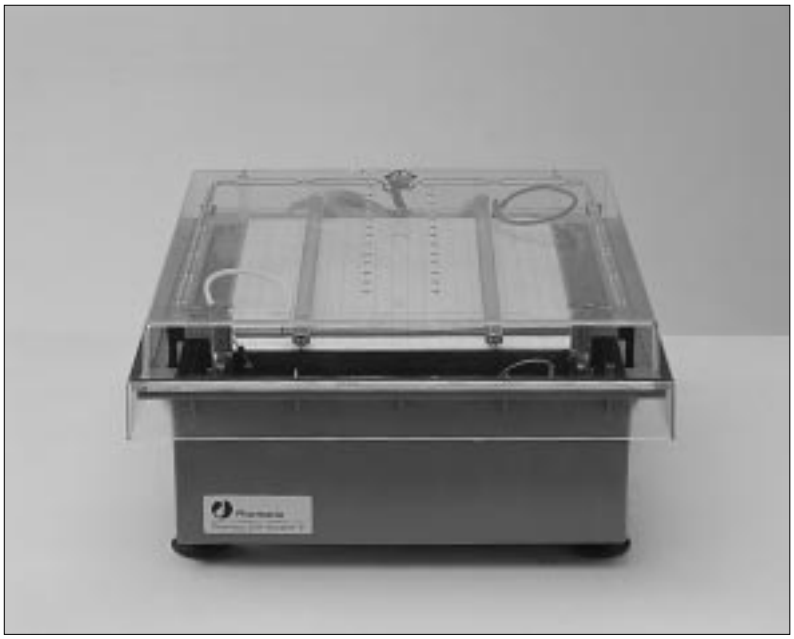
Multiphor™ II Electrophoresis System



User Manual

18-1103-43
Edition AF

Pharmacia Biotech

Important user information

Meaning: Consult the insruction manual to avoid persoanl injury, or damage to the product or other equipment.

WARNING! The Warning sign is used to call attention to the necessity to follow an instruction in detail to avoid personal injury. Do not proceed until the instructions are clerarly understood and all stated conditions are met.

CAUTION! The Caution sign is used to call attention to instructions or conditions that are to be followed to avoid damage to the product or other equipment. Do not proceed until the instructions are clerarly understood and all stated conditions are met.

Note: The Note sign is used to indicate information important for trouble free or optiamal use of the prodct. Should you have any comments on this instruction, we will be pleased to receive them at:

Pharmacia Biotech AB
S-751 82 Uppsala
Sweden

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Pharmacia Biotech AB guarantees that the product delivered has been thoroughly tested to ensure that it meets its published specifications. The warranty included in the conditions of delivery is valid only if the product has been installed and used according to the instructions supplied by Pharmacia Biotech AB.

Pharmacia Biotech AB shall in no event be liable for incidental or consequential damages, including without limitation, lost profits, loss of income, loss of business opportunities, loss of use and other related exposures, however caused, arising from the faulty and incorrect use of the product.

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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, a unique range of precast gels for all major electrophoretic techniques is available with Multiphor II:

Technique	**Precast Gel**
SDS and Native PAGE	ExcelGel SDS Gradient ExcelGel SDS Homogeneous CleanGel
IEF	Ampholine PAGplate Immobiline DryPlate CleanGel IEF
2-D electrophoresis	Immobiline DryStrip ExcelGel XL SDS gradient

Multiphor II is available in two basic configurations: Multiphor II Electrophoresis Unit for electrophoresis and isoelectric focusing, and Multiphor II NovaBlot Unit for electrophoretic transfer.
If laboratory cast gels are preferred, application kits and accessories can be added to either of the basic configurations.

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 0.5x125x260 mm homogeneous and gradient gel	SDS and Native PAGE, IEF Kit Gradient Maker	18-1102-45 18-1013-72
SDS and Native PAGE 0.5x200x260 mm homogeneous and gradient gel	Large Scale SDS and Native PAGE Kit Gradient Maker	18-1102-46 18-1013-72
IEF in polyacrylamide 0.1-0.5x125x260 mm	UltroMould	18-1018-16
IEF in polyacrylamide 0.5x125x260 mm	SDS and Native PAGE, IEF Kit	18-1102-45
IEF in agarose	Agarose IEF Kit	18-1016-82
Preparative IEF in granulated gel 5x125x260 mm	Preparative IEF Kit	18-1018-05
Immunoelectrophoresis - Grabar & Williams, Laurell and fused rocket, crossed and tandem crossed, double diffusion	Immunoelectrophoresis Kit	18-1016-87
2-D, first dimension: Immobiline DryStrip	Immobiline DryStrip Kit Reswelling Cassette	18-1004-30 18-1013-74
2-D, second dimension: 0.5x125x260 mm	SDS and Native PAGE, IEF Kit Gradient Maker	18-1102-45 18-1013-72
2-D, second dimension: 0.5x200x260 mm	Large Scale SDS and Native PAGE Kit Gradient Maker	18-1102-46 18-1013-72
Electrophoretic transfer	NovaBlot Kit FilmRemover	18-1016-86 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 and Multiphor II NovaBlot 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 basic configurations, application kits, accessories and replacement parts
- MultiTemp III, EPS and GPS Power Supplies
- Precast gels and buffer strips
- Molecular weight and pI markers
- Ampholine and 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! 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.

WARNING! NEVER EXCEED the maximum pressure 0.5 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 (p-p).

3. Description of parts

3.1 Multiphor II Electrophoresis Unit

This Multiphor II configuration 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) and card-mounted EPH electrodes (for electrophoresis using buffer chambers).

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, 210x270 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	80-1121-52
EPH/IEF Electrode anode	80-1121-53
User Manual and Application Package	18-1103-44

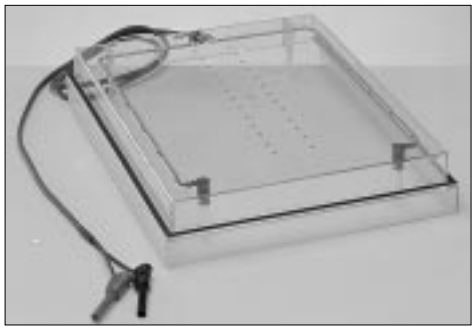
The buffer tank is made of polypropylene, which is resistant to nearly all chemicals at room temperature.

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.

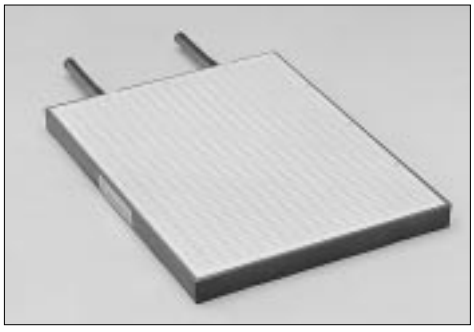
The small left land pin is used to connect the EPH/IEF or card-mounted cathode electrodes. The small pin on the right connects to the card-mounted anode or the EPH/IEF cathode via the red lead mounted on the unit.

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.

Note: Polycarbonate is not resistant to concentrated acids and bases, or to halogenated and aromatic hydrocarbons.



The ceramic (aluminium oxide) cooling plate measures 210x270 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 (p-p).

To facilitate the correct positioning of electrophoresis gels, the surface of the cooling plate is screened with a template measuring 190x250 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 III.

WARNING! Only use water with high electrical resistance as coolant and NEVER EXCEED maximum pressure of 0.5 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 III.

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.

Note: Polysulfone is not resistant to ketones, esters, halogenated and aromatic hydrocarbons.

3.2 Multiphor II NovaBlot Unit for electro-phoretic transfer

This Multiphor II configuration is a dedicated unit for electrophoretic transfer and includes a buffer tank with four levelling feet, a safety lid, NovaBlot electrodes, electrode paper and cellophane sheets.
The unique NovaBlot electrodes made of graphite (210 x 260 mm), provide an even distribution of current across the entire surface, thus ensuring uniform electrophoretic transfer.
Both electrodes are bound to acrylic plates. The lower anode electrode forms the base and the upper cathode electrode forms the lid of the transfer assembly.
The NovaBlot electrodes are designed so that when cathode and anode electrodes are fitted together, they will not touch each other. This prevents damage to the graphite electrodes when they are not in use.

By addition of the cooling plate, electrode holder with EPH/IEF electrodes and EPH electrodes, the NovaBlot unit can be expanded into a horizontal electrophoresis system (see 2.1 Multiphor II Electrophoresis System).

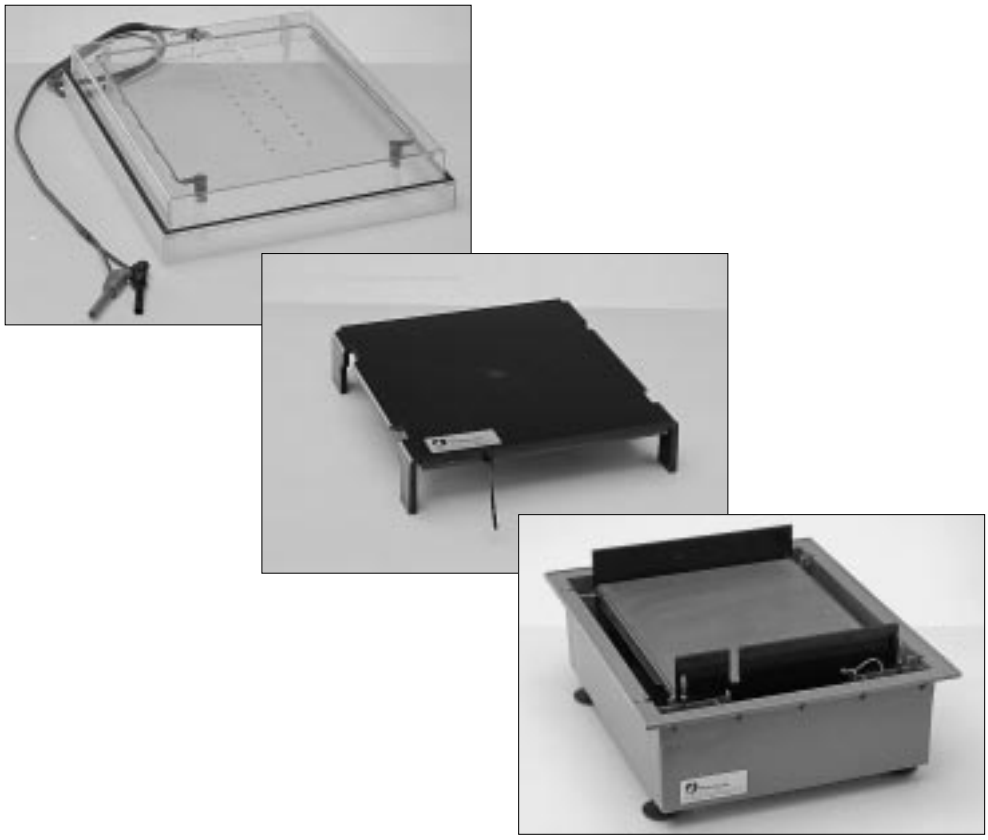
Unit contents - Code No. 18-1016-85

Designation	Code No.
Buffer Tank	18-1122-25
Levelling Feet (4/pkg)	18-1026-40
Safety Lid	18-1122-26
NovaBlot Electrode, cathode	18-1019-86
NovaBlot Electrode, anode	80-1257-87
Electrode Paper NovaBlot, 200x250 mm (500/pkg)	80-1106-19
Cellophane Sheets, 210x320 mm (50/pkg)	80-1129-38
Manual and Application Package	18-1103-44

Optional accessories

Designation	Code No.
FilmRemover	18-1013-75
Nitrocellulose 0.20 µm, 150x200 mm (15/pkg)	80-1098-91
Nitrocellulose 0.45 µm, 150x200 mm (15/pkg)	80-1098-90
ProBind 45 NC 0.45 µm, roll 0.2x3.0 m	80-1247-86
GeneBind 45 nylon 0.45 µm, roll 0.2x3.0 m	80-1247-87

The Multiphor II buffer tank with the four adjustable levelling feet supports the NovaBlot electrodes.



Two electrode connections are provided for each pole, one small pin and one large banana plug. The larger banana plugs connect the buffer tank to the safety lid. The NovaBlot electrodes are connected to the small pins.
The buffer tank is made of polypropylene, which is resistant to nearly all chemicals at room temperature.
The safety lid, made of polycarbonate, includes the electrode leads and the safety interlock.

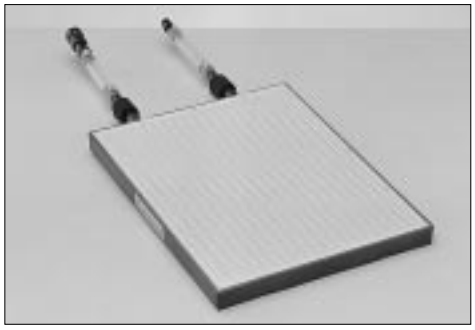
WARNING! Polycarbonate is not resistant to concentrated acids and bases, or to halogenated and aromatic hydrocarbons.

Precut electrode paper 200x250 mm and cellophane sheets, 210x320 mm, are included with Multiphor II NovaBlot Unit.

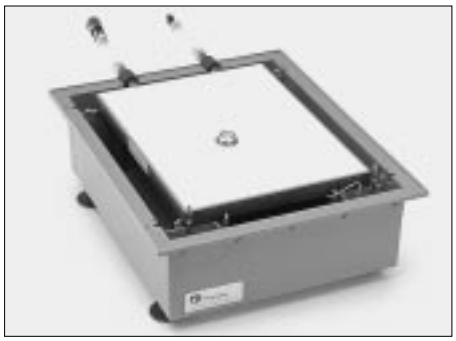
4. Installation

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

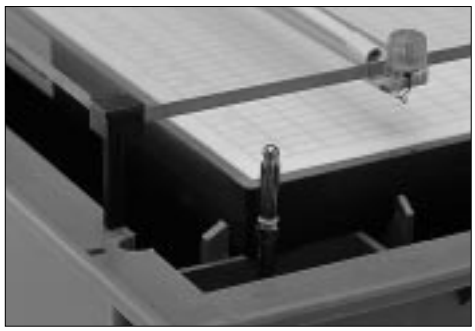
4.1 Multiphor II Electro-phoresis Unit

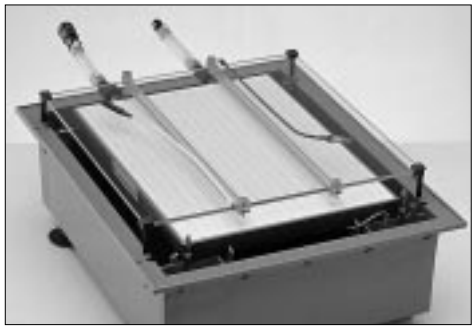


For easy connection of Multiphor II Electrophoresis Unit to the cooling device, install tubing connectors on both inlet and outlet tubings. Use one male and one female connector on the Multiphor II unit side and thermostat unit side respectively. The cooling plate tubings and thermostatic circulator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

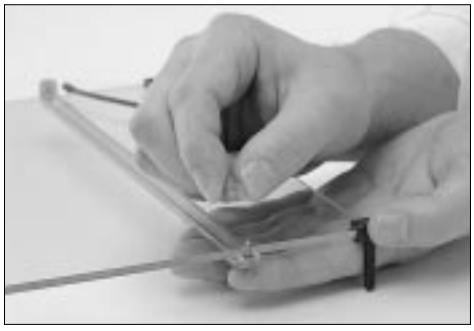


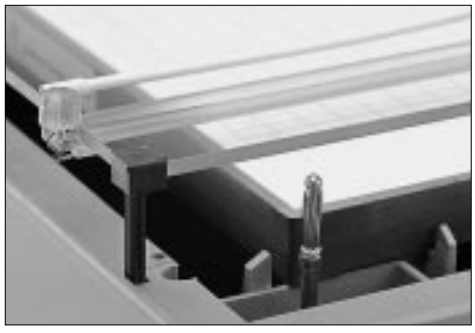
Screw one levelling foot into each corner of the buffer tank. Place the buffer tank on the lab bench where it will be used. Place the cooling plate on the unit, using the moulded guides to position it correctly. Fit the grommets into the cutouts in the back of the unit. Place a spirit level on the cooling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

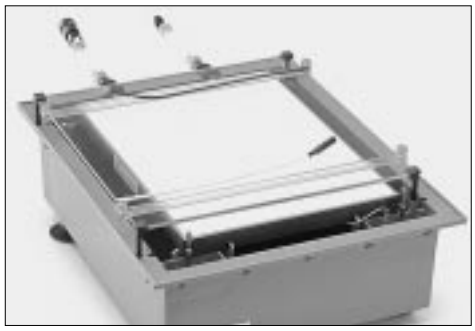




To mount the EPH/IEF electrodes on the electrode holder, unscrew the clamping nut from each electrode.
When running electrophoresis across the width of the cooling plate, mount the electrodes as illustrated. Place the electrode under the electrode holder. Replace the nut and lightly tighten until the 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.





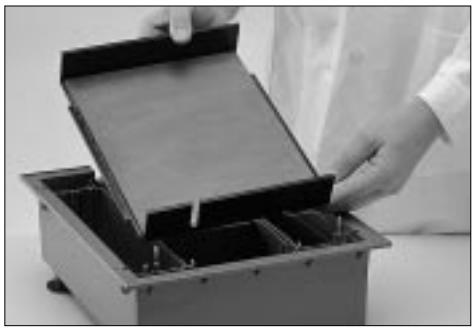


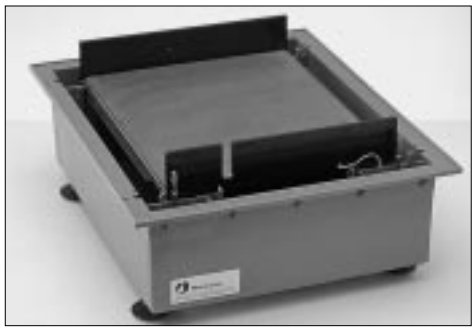
When running electrophoresis along the length of the cooling plate, mount the electrodes as illustrated. Place the electrodes under the electrode holder.

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.

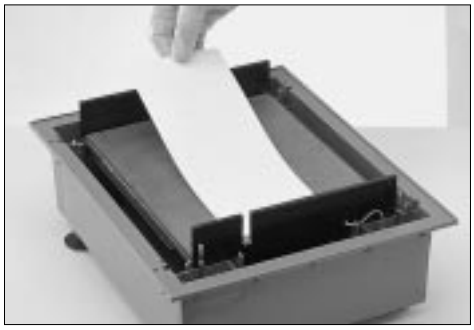
Install the safety lid.

4.2 Multiphor II NovaBlot Unit for electro-phoretic transfer

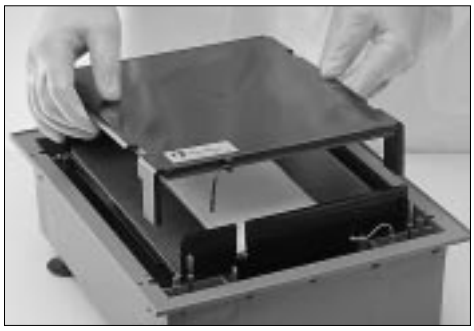




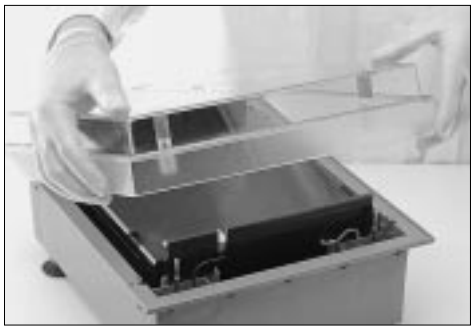
Multiphor buffer tank should be assembled with the four levelling feet.
Make sure the buffer tank is rinsed with water and dried.
Saturate the anode NovaBlot electrode with distilled water and remove the excess water with absorbent paper. With the electrode lead to the front of the instrument, fit the anode electrode onto the buffer tank.
Connect the anode socket (red lead) to the anode pin on the right side of the buffer tank.
Place a spirit level on the anode electrode and adjust the levelling feet until the unit is levelled.



The transfer sandwich can now be assembled on the bottom anode electrode. Full assembly instructions are given in Section 4.8.



After assembling the transfer sandwich, saturate the upper cathode electrode with distilled water and remove any excess with absorbent paper. With the electrode lead to the front of the instrument, place the cathode electrode on top of the stack.
Connect the cathode socket (black lead) to the cathode pin on the left of the buffer tank.



Install the safety lid.

WARNING! Check that the electrodes are properly connected before closing the lid. Always check that the lid is properly before turning the power supply ON.

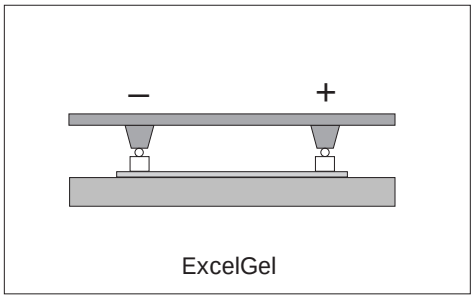
5. Operation

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.

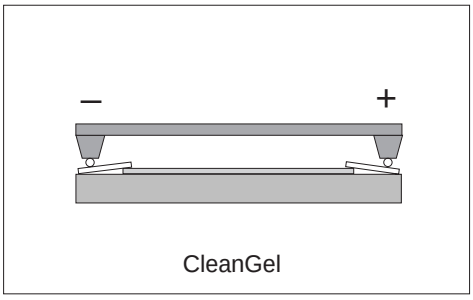
Multiphor II contains two alternative electrode configurations.

- EPH/IEF electrodes for use with buffer strips or electrode strips
- EPH electrodes for use with electrode wicks and buffer chambers

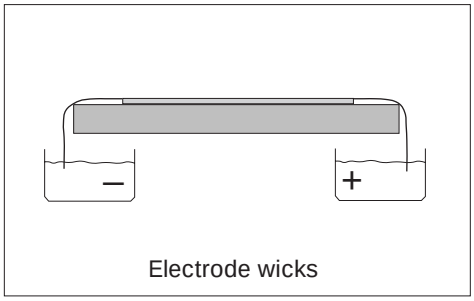
IEF, SDS-PAGE and native PAGE are most conveniently performed using EPH/IEF electrodes and buffer strips. There are two types of buffer strips: ExcelGel buffer strips made of polyacrylamide and CleanGel buffer strips made of special paper. The buffer strips are applied on the gel edges with the electrodes on top.



ExcelGel



CleanGel



Electrode wicks

Immunoelectrophoresis is run using EPH electrodes and buffer chambers. 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 can also be used for SDS-PAGE and native PAGE.
The optional car-mounted EPH electrodes (80-1106-62 and 80-1106-63) - 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

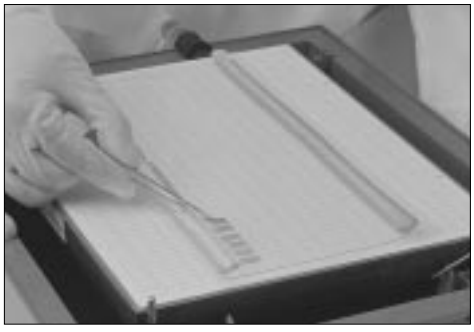
This section describes the running procedure for SDS PAGE using buffer strips. The running of ExcelGel SDS, gradient 8-18 using ExcelGel SDS buffer strips is chosen as an example, but the basic method is applicable to all SDS PAGE and native PAGE gels.
ExcelGel SDS, gradient 8-18, is a 0.5 mm-thin, precast 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.

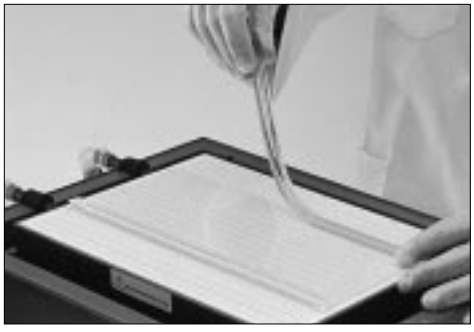
ExcelGel SDS, ExcelGel Homogeneous 7.5, 12.5, 15 and CleanGel are available with various numbers of sample wells for various volumes. Samples are applied directly into the wells immediately prior to the run.

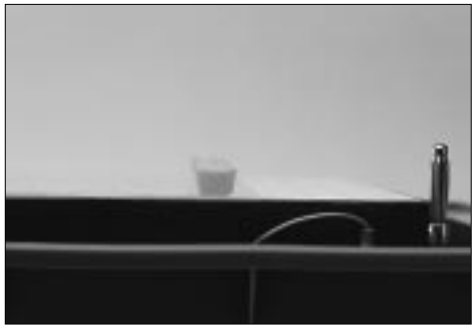
Electrophoresis

Connect Multiphor II to MultiTemp II thermostatic circulator. Switch on MultiTemp II 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.

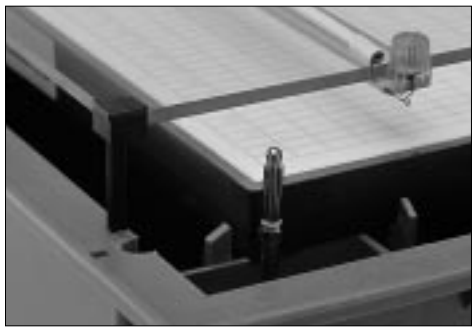
Open the ExcelGel SDS strip packages and apply the cathodic and the anodic SDS buffer strips on the respective sides of the gel.





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.

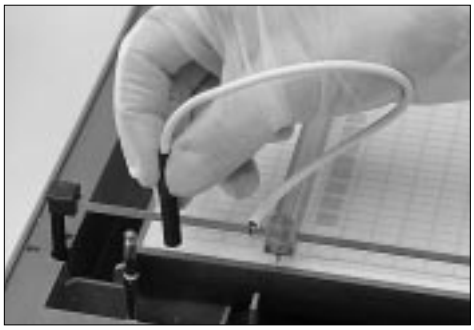


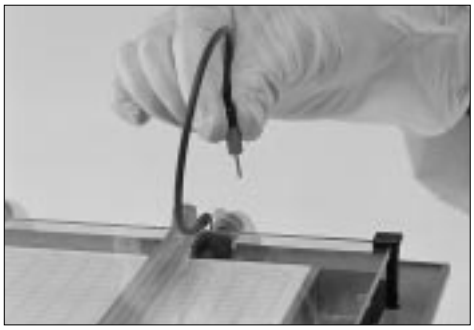
Place the electrode holder on the electrophoresis unit in the shallow depressions.

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

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

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

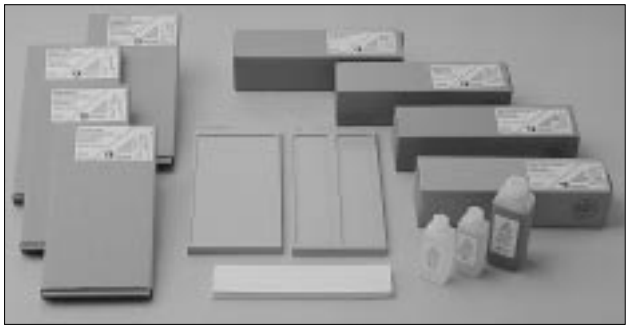
Step No.	Solution	Time (min)
1	Fixing solution C	30
2	Incubation solution D	30
3	Distilled water	3x5
4	Silver solution E	40
5	Developing solution F	5-15 *
6	Stop solution G	10
7	Distilled water	3x5
8	Preserving solution L	20

*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 CleanGel and buffer strips

This section gives a brief description of the running procedure for native PAGE and SDS PAGE using CleanGel and CleanGel buffer strips. CleanGel is a washed and dried polyacrylamide gel. Depending on the application, the gels are rehydrated in different buffers to a gel thickness of 0.5 mm. The gels are available with various numbers and sizes of sample wells. There are various application kits with prepared buffers. For more information, see Ordering information and the information supplied with the CleanGel.



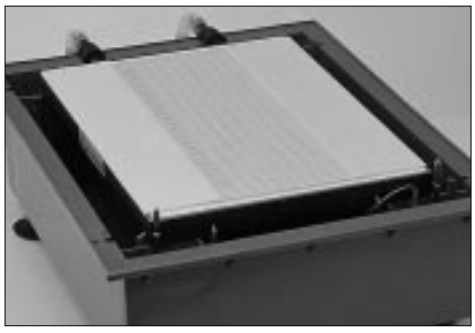
Preparing the experiment

Connect Multiphor II to MultiTemp II thermostatic circulator. Switch on MultiTemp II 20 minutes before starting the experiment and set the temperature to 15 °C.

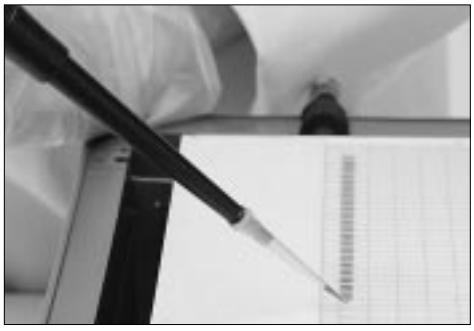
Note: Always wear clean gloves when working with polyacrylamide gels and buffer strips, particularly when using sensitive staining methods.

Rehydrate a CleanGel in an appropriate buffer using the GelPool, according to instructions supplied with the CleanGel.
Pipette 1 ml kerosene onto the cooling plate. Position the gel in the middle of the cooling plate with the stiff plastic support film facing down. Position the sample wells at the cathodic or anodic side depending on the buffer system used. No air bubbles should be trapped beneath the gel.

Place the electrode strips in the two compartments of the PaperPool. If a smaller gel size is used, cut the strips to the correct size. Apply 20 ml of the cathode buffer to the cathodic buffer strip using a pipette (use less buffer volume for shorter strips). Follow the same procedure with the anode buffer and the anode buffer strip.



Place the buffer strips onto the edges of the gel so that the strips overlap the gel by 5 mm and there is a distance of 4 mm between the edge of the strip and the sample wells. Place the anode buffer strip on the anode side and the cathode buffer strip on cathode side.

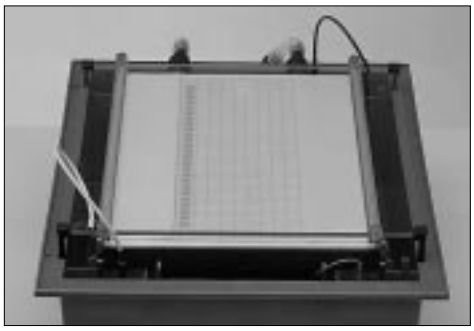


Sample application

Apply 5-100 µl sample/well depending on gel type and sample concentration.

Running conditions

Place the electrode holder with the EPH/IEF electrodes on the Multiphor II unit. Align the electrodes so that they rest on the outer edges of the electrode strips.



Connect the cables of the electrodes to the unit. Place the safety lid in position. Connect the power supply. A low starting voltage for smooth sample entry generally improves the result.

Recommended electrical settings for anodal electrophoresis with Native Buffer Kit pH 8.9

Phase	Voltage (V)	Current (mA)	Power (W)	Time (min)
Pre-run	300	18	10	10
Run	900	50	30	60*

* Approximate time, or until the buffer front (dye marker Bromophenol Blue) reaches the anodic wick.

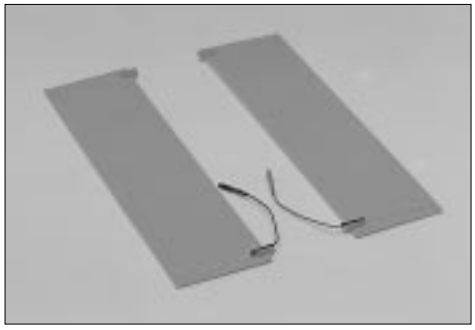
Further information about the gels and running conditions are supplied with the product.

Staining and preserving

For automated silver and coomassie staining of polyacrylamaida gels see Protocol guide, Hoefer Automated Gel Stainer (80-6343-34).
CleanGel can be stained with most protein and DNA staining methods. The

5.3 Electrophoresis using buffer chambers

staining and preserving methods recommended for ExcelGel SDS (See Section 4.1) work well with CleanGel.
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.

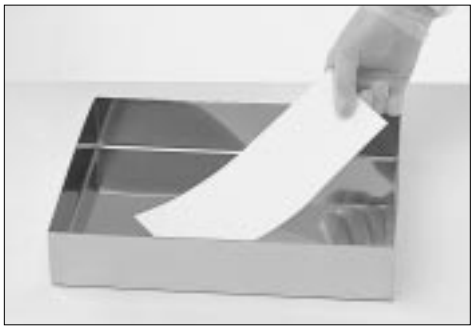


Fill each chamber to the moulded line (indicating 1 liter volume) with buffer solution. (When running 120x250 mm gels, pour 1.2 liters of buffer into each chamber to ensure adequate buffer contact with the wicks.)
Replace the cooling plate, making sure that the electrode socket connectors lie to the 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.

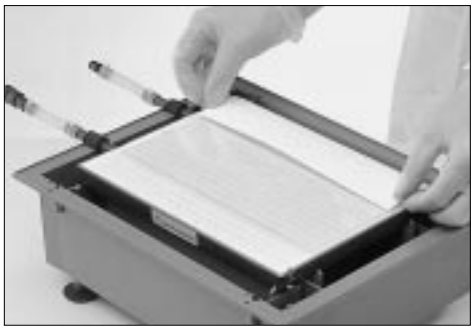
When performing immunoelectrophoresis or agarose electrophoresis, center a small (84x94 mm) glass plate on the dry cooling plate and attach a strip of tape along the width of the cooling plate in alignment with the edges of the glass plate. These stop the small gels from shifting during application of the wicks and ensure that they are centred between the two buffer chambers during electrophoresis.

Switch on MultiTemp III 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.

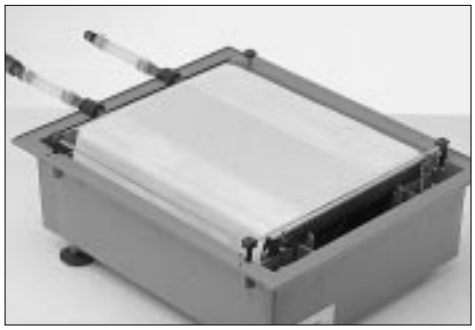


Prepare the electrode wicks by aligning 8-10 pieces of filter paper for each buffer chamber. Starting at one end, slowly immerse the electrode wicks in the buffer, using capillary action to reduce the amount of air trapped in the paper.



When running large (195x250 mm) or medium sized gels (120x250 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.

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.

5.4 Isoelectric focusing using Ampholine PAGplate

This section describes the running procedure for IEF using Ampholine PAGplate. For further information, see the instructions supplied with the product.

Switch on MultiTemp II thermostatic circulator and set the temperature (normally 10 °C for polyacrylamide IEF) 15 minutes before starting the experiment.
Cover the holes in the safety lid with tape to limit the amount of CO_2 in contact with the gel, thereby improving the basic region of the pH gradient. If desired, 100 ml of 1 M sodium hydroxide solution may be poured into the buffer chambers to absorb CO_2 and further improve gradient stability.

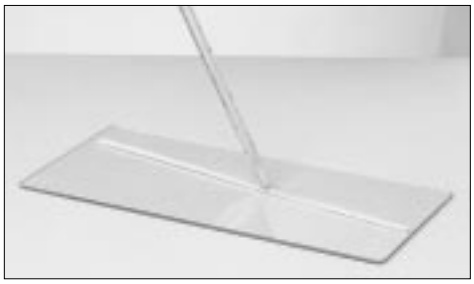
Make up about 100 mls of the required electrode solutions. (See Table).

Electrode solutions for IEF using Ampholine PAGplate

pH range	Anode solution	Cathode solution
3.5-9.5	1 M Phosphoric acid	1.0 M NaOH
4.0-6.5	0.1 M Glutamic acid	0.1 M ß-alanine
5.5-8.5	0.4 M HEPES	0.1 M NaOH
4.0-5.0	1 M Phosphoric acid	1.0 M Glycine
5.0-6.5	0.01 M Acetic acid	0.01 M NaOH

Note: Wear clean gloves when working with polyacrylamide gels.

Pipette 1 ml of insulating fluid (kerosene or light paraffin oil) onto the cooling plate. Open one package of Ampholine PAGplate and position the gel with the stiff plastic film facing down on the cooling plate. Make sure no air bubbles are trapped under the gel. Use the screened template on the cooling plate to centre the gel. Remove any excess solution with a paper tissue.
Soak the electrode strips evenly in the appropriate electrode solution (see table above), approximately 3 ml/strip. The surface of the strips should look wet. Remove excess solution with a tissue paper.

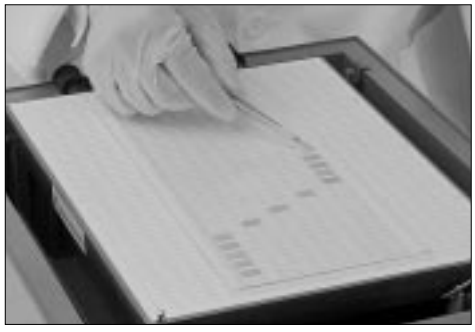


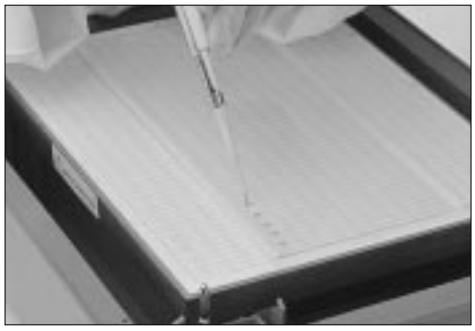
Apply the electrode strips to the long edges of the gel. Make sure the electrode strips are applied with the correct polarity. Use sharp scissors to cut off the strips which protrude beyond the ends of the gel.

Sample application

Some proteins may be pH sensitive (they may precipitate at specific pH values) and better separation may be obtained when the sample is applied at a specific position in the pH gradient. This can be tested by applying the sample at various positions across the pH gradient.
There are three different methods for sample application. The method of choice depends primarily by the sample and the volume to be applied.

1. IEF/SDS applicator strip for 5–20 µl sample volumes. This applicator strip makes sample loading quick and simple, especially when a large number of samples are to be applied. Check that the contact between the gel and the applicator strip is uniform. Leave the applicator strip on the gel during focusing.





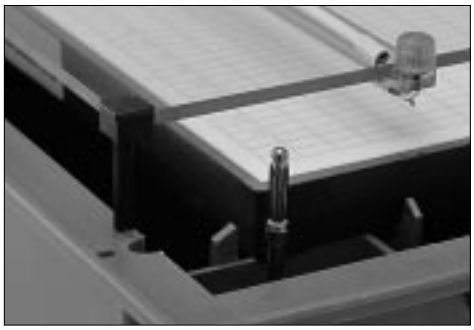
2. Sample application pieces. Place the dry pieces on the Ampholine PAGplate surface at the desired positions(s) in the gradient. Using a micropipette, apply 15–20 µl volumes of sample solution on each piece. To apply larger volumes, use 2 or 3 pieces, stacked or placed end to end, for each sample applied. If smaller volumes are used, trim the paper proportionally before applying it to the gel. Remove the pieces after completing approximately half the total focusing time.

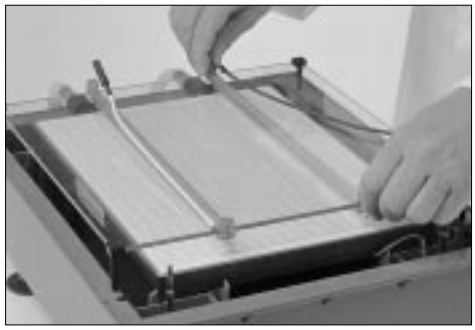
3. Very small sample volumes (e.g. 2 µl) can be applied as droplets directly onto the dry gel surface.

To determine the pH gradient, prepare and apply pI markers according to the instruction sheet included in the pI Marker Kit.

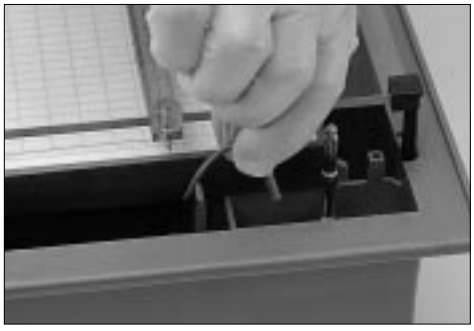
Starting the IEF run

Place the electrode holder in the shallow depressions on the Multiphor II unit.



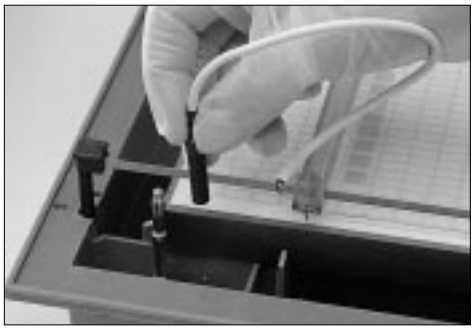


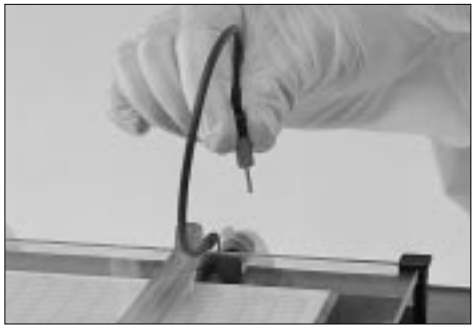
Align the electrodes with the centre of the electrode strips by loosening the clamping nuts and sliding the electrodes to the appropriate position. Retighten the clamping nuts.
Lift the electrode holder slightly and reposition the supporting feet over the deep holes. Lower carefully so that the electrodes rest on the electrode strips. As the anodic pin and socket connectors are different for electrophoresis using buffer chambers and isoelectric focusing, a red bridging cable has been provided.



During isoelectric focusing, the socket on the bridging cable MUST be attached to the pin connector.

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 electrode pin to the socket 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.

Running conditions

Connect Multiphor II to the power supply. Set the running conditions given in the table below. Start the isoelectric focusing by turning on the power supply. Observe the time limits closely. If the isoelectric focusing is run too long, the pH gradient will begin to drift towards the cathode.

Suggested running conditions for Ampholine PAGplate

pH range	Voltage (V)	Current (mA)	Power (W)	Time (h)
3.5–9–5	1 500	50	30	1.5
4.0–6.5	2 000	25	25	2.5
5.5–8.5	1 600	50	25	2.5
4.0–5.0	1 400	50	30	3.0
5.0–6.5	2 000	15	20	3.0

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

Remove the sample application pieces with forceps after approximately half the focusing time has expired. When a sample application strip is used, let it remain on the gel during the focusing, but remove it before placing the gel in the fixing solution.

Ending the run

Turn off the power supply and disconnect Multiphor II from the power supply. Remove the safety lid and electrode holder from the unit. Carefully remove the gel and proceed with staining or preparation for the second dimension as required.

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

Detection methods

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

PhastGel Blue staining

On completion of isoelectric focusing, remove the electrode strips from the gel. Immerse the gel in 250 ml of solution according to the schedule below (See section 4.9 for stock solutions). The staining and destaining steps should be carried out on a shaking table.

Step No.	Solution	Time (min)	Temp (°C)
1	Fixing solution N	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 O	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 (I) and lay 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.

5.5 Isoelectric focusing using CleanGel IEF

CleanGel IEF is a washed and dried polyacrylamide gel optimized for analytical isoelectric focusing.

Prior to use, the dried gel is rehydrated in a solution containing Ampholine or/and Pharmalyte. Additives such as urea and/or detergents can also be added at this stage. CleanGel IEF is rehydrated in a flat tray (GelPool) to a thickness of 0.43 mm. Instructions are supplied with the product.

Sample application

Use the same methods as for Ampholine PAGplate (see Section 4.4).

Running conditions

In principle, CleanGel is run in the same way as Ampholine PAGplate except no electrode strips are used. The electrodes rest directly on the gel surface.

Recommended running conditions for one CleanGel IEF 3-10

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

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

Detection Methods

Use the detection methods described for Ampholine PAGplate (see Section 4.4).

5.6 Isoelectric focusing using Immobiline DryPlate

Immobiline DryPlate is a dried polyacrylamide gel containing an immobilized pH gradient.

Prior to use, the gel is rehydrated in distilled water or 1% Pharmalyte solution to a thickness of 0.5 mm in a special reswelling cassette. Additives such as urea and/or detergents can also be included at this stage. Instructions are supplied with Immobiline DryPlate gels.

Immobiline DryPlate is run with electrode strips wetted in distilled water.

Recommended running conditions for one Immobiline DryPlate

pH gradient	Voltage (V)	Current (mA)	Power (W)	Time (h)
4.0- 7.0	3500	1	5	20
4.2-4.9	3500	2	5	20
4.5-5.4	3500	2	5	20
5.0-6.0	3500	1	5	20
5.6-6.6	3500	1	5	20

5.7 2-D electrophoresis using Immobiline DryStrip and ExcelGel SDS gradient

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

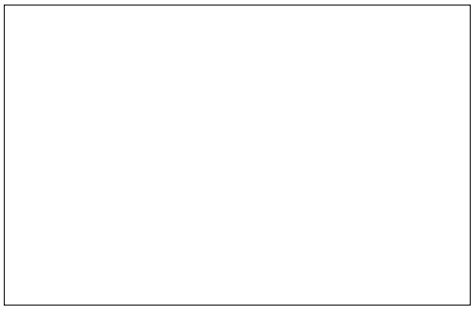


Fig in här, se gamla versionen!

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. The high sample capacity allows the application of up to 100 µl on each Immobiline DryStrip.

First dimension:
Immobiline DryStrip are precast polyacrylamide gels (T=4%, C=3%) containing an immobilized pH gradient, cast on plastic support film.
The strips are delivered in a dehydrated form. After rehydration, the gel thickness of the strips is 0.5 mm.
Immobiline DryStrip gels are available in different sizes (110 x 3 mm and 180 x 3 mm) and pH ranges (4-7 and 3-10). The shapes of the pH gradients are described in the following table.

Immobiline DryStrip pH gradient shapes

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

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

Second dimension:
ExcelGel SDS polyacrylamide gradient gels are 0.5 mm thin precast polyacrylamide gradient gels cast on plastic support film. The gels are available in two different sizes with two different polyacrylamide gradients, consisting of a stacking gel zone followed by the polyacrylamide gradient.

ExcelGel SDS gels

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

ExcelGel SDS buffer strips supply the gel with buffer ions and form a discontinuous buffer system together with the buffer in the gel.

A summary of the 2-D method

Day 1: Rehydration of Immobiline DryStrip

Activities:
- Prepare solutions
- Wash reswelling cassette
- Assemble cassette with dry strips
- Fill Cassette with rehydration solution

Hands-on: 1 Hour

Run Time: Overnight

Day 2: 1st Dimension IEF in Immobiline DryStrip

Activities:
- Prepare samples
- Remove strips from rehydration cassette
- Assemble running tray with strips and electrodes
- Overlay oil and load samples
- Turn on power supply

Hands-On: 1-2 Hours

Run Time: Overnight

Day 3: Second Dimension SDS-PAGE in ExcelGel gradient gels

Activities:
- Prepare equilibration solutions
- Remove strips from 1st dimension
- Equilibrate strips 2x10 minutes
- Lay strips on 2nd dimension with SDS Markers
- Run for approx. 3 hours
- Add 1-2 hours for Coomassie staining and 4 hours for Silver staining.

Hands-On: 2 Hours

Run Time: 3 Hours (Including strip equilibration)

Total Hands-On Time: 3-5 Hours over three days

For more detailed information on sample preparation, running conditions and detection, please refer to the instructions supplied with Immobiline DryStrip Kit.

5.8. Electro-phoretic 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, re-wetting 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:

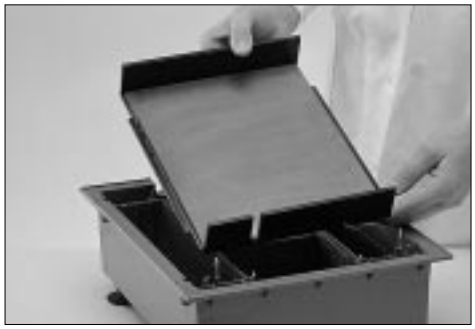
- High binding capacity for the molecules of interest
- Preservation of the biologic activity of the molecules of interest
- No interference with subsequent detection methods
- Chemical and mechanical stability to assay conditions
- Provision of an accurate reflection of the original separation

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.

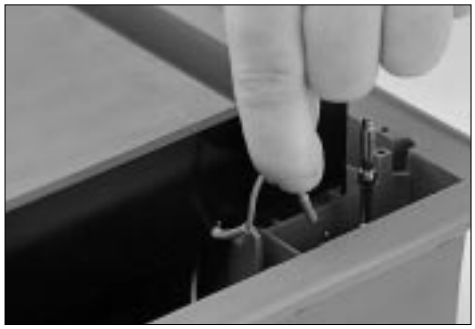
Nitrocellulose membranes can be probed several times. The membranes require no activation and the functional groups have an extended lifetime. Protein patterns on nitrocellulose membranes can be easily detected with most conventional stains, as well as by autoradiographic, immunoenzymatic and fluorescent methods.
Other membranes are: nylon-based membranes, diazobenzyloxymethyl (DBM) and diazophenylthioether (DPT) papers.

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.

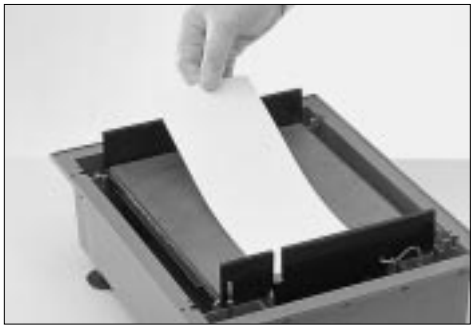


1. Saturate the graphite anode plate with distilled water and remove the excess water with absorbent paper. With the electrode lead to the front of the instrument, fit the lower anode plate onto the buffer tank.



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.

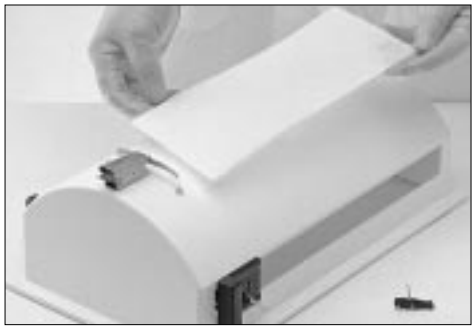


3. Carefully place the filter papers onto the anode electrode. When forming the first transfer sandwich, soak a further layer of three filter papers in anode solution S (see Section 4.9, Stock solutions), using the same method as above. Place them on top of the six filter papers on the anode electrode 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.

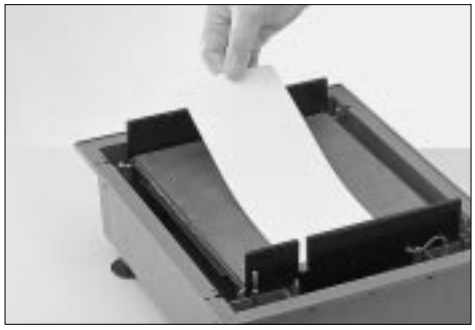
Note: To obtain optimal transfer of molecules from the gel, care should be taken to avoid trapping air bubbles at all stages of the assembly of the transfer sandwiches.

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.

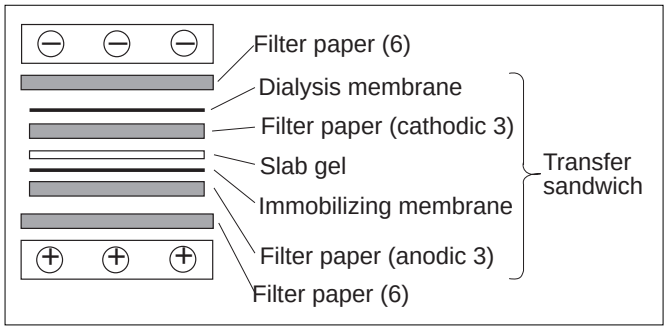
Note: Wear gloves to avoid contamination of the membrane.



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.

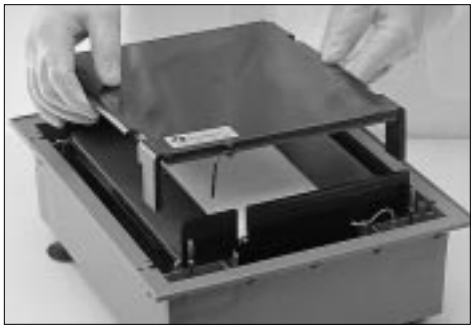


6. Immerse nine filter papers in cathode solution T. Place these filter papers on top of the gel to complete the transfer sandwich.

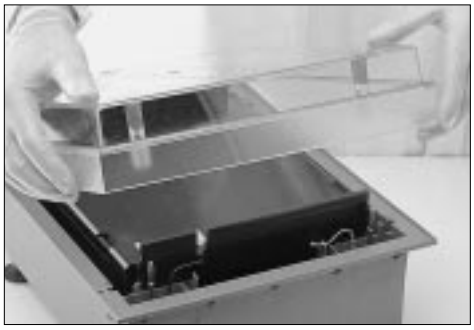


7. Several gels of the same type and size can be transferred simultaneously. Two transfer sandwiches can be put on top of each other. The cellophane dialysis membrane placed between each transfer sandwich prevents cross-contamination between transfer sandwiches.
The maximum gel size is 200 x 250 mm. 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.



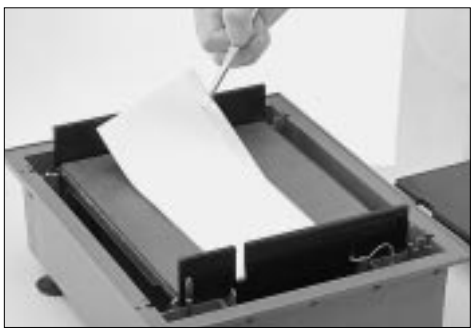
8. Saturate the cathode electrode plate with distilled water and remove any excess with absorbent paper. Place the cathode on top of the transfer sandwich and connect the socket on the black cathode lead to the cathode pin in the Multiphor II base.



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.

Note: 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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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)*

5.9 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.

5.10 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 with Native Buffer Kit pH 8.9

Running conditions for CleanGel anodal electrophoresis with Native Buffer Kit, pH 8.9.

Phase	Voltage (V)	Current (mA)	Power (W)	Time (min)	Temp °C
pre-run	300	18	10	10	10
run	900	50	30	60*	10

* Or until the Bromophenol Blue front reaches the anodic wick.

CleanGel with SDS Buffer Kit pH 8.0

Running conditions for CleanGel anodal electrophoresis with SDS Buffer Kit, pH 8.0.

Phase	Voltage (V)	Current (mA)	Power (W)	Time (min)	Temp °C
pre-run	200	70	40	10	10
run	600	100	40	80*	10

* Or until the Bromophenol Blue front reaches the anodic wick.

CleanGel with Cathodal Native Buffer Kit, pH 5.5.

Running conditions for CleanGel cathodal electrophoresis with Native Buffer Kit, pH 5.5.

Phase	Voltage (V)	Current (mA)	Power (W)	Time (min)	Temp °C
pre-run	500	10	10	10	10
run	1200	28	28	50*	10

* Or until the Pyronin front reaches the cathodic wick.

CleanGel with Native Buffer Kit, pH 4.8.

Running conditions for CleanGel anodal electrophoresis with Native Buffer Kit, pH 4.8.

Phase	Voltage (V)	Current (mA)	Power (W)	Time (min)	Temp °C
pre-run	300	40	20	20	10
run	1300	40	20	50*	10

* Or until the Orange G front reaches the anodic wick.

Ampholine PAGplate for IEF

Running conditions for Ampholine PAGplate.

pH range	Voltage (V)	Current (mA)	Power (W)	Time (h)	Temp °C
3.5–9–5	1 500	50	30	1.5	10
4.0–6.5	2 000	25	25	2.5	10
5.5–8.5	1 600	50	25	2.5	10
4.0–5.0	1 400	50	30	3.0	10
5.0–6.5	2 000	15	20	3.0	10

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

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.

Immobiline DryPlate

Running conditions for one Immobiline DryPlate.

pH gradient	Voltage (V)	Current (mA)	Power (W)	Time (min)	Temp °C
4.0- 7.0	3500	1	5	20	10
4.2-4.9	3500	2	5	20	10
4.5-5.4	3500	2	5	20	10
5.0-6.0	3500	1	5	20	10
5.6-6.6	3500	1	5	20	10

2-D electrophoresis using Immobiline DryStrip and ExcelGel SDS gradient

First dimension

Option 1: EPS 3500 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*

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.

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

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.

6. 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.

7. Technical specifications

Maximum Voltage	3500 Vp-p (+/– 1750 V with reference to Ground)
Maximum Power	100 W
Max pressure cooling plate	0.5 bar
Dimensions	16 x 31 x 40 cm
Environment	+4 – +40°C, 20–95% 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.
Safety standards	This products meets the requirements of the Low Voltage Directive (LVD) 73/23/EEC through the harmonized standards EN61010-1 *Note:* The declaration of conformity is valid for the instrument when it is – used in laboratory locations – used in the same state as it was delivered from Pharmacia Biotech AB, excepting alterations described in the user manual. – used as a “stand alone” unit or connected to other CE labelled Pharmacia Biotech AB instruments or other products as recommended

8. Trouble shooting

WARNING! 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.
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

Symptom	Cause	Remedy
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 flow of water towards the cathode	Occasionally remove the excess 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)
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

Symptom	Cause	Remedy
	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

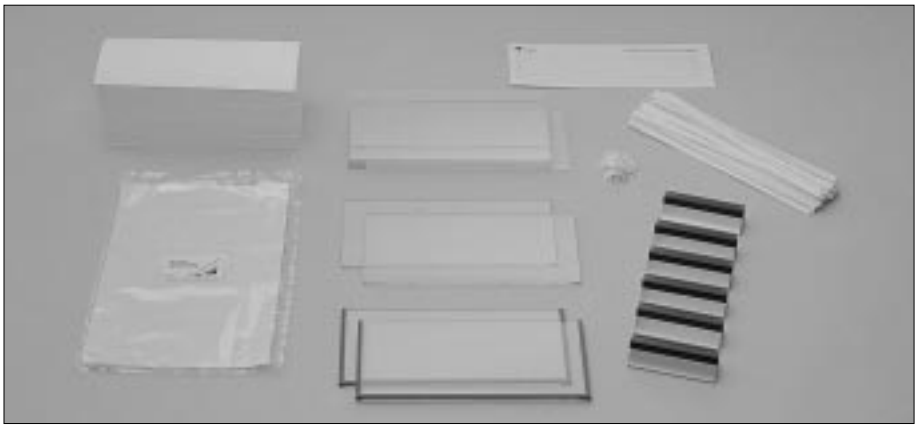
Symptom	Cause	Remedy
Incomplete transfer	Gel concentration too high	Use reversible cross-linkers (e.g. DATD, BAC, DHEBA) and depolymerize gel before transfer. Lower monomer concentration. Convert molecule to smaller form by limited digestion with proteases (for proteins) or with nucleases or acid hydrolysis (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
	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 and “Acrylamide Gel Casting Handbook” - Code No. 18-1102-95.

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 (125x260 mm). Alternatively, casting can be done on GelBond PAGfilm (124x258 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.



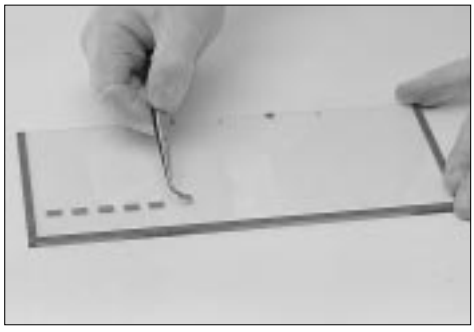
Kit contents - Code No. 18-1102-45

Designation	Code No.
Glass Plate, 125x260, 0.5 mm U-frame (2/pkg)	80-1106-89
Glass Plate, 125x260x1 mm (15/pkg)	80-1106-29
Glass Plate, 125x260x3 mm (2/pkg)	80-1106-99
FlexiClamp (6/pkg)	18-1013-73
IEF Electrode Strip (100/pkg)	18-1004-40
Electrophoresis Wick 104x253 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, 124x258 mm (50/pkg)	80-1129-36
Bind-Silane, 100 ml	17-1330-01
Repel-Silane, 500 ml	17-1331-01
Sample Application Syringe, 15 µl	80-1106-48
SDS Sample Application Strip, 26 samples, 40 µl	18-1002-74
Levelling Set	18-1018-88
Gradient Maker	18-1013-72
Glass Plate, 125x260 mm, 1,0 mm U-frame (2/pkg)	80-1106-91
Glass Plate, 125x260 mm, 2,0 mm U-frame (2/pkg)	80-1106-92
Tape, Dymo 0,25x9 mm, 3 m	80-1129-50
Gel Knife	80-1106-37
Blades (5/pkg)	80-1106-38
Template, 125x260 mm (10/pkg)	80-1129-55
Staining Tray 1, 60x150x300 mm	18-1018-08

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 U-frame, tape, 0,25x9 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 5x3 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. Using the template as a guide, apply the 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.

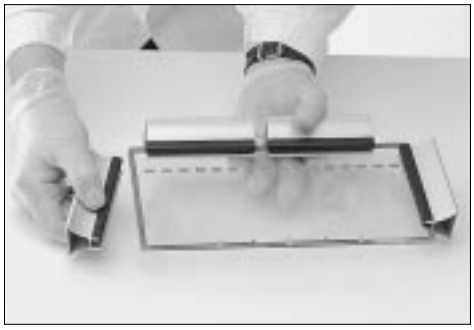
Pour about 2 ml of Repel-Silane onto the glass plate and distribute it evenly with a tissue. Leave it to dry for a few minutes. Rinse the glass plate with distilled water and remove water drops by shaking or wiping lightly with a tissue. Leave the glass plate to dry.

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.

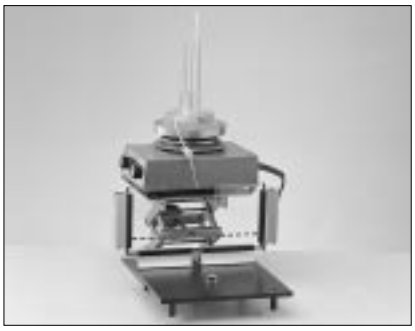
Note: For this operation use gloves and a fume hood.

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 Large Scale SDS and Native PAGE Kit

This kit is used for casting 0.5 mm homogeneous or gradient polyacrylamide gels. The gels are cast only on GelBond PAGfilm. The kit is recommended for casting large format gels for 2-D electrophoresis.

The kit includes sample application strips for applying the sample onto the gel surface. Optional tape and template allow preparation of a slot-former. Optional glass plates with U-frame allow casting of 1,0 and 2,0 mm gels.

Kit contents - Code No. 18-1102-46

Designation	Code No.
Glass Plate, 200x260, 0.5 mm U-frame (2/pkg)	80-1106-87
Glass Plate, 200x260x4 mm (2/pkg)	18-1102-47
FlexiClamp (6/pkg)	18-1013-73
Electrophoresis Wick, 104x253 mm (500/pkg)	80-1129-52
IEF/SDS Sample Application. Strip, 52 samples 5-20 µl (5/pkg)	18-1002-26

Optional accessories

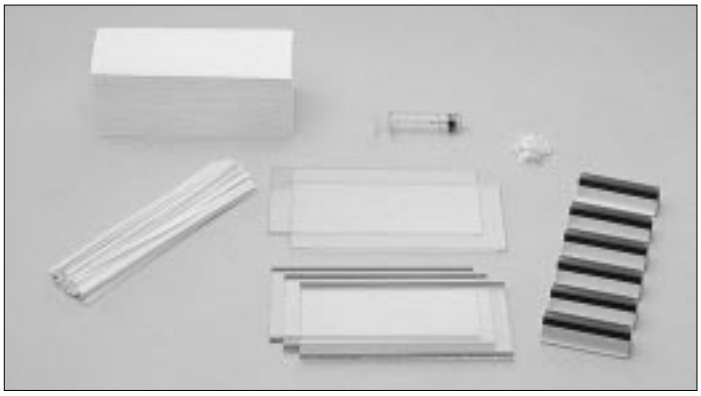
Designation	Code No.
Roller	80-1106-79
GelBond PAGfilm, 203x260 mm (50/pkg)	80-1129-37
Bind-Silane, 100 ml	17-1330-01
Repel-Silane, 500 ml	17-1331-01
Sample Application Syringe, 15 µl	80-1106-48
SDS Sample Application Strip, 26 samples, 40 µl	18-1002-74
Levelling Set	18-1018-88
Gradient Maker	18-1013-72
Glass Plate, 200x260 mm, 1,0 mm U-frame (2/pkg)	80-1106-88
Glass Plate, 200x260 mm, 2,0 mm U-frame (2/pkg)	80-1106-93
Tape, Dymo 0,25x9 mm, 3 m	80-1129-50

Gel Knife	80-1106-37
Blades (5/pkg)	80-1106-38
Template, 125x260 mm (10/pkg)	80-1129-55
Staining Tray 2, 60x260x320 mm	18-1018-09
Cellophane Sheets (50/pkg)	80-1129-38

The 4 mm glass plate is used as a support for GelBond PAGfilm.
The mould comprising a 4 mm glass plate, GelBond PAGfilm and glass plate with U-frame is clamped together using six FlexiClamps.
See Section 7.1 SDS- and Native PAGE, IEF Kit for information on preparation of slot-former, glass plate treatment, gel casting etc.

9.3 Agarose IEF Kit

This kit is used for casting a 0,5 mm agarose gel on GelBond film for IEF with the carrier ampholytes - Ampholine or Pharmalyte.
The sample is applied by soaking IEF Sample Application Pieces in 15-20 µl of sample solution and placing them on the gel surface. Alternatively, smaller volumes, 2-4 µl, can be applied directly onto the gel surface with the aid of the EPH/IEF Sample Application Foil.



Kit contents - Code No. 18-1016-82

Designation	**Code No.**
Glass Plate, 125x260x3 mm (2/pkg)	80-1106-99
Glass Plate, 125x260 mm, 0,5 mm parallel spacer (3/pkg)	80-1106-94
Syringe, 20 ml	56-1135-01
FlexiClamp (6/pkg)	18-1013-73
IEF Electrode Strips (100/pkg)	18-1004-40
IEF Sample Application. Pieces (200/pkg)	80-1129-46
Filter paper - Electrophoresis Wick 104x253 mm (500/pkg)	80-1129-52

Optional accessories

Designation	Code No.
Roller	80-1106-79
GelBond film, 124x258 mm (50/pkg)	80-1129-32
Sample Application Syringe, 15 µl	80-1106-48
EPH/IEF Sample Application Foil, 24 samples 2-4 µl (5/pkg)	80-1129-47
Humidity Chamber 125x260 mm (4/pkg)	18-1016-78

The 3 mm thick glass plate is used as a support for GelBond film. Clean the glass plate with a mild detergent. Rinse it with distilled water and dry.

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 over the glass plate. Beginning at one end, use the roller to apply an 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.

Position the glass plate with spacer on top of the film, leaving 1 cm free at both ends. Hold the plates securely together by placing one FlexiClamp on each of the long sides. Place the mould in a heating cabinet at 70 °C for 10 minutes.

Prepare the agarose solution in a water bath and cool to 75 °C before adding the carrier ampholytes. Take the mould out of the heating cabinet. Slowly fill a 20 ml syringe (with flexible tubing attached) with the molten agarose solution. Avoid drawing air bubbles into the syringe.
Hold the mould at a 20° angle. Inject the solution between the glass plate with spacer and the film. The gel solution will flow into and fill the mould by capillary action. As the mould fills, lower the angle to decrease the flow of the gel solution. When completely filled, place the mould in a horizontal position and allow the agarose solution to gel for 15 minutes at room temperature.

Before opening, remove the excess gel from the ends of the mould with a scalpel. Remove the two FlexiClamps. Stand the mould on one long side.

Grasp the GelBond film and glass plate with spacer with one hand and the 3 mm glass plate with the other. Pull apart gently. Remove the 3 mm glass plate and then the glass plate with spacer from the gel. Place the gel in the Humidity Chamber at 4 °C for at least 1 hour. The gel is now ready for use. Alternatively it can be stored in the Humidity Chamber at 4 °C up to three days.

Before use, lay a sheet of filter paper on the gel surface. Check that there are no air bubbles trapped. Leave for l minute and then carefully peel off the filter paper. The gel is now ready for IEF.

9.4 Immuno-electro-phoresis Kit

This kit is used for casting agarose gels on a 84x94 mm glass plate or GelBond film for immunoelectrophoresis or immunodiffusion. Templates are included for the following techniques

- Grabar & Williams immunoelectrophoresis
- Laurell rocket and fused rocket immunoelectrophoresis
- Crossed and tandem crossed immunoelectrophoresis
- Intermediate gel in fused and crossed immunoelectrophoresis
- Double-Diffusion (Ouchterlony)

Sample wells are punched in a pattern depending on the technique being performed. Gel punchers are available with outer diameters of 4.0, 2.5 and 2.0 mm, corresponding to 10, 5 and 2 µl sample volumes.

Note: GelBond film is recommended for all one-dimensional immunoelectrophoretic techniques and as the second dimension in crossed immunoelectrophoresis. However, for ease of handling the gel in two dimensional techniques, the gel for the first dimension should always be cast on a glass plate.

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Kit contents - Code No. 18-1016-87

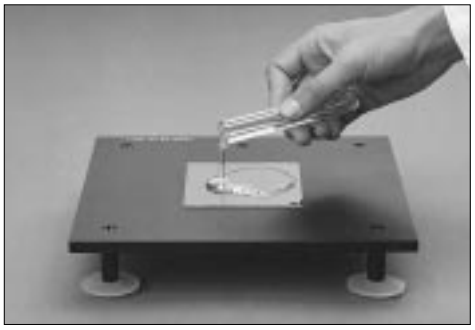
Designation	Code No.
Glass Plate, 84x94x1 mm (50/pkg)	80-1106-69
Plate Holder	18-1019-92
Template, Laurell and fused rocket	80-1109-60
Template, Grabar & Williams	80-1109-61
Template, crossed and tandem crossed	80-1109-62
Template, double and counter diffusion	80-1109-63
Gel Puncher, 2.0 mm	80-1109-69
Gel Puncher, 2.5 mm	80-1109-70
Gel Puncher, 4.0 mm	80-1109-71
Levelling Set	18-1018-88

Gel Knife	80-1106-37
Blade (5/pkg)	80-1106-38
Tape, Dymo 0,25x9 mm, 3 m	80-1129-50
Electrophoresis Wick, 82x130 mm (500/pkg)	80-1129-53
Tygon Tubing, 2.4/4.0 mm, 2 m	80-1006-70
Adaptor Connector	80-1011-88

Optional accessories

Designation	**Code No.**
Roller	80-1106-79
GelBond film 84x94 mm (50/pkg)	80-1129-33
Sample Application Syringe, 15 µl	80-1106-48
Voltage Probe	18-1018-15
Anti-condensation Lid	80-1106-46
Humidity Chamber (4/pkg)	18-1016-78
Staining Tray 1, 60x150x300 mm	18-1018-08

Clean the glass plates. Pre-coat the glass plates with agarose so that the gels adhere during staining and destaining. Apply a thin layer of hot agarose solution (0.l-0.3%) onto each warmed glass plate, distribute it evenly and leave the plate to dry. Lay the prepared glass plate on a levelled table.



Prepare the agarose-buffer gel solution and divide it into 8 or l2 ml aliquots. With one movement, pour each aliquot of molten gel solution onto the center of the gel support. If necessary, use the tip of a pipette or the lip of the test tube to ensure that the gel solution is distributed evenly over the entire gel support. Surface tension should stop the gel solution from overflowing.

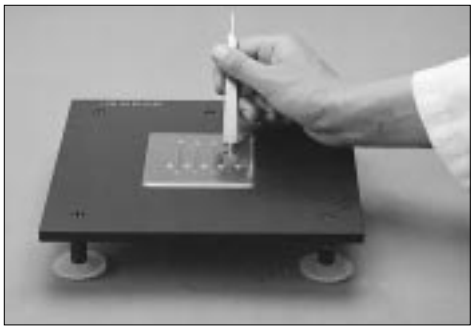
Leave the gels to solidify for l5 minutes at room temperature. For the best results, store the gels in Humidity Chamber at 4 °C for one hour to allow complete solidification. Gels may be stored in this manner for several days.

Select the appropriate Template and Gel Puncher. Slide one end of the Tygon Tubing over the ridged section on the Gel Puncher. Using the Adapter Connector, attach the Tygon Tubing to the vacuum tubing from a vacuum source.

Select the appropriate Template and place it in the Plate Holder. One of the corners of the Plate Holder is rounded to ensure that the Template is in the correct orientation. Slide the gel underneath the Template.

Turn on the vacuum source. Place the Gel Puncher in the appropriate hole in the Template and press it into the gel. The sharp edge of the Gel Puncher will make a clean cut in the gel. Continue to press on the Gel Puncher until the metal sleeve slides down, creating a vacuum which removes the gel plug.

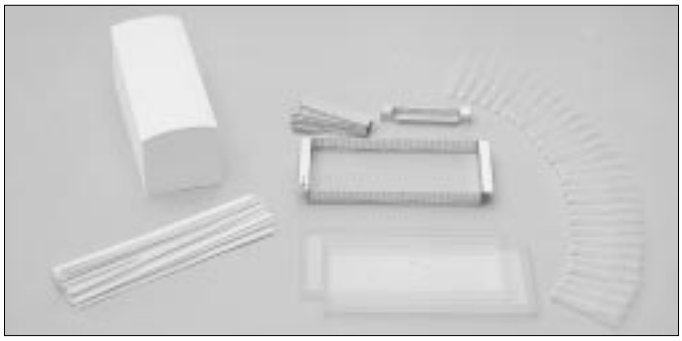
Using the Sample Application Syringe, carefully fill the wells making sure not to damage the gel. For immunoelectrophoretic techniques, begin the electrophoresis as soon as possible to minimize diffusion. Diffusion techniques can be performed in the Humidity Chamber.



7. For Grabar & Williams immunoelectrophoresis, troughs must be formed in the gel after the first dimension electrophoresis. Using the Template, outline the troughs with two parallel cuts through the gel with the Gel Knife, and then remove the middle section using the projection on the handle.

9.5 Preparative IEF Kit

This kit is used for preparative IEF in a granulated gel, Ultrodex. The separation is performed in a tray measuring 125x260x5 mm.
The maximum capacity of the tray is 150 ml of initial Ultrodex gel slurry. Using the sample applicator, sample volumes of 3 ml for each 100 ml of initial Ultrodex gel slurry can be applied.
The sample can be conveniently applied and separated, paper prints taken and the gel bed fractionated. The separated proteins can then be eluted from the granulated gel. For sample volumes larger than 5 ml, the sample can be included in the initial gel slurry.

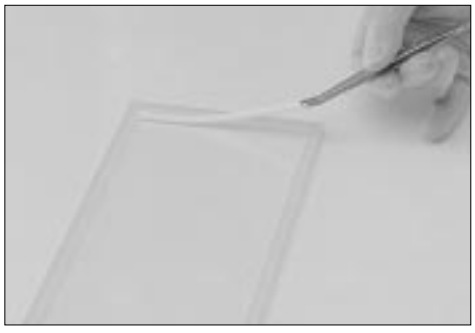


Kit contents - Code No. 10-1018-05

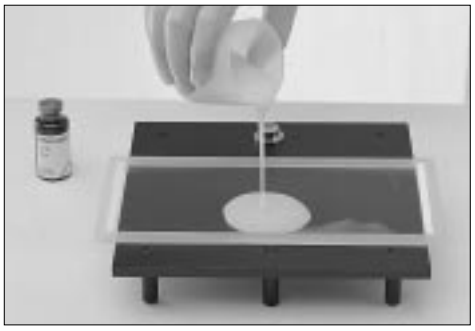
Designation	Code No.
Tray, 125x260 mm, rim 5 mm (2/pkg)	80-1107-03
IEF Electrode Strip (100/pkg)	18-1004-40
Sample Applicator	80-1107-15
PEGG Print Paper - Electrophoresis Wick 104x253 mm (500/pkg)	80-1129-52
Fractionating Grid Frame	80-1317-86
Fractionating Grid Blade (20/pkg)	80-1108-36
PEGG Elution Column (30/pkg)	80-1106-75

Optional accessories

Designation	Code No.
Ultrodex granulated gel, 50 g	80-1130-01
Levelling Set	18-1016-88
Staining Tray 1, 60x150x300 mm	18-1018-08

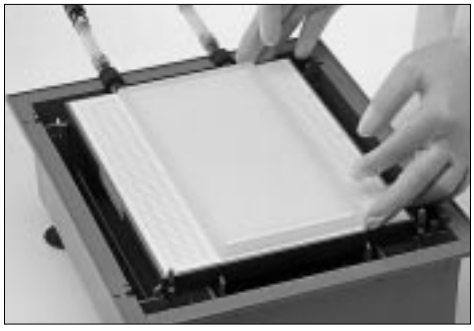


Clean the tray with the silicone rubber rim. Cut IEF Electrode Strips into six 10.5 cm lengths, soak the strips in a 2% Ampholine solution and place a package of three strips at each of the short ends of the tray. Weigh the tray and strips. Place the tray on a table levelled using the Levelling Set.

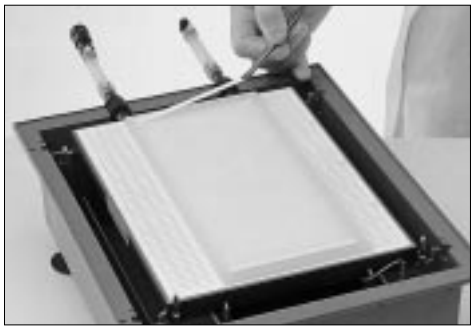


Prepare the initial Ultrodex granulated gel slurry in Ampholine solution in a beaker. Weigh the beaker and its contents. Mix the suspension by gently stirring and immediately pour it into the tray. If the suspension does not spread evenly gently tap the ends of the tray. Weigh the beaker and the remaining of the gel slurry.

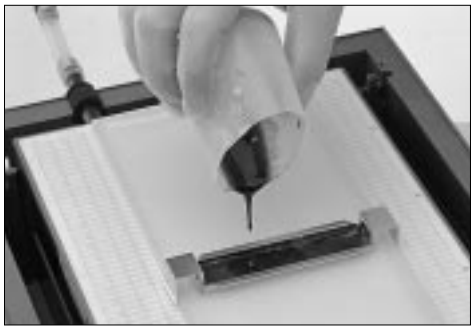
Mount a small fan about 70 cm above the tray and evaporate excess water from the suspension with a gentle stream of air. The speed and distance of the fan should be adjusted so that no ripples are formed on the surface of the suspension.
Calculate the final weight of the prepared gel bed using the evaporation limit shown on the side of the bottle of Ultrodex. Monitor the weight loss until the calculated final weight is reached.



Place the prepared tray containing gel onto the cooling plate (cooled to 10 °C) of Multiphor II. A film of water containing a non-ionic detergent (e.g. 0.1% Triton X-100) is used to aid thermal conductivity.



Place IEF Electrode Strips soaked in the appropriate electrode solution at the anodic and cathodic side, each on top of the strips already placed in the tray. Cut off the protruding parts so that the strips fit exactly into the tray. If desired, prefocus the gel.



To apply the sample with the Sample Applicator, press the applicator through the gel bed at the desired position. Scrape out the gel in the applicator and mix it with the prepared sample in a small beaker.
Pour the gel slurry with sample back into the applicator, remove the applicator and allow the bed to equilibrate hydrostatically for a few minutes. Place the electrode holder with the EPH/IEF electrode onto the Electrode Strips and start the isoelectric focusing.

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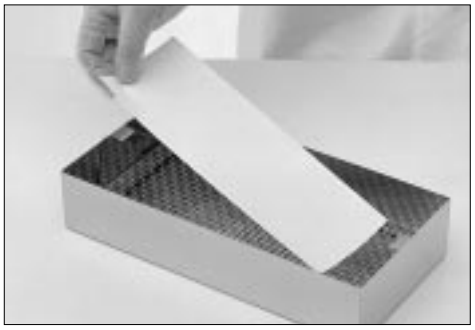
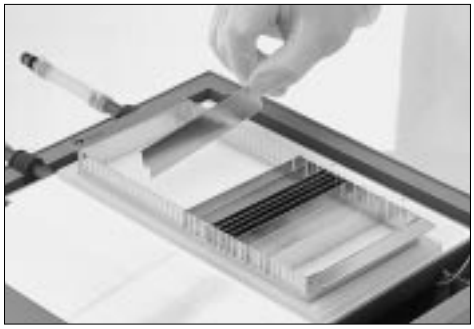
When the separation is complete, take a paper print of the separated proteins. Mark the anode on a sheet of PEGG Print Paper. Carefully roll the paper onto the gel surface. Avoid trapping air bubbles between the paper and gel. Allow the paper to remain in contact with the gel for 30 to 60 seconds. Remove the wet print carefully by peeling. Place the paper print, gel side down, on a glass plate and dry immediately from above with a hot stream of air from a hair dryer.



Stain the entire paper print or, if required, cut it into parallel strips for treatment with different staining techniques (e.g. general protein stain, zymogram stain or glycoprotein stain).
If the staining procedure is time-consuming, reapply the power to the gel to avoid diffusion or insert the fractionating grid frame with blades immediately after the paper print has been taken.



Locate the protein zones of interest by placing the stained print paper directly along the tray. Press the fractionating grid frame into the gel bed. The frame is slightly shorter than the tray which makes it possible to match the slots for the blades with the protein zone pattern.

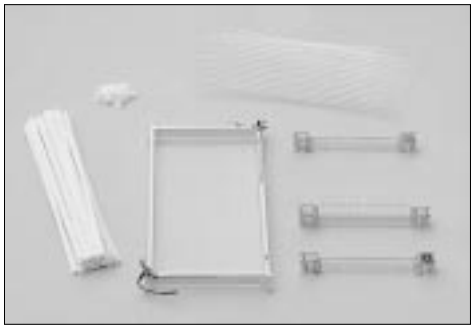
Select the best positions for inserting the blades. To insert a blade in the frame, align the two extensions of a blade with the appropriate slots in the frame. Then, lower the blade evenly. The extensions guide the narrow blade as it cuts through the gel. Repeat this procedure for up to 20 blades.

Using a surface pH electrode, measure the pH gradient with the tray still on the cooling plate.

Using a spatula, transfer each gel section to a PEGG Elution Column. Add just enough of a suitable elution buffer to each column to resuspend the gel, and let the mixture settle until all of the buffer has entered the gel bed. Then, carefully add one gel volume of buffer and continue the elution. The volumes of the individual fractions must be equalized in order to measure the UV or enzymatic profile of the separation after the elution.

9.6 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

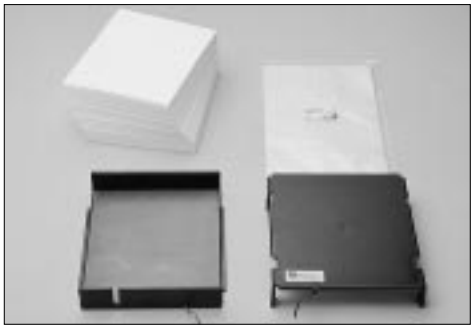
Designation	Code No.
Tray and Electrode Holder	18-1004-31
DryStrip Aligner (4/pkg)	18-1004-34
DryStrip Kit Electrode, cathode	18-1018-67
DryStrip Kit Electrode, anode	18-1018-66
Sample Cup Bar	18-1004-33
Sample Cup (6x10/pkg)	18-1004-35
IEF Electrode Strip (100/pkg)	18-1004-40
IEF Sample Application. Piece (200/pkg)	80-1129-46
Instruction Manual	18-1038-63

Optional accessories

Designation	Code No.
Repel-Silane, 500 ml	17-1331-01
Reswelling Cassette	18-1013-74

9.7 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 200x250 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 125x250 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

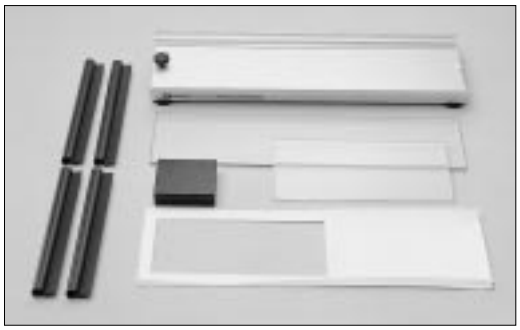
Designation	Code No.
NovaBlot Electrode, cathode	18-1019-86
NovaBlot Electrode, anode	80-1257-87
Electrode Paper NovaBlot, 200x250 mm (500/pkg)	80-1106-19
Cellophane Sheets, 210x320 mm (50/pkg)	80-1129-38

Optional accessories

Designation	Code No.
FilmRemover	18-1013-75
Nitrocellulose 0.20 µm, 150x200 mm (15/pkg)	80-1098-91
Nitrocellulose 0.45 µm, 150x200 mm (15/pkg)	80-1098-90
ProBind 45 NC 0.45 µm, roll 0.2x3.0 m	80-1247-86
GeneBind 45 nylon 0.45µm, roll 0.2x3.0 m	80-1247-87

9.8 UltroMould

UltroMould provides a simple and reliable method for casting ultrathin polyacrylamide gels with a size of 125x258 mm. Using the patented sliding plate technique, gels with a uniform thickness of 0.1, 0.2, 0.3, 0.4 or 0.5 mm can be prepared. The required gel volumes are 5, 10, 15, 20 and 25 ml, respectively. Urea or other gel additives may be included in the gel solution.



Unit contents - Code No. 18-1018-16

Designation	Code No.
Gel Casting Table	80-1115-78
Glass Plate long, 6x125x490 mm	80-1115-80
Glass Plate short, 6x125x270 mm	80-1115-81
Holding Guides 4 pcs	80-1318-35
Weight	80-1115-88
Gel Spacer Set (5 units/pkg)	56-1154-98

Optional accessories

Designation	Code No.
Roller	80-1106-79
GelBond PAGfilm, 124x258 mm (50/pkg)	80-1129-36
Repel-Silane 500 ml	17-1331-01

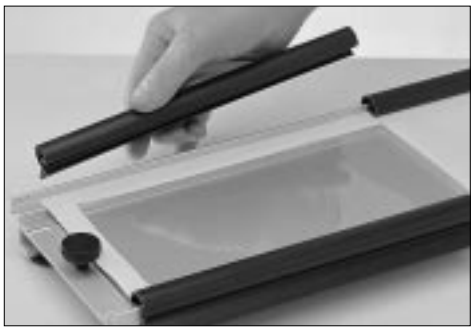
Pour about 2 ml of Repel-Silane onto one side of the clean short glass plate and distribute it evenly over the surface with a tissue. Leave it to dry for a few minutes. Rinse the glass plate with distilled water and remove water droplets by shaking or wiping lightly with a tissue. Leave the plate to air dry. The coating of Repel-Silane will stop the polyacrylamide gel from adhering to the surface of the glass plate.

Place the Gel Casting Table in a fume cupboard. (It is very important to work in a fume cupboard as acrylamide and bis are neurotoxins). Place the clean long glass plate on the lower shelf of the Gel Casting Table so that one end touches the white end screw. Pour a few ml of water close to the opposite end of the plate and lay a GelBond PAGfilm over it with the hydrophilic side up (see instructions supplied with the film). Align the film with the edges of the glass plate. Beginning at one end of the film, use the roller to apply an even pressure over the surface in order to eliminate air bubbles and seal the film to the plate with a minimum amount of water. Remove any excess water with a tissue.

Place the gel spacer of the desired thickness onto the glass plate with the window in the spacer over GelBond PAGfilm.

Note: The thickness of each gel spacer is easily determined. A number of holes relating to the thickness are punched at the end of the spacer, 5 holes = 0.5 mm, 4 holes = 0.4 mm etc.

Hold the spacer in place by screwing the black end screw into the corresponding hole in the Gel Casting Table. The long glass plate should now be held firmly between the black and whites screw at the two ends of the Gel Casting Table.

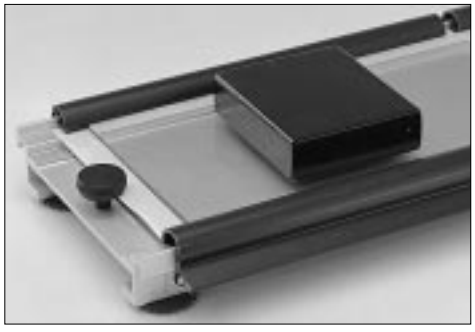


Making sure that the gel spacer is completely flat, attach the four holding guides to the rails along the sides of the Gel Casting Table. The flat side holds the spacer securely in place and guides the movement of the short glass plate when filling the cassette. Level the assembled table using a spirit level. This ensures that the gel solution does not collect on one side, as this may cause leakage.

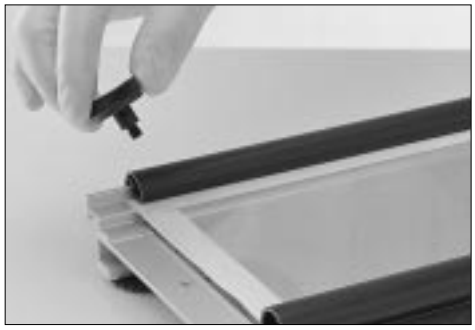
Place the short glass plate (with the Repel-Silane coated side downwards) on the gel spacer, but not overlapping the window. Contact between the Repel-Silane coated plate and GelBond PAGfilm should be avoided as this may affect the film's adhesive properties. Prepare the appropriate volume of gel solution (5, 10, 15, 20 or 25 ml) and transfer it to a beaker.

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

To obtain the correct gel thickness, apply an even and continuous pressure to the short glass plate during the entire casting procedure. With one hand pressed firmly on the plate, slide it so that it overlaps the window by a few mm.
Immediately pour a few ml of gel solution onto the GelBond PAGfilm just in front of the short glass plate. Allow the solution to completely fill the space under the plate. Then, begin to simultaneously pour the gel solution onto the film and slide the short glass plate over it.
If air bubbles appear, draw the plate back just far enough to release them and then continue pouring the gel solution.



When the mould is filled and the short glass plate touches the black end screw, place the weight on the plate to maintain pressure during polymerization. Check that there are no air bubbles in the gel or at the edges. Polymerization will be complete after one hour at room temperature.



Before disassembling the mould, carefully remove any unpolymerized gel solution. Remove the weight, the holding guides and the black end screw. To remove the gel, insert a spatula under the gel spacer and between the film and the long glass plate and twist slightly to separate them. This introduces air between the gel and the short glass plate and allows the plate to be removed without disturbing the gel surface.

Holding the gel spacer at one corner, carefully peel it away. Remove the prepared gel from the Gel Casting Table. The gel may be used immediately or covered with a thin polyester film and stored in a sealed plastic bag at 4 °C for several days.

Wash the Gel Casting Table and accessories with a mild detergent and rinse well with water before using again.

9.9 Gradient Maker

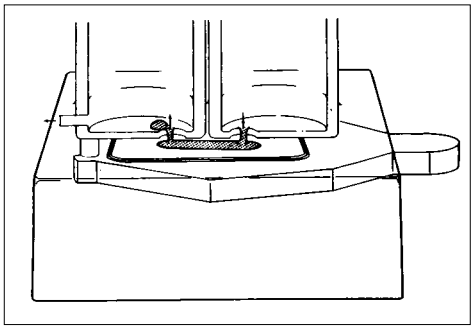
This Gradient Maker comprises two compartments, the mixing chamber, closest to the outlet, and the reservoir.
The Compensating Stick is used for linear gradients and corrects for the density difference between the light and dense solutions and the volume of the magnetic stirrer. The plunger is used to give a fixed volume to the mixing chamber for preparing exponential gradients.

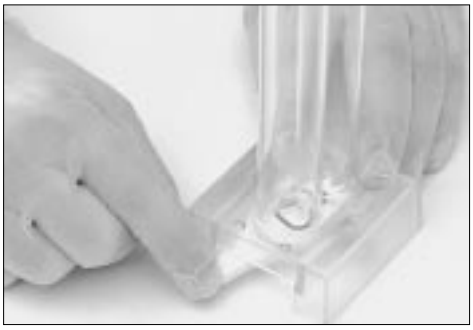
When using the Gradient Maker for the first time, or after it has been disassembled for cleaning, check that it works properly by filling it with water.

Unit contents - Code No. 18-1013-72

Designation	Code No.
Gradient Maker with mixing chamber and reservoir	80-1315-58
Magnetic Stir Bar (2/pkg)	18-1039-88
Plunger	80-1108-68
Compensating Stick	80-1108-67
Silicone Tubing 1.35/3.35 mm, 2 m	80-1065-91
Pinch Cock (2/pkg)	80-1106-41
Polyethylene Tubing, 1/2 mm, 5 m	80-1065-75

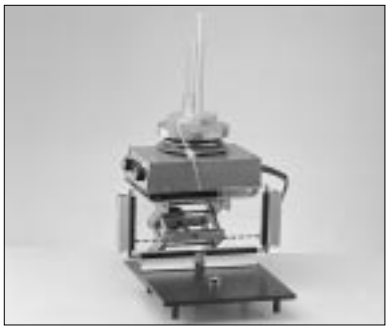
Cut a 5 cm length of the silicone tubing, attach the pinch cock and attach one end to the outlet of the Gradient Maker. At the other end of the tubing attach a 10 cm length of the polyethylene tubing. With the pinch cock open, check that the channel between the mixing chamber and reservoir, the outlet and the tubing are free of water.
To move water to a position where it can easily be removed, create additional air pressure in one of the chambers by covering the opening completely with a thumb or heel of the hand and press down. Alternatively, use the plunger to force air through the channel. Remaining water droplets are removed by using a tissue.
Insert the magnetic stir bar into the mixing chamber.





If the channel is blocked with polymerized gel solution, disassemble and clean the Gradient Maker as described below.

Close the channel by pushing the valve to closed (c) position. Check that the pinch cock is closed.



Prepare the dense and light solutions.

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

Fill the reservoir with the light solution. To fill up the channel with light solution, open the valve carefully until a drop emerges in the mixing chamber and close the valve immediately. If light solution has entered the mixing chamber, use a pipette to transfer it back to the reservoir.
Fill the mixing chamber with the dense solution. Center the mixing chamber over the magnetic stirrer. Check that the gradient maker is levelled. Place the prepared mould on a levelled table and insert the end of the tubing between the glass plates in the centred groove. The tubing should lie against the gel support (glass plate or film), not the glass plate with U-frame. The slot former will otherwise disturb the flow of the solution.

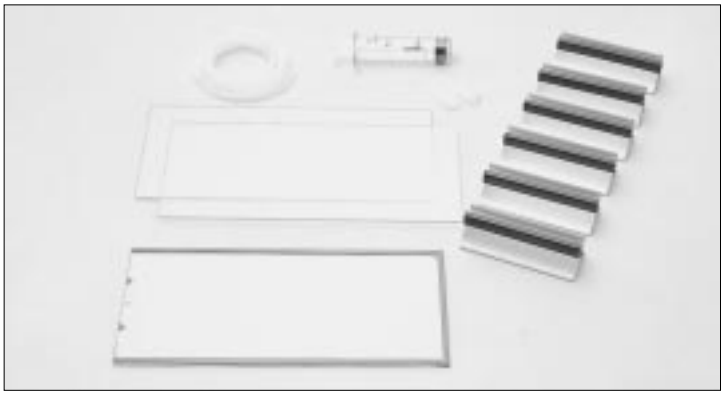
When preparing a linear gradient, place the compensating stick in the reservoir. For good linear gradient formation, the difference in height between the Gradient Maker outlet and the end of the tubing inserted in the mold should be about 5 cm for a total gel volume of 15 ml and 7 cm for a total gel volume of 60 ml.
When preparing an exponential gradient, insert the plunger in the mixing chamber until it is 0.5-1 cm above the level of the solution.
Turn on the magnetic stirrer and check that the bar rotates smoothly, creating a small vortex. Set the stirrer to 250 rpm for 15 ml gels and to 300 rpm for 60 ml gels.

Open the valve between the chambers and then the pinch cock on the outlet tubing to begin gradient formation. Check that the solution begins to flow into the mould.

When the mould has been filled, rinse the Gradient Maker with water to avoid polymerization of remaining gel solution in the channel.
If polymerization has occurred, remove the valve by pulling it straight out. Clean the channel with a few long bristles from a scrubbing brush and rinse it with water. Also, check the outlet and tubing for small pieces of gel. Dry the components with a tissue and reassemble.

9.10 Reswelling Cassette

The Reswelling Cassette is used for reproducible rehydration of precast Immobiline DryPlate and Immobiline DryStrip gels.



Unit contents - Code No. 18-1013-74

Designation	Code No.
Glass Plate, 125x260 mm, 0.5 mm U-frame	80-1106-95
Glass Plate, 3x125x260 mm (2/pkg)	80-1106-99
FlexiClamp (6/pkg)	18-1013-73
Silicone Tubing, 3.0/5.0 mm, 2 m	80-1065-94
Pinch Cock (2/pkg)	80-1106-41
Syringe, 20 ml	56-1135-01

Optional accessories

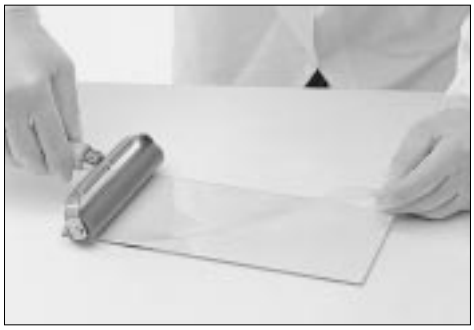
Designation	Code No.
Roller	80-1106-79
Repel-Silane	17-1331-01

Cut a 10 cm length of silicone tubing and make a 2 cm long cut across one end of the tubing.

Note: It is important that the cut is 2 cm long since it may be difficult to get the tubing through the hole in the glass plate with a shorter cut.

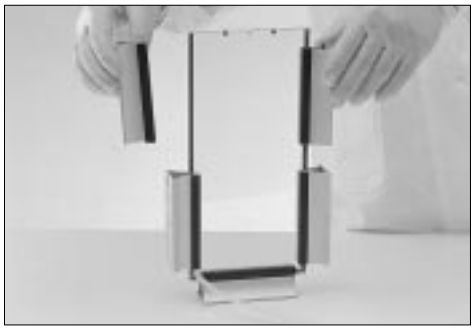
Hold the U-framed glass plate with the spacer facing the user. Push the cut end of the tubing through the hole in the bottom corner of the plate. Pull the tubing through the hole until the other end is flush with the inner face of the plate. Apply the pinch cock to the silicone tubing. If required, the tubing can be permanently fixed using a silicone glue, such as General Electric RTV Silicone Rubber Adhesive Sealant 108.

The following instructions apply to Immobiline DryPlate. If using Immobiline DryStrip, see the instruction manual supplied with Immobiline DryStrip Kit.

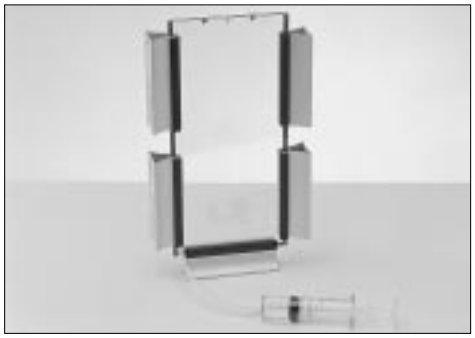


Clean the 3 mm thick glass plate and lightly wet it with a few drops of water. Place the dry gel on the glass plate with the gel side up. Roll the gel flat with the roller to remove air bubbles. Keep water from getting onto the

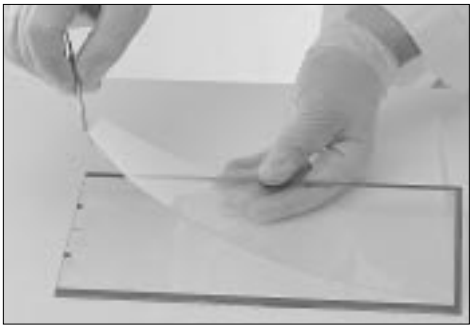
gel surface as this may start rehydration and prevent proper rehydration of the entire gel. Sticky spots on the gel will form lead to bubble formation during addition of rehydrating solution.
To prevent the gel from adhering to the U-framed glass plate, coat the plate with Repel-Silane. Pour about 2 ml of Repel-Silane onto the glass and distribute it evenly with a tissue. Leave it to dry for a few minutes and then rinse it with distilled water. Remove water droplets by shaking or wiping lightly with a tissue. Leave the plate to dry.



Place the U-framed glass plate on top of the dry gel and glass plate. Clamp the cassette together using five FlexiClamps. Stand the assembled Reswelling Cassette vertically.



Fill the syringe with approximately 20 ml of the rehydration solution and remove air bubbles from the syringe. Connect the syringe to the tubing and fill the cassette to the upper edge of the gel. Close the pinch cock.
If minor leakage occurs, the cassette may be leaned at a 45° angle. If major leakage occurs (more than a few drops), remove the rehydration solution, take the cassette apart and start again.



Before opening the cassette, open the pinch cock and allow the remaining rehydration solution to flow out through the tubing by tilting the cassette.

When opening the Reswelling Cassette, first remove the 3 mm thick glass plate and then separate the gel and the U-framed glass plate. Check the rehydrated gel and remove excess rehydration solution with a paper tissue.

9.11 Levelling Set

This set is recommended for casting gradient polyacrylamide gels, agarose gels and when preparing granulated gel beds for preparative IEF.

Unit contents - Code No. 18-1016-88

Designation	Code No.
Levelling Table	80-1316-58
Spirit Level	56-2019-48

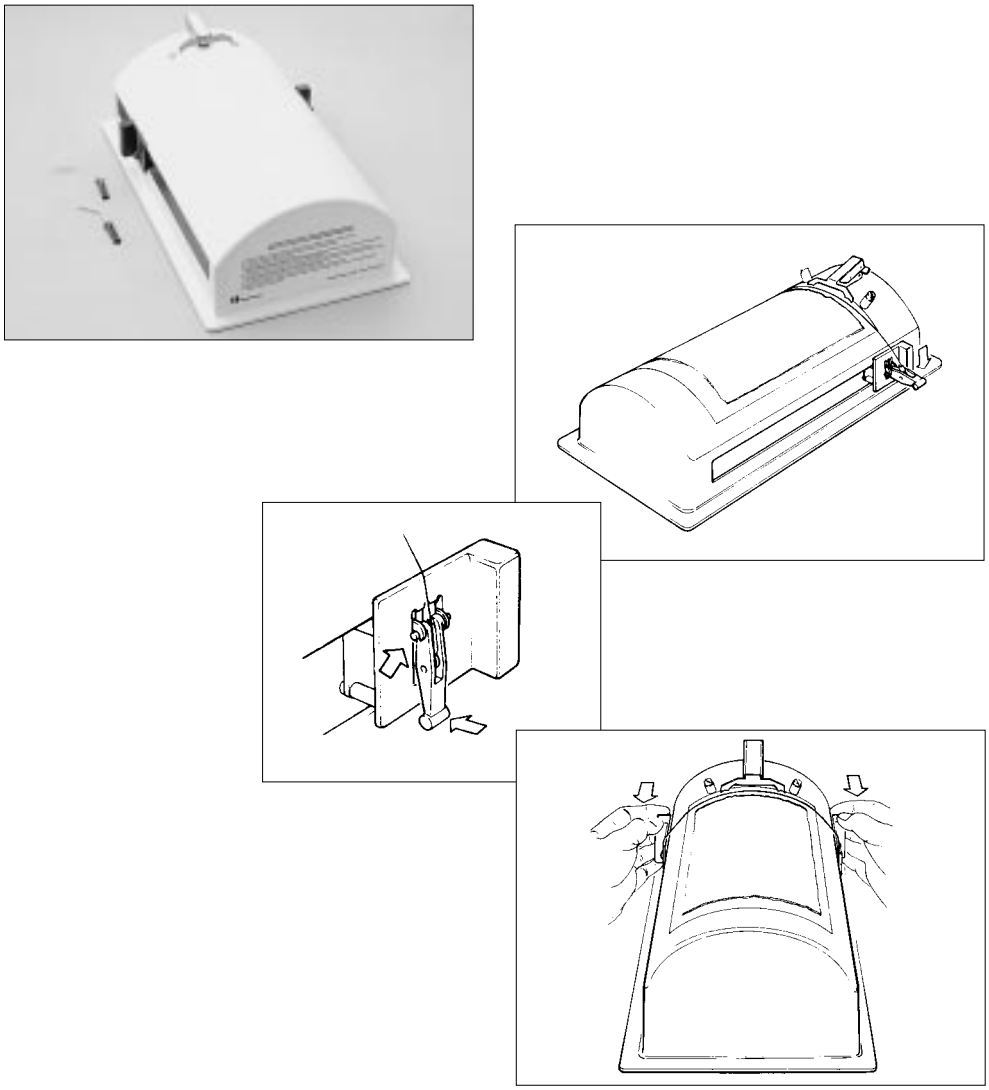
9.12 Humidity Chamber

The Humidity Chamber allows gels to be stored in a humid atmosphere. One chamber holds 3 agarose gels (84x94 mm) or one polyacrylamide or agarose gel (125x260 mm).

Designation	Code No.
Humidity Chamber (4/pkg)	18-1016-78

9.13 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 200x245 mm can be used.



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

Unit contents - 18-1013-75

Designation	Code No.
FilmRemover basic unit	80-1316-21
Lever and Wire Assembly (3/pkg)	18-1013-79
Instruction Manual	80-1316-37
Nitrocellulose 0.20 µm, 150x200 mm (15/pkg)	80-1098-91
Nitrocellulose 0.45 µm, 150x200 mm (15/pkg)	80-1098-90
ProBind 45 NC 0.45 µm, roll 0.2x3.0 m	80-1247-86
GeneBind 45 nylon 0.45µm, roll 0.2x3.0 m	80-1247-87

9.14 Staining Tray 1 and 2

Both trays have lids with a recessed handle allowing several trays to be stacked.
Please note that only Staining Tray 1 has a removable gel holder.

Designation	Code No.
Staining Tray 1, 60x150x300 mm with gel holder	18-1018-08
Staining Tray 2, 60x260x320 mm	18-1018-09

9.15 Roller

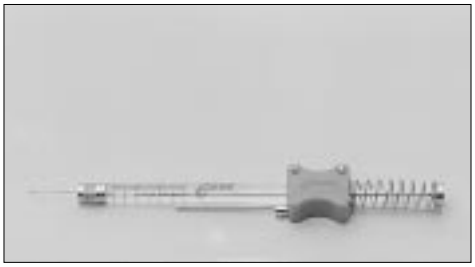
For use when applying plastic support films onto glass plates with an interfacing fluid. The roller is used to provide even pressure over a large area, ensuring adhesion with a minimum amount of fluid and elimination of bubble formation.

Designation	Code No.
Roller	80-1106-79

9.16 Sample application accessories

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

Designation	Code No.
Sample Application Syringe, 15 µl	80-1106-48

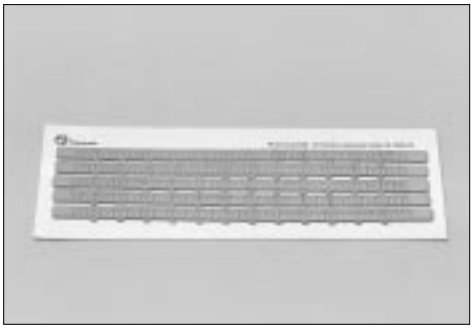


The glass syringe accurately dispenses up to 15 µl of fluid and is supplied with a short needle for convenient sample application.

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

The 5x10 mm sample application piece made of Paratex can be used for sample volumes in the range 15-20 µl.

Designation	Code No.
Immobiline Sample Application Strips 52 samples, 5-20 µl	18-1002-76



This strip is recommended for use in Immobiline isoelectric focusing and is designed to counteract lateral band spreading.

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

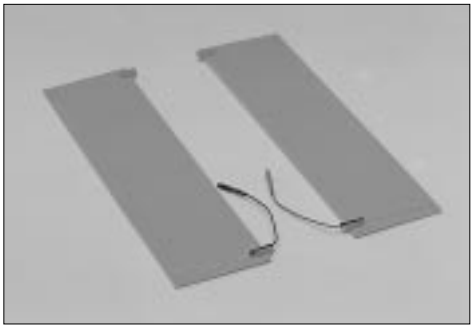
This applicator strip is recommended for use with PAGIEF gels, and SDS gradient gels without preformed slots, e.g. ExcelGel SDS, gradient 8-18.
Up to 52 samples with a sample volume of 5-20 µl can be applied in each well.
The applicator strip is made of flexible silicone and is applied directly on the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

9.17 Miscellaneous

Designation	Code No.
EPH Electrode anode, long	80-1122-20
EPH Electrode cathode, long	80-1122-19



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 Pharmacia Biotech AB 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
Multiphor II NovaBlot Unit	1	18-1016-85
Application kits		
SDS and Native PAGE, IEF Kit	1	18-1102-45
Large Scale SDS and Native PAGE Kit	1	18-1102-46
Immobiline DryStrip Kit for running 1 to 12 Immobiline DryStrip gels (for use with Multiphor II only)	1	18-1004-30
Immunoelectrophoresis Kit for immunoelectrophoretic techniques (Grabar and Williams, Laurell and fused rocket, crossed and tandem crossed and immunodiffusion)	1	18-1016-87
Agarose IEF Kit for casting 0.5x125x260 mm gels	1	18-1016-82
Preparative IEF Kit for preparative IEF in granulated gel, 5x125x260 mm	1	18-1018-05
NovaBlot Kit for electrophoretic transfer	1	18-1016-86
Accessories and replacement parts		
UltroMould for casting 124x258 mm ultrathin (0.1, 0.2, 0.3, 0.4 and 0,5 mm) polyacrylamide gels	1	18-1018-16
Gradient Maker for casting linear or exponential gradient gels, maximum gel volume 100 ml	1	18-1013-72
Reswelling Cassette for Immobiline DryStrip and DryPlate	1	18-1013-74
Levelling Set	1	18-1016-88
FlexiClamps	6	18-1013-73
Humidity Chambers for storage of agarose and polyacrylamide gels, 125x260 mm	4	18-1016-78
FilmRemover for removing plastic gel backing before electrophoretic transfer	1	18-1013-75
Staining Tray 1 with lid and removable gel holder, 60x150x300 mm	1	18-1018-08

Product	Quantity	Code No.
Staining Tray 2 with lid, no gel holder, 60x260x320 mm	1	18-1018-09
Roller	1	80-1106-79
Template, 125x260 mm	10	80-1129-55
PEGG Elution Columns	30	80-1106-75
Gel Knife (no blades)	1	80-1106-37
Blades (for Gel Knife)	5	80-1106-38
Gel Puncher, 2.0 mm	1	80-1109-69
Gel Puncher, 2.5 mm	1	80-1109-70
Gel Puncher, 4.0 mm	1	80-1109-71
Holding Guide (UltroMould)	1	80-1385-35
Gel Spacer (UltroMould), 0.1 mm	5	80-1115-22
Gel Spacer (UltroMould), 0.2 mm	4	80-1115-23
Gel Spacer (UltroMould), 0.3 mm	3	80-1115-24
Gel Spacer (UltroMould), 0.4 mm	2	80-1115-25
Gel Spacer (UltroMould), 0.5 mm	1	80-1115-26
Lever and Wire Assemblies (for Film-Remover)	3	18-1013-79
Levelling Feet	4	18-1026-40
Magnetic Stir Bars	2	18-1039-88
Pinch Cocks	2	80-1106-41
Cooling plates		
Cooling Plate ceramic, 210x270 mm	1	18-1103-46
Grommets	2	80-1106-58
Hose Clamps	10	18-1104-27
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

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		
84x94x1 mm	50	80-1106-69
125x260x3 mm	2	80-1106-99
125x260x1 mm	15	80-1106-29
200x260x4 mm	2	18-1102-47
125x260 mm, 0.5 mm U-frame	2	80-1106-89
125x260 mm, 1.0 mm U-frame	2	80-1106-91
125x260 mm, 2.0 mm U-frame	2	80-1106-92
125x260 mm, 0.5 mm U-frame (Reswelling Cassette)	1	80-1106-95
125x240 mm, 0.5 mm parallel spacer	3	80-1106-94
200x260 mm, 0.5 mm U-frame	2	80-1106-87
200x260 mm, 1.0 mm U-frame	2	80-1106-88
200x260 mm, 2.0 mm U-frame	2	80-1106-93
Tray 125x260 mm, 5 mm silicone rim	2	80-1107-03

Product	Quantity	Code No.
125x270x6 mm (UltroMould)	1	80-1115-81
125x490x6 mm (UltroMould)	1	80-1115-80
Glass plate treatment		
Bind-Silane	100 ml	17-1330-01
Repel-Silane	500 ml	17-1331-01
Gel support		
GelBond PAGfilm,		
124x258 mm	50	80-1129-36
203x260 mm	50	80-1129-37
GelBond Film (agarose),		
84x94 mm	50	80-1129-33
124x258 mm	50	80-1129-32
Paper electrode strip and wicks		
IEF Electrode Strip	100	18-1004-40
EPH Electrode Wick,		
82x130 mm	500	80-1129-53
EPH Electrode Wick		
104x253 mm (also used as PEGG print paper and with agarose IEF)	500	80-1129-52
Electrode Paper NovaBlot,		
200x250 mm	500	80-1106-19
Sample application		
Syringe, 15 µl	1	80-1106-48
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
Immobiline Sample Application Strip,		
52 samples, 5-20 µl	5	18-1002-76
IEF Sample		
Application Pieces	200	80-1129-46
EPH/IEF Sample		
Application Foil (agarose),		
24 samples, 2-4 µl	5	80-1129-47
Sample Cups,		
Immobiline DryStrip Kit	60	18-1004-35
Preserving		
Cellophane Sheets,		
210x320 mm	50	80-1129-38
Mylar Sheets,		
125x260 mm	50	80-1129-39

	Product	Quantity	Code No.
	Membranes, electrophoretic transfer		
	Nitrocellulose, 0.20 µm, 150x200 mm	15	80-1098-91
	Nitrocellulose, 0.45 µm, 150x200 mm	15	80-1098-90
	ProBind 45 NC 0.45 µm, roll 0.2x3.0 m	1	80-1247-86
	GeneBind 45 nylon 0.45 µm, roll 0.2x3.0 m		80-1247-87
	Tapes		
	Dymo, 0.25x9 mm, 3 m	1	80-1129-50
10.2 MultiTemp III	MultiTemp III thermostatic circulator, 100-120 V	1	18-1102-77
	MultiTemp III thermostatic circulator, 220-220 V	1	18-1102-78
	Hose Clamps	10	18-1104-27
	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 Supplies	EPS 3500 XL 35-3500 V, 1-150 mA,	1	19-3500-01
	EPS 3500 35-3500 V, 1-150 mA,	1	19-3500-00
	EPS 600 6-600 V, 1-400 mA	1	19-0600-00
	EPS 200 0-200 V, 1-400 mA,	1	19-0200-00
10.4 Hoefer Automated Gel Stainer	230 V version	1	80-6330-04
	115 V version	1	80-6330-23

10.5 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
CleanGel, 16 S	5	18-1031-57
CleanGel, 25 S	5	18-1031-54
CleanGel, 36 S	5	18-1031-55
CleanGel, 48 S	5	18-1031-56
SDS -Buffer Kit	for 5 gels	18-1031-60
Native-Buffer Kit, pH 5.5	for 5 gels	18-1031-61
Native-Buffer Kit, pH 8.9	for 5 gels	18-1031-62
Native-Buffer Kit, pH 4.8	for 5 gels	18-1031-63
GelPool for gel rehydration	1	18-1031-58
PaperPool for electrode strips	1	18-1031-59
IEF		
CleanGel IEF	5	18-1035-32
Ampholine PAGplate pH 3.5-9.5	5	80-1124-80
Ampholine PAGplate pH 4.0-6.5	5	80-1124-81
Ampholine PAGplate pH 5.5-8.5	5	80-1124-82
Ampholine PAGplate pH 4.0-5.0	5	80-1124-83
Ampholine PAGplate for PGM	5	17-0996-01
Immobiline DryPlate pH 4.0-7.0	3	80-1128-28
Immobiline DryPlate pH 4.2-4.9	3	80-1128-29
Immobiline DryPlate pH 4.5-5.4	3	80-1128-30
Immobiline DryPlate pH 5.0-6.0	3	80-1128-31
Immobiline DryPlate pH 5.6-6.6	3	80-1128-32
2-D First dimension		
110 mm		
Immobiline DryStrip pH 4-7	12	18-1016-60
Immobiline DryStrip pH 3-10	12	18-1016-61
180 mm		
Immobiline DryStrip pH 4-7	12	17-1233-01
Immobiline DryStrip pH 3-10 L	12	17-1234-01
Immobiline DryStrip pH 3-10 NL	12	17-1235-01

Product	Quantity	Code No.
Second dimension		
ExcelGel SDS, gradient 8-18 110x245x0.5 mm	6	80-1255-53
ExcelGel XL SDS, gradient 12-14 180x245x0.5 mm	3	17-1236-01
ExcelGel SDS Buffer Strips anode and cathode	6 each	17-1342-01

10.6 Molecular weight and pI markers

MW range 2.512-16.949, 2 mg	1	80-1129-83
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		17-0472-01
High pI kit pH 5.2-10.3		17-0473-01
Carbamylyte calibration kit		17-0582-01

10.7 Carrier ampholytes

Ampholine		
Ampholine, preblended pH 3.5-9.5	25 ml	80-1127-15
Ampholine, preblended pH 4.0-6.5	25 ml	80-1127-17
Ampholine, preblended pH 5.0-8.0	25 ml	80-1127-19
Ampholine for PGM	25 ml	17-0997-01
Ampholine pH 3.5-10.0	25 ml	80-1125-87
Ampholine pH 3.5-5.0	25 ml	80-1125-89
Ampholine pH 4.0-6.0	25 ml	80-1125-90
Ampholine pH 5.0-7.0	25 ml	80-1125-91
Ampholine pH 5.0-8.0	25 ml	80-1125-92
Ampholine pH 6.0-8.0	25 ml	80-1125-93
Ampholine pH 7.0-9.0	25 ml	80-1125-94
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

Product	Size	Code No.
Immobiline		
Immobiline II pK 3.6	10 ml	80-1255-70
Immobiline II pK 4.6	10 ml	80-1255-71
Immobiline II pK 6.2	10 ml	80-1255-72
Immobiline II pK 7.0	10 ml	80-1255-73
Immobiline II pK 8.5	10 ml	80-1255-74
Immobiline II pK 9.3	10 ml	80-1255-75

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

10.8 Agarose, Ultrodex and PlusOne electro-phoresis chemicals

Agarose and Ultrodex

	Type of agarose					
Application	**H**	**M**	**L**	**NA**	**IEF**	**Prep**
Zone electrophoresis	◎	n	n			
Immunoelectrophoresis (Grabar-Williams)	◎	n	n			
Electroimmunoassay (Laurell rockets)		n				
Crossed immunoelectrophoresis (Clarke-Freeman)		n	n			
Immunodiffusion (Ouchterlony)	◎	n	◎			
Counter immunoelectrophoresis	n					
Nucleic acid electrophoresis			◎	n		n
Cell culture and cloning techniques		n	n			
Isoelectric focusing					n	

n = recommended, ◎ = often used

Agarose M	10 g 100 g	17-0422-01 17-0422-02
Agarose H	10 g 100 g	17-0423-01 17-0423-02
Agarose L	10 g 100 g	17-0424-01 17-0424-02
Agarose IEF	10 g	17-0468-01
Agarose NA	10 g 100 g 1,000 g	17-0554-01 17-0554-02 17-0554-03
Agarose Prep	50 g	80-1130-07
Ultrodex granulated gel	50 g	80-1130-01

PlusOne electrophoresis chemicals

Product	Use	Quantity	Storage	Code No.
Gel casting chemicals				
Acrylamide IEF	IEF, PAGE, Sequencing	250 g	A	17-1300-01
Acrylamide IEF	IEF, PAGE, Sequencing	1000 g	A	17-1300-02
Acrylamide IEF 40% solution	IEF, PAGE	1000 ml	D	17-1301-01
ReadyMix IEF	IEF	41.5 g1	C	17-1309-01
ReadySol IEF T40 C3	IEF	1000 ml	D	17-1310-01
Acrylamide PAGE	PAGE	250 g	A	17-1302-01
Acrylamide PAGE	PAGE	1000 g	A	17-1302-02
Acrylamide PAGE 40% Solution	PAGE	1000 ml	D	17-1303-01
ReadyMix DNA PAGE	PAGE, Sequencing	207 g2	C	17-1307-01
ReadySol DNA PAGE T40 C5	PAGE, Sequencing	1000 ml	D	17-1308-01
N,N-Methylene bis-acrylamide	IEF, PAGE, Sequencing	25 g	C	17-1304-01
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
Boric acid	PAGE, Sequencing	500 g	A	17-1322-01
EDTA, di-sodium salt	PAGE, Sequencing	100 g	A	17-1324-01
Glycine	PAGE, Sequencing	500 g	A	17-1323-01
Barbitone-acetate buffer	Serum electrophoresis	1000 ml	A	17-1333-01
Non-barbitone buffer	Serum electrophoresis	250 ml	A	17-1334-01
Additives and sample treatment				
Urea	IEF, PAGE, Sequencing	500 g	B	17-1319-01
Formamide	IEF, PAGE, Sequencing	250 ml	B	17-1320-01
Dithiothreitol	IEF, SDS-PAGE	0.5 g	F	17-1318-01
Dithiothreitol	IEF, SDS-PAGE	5 g	F	17-1318-02
Mercaptoethanol	IEF, SDS-PAGE	25 ml	B	17-1317-01
Glycerol 87%	IEF, PAGE, Sequencing	1000 ml	A	17-1325-01
Detergents				
Sodium dodecylsulphate	PAGE	100 g	A	17-1313-01
Triton X-100	IEF, PAGE, Sequencing	500 ml	G	17-1315-01
CHAPS	IEF, PAGE, Sequencing	1 g	F	17-1314-01
Tween 20	IEF, PAGE, Sequencing	500 ml	G	17-1316-01
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
Ethidium bromide solution 10 mg/ ml	DNA/RNA detection	10 ml	A	17-1328-01
Bromophenol Blue	IEF, PAGE, Sequencing	10 g	A	17-1329-01
Glass plate treatment				
Repel-Silane	IEF, PAGE, Sequencing	500 ml	C	17-1331-01
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
Amberlite IRN-150L	Purifying solutions	500 g	A	17-1326-01

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.

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