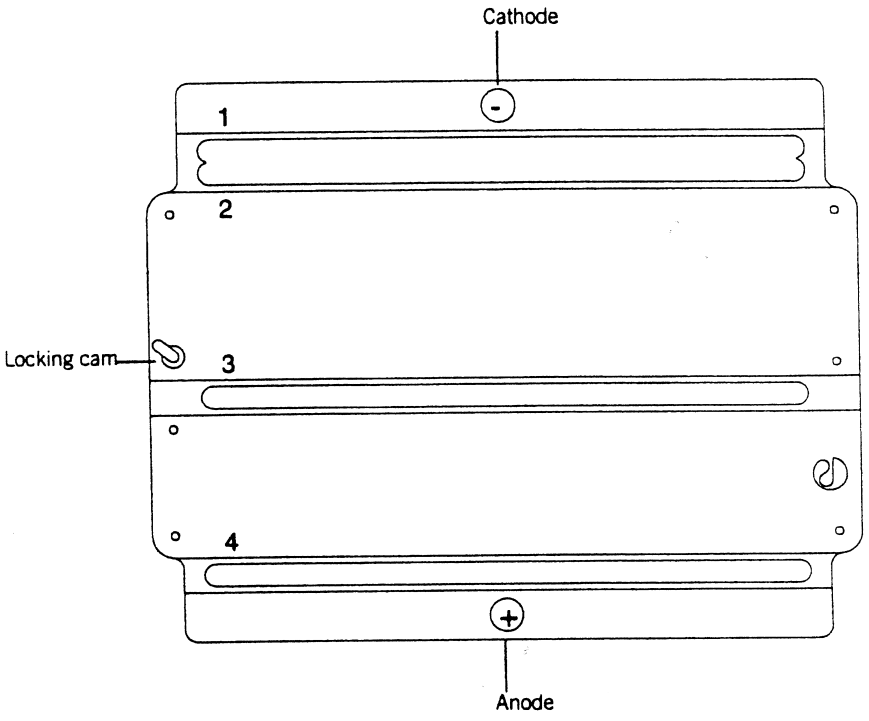



**Fig. 1.** Features of the Multiphor II Buffer Strip Positioner

| Slot# | Use for placing |
|---|---|
| 1 | Cathodic buffer strip, Phase 1 or entire ruun |
| 2 | Sample wells<br>Immobiline DryStrip gels (IPG strips), Phase 1<br>Cathodic buffer strip, Phase 2 |
| 3 | Anodic buffer strip (with 11 x 25 cm ExcelGel SDS gels) |
| 4 | Anodic buffer strip (with 18 x 25 cm ExcelGel SDS gels |

| Designation | Code No. |
|---|---|
| Multiphor II Buffer Strip Positioner | 80-6442-90 |

## *9.3 Immobiline DryStrip Kit*

This kit is used for running IEF with Immobiline DryStrip for the first dimension in 2-D electrophoresis. Twelve strips can be focused simultaneously under a protective layer of silicone oil. The high sample capacity allows the application of up to 100 µl on each Immobiline DryStrip. Detailed instructions for use are available in the instruction manual provided with this kit.

**Kit contents - Code No. 18-1004-30**

| Designation | Code No. |
|---|---|
| Tray and Electrode Holder | 18-1004-31 |
| DryStrip Aligner (4/pkg) | 18-1004-34 |
| DryStrip Kit Electrode, cathode | 18-1018-67 |
| DryStrip Kit Electrode, anode | 18-1018-66 |
| Sample Cup Bar | 18-1004-33 |
| Sample Cup (6 x 10/pkg) | 18-1004-35 |
| IEF Electrode Strip (100/pkg | 18-1004-40 |
| IEF Sample Application. Piece (200/pkg) | 80-1129-46 |
| Instruction Manual | 18-1038-63 |

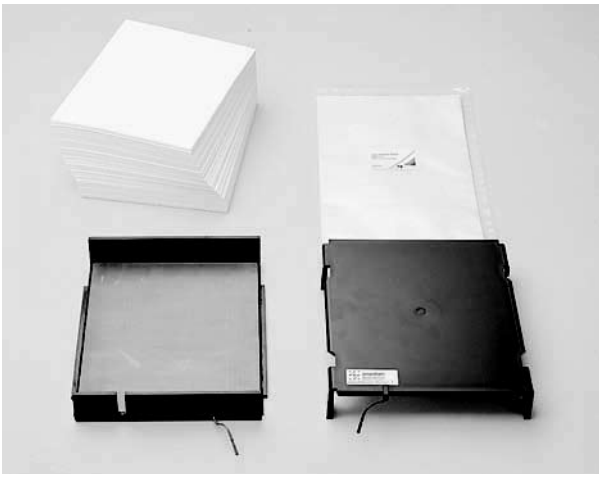
## *9.4 NovaBlot Kit*

This kit is used for electrophoretic transfer of proteins from polyacrylamide or agarose gels to an immobilizing membrane. The maximum gel size is 200 x 250 mm.

By building transfer sandwiches, simultaneous transfer from several gels of the same type can be achieved. Up to six transfer sandwiches can be stacked one on top of the other.

If 125 x 250 mm gels are to be transferred, NovaBlot accepts up to six gels for simultaneous transfer by assembling two transfer sandwiches side by side.

The operating procedures for NovaBlot Kit and FilmRemover are described and illustrated in Sections 4.8 and 7.13 respectively.



**Kit contents – Code No. 18-1016-86**

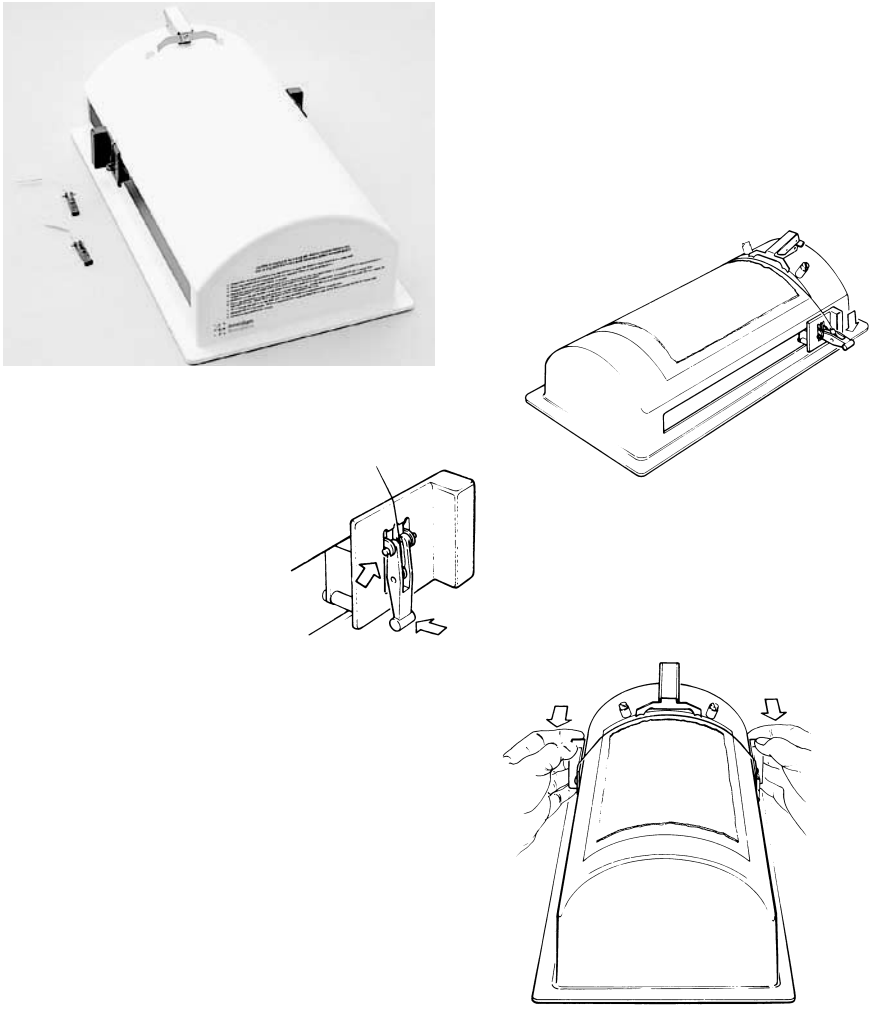
| Designation | Code No. |
|---|---|
| NovaBlot Electrode, cathode | 18-1019-86 |
| NovaBlot Electrode, anode | 80-1257-87 |
| Electrode Paper NovaBlot, 200 x 250 mm (500/pkg) | 80-1106-19 |
| Cellophane Sheets, 210 x 320 mm (50/pkg) | 80-1129-38 |

**Optional accessories**

| Designation | Code No. |
|---|---|
| FilmRemover | 18-1013-75 |

## *9.5 FilmRemover*

FilmRemover is used for removing backing from a gel before electrophoretic transfer. Polyacrylamide or agarose gels with a thickness between 0.1 mm and 5.0 mm and a maximum gel size of 200 x 245 mm can be used.



Detailed instructions for the use of FilmRemover are available in the instruction manual provided with the product.

## 9.6 Roller

For use when applying plastic support films onto glass plates with an interfacing fluid. The roller is used to provide even pressure over a large area, ensuring adhesion with a minimum amount of fluid and elimination of bubble formation.

| Designation | Code No. |
|---|---|
| Roller | 80-1106-79 |

Several accessories are available for simple and convenient sample application with Multiphor II.

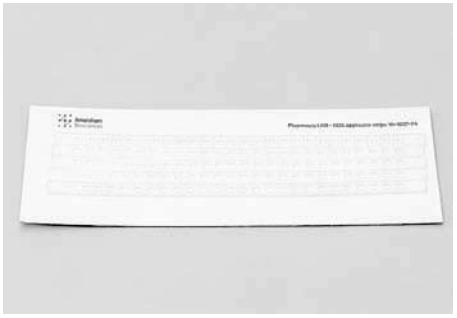
## 9.8 Sample application accessories

| Designation | Code No. |
|---|---|
| IEF Sample Application Pieces (200/pkg) | 80-1129-46 |

The 5 x 10 mm sample application piece made of Paratex can be used for sample volumes in the range 15–20 µl.

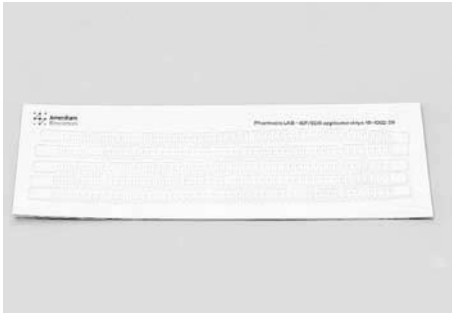
Up to 52 samples can be applied with this strip. Each well holds up to 20 µl of sample. The applicator strip is made of flexible silicone and is applied directly onto the gel surface.

| Designation | Code No. |
|---|---|
| SDS Sample Application Strip 26 samples, 40 µl | 18-1002-74 |



This strip is recommended for sample application on SDS gradient gels without preformed slots, e.g. ExcelGel SDS, gradient 8–18. Up to 26 samples can be applied and each well holds up to 40 µl of sample. The strip is made of transparent flexible silicone and is applied directly onto the gel surface.

| Designation | Code No. |
|---|---|
| IEF/SDS Sample Application Strip 52 samples, 5–20 µl | 18-1002-26 |



This applicator strip is recommended for use with PAGIEF gels, and SDS gradient gels without preformed slots, e.g. ExcelGel SDS, gradient 8–18. Up to 52 samples with a sample volume of 5–20 µl can be applied in each well.

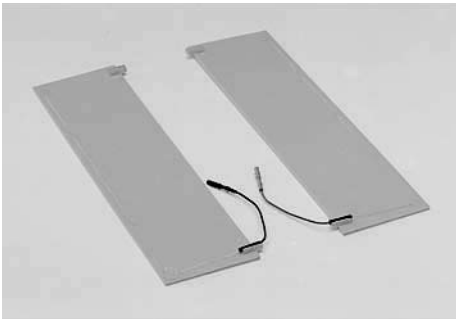
The applicator strip is made of flexible silicone and is applied directly on the gel surface.

| Designation | Code No. |
|---|---|
| EPH/IEF Sample Application Foil 24 samples, 2–4 µl | 80-1129-47 |



This application foil with narrow slits is recommended for electrophoresis and IEF in agarose gels. Up to 24 samples can be applied, with a sample volume of 2–4 µl in each slit. The foil is applied directly on the gel surface.

| Designation | Code No. |
|---|---|
| EPH Electrode anode, long | 18-1122-20 |
| EPH Electrode cathode, long | 18-1122-19 |



The electrophoresis electrodes are designed for use with the buffer vessels at the side of the buffer tank, allowing electrophoresis along the width of the cooling plate.

# 10 Ordering information

**WARNING!** Only spare parts approved or supplied by GE Healthcare may be used for maintaining and servicing of MULTIPHOR II

## *10.1 Multiphor II*

| Product | Quantity | Code No. |
|---|---|---|
| **Basic configuration** | | |
| Multiphor II Electrophoresis Unit | 1 | 18-1018-06 |
| **Application kits and accessories** | | |
| SDS and Native PAGE, IEF Kit | 1 | 18-1102-45 |
| Immobiline DryStrip Kit for running 1 to 12 Immobiline DryStrip gels (for use with Multiphor II only) | 1 | 18-1004-30 |
| NovaBlot Kit for electrophoretic transfer | 1 | 18-1016-86 |
| Multiphor II Buffer Strip Positioner, complete | 1 | 80-6442-90 |
| FlexiClamps | 6 | 18-1013-73 |
| FilmRemover for removing plastic gel backing before electrophoretic transfer | 1 | 18-1013-75 |
| Gradient Maker, 100 ml | 1 | 80-6196-09 |
| Roller | 1 | 80-1106-79 |
| Lever and Wire Assemblies (for Film-Remover) | 3 | 18-1013-79 |
| Levelling Feet | 4 | 18-1026-40 |
| **Cooling plates** | | |
| Cooling Plate ceramic, 210 x 270 mm | 1 | 18-1103-46 |
| Grommets | 2 | 80-1106-58 |
| Cooling Tubing, 8/12 mm | 4 m | 80-1106-56 |
| Tubing Connector Set, female and male | 4 | 18-1104-26 |
| Insulation for Cooling Tubing 14/27 mm | 8 m | 80-1116-11 |
| Anade and Cathode Electrode | 1 | 18-1037-44 |
| Leads (for 18-1004-31 Immobline DryStrip Kit Tray) | | |

| Product | Quantity | Code No. |
|---|---|---|
| **Electrodes and electrode holders** | | |
| EPH/IEF Electrode, anode | 1 | 18-1121-53 |
| EPH/IEF Electrode, cathode | 1 | 18-1121-52 |
| Electrode Holder (for 18-1106-60/-61) | 1 | 80-1106-55 |
| EPH Electrode (long), anode | 1 | 18-1122-20 |
| EPH Electrode (long), cathode | 1 | 18-1122-19 |
| Electrode (Immobiline DryStrip Kit), anode | 1 | 18-1018-66 |
| Electrode (Immobiline DryStrip Kit), cathode | 1 | 18-1018-67 |
| Tray and Electrode Holder for 18-1018-66/-67 | 1 | 18-1004-31 |
| NovaBlot Electrode, anode | 1 | 80-1257-87 |
| NovaBlot Electrode, cathode | 1 | 18-1019-86 |
| **Glass plates and trays** | | |
| 125 x 260 x 3 mm | 2 | 80-1106-99 |
| 125 x 260 x 1 mm | 15 | 80-1106-29 |
| 125 x 260 mm, 0.5 mm U-frame | 2 | 80-1106-89 |
| 125 x 260 mm, 1.0 mm U-frame | 2 | 80-1106-91 |
| 125 x 260 mm, 2.0 mm U-frame | 2 | 80-1106-92 |
| **Glass plate treatment** | | |
| Bind-Silane | 100 ml | 17-1330-01 |
| Repel-Silane ES | 500 ml | 17-1332-01 |
| **Gel support** | | |
| GelBond PAGfilm, 124 x 258 mm | 50 | 80-1129-36 |
| **Paper electrode strip and wicks** | | |
| IEF Electrode Strip | 100 | 18-1004-40 |
| EPH Electrode Wick, 82 x 130 mm | 500 | 80-1129-53 |
| EPH Electrode Wick 104 x 253 mm (also used as PEGG print paper and with agarose IEF) | 500 | 80-1129-52 |
| Electrode Paper NovaBlot, 200 x 250 mm | 500 | 80-1106-19 |

| Product | Quantity | Code No. |
|---|---|---|
| **Sample application** | | |
| SDS Sample Application Strip, 26 samples, 40 µl | 5 | 18-1002-74 |
| IEF/SDS Sample Application Strip, 52 samples, 5–20 µl | 5 | 18-1002-26 |
| IEF Sample Application Pieces | 200 | 80-1129-46 |
| Sample Cups, Immobiline DryStrip Kit | 60 | 18-1004-35 |
| **Preserving** | | |
| Cellophane Sheets, 210 x 320 mm | 50 | 80-1129-38 |
| Mylar™ Sheets, 125 x 260 mm | 50 | 80-1129-39 |

## *10.2 MultiTemp IV*

| Product | Quantity | Code No. |
|---|---|---|
| MultiTemp IV thermostatic circulator, 115 V/60 Hz | 1 | 28-9941-72 |
| MultiTemp IV thermostatic circulator, 230 V/50 Hz | 1 | 28-9941-71 |
| MultiTemp IV thermostatic circulator 100 V, 50/60 Hz | 1 | 29-0096-30 |
| Cooling Tubing, 8/12 mm | 4 m | 80-1106-56 |
| Tubing Connector Set, female and male | 4 | 18-1104-26 |
| Insulation for Cooling Tubing 14/27 mm | 2 m | 80-1116-11 |
| 3-way Valve Set | 1 | 18-1106-39 |

## *10.3 EPS Power Supplies*

| Product | Quantity | Code No. |
|---|---|---|
| EPS 3501 XL 35-3500 V, 1–400 mA | 1 | 18-1130-05 |
| EPS 3501 35-3500 V, 1–150 mA | 1 | 18-1130-04 |
| EPS 1001 5-1000 V, 1–400 mA | 1 | 18-1130-03 |
| EPS 601 6-600 V, 1–400 mA | 1 | 18-1130-02 |
| EPS 301 5-300 V, 1-400 mA | 1 | 18-1130-01 |

## *10.4 Precast gels and buffer strips*

| Product | Quantity | Code No. |
|---|---|---|
| **SDS-PAGE and Native PAGE** | | |
| ExcelGel SDS Homogeneous 7.5 | 6 | 80-1260-01 |
| ExcelGel SDS Homogeneous 12.5 | 6 | 80-1261-01 |
| ExcelGel SDS Homogeneous 15 | 6 | 80-1262-01 |
| ExcelGel SDS, gradient 8–18 | 6 | 80-1255-53 |
| ExcelGel XL SDS, gradient 12–14 | 3 | 17-1236-01 |
| ExcelGel SDS Buffer Strips anode and cathode | 6 each | 17-1342-01 |
| **IEF** | | |
| CleanGel IEF | 5 | 18-1035-32 |
| GelPool for gel rehydration | 1 | 18-1031-58 |
| **Immobiline DryStrip Gels** | | |
| Immobiline DryStrip pH 3.5–4.5, 24 cm | 12 | 17-6002-38 |
| Immobiline DryStrip pH 6–9, 24 cm | 12 | 17-6002-47 |
| Immobiline DryStrip pH 3–7 NL, 24 cm | 12 | 17-6002-43 |
| Immobiline DryStrip pH 3–10, 24 cm | 12 | 17-6002-44 |
| Immobiline DryStrip pH 3–10 NL, 24 cm* | 12 | 17-6002-45 |
| Immobiline DryStrip pH 4–7, 24 cm | 12 | 17-6002-46 |
| Immobiline DryStrip pH 4–7, 18 cm | 12 | 17-1233-01 |
| Immobiline DryStrip pH 6–9, 18 cm | 12 | 17-6001-88 |
| Immobiline DryStrip pH 6–11, 18 cm | 12 | 17-6001-97 |
| Immobiline DryStrip pH 3–10 NL, 18 cm* | 12 | 17-1235-01 |
| Immobiline DryStrip pH 3–10, 18 cm | 12 | 17-1234-01 |
| Immobiline DryStrip pH 4–7, 13 cm | 12 | 17-6001-13 |
| Immobiline DryStrip pH 6–11, 13 cm | 12 | 17-6001-96 |
| Immobiline DryStrip pH 3–10 NL, 13 cm* | 12 | 17-6001-15 |
| Immobiline DryStrip pH 3–10, 13 cm | 12 | 17-6001-14 |

| | | |
|---|---|---|
| Immobiline DryStrip pH 4–7, 11 cm | 12 | 18-1016-60 |
| Immobiline DryStrip pH 6–11, 11 cm | 12 | 17-6001-10 |
| Immobiline DryStrip pH 3–10, 11 cm | 12 | 18-1016-61 |
| Immobiline DryStrip pH 4–7, 7 cm | 12 | 17-6001-10 |
| Immobiline DryStrip pH 6-11, 7 cm | 12 | 17-6001-94 |
| Immobiline DryStrip pH 3-10 NL, 7 cm* | 12 | 17-6001-12 |
| Immobiline DryStrip pH 4-7, 7 cm | 12 | 17-6001-10 |
| Immobiline DryStrip pH 3-10, 7 cm | 12 | 17-6001-11 |
| **IPG Buffer** | | |
| IPG Buffer pH 3.5–5.0† | 1 ml | 17-6002-02 |
| IPG Buffer pH 5.5–6.7 | 1 ml | 17-6002-06 |
| IPG Buffer pH 4–7§ | 1 ml | 17-6000-86 |
| IPG Buffer pH 6–11‡ | 1 ml | 17-6001-78 |
| IPG Buffer pH 3–10 NL* | 1 ml | 17-6000-88 |
| IPG Buffer pH 3–10 | 1 ml | 17-6000-87 |
| **Second dimension** | | |
| ExcelGel 2-D Homogeneus | 6 | 17-6002-21 |
| ExcelGel SDS, gradient 8–18<br>110 x 245 x 0.5 mm | 6 | 80-1255-53 |
| ExcelGel XL SDS, gradient 12–14<br>180 x 245 x 0.5 mm | 3 | 17-1236-01 |
| ExcelGel SDS Buffer Strips<br>anode and cathode | 6 each | 17-1342-01 |

* NL= increased resolution between pH 5-7
† Use IPG Buffer pH 3.5-5.0 for pH 3.5-4.5 and 4.0-5.0 IPG strips
‡ Use IPG Buffer pH 6-9 and pH 6-11 IPG strips.
§ Use IPG Buffer pH 4-7 for pH 3-7 IPG strips.

## *10.5 Molecular weight and pI markers*

| Product | Quantity | Code No. |
|---|---|---|
| MW range 14.000–94.000, 200 µg/vial | 10 | 17-0446-01 |
| MW range 53.000–212.000, 200 µg/vial | 10 | 17-0615-01 |
| MW range 67.000–670.000, 200 µg/vial | 10 | 17-0445-01 |
| Broad pI kit pH 3.5–9.3 | | 17-0471-01 |
| Low pI kit pH 2.8–6.5<br>High pI kit pH 5.2–10.3 | | 17-0472-01<br>17-0473-01 |

## 10.6 Carrier ampholytes

| Product | Quantity | Code No. |
|---|---|---|
| **Pharmalyte** | | |
| Pharmalyte pH 3–10 | 25 ml | 17-0456-01 |
| Pharmalyte pH 2.5–5 | 25 ml | 17-0451-01 |
| Pharmalyte pH 4–6.5 | 25 ml | 17-0452-01 |
| Pharmalyte pH 5–8 | 25 ml | 17-0453-01 |
| Pharmalyte pH 8–10.5 | 25 ml | 17-0455-01 |
| Pharmalyte pH 4.2–4.9 | 25 ml | 17-0562-01 |
| Pharmalyte pH 4.5–5.4 | 25 ml | 17-0563-01 |
| Pharmalyte pH 5–6 | 25 ml | 17-0564-01 |
| Pharmalyte pH 6.7–7.7 | 25 ml | 17-0566-01 |

Each bottle contains a ready-to-use 0.200±0.004 M solution.

## 10.7 PlusOne electrophoresis chemicals

| Product | Use | Quantity | Storage | Code No· |
|---|---|---|---|---|
| ***Gel casting chemicals*** | | | | |
| Acrylamide IEF 40% solution | IEF, PAGE | 1000 ml | D | 17-1301-01 |
| ReadySol IEF T40 C3 | IEF | 1000 ml | D | 17-1310-01 |
| Acrylamide PAGE | PAGE | 1000 g | A | 17-1302-02 |
| Acrylamide PAGE 40% Solution | PAGE | 1000 ml | D | 17-1303-01 |
| N,N-Methylene-bis-acrylamide | IEF, PAGE, Sequencing | 100 g | C | 17-1304-02 |
| N,N-Methylene-bis-acrylamide 2% solution | IEF, PAGE, Sequencing | 1000 ml | D | 17-1306-01 |
| Ammonium persulphate | IEF, PAGE, Sequencing | 25 g | C | 17-1311-01 |
| TEMED | IEF, PAGE, Sequencing | 25 ml | C* | 17-1312-01 |
| ***Buffers*** | | | | |
| Tris | PAGE, Sequencing | 500 g | A | 17-1321-01 |
| Glycine | PAGE, Sequencing | 500 g | A | 17-1323-01 |
| ***Additives and sample treatment*** | | | | |
| Urea | IEF, PAGE, Sequencing | 500 g | B | 17-1319-01 |
| Dithiothreitol | IEF, SDS-PAGE | 1.0 g | F | 17-1318-01 |
| Dithiothreitol | IEF, SDS-PAGE | 5 g | F | 17-1318-02 |
| Glycerol 87% | IEF, PAGE, Sequencing | 1000 ml | A | 17-1325-01 |
| ***Detergents*** | | | | |
| Sodium dodecylsulphate | PAGE | 100 g | A | 17-1313-01 |
| CHAPS IEF, | PAGE, Sequencing | 1 g | F | 17-1329-01 |
| ***Stains*** | | | | |
| Silver Staining Kit, Protein | Protein detection | For 10–20 gels | D | 17-1150-01 |
| Silver Staining Kit, DNA | Nucleic and detection | For 10–20 gels | D | 17-6000-30 |
| Bromophenol Blue IEF, | PAGE, Sequencing | 10 g | A | 17-1329-01 |
| ***Glass plate treatment*** | | | | |
| Repel-Silane ES | IEF, PAGE, Sequencing | 500 ml | C | 17-1332-01 |
| Bind-Silane | IEF, PAGE, Sequencing | 25 ml | C | 17-1330-01 |
| ***Others*** | | | | |
| DryStrip Cover Fluid 2-D Immobiline | DryStrip | 1000 ml | G | 17-1335-01 |

**Storage:** A, room temp. B, dry at room temp. C, dry & dark at room temp. D, dark at 4 °C to 8 °C. E, dry & dark at 4 °C to 8 °C. F, dry at 4 to 8 °C. G, dark at room temp.
*Store well sealed.

1. Add 100 ml. 2. Add 500 ml.

For local office contact information, visit
www.gelifesciences.com/contact

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

www.gelifesciences.com

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GE Healthcare UK Ltd
Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK

GE Healthcare Bio-Sciences Corp
800 Centennial Avenue, P.O. Box 1327, Piscataway, NJ 08855-1327, USA

GE Healthcare Europe GmbH
Munzinger Strasse 5, D-79111 Freiburg, Germany

GE Healthcare Japan corporation
Sanken Bldg. 3-25-1, Hyakunincho, Shinjuku-ku, Tokyo 169-0073, Japan

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