GE Healthcare
Life Sciences

Ettan™ IPGphor™ Cup Loading Manifold

User Manual

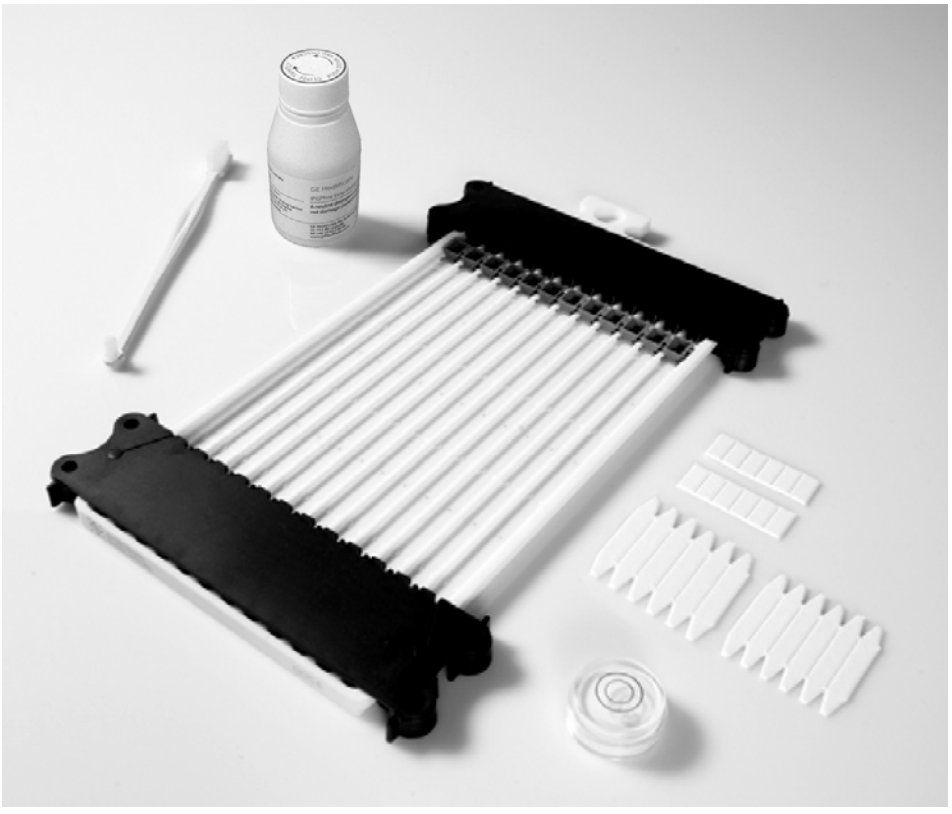


Table of Contents

1 Introduction

Purpose of the User Manual

The User Manual provides you with the instructions needed to use Ettan IPGphor Cup Loading Manifold together with an Ettan IPGphor unit.

In this chapter

1.1 Important user information

Read this before operating Ettan IPGphor unit

All users must read the entire *Ettan IPGphor Operating Instructions* before installing, operating or maintaining the instrument. Always keep the *Operating Instructions* at hand when using Ettan IPGphor.

Do not operate Ettan IPGphor in any other way than described in the user documentation. If you do, you may be exposed to hazards that can lead to personal injury and you may cause damage to the equipment.

Intended use

The Ettan IPGphor Cup Loading Manifold in combination with GE Healthcare's Ettan IPGphor, Ettan IPGphor II and Ettan IPGphor 3 Isoelectric Focusing Units, is suitable for first-dimension isoelectric focusing (IEF) of proteins on Immobiline™ DryStrip immobilized pH gradient (IPG) gel strips 3 mm wide, from 7 cm to 24 cm long.

Ettan IPGphor is intended to be used as a laboratory device for research purposes.

Ettan IPGphor shall not be used in any clinical procedures, or for diagnostic purposes.

Safety notices

This user documentation contains WARNINGS, CAUTIONS and NOTICES concerning the safe use of the product. See definitions below.

Warnings

WARNING

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

Cautions

CAUTION

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

Notices

NOTICE

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

Notes and tips

Note: *A Note is used to indicate information that is important for trouble-free and optimal use of the product.*

Tip: *A tip contains useful information that can improve or optimize your procedures.*

Typographical conventions

Software items are identified in the text by ***bold italic*** text. A colon separates menu levels, thus ***File:Open*** refers to the ***Open*** command in the ***File*** menu. Hardware items are identified in the text by **bold** text (e.g., **Power** switch).

2 Description

About this chapter

This section provides an overview of the Ettan IPGphor Cup Loading Manifold unit.

In this chapter

This chapter contains the following sections:

2.1 Overview

Introduction

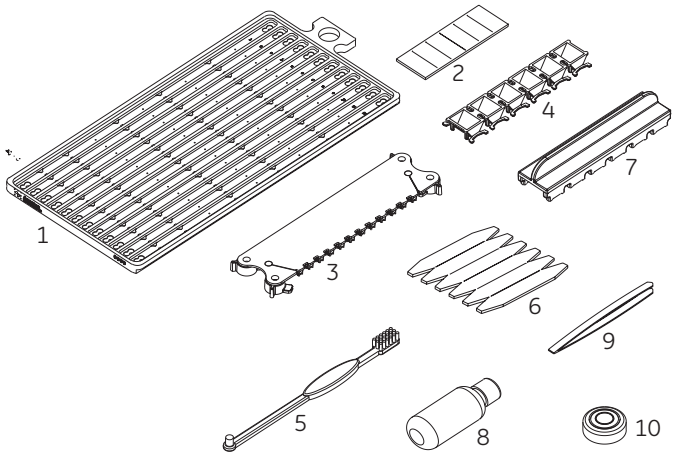
The Ettan IPGphor Cup Loading Manifold, in combination with GE Healthcare's Ettan IPGphor, Ettan IPGphor II and Ettan IPGphor 3 Isoelectric Focusing Units, is suitable for first-dimension isoelectric focusing (IEF) of proteins on Immobiline DryStrip immobilized pH gradient (IPG) gel strips 3 mm wide, from 7 cm to 24 cm long. IEF is performed with the gel side facing up.

Samples up to 150 µl are applied in an open-bottom loading cup. Cup loading has been found to improve protein focusing patterns, particularly on basic IPG strips (pH 6–9 and 6–11). The manifold can accommodate anodic and cathodic cup loading.

Larger volumes of the sample, 375-500 µl, are applied with paper bridge application. Sample volumes up to the volume of the strip (18 cm strip = 340 µl) can be applied by in gel rehydration loading.

Illustration of the Ettan IPGphor Cup Loading Manifold

The illustration below shows the main components of the Ettan IPGphor Cup Loading Manifold:



Part	Function	Part	Function
1	Manifold	6	Pre-cut paper bridges
2	Pre-cut electrode wicks	7	Sample cup insertion tool
3	Electrode assembly	8	Cleaning solution
4	Sample cups	9	Forceps
5	Cleaning brush	10	Spirit level

Manifold

Ceramic Manifold

The manifold (see figure below) is made of aluminum oxide ceramic for efficient heat transfer and temperature control during IEF. Protrusions along the channel inside the manifold align the rehydrated IPG strip, keeping it straight and centered when placed inside the manifold. The manifold has a coating to minimize protein adsorption. Because some cleaning agents can remove this coating, clean the manifold only with the Ettan IPGphor Strip Holder Cleaner.

Note: *The manifold is fragile and should be handled with care.*

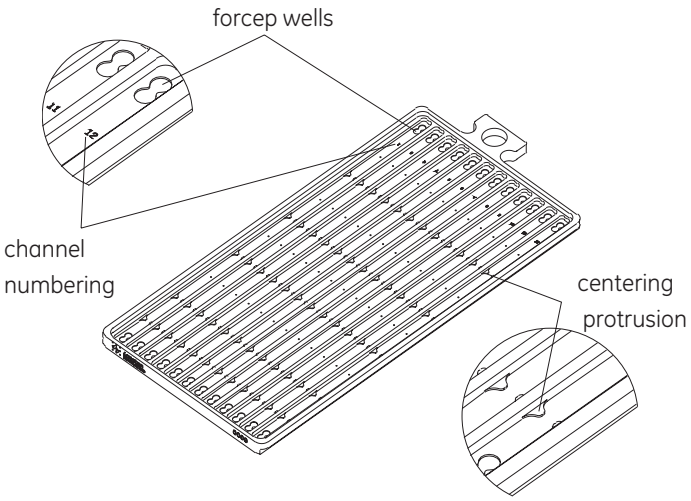
Manifold Light

The manifold light is made of the polymers PC (polycarbonate) and ABS (acrylonitrile-butadiene-styrene).

The manifold light's flat design is important for the heat transfer. If exposed to very high temperatures, the flatness could be affected. Refer to *Manifold Light, on page 26* regarding cleaning temperature.

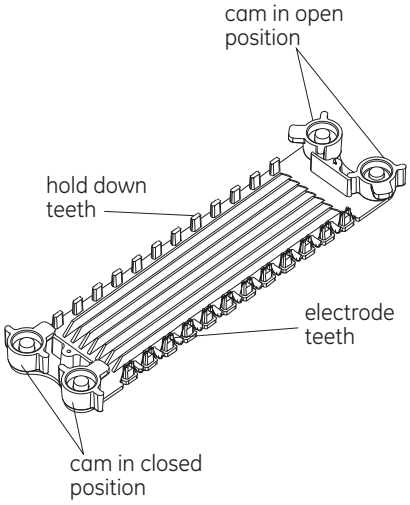
General information

IPG strips from 7 cm to 24 cm long may be used. The unit may also be used for IPG strip equilibration prior to second dimension electrophoresis. Forcep wells are located at the ends of the channels to aid strip removal after electrophoresis.



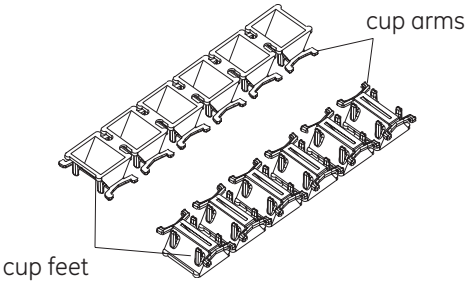
Electrode assembly

The movable electrodes, as shown below, can be placed along the manifold to fit the various length of Immobiline Dry Strip. Electrode pins make electrical contact with the power supply pads on the bed of the Ettan IPGphor. The electrodes have a platinum wire that is strung across the bottom of the electrode teeth to provide electrical contact to the IPG strips. The hold down teeth are found along the opposite length of the electrode. They are used to apply pressure on the paper bridges when the manifold is used for bridge loading. Cams on the sides of the electrode secure the electrodes in place and must be in the closed position during IEF.



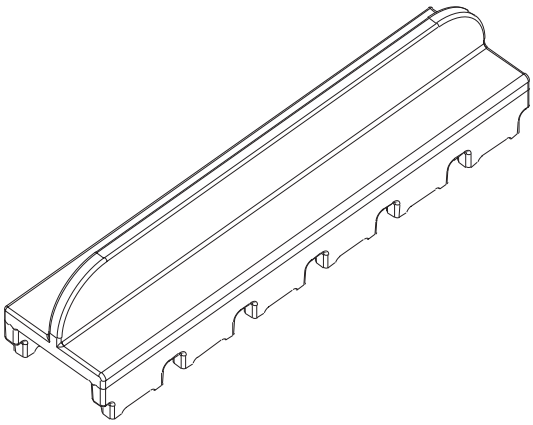
Sample cups

Sample cups, as shown in the figure below, can be placed along the length of the Ettan IPGphor manifold that is not blocked by a centering protrusion. The sample cups are supplied in strips of 6 for easy handling and placement. If fewer than 6 cups are required, they may be easily separated by cutting the thin plastic bridging the strip of cups together. The sample cups can accommodate sample volumes of up to 150 µl. For proper sealing of the cup to the gel, all of the feet of the sample cup must rest on the bottom of the channel and all cup arms must be fully pressed down into the channel.



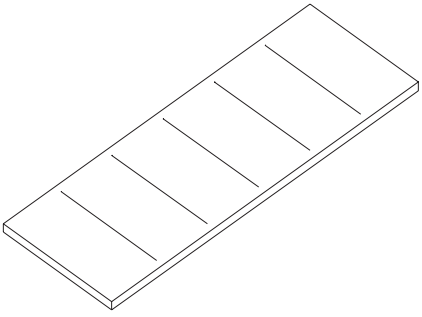
Sample cup insertion tool

The sample cup insertion tool, shown below, is supplied with the manifold kit and refill packages of sample cups. It is used for proper placement and sealing of the cup over the IPG strips.



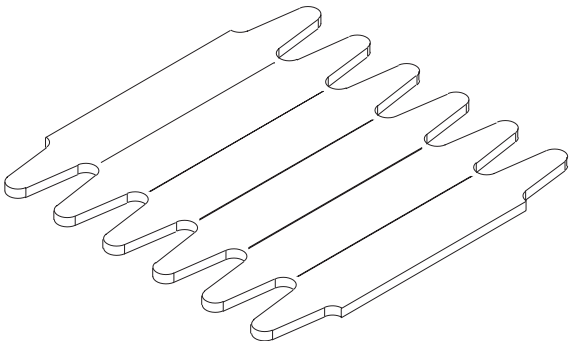
IEF electrode paper wicks

The small, precut rectangular wicks, shown below, must be placed at both the anodal and cathodal ends of the rehydrated IPG strips just under the electrodes. The wicks absorb excess water, salts, and proteins with pIs that lie outside the pH range of the IPG strip.



Paper bridge loading pads

Paper bridge loading pads, as shown below, are used to load large volumes of sample (375–500 µl). The paper bridges are 0.8 cm × 5.0 cm in size with pointed ends. One end of the pad contacts the IPG strip and is held in place by the hold down teeth of the electrode assembly. The other end of the paper bridge loading pad makes contact with a paper wick placed under the platinum wire of the electrode assembly.



2.2 Accessories

Introduction

The following accessory items are not supplied with the unit and need to be ordered separately.

Immobiline DryStrip Cover Fluid

Immobiline DryStrip Cover Fluid is required to ensure that the rehydrated IPG strip gels do not dry out during electrophoresis. Without cover fluid, the strips will dry out, urea will crystallize, and the sample will not focus properly.

Immobiline DryStrip Reswelling Tray

The Immobiline DryStrip Reswelling Tray is required for proper strip rehydration. The channel in the Cup Loading Manifold is too wide to ensure proper absorption of the required volumes of rehydration solution. Two trays are available - one for 7–18 cm strip lengths and a second for 7–24 cm strip lengths.

3 Operation

About this chapter

This section provides instructions on how to operate the Ettan IPGphor Cup Loading Manifold unit.

In this chapter

This chapter contains the following sections:

3.1 Instructions

Rehydrating the IPG strips

Follow the instructions below to rehydrate the IPG strips.

Step	Action
1	Rehydrate the IPG strips with the gel side down in the appropriate volume of rehydration solution using the Immobiline DryStrip Reswelling Tray. Rehydration in the Cup Loading Manifold is not recommended: the channel is too wide to ensure proper rehydration.

Step	Action
2	Follow the instructions included with the Immobiline DryStrips. The table below is provided as a reference.

IPG strip length (cm)	Rehydration volume (µl)
7	125
11	200
13	250
18	340
24	450

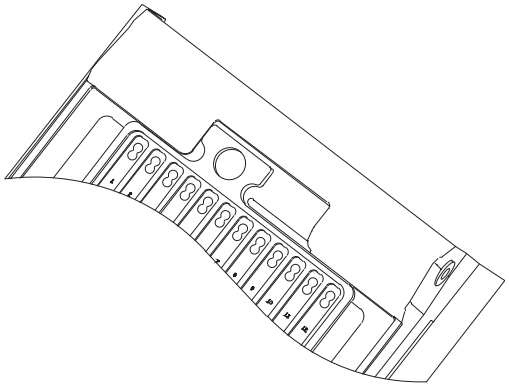
3	Cover the IPG strips with Immobiline DryStrip Cover Fluid and allow the strips to rehydrate overnight (10–20 hours).

Placing the Manifold on the IEF unit

Follow the instructions below to place the manifold on the IEF unit.

Step	Action
1	Clean and dry the IPGphor bed before placing the manifold on the unit. Position the manifold on the IPGphor platform.

Step	Action
2	The small T-shaped protrusion fits into a cutout section of the IPGphor bed near the lid hinge, see figure below. Ensure that the manifold is level by placing the round spirit level on the center of the manifold after it is placed on the Ettan IPGphor unit.



Transferring the IPG strips to the Manifold

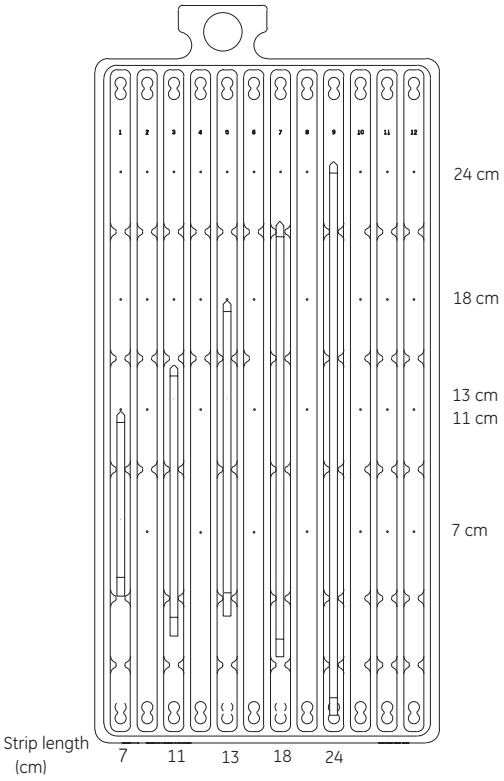
NOTICE

Make sure that the Ettan IPGphor unit is placed on a stable flat surface.

Follow the steps below to transfer the IPG strips to the manifold.

Step	Action
1	Measure out 108 ml of Immobiline DryStrip Cover Fluid (even if fewer than 12 strips will be loaded into the manifold). Add the cover fluid evenly in the 12 manifold channels.

Step	Action
2	Transfer the strips to the Ettan IPGphor Cup Loading Manifold. Place the strips under the cover fluid with the gel surface face up and the anodic (+, pointed) end to the anode of the Manifold. Place the strip so that the electrode assembly accommodates at each end of the strip without hiding the strip, see figure below, to be able to see the brome phenol blue moving away at start.
3	Center the strip down the length of the manifold channel. Protrusions along the sides guide the strip approximately straight, although some manual adjustment of the strip may be necessary, as shown below.



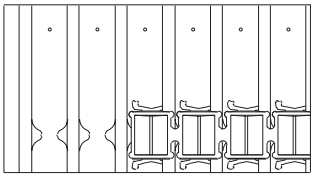
Note:

Using 24 cm long Immobiline Dry Strip and cathodic cup loading is going to be used, the strips should be placed 3–4 cm further up compared to the figure above to make the cathode electrode assembly accommodated at the cathode end, not hiding the sample application place.

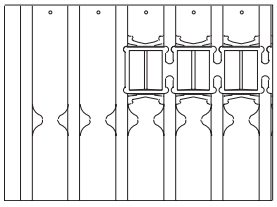
Placing sample cups

Place the sample cups following the instructions below.

Step	Action
1	Place a strip of cups in the appropriate position as shown in the figure below, for example ~1 cm from the end of the gel portion of the IPG strip. Do NOT place the cup with the feet over a center protrusion. Push the cups into the channels with gloved fingers, starting at one end of the strip and working towards the other.
2	Align the insertion tool over the cups and push down to ensure that the feet of the cups are properly seated at the bottom of the channel (wiggle the tool gently while pushing down in order to ensure that the cups are seated as far down as they will go). Take care not to move the cups while removing the insertion tool.
3	Fill the cups with cover fluid to test for proper seating of the cups. Remove the cover fluid after 10 minutes.



incorrect cup position



correct cup position

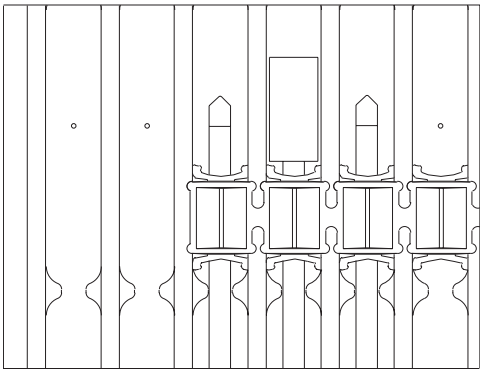
Note:

Cups must not straddle the centering protrusions on the bottom of the channels.

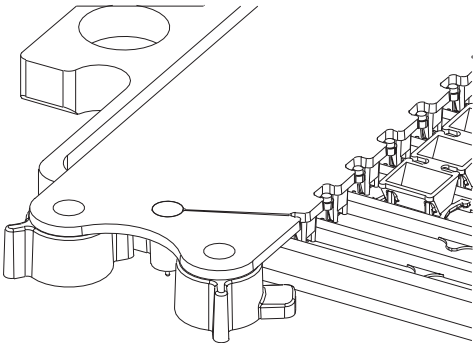
Placing paper wicks

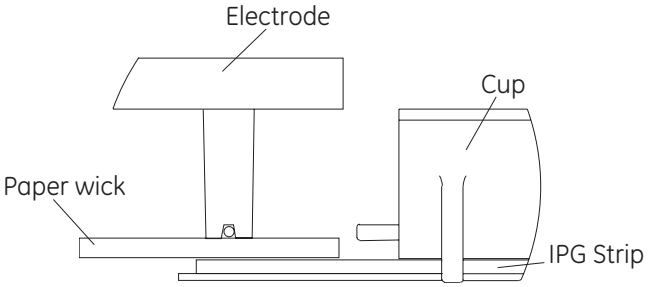
Follow the instructions below to place the paper wicks.

Step	Action
1	Count out the appropriate number of precut paper wicks. Two wicks per strip are required. Separate the wicks from each other. Add 150 µl distilled water to each wick.
2	Place the wicks on the IPG strips such that one end of the wick overlaps the end of the gel on the IPG strip, as shown in figure below. The electrode must contact the wick.



Step	Action
3	With the electrode cams in the open position, place the electrode assembly on top of all the wicks. Swivel the cams into the closed position under the external lip of the tray. The electrodes should not be moved while the cams are in the closed position, see figure below.





Sample application (loading)

Follow the instructions below to load the sample.

Step	Action
1	Briefly centrifuge the protein sample prior to loading to remove insoluble material and particulate matter. These materials will impede sample entry and result in vertical streaks in the second-dimension gel.

Step	Action
2	Load samples into the sample cups. A maximum of 150 µl of sample may be placed in these cups. Check to make sure that there is cover fluid over the samples. When the cups are initially placed on the manifold, cover fluid will flow into the cups as they are seated. When sample is introduced into the cups, the sample will sink to the bottom of the cup and contact the IPG strip. ***Note:*** *For basic IPG strips, superior focusing patterns are generally obtained when the sample cup is placed at the anodic (+) end of the Immobiline Dry Strip.*
3	Close the Ettan IPGphor lid.

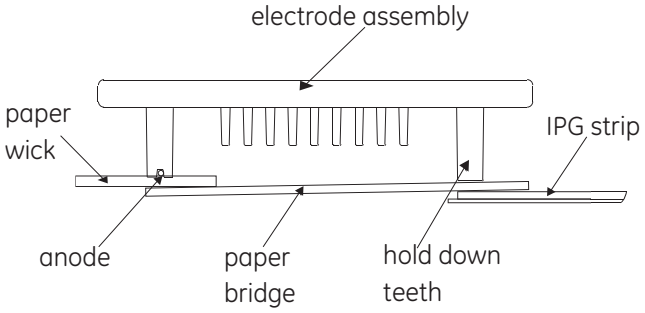
Paper-bridge loading

Large sample volumes and large protein amounts can be applied using paper bridge loading. A paper pad (paper bridge) is soaked with sample and placed between the anodic end of the Immobiline DryStrip gel and the electrode 375– 500 µl sample can be applied using the paper bridge pads.

Solutions containing up to 5 mg protein have been loaded on a 18 cm-long narrow pH range Immobiline DryStrip gel. The rehydrated Immobiline DryStrip gel is first positioned in the bottom of the manifold channel. Then the paper bridge with sample is positioned, followed by a paper wick (see figure below).

With anodic application the anode electrode is positioned as far out as possible in the electrode assembly, while the cathode electrode is positioned close to the end of the Immobiline DryStrip gel to ensure good contact between the paper wick and Immobiline DryStrip gel.

Note: *The anodic application point is an important factor for obtaining good results.*



3.2 Run conditions

Introduction

Program the Ettan IPGphor with the recommended run parameters. Ramping the voltage slowly while the sample is entering the IPG strip will improve results.

Instructions for optimal ramp, voltages and times, or Vhrs (volt-hours) totals are attached to each package of Immobiline Dry Strip.

WARNING

Ettan IPGphor is a high voltage instrument that can cause fatal electrical shock if the safety features are disabled. The safety lid must be securely closed before starting a protocol.

WARNING

The Ettan IPGphor unit is capable of producing thousands of volts. Before operating the unit, read and fully understand the Ettan IPGphor operating instructions and warnings.

Ceramic manifold

Follow the recommended run conditions found in the Instructions attached to each Immobiline DryStrip package.

Manifold Light

Follow the recommended run conditions found in the Instructions attached to each Immobiline DryStrip package except for focusing time.

CAUTION

Do not use any organic solvents during cleaning.

Focusing time

As a guideline, use the lower number of recommended volt-hours to avoid overfocusing. Normally the focusing results does not improve after this time.

WARNING

Check that the high voltage is turned off before opening the safety lid. The **HV ON** indicator on the front panel should not light.

4 Maintenance

Precautions

WARNING

Disconnect power. Always disconnect power from the instrument before performing any maintenance task.

NOTICE

The ceramic strip holders and manifold are very brittle and fragile. Take care in handling and do not subject to impacts.

NOTICE

Avoid scratching the electrode surfaces.

NOTICE

Keep the instrument dry and clean. Wipe regularly with a soft damp tissue and, if necessary, a mild cleaning agent. Let the instrument dry completely before use.

Ceramic Manifold

NOTICE

The ceramic strip holders and manifold trays are very brittle and fragile. Handle carefully: Do not bump or drop.

Gently clean the manifold after each use. Wash the manifold channels with the Ettan IPGphor Strip Holder Cleaner. Rinse thoroughly with water and then deionized water and allow the manifold to air dry. Do not use abrasive cleaning agents or pads. Take care to avoid chipping the manifold.

Manifold Light

Clean the Ettan IPGphor Manifold Light with water, no warmer than 40 °C, and with IPG Strip Holder cleaning solution.

NOTICE

Do not use any organic solvents.

Electrodes

Wash the electrode assemblies with the Ettan IPGphor Strip Holder Cleaner. Rinse thoroughly with water then deionized water and allow to air dry.

NOTICE

Do not use strong acids, bases, ketones, alcohols, or other reagents to clean the covers, sample cups, or electrodes or the parts may be damaged. Cups may be briefly rinsed with ethanol if desired.

5 Troubleshooting

Troubleshooting first-dimension IEF

The following table specifies the troubleshooting situations that you might encounter while using the Ettan IPGphor Isoelectric Focusing System employing the Cup Loading Manifold.

Symptom	Possible cause	Remedy
Current is too low or zero	Electrical continuity is impeded.	Check the external electrode contacts: The electrodes at the bottom of the strip holder (one at each end) must make metal to-metal contact with the appropriate electrode contact area.
		Check the internal electrode contacts: The gel (which becomes visible because of the dye in the rehydration solution) must contact both electrodes in the manifold through the paper wicks and/or paper bridge parts.
		Check that the IPG strip is fully rehydrated along its entire length. Electrical contact at the electrodes is reduced by incomplete rehydration.
		Check that the paper wicks are present, wet and properly positioned.

Symptom	Possible cause	Remedy
Voltage too low or does not reach maximum set value	Ettan IPGphor protocol settings are incorrect for the experiment.	Check that the current limit is properly set.
		Check that the actual number of Immobiline DryStrip gels on the Ettan IPGphor platform is the same as the number of gels entered in the protocol.
	Conductivity/ionic strength is too high.	Prepare the sample to yield a salt concentration less than 10 mM. The recommended IPG Buffer concentration is 0.5%. A maximum of 2% is advisable only if sample solubility is a problem. High conductivity can also arise from the use of poor quality urea or other denaturants. Urea is also prone to decompose to charged breakdown products. Salts and ionic impurities in the sample can raise the conductivity of the strip.
		Shorter length IPG strips (e.g. 7 cm strips) will not reach 8000 V. The distance between the electrodes is shorter so that the voltage gradient (V/cm) required to reach the 50 µA current limit is reached at a lower overall voltage.

Symptom	Possible cause	Remedy
Sample leaks from cup	Incorrect cup placement.	Check that the feet of the cups are resting on the bottom of the manifold channel.
		Check for correct positioning of sample cup arms.
		Check that the feet of the cups are not resting on a centering protrusion in the channel.
	Incorrect strip placement.	Check that the strip is centered inside of the channel.
Sparking or burning in the Immobiline DryStrip gels	Current limit setting is too high.	Do not exceed the maximum recommended setting of 75 µA to 18 and 24 cm strips and 50 µA to 7, 11 and 13 cm strips per Immobiline DryStrip gel.
	Immobiline DryStrip gel is not fully rehydrated.	Ensure that the Immobiline DryStrip gels are rehydrated with a sufficient volume of rehydration solution. Remove any large bubbles trapped under the Immobiline DryStrip gel after placing it on rehydration solution.
	Immobiline DryStrip gels dried out during IEF.	Always apply Immobiline DryStrip Cover Fluid to prevent dehydration of rehydrated Immobiline DryStrip gels.
Immobiline DryStrips turn white and opaque after focusing	Immobiline DryStrip gels dried out during IEF.	Always apply recommended amount of Immobiline DryStrip Cover Fluid to prevent dehydration of rehydrated Immobiline DryStrip gels.

Symptom	Possible cause	Remedy
Immobiline DryStrip Cover Fluid overflows from Cup Loading Manifold	Excess cover fluid added.	Do not add more than the recommended volume. Ensure that the outside rim of the tray does not have any oil on it.

6 Reference information

Technical service and repair

GE Healthcare offers complete technical support for all our products. If you have any questions about how to use this product, please contact your local GE Healthcare representative.

Ordering information

The following table provides you with information about replacement parts and their code numbers.

Replacement part	Code number
Ettan IPGphor Cup Loading Manifold, Ceramic	80-6498-57
Ettan IPGphor Cup Loading Manifold Light	11-0025-80
Sample cups, pack of 20 (6x)	80-6498-95
Paper electrode wicks, pack of 40 (6x)	80-6499-14
Paper bridge pads, pack of 20 (6x)	80-6499-33
Electrode set	80-6498-76
Cleaning brush	80-6505-98
Spirit level	80-6194-19
Forceps SS	80-6506-17

The following table provides you information about accessory parts and code number.

Accessory part	Quantity	Code number
Immobiline DryStrip Reswelling Tray, 7–18 cm	1	80-6371-84
Immobiline DryStrip Reswelling Tray, 7–24 cm	1	80-6465-32
Immobiline DryStrip Cover Fluid	1 liter	17-1335-01
IPGphor Strip Holder Cleaning Solution	1 liter	29-0119-27

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

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

www.gelifesciences.com/2DE

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

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

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

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

imagination at work

11-0034-60 AD 04/2012