

# Ettan<sup>™</sup> IPGphor<sup>™</sup> 3 Operating Instructions

**Original instructions** 





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# 1 Introduction

# About this chapter

This chapter contains information about these Operating Instructions, important user information, and lists of associated documentation.

# In this chapter

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# 1.1 Important user information

## Introduction

This section contains important user information about the product and this manual.

# Read this before operating the product



All users must read the entire *Operating Instructions* before installing, operating, or maintaining the product.

Always keep the Operating Instructions at hand when operating the product.

Do not install, operate, or perform maintenance on the product in any other way than described in the user documentation. If you do, you may be exposed or expose others to hazards that can lead to personal injury and you may cause damage to the equipment.

#### **Intended** use

Ettan<sup>™</sup> IPGphor<sup>™</sup> 3 Isoelectric Focusing System performs the first-dimension isoelectric focusing (IEF) step in 2-dimensional (2-D) electrophoresis. The system comprises Cytiva's Ettan IPGphor 3, combined with separate strip holders or a manifold. Two types of manifolds are available; the ceramic manifold and the light manifold. The IEF separation protocols are programmed, run and controlled from the Ettan IPGphor 3 control panel or from a PC running the Ettan IPGphor 3 control software.

Ettan IPGphor 3 is intended to be used as a laboratory unit for research purposes and must only be used by properly trained personnel.

Ettan IPGphor 3 shall not be used in any clinical procedures, or for diagnostic purposes.

For larger sample volumes the *Ettan IPGphor 3* Cup Loading Manifold facilitates paper bridge loading and in gel rehydration loading, for more information see *Ettan IPGphor Cup Loading Manifold User Manual*.

# 1.2 About this manual

#### Introduction

This section contains information about the purpose and scope of this manual, notes and tips, and typographical conventions.

#### **Purpose of this manual**

This manual provides information needed to install, operate and maintain the product in a safe way.

#### Scope of this manual

The document provides you with the instructions needed to use the Ettan IPGphor 3 Isoelectric Focusing System together with Ettan IPGphor 3.

## **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.

Hardware items are identified in the text by **bold** text.

Text that the user must either type exactly as shown in the manual, or that the software displays as a response (not a regular part of the graphic user interface), is shown by a monospaced typeface (for example, Recipe Information).

*Tip:* The text can include clickable hyperlinks to reference information.

# 1.3 Associated documentation

# Introduction

This section describes associated documentation and how to find related literature that can be downloaded or ordered from Cytiva.

- *Ettan IPGphor Cup Loading Manifold User Manual* contains instructions for firstdimension isoelectric focusing of proteins on IPG strips.
- 2-D Electrophoresis, principles and methods guide
- Instructions attached to each package of Immobiline<sup>™</sup> DryStrip.

# 2 Safety instructions

### About this chapter

This chapter describes safety precautions, labels and symbols that are attached to the instrument. In addition, the chapter describes emergency and recovery procedures.

## In this chapter

Section		Seepage
2.1	Safety precautions	9
2.2	Labels	15
2.3	Emergency procedures	16

# Important



#### WARNING

All users must read and understand the entire contents of this general safety chapter, and the specific safety precautions information in each subsequent chapter of this manual to become aware of the hazards involved.

# 2.1 Safety precautions

## Introduction

Before operating the instrument, you must be aware of the hazards described in the user documentation. Follow the instructions provided to avoid personal injury or damage to the equipment.

The safety precautions in this section are grouped into the following categories:

- General precautions, on page 10
- Personal protection, on page 10
- Using flammable liquids, on page 11
- Maintenance, on page 14
- System operation, on page 12
- Maintenance, on page 14

### Definitions

This user documentation contains safety notices (WARNING, CAUTION, and NOTICE) concerning the safe use of the product. See definitions below.



#### 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.



#### CAUTION

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



#### NOTICE

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

# **General precautions**



#### WARNING

**Risk assessment**. Perform a risk assessment for the process or process environment. Evaluate the effects the use of the product and the operational processes may have on the classification of the hazardous area. The process can cause the hazardous area to increase or the zone classification to change. Implement the risk reduction measures needed, including use of personal protective equipment.



#### WARNING

The customer must make sure that all installation, maintenance, operation and inspection is carried out by qualified personnel who are adequately trained, understand and adhere to local regulations and the operating instructions, and have a thorough knowledge of the product and the entire process.



#### WARNING

Do not operate the product in any other way than as described in the user documentation.



#### WARNING

Only properly trained personnel are allowed to operate and maintain the product.



#### WARNING

**Use only approved parts**. Only spare parts and accessories that are approved or supplied by Cytiva may be used for maintaining or servicing the product.

# **Personal protection**



#### WARNING

Always use appropriate Personal Protective Equipment (PPE) during operation and maintenance of this product.



#### WARNING

**Hazardous substances and biological agents**. When using hazardous chemical or biological agents, take all suitable protective measures, such as wearing protective clothing, glasses and gloves resistant to the substances used. Follow local and national regulations for safe operation and maintenance of this product.



#### WARNING

**Spread of biological agents**. The operator must take all necessary actions to avoid spreading hazardous biological agents. The facility must comply with the national code of practice for biosafety.

# **Using flammable liquids**



#### WARNING

**Fire Hazard**. Before starting the system, make sure that there is no leakage.



#### WARNING

**Ventilation system.** A fume hood or similar ventilation system must be used when working with flammable or noxious substances.

#### Installation



#### WARNING

**Supply voltage.** Before connecting the power cord, make sure that the supply voltage at the wall outlet corresponds to the requirements for the instrument.



#### WARNING

**Protective ground.** The instrument must be connected to a grounded power outlet.



#### WARNING

**Power cord**. Only use power cords with plugs delivered or approved by Cytiva.



#### WARNING

Access to power switch and power cord. The power switch must always be easy to access. The power cord must always be easy to disconnect.



#### NOTICE

Any computer used with the equipment must comply with IEC 60950 or IEC 62368-1 and be installed and used according to the manufacturer's instructions.

# System operation



#### WARNING

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



#### WARNING

Do not use the product if it is not working properly, or if it has suffered any damage including:

- · damage to the power cord or its plug,
- damage caused by dropping the product,
- damage caused by splashing liquid onto the product.



#### WARNING

The instrument must never be used if any safety functions are out of order. Contact your Cytiva service operator for more information.



#### WARNING

Ettan IPGphor 3 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

In a situation where there is a risk of injury, turn off the instrument by switching the mains power switch (located on the rear panel) to position **0**. Malfunctions must be rectified before Ettan IPGphor 3 Isoelectric Focusing System is restarted.



#### WARNING

The vents at the front, back, and bottom of Ettan IPGphor 3 must not be obstructed. Air must be free to circulate for the cooling apparatus to function correctly. There should be a minimum of 10 cm unobstructed space behind the rear panel of the instrument.



#### WARNING

Avoid spilling liquids on the body of the instrument. If large volumes of liquid have penetrated the casing of the instrument and come into contact with the electrical components, switch off the instrument immediately and contact an authorized service technician.



#### CAUTION

Always wear protective gloves when working with rehydration solution.



#### CAUTION

Always wear gloves when handling IPG strips and the equipment that comes in contact with them. This will help minimize protein contamination which can result in artifactual spots in stained 2-D gel patterns.



#### CAUTION

The safety lid must be properly closed before power is applied.

## Maintenance



#### WARNING

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



#### WARNING

Ettan IPGphor 3 covers must not be opened by the user. It contains electrical circuits which can give a lethal electric shock. Service and planned maintenance should be performed by personnel authorized by Cytiva.



#### WARNING

Do not remove the main cover. There are no user serviceable components inside, and you can be exposed to high voltage.



#### WARNING

**Disconnect power.** The power must be disconnected before replacing fuses.



#### CAUTION

Do not use any organic solvents during cleaning.

# 2.2 Labels

### Introduction

This section describes the system label and the safety labels on Ettan IPGphor 3.

## System label

The system label is located on the back of the equipment. The system label identifies the equipment and shows electrical data, regulatory compliance, and warning symbols.

## System label description

Label	Description
Code no.	Instrument Assembly Number
Serial no.	Instrument serial number
Mfg Year	Manufacturing year and month
Voltage	Voltage
Frequency	Frequency
Max Power	Max Power rating
Fuse	Fuse
$\triangle$	<b>Warning!</b> Read the user documentation before using the system. Do not open any covers or replace parts unless specifically stated in the user documentation.

# **Safety label description**

Safety labels (see figure below) are attached to both the light protective cover and the transparent lid of the safety lid. The safety label warns the user of risk for personal injury. Do not proceed until the instructions are clearly understood and all stated conditions are met.



# 2.3 Emergency procedures

## Introduction

This section describes how to perform an emergency shutdown of Ettan IPGphor 3 and the result in the event of power failure.

#### **Emergency shutdown**

Switch off power to the instrument by pressing the Power switch to the **0** position (1). If required, disconnect the mains power cord (2). The run is interrupted immediately.

This will ensure that the power is cut immediately even if an uninterruptible power supply (UPS) is used.



### **Power failure**

In the event of system shutdown due to power failure, emergency stop or process interruption, malfunctions must be rectified before Ettan IPGphor 3 is restarted.

The result of a power failure depends on which unit is affected.

Power failure to	will result in
Ettan IPGphor 3 instru- ment	<ul> <li>Any experiments that are running will stop.</li> <li>The time for the stop will be logged in the instrument.</li> <li>The run is interrupted in an undefined state.</li> </ul>
Computer	<ul> <li>The Ettan IPGphor computer shuts down.</li> <li>Any running experiments will be continued and completed.</li> <li>Scanned images will be saved and uploaded to the computer when computer contact has been reestablished.</li> </ul>

**Note:** A UPS can eliminate data loss during and after a power failure, and allow time for a controlled shut-down of Ettan IPGphor 3.

# Restart after emergency shutdown/ power failure

When the power returns to normal no experiments that were running at the time of emergency shutdown will be continued. Proceed as follows:

Step	Action	
1	Start the instrument, see <i>Turn on power and run diagnostic program, on page 54</i> .	
2	Restart the software. When the system is connected, the log event will be stored in the system log.	
3	If you wish to continue a run, set up and run a new experiment with the appropriate/remaining steps to complete the run in the best way.	

# 3 System description

## **About this chapter**

This section provides a description of Ettan IPGphor 3 Isoelectric Focusing System and an overview of its components.

# In this chapter

Section	1	See page
3.1	Illustrations of Ettan IPGphor 3	19
3.2	Ettan IPGphor 3 instrument	23
3.3	Ettan IPGphor 3 control software	24
3.4	Strip holders	25

# 3.1 Illustrations of Ettan IPGphor 3

# Ettan IPGphor 3 Isoelectric Focusing System



Part	Function
1	Ettan IPGphor 3 control software
2	Ettan IPGphor 3 instrument

**Note:** Ettan IPGphor 3 instrument is used together with separate strip holders or a manifold. Two types of manifolds are available; the ceramic manifold and the light manifold.

*Ettan IPGphor 3 Isoelectric Focusing System also includes Immobiline DryStrip gel strips, which contain an immobilized pH gradient (IPG).* 

For more information about the manifolds, see Ettan IPGphor Cup Loading Manifold User Manual.

# **Ettan IPGphor 3 platform**



Part	Function
1	Safety lid
2	Positive (anode) electrode area
3	Negative (cathode) electrode area
4	Control panel

# **Ettan IPGphor 3 control panel**

Ettan IPGphor 3 is controlled by a 7-key membrane keypad. The instrument status is indicated on the LCD screen.



Part	Function
1	START button
2	STOP button
3	LCD screen (Main screen)
4	Left arrow button
5	Up arrow button
6	Right arrow button
7	Down arrow button
8	<b>EDIT</b> button
9	HV ON indicator lamp

# **Ettan IPGphor 3 rear panel**

Electrical and communication connections are placed on the rear panel of Ettan IPGphor 3.



Part	Function
1	Power switch
2	Fuse holder
3	USB Type-C port for connection to computer
4	Power inlet

# 3.2 Ettan IPGphor 3 instrument

## Platform

The platform accommodates one manifold for the first dimension isoelectric focusing (IEF) run. Up to 12 fixed-length strip holders can be placed on the platform for rehydration and IEF. The embedded electrode areas provide the electrical connection between strip holder or manifold electrodes and an integrated high-voltage DC power supply delivering up to 10 kV. Platform temperature is controlled by Peltier thermoelectric modules.

Both electrode areas are gold-plated copper. Placing a manifold or a strip holder so that it bridges both electrode areas completes the electrical circuit (once the safety lid is in position).

#### **Protective cover**

When analyzing proteins labeled with light sensitive stains like CyDye<sup>™</sup>, a light protective cover is used. The cover is applied by magnetic force but is easy to partially remove for checking the isoelectric focusing procedure, especially the migration of bromophenol-blue at start.



The voltage is cutoff when the safety lid is opened.

# Lid adapter for strip holders

When using Ettan IPGphor 3 standard strip holders a lid adapter must be used to apply the correct amount of pressure to the lids of the Ettan IPGphor 3 strip holders.



# 3.3 Ettan IPGphor 3 control software

#### **Control software**

Ettan IPGphor 3 control software is used to control the Ettan IPGphor 3 Isoelectric Focusing System. The software provides data presentation, data storage, and protocol handling. The PC is connected to Ettan IPGphor 3 by a USB Type-C cable.

Ettan IPGphor 3 control software controls up to four Ettan IPGphor 3 systems at one time, each running a different set of run parameters.

The software allows programming and recommended protocols are generated by providing instrument configuration, IPG strip length and pH gradient.

The software records the run parameters over time and presents data as graphs and log files. Data is saved or can be exported to Microsoft Excel.

Ettan IPGphor 3	-
He Protocol Communication Help Instri Instri2 Instri3 Instri4 ····· Fast Adv ····· II III I : Instri I · Instri I ···· Fast Adv ····· III Run settings & details Session log	Cytiva Ettan IPGphor 3
Strip length pH range Number of strips	
	Jun 07, 2022
Protocol details	
1-	1 - Instr1
0,8-	
0,6-	Step 0/0
0,4	140- Measured Setpoint
	120 4 100- ξ <b>Ο μΑ</b> Ο μΑ
<sup>45</sup> -0,2-	80- <sup>1</sup>
-0,4-	0 Vh
-0,6-	40-
-0,8-	
-1-1 -01 -01	01 0-
Ime Measured current [µA]	Total time Total Vh 00:00 0

# 3.4 Strip holders

## Two models of strip manifolds

IPG strip manifolds are available in two models:

- One produced from a ceramic material providing greater temperature uniformity
- One produced from a light polymeric material for less critical experimentation

Both are designed for analytical analysis using cup application and preparative runs applying sample to the gel during the rehydration step or by paper bridge loading.

#### **Strip holder**

The strip holders are made of aluminum oxide ceramic with electrodes of platinum on titanium. The ceramic is very brittle so the holders are fragile and must be handled with care.

The ceramic is treated with a special coating to minimize protein binding and must be cleaned only with the Ettan IPGphor Cleaning Solution supplied to retain that coating.

Each fixed-length strip holder base holds a single IPG strip throughout rehydration and isoelectric focusing. A matching length holder is available for each size strip. All strips (and holders) used at any one time must be of the same length.

Five strip holder lengths are available, 7, 11, 13, 18, and 24 cm, one for each IPG strip length and a number of pH ranges, several linear and non-linear. Refer to *Section 8.4 Ordering information, on page 108*.



Part	Function
Sample appli- cation wells	Each of the two wells can contain to 15 $\mu$ L of sample (7.5 $\mu$ L on either side of the strip) in excess of the rehydration volume (i.e. 30 $\mu$ L maximum for both wells). Introduce the sample through the wells if it is not included in the rehydration solution.

Part	Function
Cover	The cover is made of acrylic and protects the IPG strip during rehydration. The shape of the underside of the cover ensures electrical continuity during IEF by pressing the strip against the electrode while allowing electrolysis gases to escape. Rehydra- tion and separation progress can be monitored visually through the clear cover.

### Strip holder cover

The cover is made of acrylic and protects the IPG strip during rehydration. The shape of the underside of the cover ensures electrical continuity during IEF by pressing the strip against the electrode while allowing electrolysis gases to escape.

Rehydration and separation progress can be monitored visually through the transparent cover.



Part	Function
1	Pressure block
2	Cover
3	Base
4	Sample application walls

# 4 Installation

# Introduction

This chapter provides information about safe installation of Ettan IPGphor 3 Isoelectric Focusing System.

# In this chapter

Section	on	Seepage
4.1	Site requirements	28
4.2	Unpacking and transport	30
4.3	Setup	31

# 4.1 Site requirements

# **Space requirements**

Parameter	Specification
Minimum bench area (w × d)	480 × 670 mm

# Dimensions and weight of Ettan IPGphor 3

Parameter	Specification
Dimensions (h × w × d)	160 × 278 × 471 mm
Weight	8.3 kg

# **Operating environment**

Parameter	Specification
Operation site	Indooruse
Altitude	Maximum 2000 m
Ambient temperature	15°C to 32°C
Relative humidity	0% to 70% <sup>1</sup>
Pollution degree	2
Noise emission	<70 dB A

<sup>1</sup> High relative humidity may result in condensation forming on the platform surface. Runs conducted at a platform temperature of 20°C and relative humidity less than 70% will generally not cause condensation. Platform temperatures greater than 20°C allow for higher relative humidity without condensation. Environments for which condensation occurs on the platform surface may vary. If condensation occurs, the unit may require conditioned air to reduce temperature and relative humidity.

# **Power requirements**

Parameter	Specification
Mains supply voltage	100-240 V AC ±10%
Frequency	50/60 Hz
Power consumption	230 VA

Parameter	Specification
Transient overvoltages	Overvoltage category II

# **Computer requirements**

Ettan IPGphor 3 control software is compatible with computers that have Microsoft Windows 10 operating system and a USB port.

The computer must have Microsoft Excel (Microsoft Excel for Microsoft 365 or later) installed to be able to export and save run data, graphs and log files.

# 4.2 Unpacking and transport

# Unpacking

Carefully unpack all items delivered and make sure that all items are present by comparing the contents with the packing list. If any part is missing, please contact your local Cytiva sales office.

Inspect all components. If any part appears damaged, please contact the courier immediately.

Please keep all packing material and use if damaged parts need to be returned for repair or replacement.

- Check the equipment for damage before starting assembly and installation.
- Document any damage and contact your local Cytiva.
- Remove straps and packing material and stand equipment upright before starting installation.

# Transport

Before moving the system:

- Disconnect all cables.
- Lift the instrument by the base unit.

# 4.3 Setup

# **Place the instrument**

	WARNING The vents at the front, back, and bottom of Ettan IPGphor 3 must not be obstructed. Air must be free to circulate for the cooling apparatus to function correctly. There should be a minimum of 10 cm unobstructed space behind the rear panel of the instrument.
Step	Action
1	Select a place for Ettan IPGphor 3 where it is possible to access the mains power switch on the rear panel of the instrument.
2	Position the instrument on a flat surface with the spirit level in the center. Turn the leveling feet as necessary to make the instrument level and stable.

#### **Power connection**





#### WARNING

**Power cord**. Only use power cords with plugs delivered or approved by Cytiva.



#### WARNING

**Protective ground.** The instrument must be connected to a grounded power outlet.



#### WARNING

Access to power switch and power cord. The power switch must always be easy to access. The power cord must always be easy to disconnect.

Two power cords are supplied: one cord has a North American style (UL817) 3-pronged plug for 115 V AC power outlets, and the other has a Central European style (CEE7/VII) plug for 230 V AC power outlets.

Step	Action		
1	Select the cord with the appropriate plug for the power outlet in your labora- tory. If neither of these cords are suitable for your power outlets, obtain a detachable cord with an IEC/320/C13 (CEE22/V) outlet.		
2	Plug the connectors into the power cord receptacle on the rear panel and into a properly grounded power outlet.		

# Turn on power and run diagnostic program

Turn on the instrument and run a diagnostic program according to the description in *Section 5.2 Start up the instrument, on page 54.* 

#### **Software installation**

Follow the steps below to install the Ettan IPGphor 3 control software.

Step	Action		
1	Unistall the previous version of Ettan IPGphor 3 control software, if present.		
2	Insert the control software CD or download the control software from the web. Contact your Cytiva representative if you need help to download the control software.		
3	Run Setup.exe. It is found in the <i>Installation</i> folder on the CD or the download folder of the software package downloaded from the web. <i>Result:</i> During the installation the following directories are created:		
	• C\IPGphor 3\Protocols		
	• C\IPGphor 3\Run Data		
4	At the end of the installation, a dialog for adding the protocols fromCytiva t the registry appears. Click the <b>Yes</b> button in this dialog and restart the		

computer.

## **Computer connection**



#### NOTICE

Any computer used with the equipment must comply with IEC 60950 or IEC 62368-1 and be installed and used according to the manufacturer's instructions.



#### NOTICE

This equipment is not intended for use in residential environments and may not provide adequate protection to radio reception in such environments.

Ettan IPGphor 3 is connected to a computer with a USB Type-C cable. The USB Type-C port is placed at the rear of Ettan IPGphor 3. Use the USB Type-C to USB cable that comes with Ettan IPGphor 3 to connect the USB Type-C to the appropriate USB port of the computer.

If multiple Ettan IPGphor 3 units are to be controlled by the Control Software, the user must have enough USB ports on the computer or use a USB expansion box.

Parameter	Settings
Baud rate	9600
Data bits	8
Stop bit	1
Start bit	1
Parity	None
Flow control	None
Max USB cable length	3 m

The table below shows the required settings in the device receiving data.

#### **Driver installation**

Connect Ettan IPGphor 3 to the PC, using a USB Type-C cable.

When installing the driver, refer to the *Driver Installation Guide*. There is no need to download the driver using the link in the *Driver Installation Guide*, as both the driver and the *Driver Installation Guide* are provided on the CD or software package downloaded from the web.

### Set the baud rate

Follow the instructions below to set the baud rate on the LCD screen.

Step	Action		
1	Press the up and down arrows together to access the <b>baud</b> menu.		
2	Use the up or down arrows to set the baud rate.		
3	Use the right arrow to exit. <i>Result:</i> You return to the main menu.		
	Serial Port Setup Baud Rate: 9600 Set Up or Dn Exit>		

- 4
- Confirm that the baud rate on the PC matches with the baud rate on the instrument.



5 Once the driver is installed and the baud rate is matched, the control software can be connected to the instrument and protocols can be run. For further instructions on how to run protocols, see Section 5.4 Run a preprogrammed protocol from the PC, on page 73.

# Assembly

The following parts must be added to Ettan IPGphor 3 instrument before it can be used:

• Strip holder or manifold

- Strips
- Buffer and sample liquids

# **Spare parts and accessories**

For correct up to date information on spare parts and accessories visit:

cytiva.com

# 5 Operation

# Introduction

This chapter provides instructions for the use of Ettan IPGphor 3 Isoelectric Focusing System.

Before starting the instrument, rehydrate the samples according to the method selected, either fixed-length Ettan IPGphor 3 strip holders or use the Ettan IPGphor Cup Loading Manifold.

# In this chapter

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5.4	Run a pre-programmed protocol from the PC	73
# 5.1 Preparations before start

# In this section

Section		See page
5.1.1	Use the strip holders	38
5.1.2	Use the Ettan IPGphor Cup Loading Manifold	49

## Introduction

This section provides instructions on how to prepare the strip holders or the manifolds before starting the system.

# Precautions

0	<b>NOTICE</b> Solutions containing urea may be warmed briefly. Do not heat any solutions containing urea above 30°C to 40°C as isocyanate, a urea degradation product, will carbamylate the proteins in the sample, thus changing their isoelectric points.
	<b>NOTICE</b> All chemicals should be of the highest purity. Double distilled water should be used.

5 Operation 5.1 Preparations before start 5.1.1 Use the strip holders

# 5.1.1 Use the strip holders

## Introduction

Ettan IPGphor 3 Isoelectric Focusing System fixed-length strip holders allow IPG strips to be rehydrated and samples loaded in one step before proceeding automatically to perform the separation. The IPG strips are 3 mm wide and 0.5 mm thick after rehydration.

# Precautions



# Prepare the strip holder(s).



#### NOTICE

Handle the ceramic holders with care, as they are brittle and fragile.

Step	Action
1	Select the strip holder(s) corresponding to the IPG strip length chosen for the experiment (7, 11, 13, 18, or 24 cm).
2	Wash each holder with the strip holder cleaning solution supplied to remove residual protein.
3	Rinse thoroughly with double distilled water.
4	Use a cotton swab or a lint-free tissue to dry the holder or allow it to air-dry. The holder must be completely dry before use.

# Rehydrate the Immobiline DryStrip

Follow the instructions below to rehydrate the Immobiline Drystrip.

Step	Action
1	Prepare the rehydration solution and rehydrate the Immobiline DryStrip in
	the strip holder or in the rehydration tray when using the manifold.

# Step Action 2 Follow the recommended procedures found in the 2-D Electrophoresis, Principles and Methods guide. The IEF protocols are also available in the IPGphor 3 software.

## Pipet the rehydration solution



#### WARNING

Avoid spilling liquids on the body of the instrument. If large volumes of liquid have penetrated the casing of the instrument and come into contact with the electrical components, switch off the instrument immediately and contact an authorized service technician.



#### CAUTION

Always wear gloves when handling IPG strips and the equipment that comes in contact with them. This will help minimize protein contamination which can result in artifactual spots in stained 2-D gel patterns.



#### NOTICE

Use the appropriate rehydration volume for the IPG strip length.

Follow the instructions below to pipet the appropriate volume of rehydration solution into each holder. The correct volume to each strip length is found in the instructions attached to each Immobiline DryStrip package.

Step Ac	tion
---------	------

1

Pipette the solution slowly at a central point in the strip holder channel away from the sample application wells.

# Step Action 2 Remove any larger air bubbles.

# **Position the IPG strip**

Follow the instructions below to postion the IPG strip.

Step	Action
1	Remove the protective cover from the IPG strip.
2	Position it with the gel side down and the pointed (anodic) end of the strip directed toward the pointed end of the strip holder.

3 With the pointed end first, lower the strip onto the solution. To help coat the entire strip, gently lift and lower the strip and slide it back and forth along the surface of the solution, tilting the strip holder slightly as needed to assure complete and even wetting.



- 4
- Lower the cathodic (square) end of the strip into the channel, making sure that the IPG gel is in contact with the strip holder electrodes at each end. The gel can be visually identified once the rehydration solution begins to dye the gel. Be careful not to trap bubbles under the strip.



### Apply cover fluid

Follow the instructions below to apply cover fluid to minimize evaporation and urea crystallization.

Step	Action
1	Pipet the cover fluid dropwise into one end of the strip holder until one half of the strip is covered.
2	Pipet the cover fluid dropwise into the other end of the strip holder, adding fluid until the entire IPG strip is covered.

#### Place the strip holder cover

Position the cover on the holder. Pressure blocks on the underside of the cover ensure that the strip maintains good contact with the electrodes as the gel rehydrates.

#### **Rehydration proceeding**

Rehydration can proceed on the bench top or on the Ettan IPGphor 3 platform. Ensure that the holder is on a level surface. A minimum of 10 h is required for rehydration, overnight is recommended.

Alternatively, the rehydration period can be programmed as the first step of an Ettan IPGphor 3 protocol. This is especially convenient if temperature control during rehydration is a concern, or if a low voltage is applied during rehydration.

# Sample rehydration under voltage (optional)

Follow the instructions below to perform rehydration under voltage. If desired, the sample can be absorbed by the IPG strip under low voltage. This may improve the uptake of high molecular weight proteins.

Step	Action
1	Set the rehydration time to 0:00 and program <b>Step 1</b> of the protocol for low voltage (30 to 100 V) for 10 to 12 h, see <i>Edit step or gradient, on page 63</i>
2	Program additional steps as desired to achieve complete focusing.

# Apply electrode pads prior to IEF (optional)

Under certain conditions, such as prolonged focusing, water may migrate toward one end of the strip, causing the other end to begin drying out. This effect can be minimized by placing paper electrode pads between the IPG strip and each strip holder electrode just before IEF. Electrode pads may also absorb ions that would otherwise accumulate at the ends of the IPG strip and possibly interfere with the separation.

Follow the instructions below to apply electrode pads prior to IEF.

Step	Action
1	Cut two 3 mm wide electrode pads from a paper IEF electrode strip (18100440).
2	Position on a clean, flat surface such as a glass plate and soak with deionized water. Remove excess water by blotting with tissue paper.
	Note:
	Electrode pads must be damp, not saturated or dripping.
3	Lift one end of the rehydrated IPG strip using forceps or tweezers. Position an electrode pad over the electrode, then lower the strip back in place.
4	Repeat at the other end.

# Apply sample after rehydration (optional)

If the sample was not applied by inclusion in the rehydration solution, it can be applied immediately prior to IEF.

Follow the instructions below to apply electrode pads prior to IEF.

**Note:** The IPG strip backing is impermeable; do not apply the sample to the back of the strip.

Step	Action
1	Prepare the sample in a solution similar in composition to the rehydration solution used.
2	Pipet the sample into either or both of the lateral wells at either end of the strip holder. Introduce the sample below the cover fluid.
	Up to 7.5 $\mu L$ of sample solution can be added to each side (i.e. 15 $\mu L$ per well or maximum 30 $\mu L$ if both sides of both wells are used).



## **Position the strip holders**

Ettan IPGphor 3 platform has two electrode areas:

- The larger area (3) is the positive electrode (anode)
- The smaller area (4) is the negative electrode (cathode).

Position the strip holder on the platform as shown below: Electrode contacts underneath, one in each electrode area (1). The pointed end of the strip holder (2) is over the anode (pointing to the back of the unit) and the blunt end is over the cathode. Guidemarks along the sides of the platform show approximate positioning for each strip holder size (7, 11, 13, 18, and 24 cm).



#### 5 Operation 5.1 Preparations before start 5.1.1 Use the strip holders

# Using safety lid



#### NOTICE

During isoelectric focusing, do not lean on the safety lid, do not apply excess pressure or uneven weight to the lid, and do not place any items on the lid. Such pressure could cause arcing between the strip holder electrodes and the electrode areas, damaging the instrument.

When using the Ettan IPGphor standard strip holders, lid adapters must be used to apply the correct amount of pressure to the lids of the IPGphor strip holders. The pressure keeps the IPG strip in contact with the IPG strip holder electrodes.



5 Operation 5.1 Preparations before start 5.1.1 Use the strip holders

## **Close the safety lid**

$\underline{\land}$	<b>CAUTION</b> The safety lid must be properly closed before power is applied.
---------------------	---

#### NOTICE

Exceeding the recommended current limit of  $50 \,\mu\text{A}$  per IPG strip can cause the strip to burn and may damage the instrument.

Follow the instructions below to ensure good electrical contact when using the fixed length strip holder:

#### Step Action

1 Apply two lid adapters (2) right across the lids of the strip holders, one over the anodic area and the other over the cathodic area.



- 2 Use two or more strip holders (3) and place them wide apart to ensure that the lid adapters are only hanging on one strip holder and not pressing down on the other.
- 3 Carefully close the safety lid (1) by applying light downwards pressure, ensure the mechanism locks the lid.

4 To inspect the run the light protective cover can be lifted without stopping the run.



5 To open the safety lid press down the lid and the lock is released and the run is stopped. Close the lid and the run continues.

# 5.1.2 Use the Ettan IPGphor Cup Loading Manifold

# Precautions



# Introduction

For more detailed information, please refer to *Ettan IPGphor Cup Loading Manifold User Manual*.

# Rehydrate Immobiline DryStrip in the IPGbox

Rehydrate the IPG strips with the gel side down in the appropriate volume of rehydration solution, with or without sample, using the IPGbox.



Rehydration in the Cup Loading Manifold is not recommended, the channel is too wide to ensure proper rehydration.

Also, follow the instructions included with the Immobiline DryStrips or the IPGbox. Close the lid of the IPGbox and allow the strips to rehydrate overnight (10 to 20 hs).

# Position the manifold on Ettan IPGphor 3

Follow the instructions below to position the manifold on Ettan IPGphor 3.

Step	Action
1	Clean and dry the Ettan IPGphor 3 platform before placing the manifold on the system.

2 Position the manifold on the Ettan IPGphor 3 platform. The small T-shaped protrusion fits into a cutout section of the Ettan IPGphor 3 platform near the lid hinge.



- 3 Ensure that the manifold is level by placing the round spirit level on the center of the manifold tray after it is placed on Ettan IPGphor 3.
- 4 Measure out 108 mL of cover fluid (even if fewer than 12 strips will be loaded into the manifold). Add the cover fluid evenly in the 12 manifold channels.

# Position the IPG strips in the manifold

Follow the instructions below to position the IPG strips in the manifold.

StepAction1Transfer the strips to the Ettan IPGphor Cup Loading Manifold.1Image: Comparison of the strips to the Ettan IPGphor Cup Loading Manifold.2Position the strips under the cover fluid face up in the tray with the anodic (+, pointed) end of the IPG strip pointing at the anode of Ettan IPGphor 3.3Center 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. See also the Ettan IPGphor Cup Loading Manifold User Manual.

# **Place sample cups**

2

Step	Action
1	Position a strip of sample cups in the appropriate position, 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.

channel.



Follow the instructions below to place the sample cups.

Use the insertion tool, wiggle the tool gently while pushing it down.

Ensure that the feet of the cups are properly seated at the bottom of the

- 3 Fill the cups with cover fluid to test for proper seating of the cups.
- 4 Replace the cover fluid after 10 minutes.

# Place electrode paper wicks

Step	Action
1	Add 150 µL distilled water to each paper wick.

Follow the instructions below to place the electrode paper wicks.

2 Position the wicks on each end of the IPG strips so that one end of the wick overlaps the end of the gel on the IPG strip.



# **Position the electrodes**

Follow the instructions below to position the electrodes.

Step	Action
1	With the electrode cams in the open position, position the electrode assembly on top of all the wicks. The electrode must be in contact with the wick.
2	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 posi-



## Load samples into the sample cups

1

4

Follow the instructions below to load t	the samples into the sample cups.
---	-----------------------------------

Step	Action
------	--------

A maximum of 150 µL of sample may be placed in these cups.



- 2 Check to make sure that there is cover fluid over the samples. When the sample is introduced into the cups, it will sink through the oil to the bottom of the cup and come into contact with the IPG strip.
- 3 Close the Ettan IPGphor 3 lid.
  - Program and run Ettan IPGphor 3 from its own control panel display. See Section 5.3 Perform a run from the control panel, on page 55 for more details, or
    - Start Ettan IPGphor 3 from the control software. See Section 5.4.1 Setting up a run, on page 74 for more details.

# 5.2 Start up the instrument

# Precautions



#### WARNING

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



#### WARNING

In a situation where there is a risk of injury, turn off the instrument by switching the mains power switch (located on the rear panel) to position **0**. Malfunctions must be rectified before Ettan IPGphor 3 Isoelectric Focusing System is restarted.

# Turn on power and run diagnostic program

Step	Action	
1	Turn on the mains <b>Power switch</b> located on the rear panel.	
2	This activates a self diagnostic program that runs for approximately 10 seconds. A series of screens indicate progress. If any component fails, the diagnostic program will stop and a message will indicate the source of the fault. Note the fault, if any.	
3	Press <b>START</b> to skip to the next test.	
4	Call Cytiva service if any failure is detected,	
5	Once the diagnostic program is successfully completed, the LCD screen indicates that the instrument is ready, as shown in the figure.	

# 5.3 Perform a run from the control panel

# In this section

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# 5.3.1 Ettan IPGphor 3 control panel functionality

## Introduction

IEF runs are programmed from the Ettan IPGphor 3 Isoelectric Focusing System control panel with up to ten user-defined IEF protocols, each with up to 9 steps per protocol.

Programmable functions including: rehydration time, platform temperature, current limit, voltage limit for each step, voltage gradient or step and step duration.

# **Control unit keys**

The following keys on the control panel are used to program Ettan IPGphor 3.

COMMAND keys	Function
EDIT	Pressing the <b>EDIT</b> key in the edit mode toggles between the prorocol identification screen with general settings for all steps and the settings in the three first protocol steps.
	The <b>EDIT</b> key switches the display to edit mode, in which protocol steps can be programmed. (Refer to Section 5.3.3 Set protocol parameters, on page 60.
	The <b>EDIT</b> key also allows the estimated run time remaining to be viewed while the unit is operating.
Left arrow	The left arrow key moves the cursor to the left. Moving the cursor off the screen to the left repositions the cursor at the left side of the next row. From the bottom row the cursor is repositioned on the left side of the top row.
Right arrow	The right arrow key moves the cursor to the right. Moving the cursor off the screen to the right repositions the cursor at the left of the next row. From the bottom row the cursor is repositioned on the left side of the top row.
Up arrow	The up arrow key increases the value of the selected field or digit.
	Note:
	Exception is when the cursor is positioned to the left under the Step number S1-S9, the up arrow key changes the display to the previous group of three protocol steps.
	Note:
	During electrophoresis, the up/down arrow keys are used to

#### 5 Operation

5.3 Perform a run from the control panel

5.3.1 Ettan IPGphor 3 control panel functionality

COMMAND keys	Function
Down arrow	The down arrow arrow key decreases the value of the selected field or digit.
	Note:
	The exception is when the cursor is positioned to the left under the Step number S1-S9, the down arrow key changes the display to the next group of three protocol steps.
	Note:
	During electrophoresis, the up/down arrow keys are used to to toggle between <b>VhS</b> , <b>VhT</b> , and <b>Hrs</b> .
START	The <b>START</b> button begins the selected protocol.
STOP	The <b>STOP</b> button pauses the protocol. Pressing the <b>STOP</b> key a second time cancels the protocol in progress.
STOP	Press the <b>STOP</b> button a third time to reset the instrument to start another protocol.
HV ON indi- cator lamp	The <b>HV ON</b> lamp indicates that the high voltage power supply is on and isoelectric focusing is in progress. This lamp is not illumi- nated during the rehydration phase of a protocol.

#### **Protocol identification screen**

The **protocol identification screen** is the first screen that appears after the diagnostic program is completed. The frist row starts with **Protocol #1, Name**. The default location of the cursor is always under the protocol number "1".

A protocol can be selected by pressing the up and down arrow keys to display the number of a protocol. Once the selected protocol is started, the LCD screen indicates the parameters of the step currently being carried out.

The *protocol identification screen* comprises the following programmable items:

- Protocol number and name
- Rehydration time, temperature
- IEF run conditions (temperature and maximum current/strip)
- Number of strips and number of steps in the selected protocol.

#### 5 Operation

5.3 Perform a run from the control panel

5.3.1 Ettan IPGphor 3 control panel functionality

```
Prot# <u>1</u> File 1
Rehydrate 0:00Hr @ 20°C
IEF @ 20°C 50µA/Strip
1 Strips 0 Steps
```

#### **IEF steps screen**

The second screen in edit mode is the first in a series of nine programmable IEF steps in which the voltage change pattern, voltage, and step duration are set. The steps are displayed in groups of 3 steps per screen. The number of steps which have been programmed is displayed next to the protocol number.

The two options for the voltage change pattern are:

- **Step**, which sets the voltage at the selected value for the new step and then holds the voltage constant for the step duration.
- **Gradient**, which increases the voltage limit (linearly with respect to time) from the value set for the previous step to the value set for this step. If this is the first step, the value increases from 0.

The original factory programmed voltage for each step is 0. Program each step with the protocol voltage values. If fewer than nine steps are required, end the program by assigning a value of 0 V after the final step. The step duration (0 to 99:59 hours) or volthours (0 to 300 000 Vhr) are programmable for each step.

```
Prot# 1 3 Steps
S1 Step 500V 1:00 Hrs
S2 Step 1000V 4:00 Hrs
S3 Step 8000V 80000 Vhr
```

# 5.3.2 Protocol parameters

#### **Programmable parameters**

Ettan IPGphor 3 can store up to ten protocols. Programmable protocol parameters include:

- Duration and temperature of the rehydration phase.
- Duration, temperature, and power parameters for up to nine IEF steps for each protocol.
- Power parameters including maximum current, voltage, and voltage change pattern.

Refer to the 2-D Electrophoresis, principles and methods guide for sample handling recommendations and protocol guidelines. The permitted range of values for each programmable parameter is listed below.

Permitted range of values for each programmable parameter		
Parameter	Operational Range	Recommended values
Steps	1 to 9	
Step duration	Rehydration: 0 to 99:59 hrs Focusing: 0 to 99:59 hrs	10:00 to14:00 hrs 2:00 to 10:00 hrs
Volts	0 to 10 000 V	100 to 10 000 V
Max. current	1500 µA total for 12 strips max.	50–75 µA per strip
Platform tempera- ture	Rehydration: 15°C to 30°C Focusing: 15°C to30°C	20 20

# Navigating parameter "fields" and "digits"

- Each screen consists of information as well as one or more fields that can be edited. The active fields contain individually adjustable digits or characters.
- Certain digits are also linked to their neighbouring digits. For example, three of the four digits in the voltage field can each be adjusted individually, but when adjusting the value from 1000 to 990, for instance, even though only the tens place is adjusted manually, three digits are affected.
- Whether individual or linked, digits wrap around? so increasing from 9 produces 0 and decreasing from 0 produces 9 until a parameter limit is reached.

Ettan IPGphor 3 can be programmed in volt-hours (Vhr) or hours and minutes in stepn-hold or in gradient mode. Any values within the operating limits listed above can be used.

# 5.3.3 Set protocol parameters

#### Introduction

This section covers the programming, editing, or verifying of all parameter values.

All protocol parameters are set in the edit mode. Press the **EDIT** key to toggle between the first editable screen and the protocol identification screen.

**Note:** The cursor stops only in the fields that can be edited. Once the cursor is in position, press the Up or Down arrow keys to adjust the value.

#### **Create or edit protocols**

Follow the instructions below to edit the rehydration parameters, time and temperature, and the IEF parameters, temperature,  $\mu$ A/Strip and number of strips as necessary on the main screen.

Step	Action
1	Select the protocol to be created or edited from the <b>protocol identifica-</b> tion screen.
2	Position the cursor under the protocol number field using the right and left arrow keys and move to the desired protocol number using the up and down arrow keys.
	Prot# 1 File 1

```
Prot# 1 File 1
Rehydrate <u>1</u>0:00Hr @ 20°C
IEF @ 20°C 50µA/Strip
1 Strips 0 Steps
```

3 Edit the file name by using the right arrow key to move into the file name, and up and down arrows to adjust the characters as desired. To skip editing the name use the left arrow to proceed to the next line.

> To change any letter, move the cursor to that location using the right and left arrow keys and then scroll through the 37 available options using the up and down arrow keys. Once the desired letter appears, move the cursor to the next character to be set.

The original label file occupies 6 of the 16 available characters. To change the label to a more descriptive name, such as 11  $\,$  CM  $\,$ 3  $\,$ to  $\,$ 10L requires scrolling through the alphanumeric options for each of the 11 digits in the name.

```
Prot# 1 File 1
Rehydrate <u>1</u>0:00Hr @ 20°C
IEF @ 20°C 50µA/Strip
1 Strips 0 Steps
```

4 Edit time range by using the left and right arrow keys to position the cursor, and use the up and down arrow keys to set the time range.

> The available time range is 0–99:59 h, with 10 min resolution, hence, only the first three digits are editable. A minimum of 10 hrs is required for the rehydration of IPG strip gels, but overnight is recommended. This protocol step can also be used as a simple timer to start the protocol at a convenient time. If the IPG strips are already rehydrated and ready for immediate focusing, set the rehydration duration to 00:00 Hrs.

```
Prot# 1 File 1
Rehydrate 10:00Hr @ 20°C
IEF @ <u>2</u>0°C 50µA/Strip
1 Strips 0 Steps
```

Adjust the rehydration temperature by using the left and right arrow keys to position the cursor. Use the up and down arrow keys to select the value within the range of 15°C to 30°C.

The original factory programmed value is 20°C.

```
Prot# 1 File 1
Rehydrate 10:00Hr @ 20°C
IEF @ <u>2</u>0°C 50µA/Strip
1 Strips 0 Steps
```

#### Note:

5

Maintaining the actual platform temperature at <18°C or >25°C might require placement of Ettan IPGphor 3 in a location of lower or higher than ambient temperature (e.g. a cold room).

It may be necessary to operate the instrument in an air conditioned room to prevent condensation from collecting on the platform in humid environments when the platform is run below 7°C ambient temperature.

The third line contains fields to set the platform temperature and current limit for all isoelectric focusing steps.

Adjust the rehydration temperature by using the left and right arrow keys to position the cursor. Use the up and down arrow keys to select the value within the range of 15°C to 30°C.

6

To set the *platform temperature* use the left and right arrow keys to position the cursor in the active °C field and use the up and down arrow keys to select the value within the range of 15°C to 30°C.

The original factory programmed IEF temperature is 20°C.

Prot# 1 File 1 Rehydrate 10:00Hr @ 20°C IEF @ 20°C <u>5</u>0µA/Strip 1 Strips 0 Steps

Adjust the current limit for all isoelectric focusing steps by using the left and right arrow keys to position the cursor under one of the three active µA digits, and use the up and down arrow keys to select the value within the range of 0 to 200 µA per strip.

The original factory programmed value is 50  $\mu$ A per IPG strip.

```
Prot# 1 File 1
Rehydrate 10:00Hr @ 20°C
IEF @ 20°C <u>5</u>0µA/Strip
1 Strips 0 Steps
```

#### Note:

8

The total deliverable ampeerage is  $1500 \,\mu$ A.

Use the arrow keys to adjust the **numbers of strips** to run.

```
Prot# 1 File 1
Rehydrate 10:00Hr @ 20°C
IEF @ 20°C <u>5</u>0µA/Strip
1 Strips 0 Steps
```

9 Press EDIT on the control panel to move to next screen.

#### **Edit step or gradient**

Step	Action
1	Press the <b>EDIT</b> key to display the programmable parameters on the display. Position the cursor in the field to be edited using the right and left arrow keys and adjust the value by pressing the up and down arrow keys.
2	Access a <b>Step</b> by placing the cursor under the step number and use the right and left arrow keys.
	Each step is identified by "S" and the step number. The original programmed option is <b>Step</b> for each step.
	Prot# 1 3 Steps S1 Step 500V 1:00 Hrs S2 Step 1000V 4:00 Hrs S3 Step 8000V 80000 Vhr
3	To advance to the next step group press the down arrow key and to return to a previous step group press the up arrow key.
4	To toggle to <b>Gradient</b> , move the cursor to the <b>Step</b> field using the right and left arrow keys and toggle with the up or down arrow key.

```
Prot# 1 3 Steps
S1 Grad <u>5</u>00V 1:00 Hrs
S2 Grad 1000V 4:00 Hrs
S3 Step 8000V 80000 Vhr
```

5 **IEF voltage** is programmable from 0–10 000 V in increments of 10 V. Each of the first three digits in the voltage field can be set independently.

First move the cursor to the desired digit location using the right and left arrow keys and then change the value using the up and down arrow keys.

Prot# 1 3 Steps S1 Grad 500V 1:00 Hrs S2 Grad 2000V 1:00 Hrs S3 Step 8000V 2:00 Hrs

6

To select, set the cursor under the currently selected field and use the up or down arrow key to toggle between Hrs and Vhrs. Move the cursor to the desired digit location using the right and left arrow keys, and change the value using the up and down arrow keys.

The original factory programmed time for each step is 0:00, and should be set to 0:00 for all unused steps. The Vhrs value is calculated according to the explanatory note at the left, but these values can be set in the same manner as the Hrs values.

#### Note:

When toggling from Hrs to Vhr, the value in the Vhrs field is automatically calculated according to the selected voltage change pattern:

For Step-n-hold, Vhr is the product of hours and voltage.

For Gradient, Vhr is the product of hours and the mean of the voltage limit set for the current step and the previous step.

Absolute Vhr limits can be set to any value from 1 to 300 000 Vhr.

Prot# 1 3 Steps S1 Grad 500V 1:00 Hrs S2 Grad 1000V 4:00 Hrs S3 Step 8000V 80000 Vhr

# Simplified 4-step protocol and resulting voltage profile (example)

To illustrate the two voltage change patterns, a simplified example shows program steps and the resulting voltage profile. Note that step 4 ends the protocol.

For recommended IEF protocols, refer to the 2-D Electrophoresis Principles and Methods guide.





A lower voltage step may be entered at the end of the protocol, however, band sharpness will decrease.

# Verify the protocol

Step	Action
1	Scroll through each step: position the cursor in the step number field using the right and left arrow keys.
2	Advance through the steps using the up and down arrow keys.
3	Edit as required by placing the cursor with the right and left arrow keys and adjusting the value with the up and down arrow keys.
4	Make sure that the voltage field in all unused steps displays a value of 0, or that the value in the Hrs field is 00:00.

# Exit edit mode

Press **EDIT** to save any changes and exit the step edit mode. The *protocol identification* screen will appear.

# 5.3.4 Isoelectric focusing from the control panel

# Precautions



#### WARNING

Ettan IPGphor 3 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.

# **Protocol steps**

Once the protocol begins, each step is carried out automatically, and instrument status for each step is indicated on the screen. Platform temperature and time remaining can be changed while the protocol is in progress, and each such possibility is discussed in turn below.

# Select protocol number

Step	Action
1	Place the cursor in the protocol number field using the right and left arrow keys and select the protocol number using the up and down arrow keys.
	Prot# <u>1</u> MY PROTOCOL Rehydrate 10:00Hr @ 20°C IEF @ 20°C 200µA/Strip 12 Strips 3 Steps
2	Use the right and left arrow keys to position the cursor to set the number of strips to run, and the up and down arrow keys to select the number $(1-12)$

Prot# <u>1</u> MY PROTOCOL	
Rehydrate 10:00Hr @ 20°C	
EF @ 20°C 200µA/Strip	
12 Strips 3 Steps	

3 Press **START**. A screen is displayed to confirm the number of strips to run. Adjust the number of strips if necessary.

```
Number of strips :1<u>2</u>
Press START to continue
```

#### 4 Press **START** to continue.

5 If the safety lid is not properly closed the following message will indicate the required action:

Lid open step 1 Close lid to continue



#### CAUTION

During IEF:

- Do not lean on the safety lid.
- Do not apply excess pressure or uneven weight to the lid.
- Do not place any items on the lid.
- 6 Press **START** again after securing the safety lid.
- 7 The first screen will indicate the protocol, number of steps, and total hours (excluding rehydration time):

```
Starting protocol # 1
3 steps 4:00 Hr
```

If a rehydration period was programmed, the rehydration status screen will indicate the number of hours elapsed and the actual platform temperature:

```
Running Prot# 1
Rehydration for 10:00 Hr
0:01 Hr Elapsed
Rehydrate at 20°C
```

# Change rehydration duration or other parameters

To change rehydration duration or other parameters:

Step	Action
1	Press <b>EDIT</b> to display the set screen. Pressing <b>EDIT</b> again will access the step setting screen. Pressing <b>EDIT</b> again will return to the running screen.
2	The first line indicates that the protocol is running.
	Any changes made while a protocol is running are applied to the current run only, the stored protocol is not affected. Move the cursor under the digit you wish to set and change it using the up and down arrow keys. To end this step, set all digits to 0. This screen is automatically replaced by the regular status screen after a few seconds.
	Running Prot# 1 Rehydrate 10:00Hr @ 20°C IEF @ 20°C 200 µA/Strip 12 Strips 3 Steps
3	The temperature field can be changed directly (without pressing <b>EDIT</b> ) Use the right and left arrow keys to position the cursor in the temperature field and use the up and down arrow keys to adjust the value.

It may take up to 30 minutes to reach the set temperature, depending on ambient conditions.

Isoelectric focusing will begin after the rehydration step is complete. The **HV ON** lamp will light and the status screen showing the step currently in progress as well as all parameters will appear.

```
Running Prot# 1 @ 20°C
Step 1 of 3 Vhrs
Step Vhrs Elapsed
500V 40µA/Strip 0.0W
```

#### Note:

The indicated wattage is the total power delivered to all strips, and will often read 0 W because any value less than 0.5 is rounded down to 0. If the delivered power exceeds 0.4 W per strip, stop the run and reduce the V or  $\mu$ A settings.

The current platform temperature is indicated.

The average current per IPG strip ( $\mu$ A), the total instantaneous power delivered (W), and actual voltage (V) are indicated. If the current is the limiting parameter, the "A" in " $\mu$ A" will flash.

- 4
- To toggle between VhT, VhS and Hrs:
  - Move the cursor to the *Hrs/Vhrs* field of the display using the right or left arrow key.
  - Change the display to reflect *VhT*, *VhS* and *Hrs* using the up and down keys.

```
Running Prot# 1 @ 20°C
Step 1 of 3 Vhrs
Step Vhrs Elapsed
500V 40µA/Strip 0.0W
```

Ettan IPGphor 3 will display the volt-hours during the run as:

- VhS, which indicates the total volt hours for that step, or
- *VhT*, the total volt-hours accumulated during the entire run at the current time.

Hours elapsed for the current step will be indicated by Hrs.

5

To change the display to show remaining Hrs, VhS or VhT:

Move the cursor under the *Elapsed* or ~ *Remain* field and use the up or down arrow keys.

The temperature, voltage and current limits for the current step may be adjusted directly on this screen as well.

```
Running Prot# 1 @ 20°C
Step 1 of 3 Vhrs
Step Vhrs Elapsed
500V 40µA/Strip 0.0W
```

6 Pressing **EDIT** will access the step parameterscreen. Pressing **EDIT** again will return to the running screen. Use the **Up arrow** in the **S1** to **S3** fields to access the **Rehydration/IEF** screen.

> Running Prot# S1 Grad 500V 1:00 Hrs S2 Grad 2000V 1:00 Hrs S3 Step 8000V 2:00 Hrs

#### Note:

If you adjust the voltage during a gradient step the step will be converted to Step for the remainder of the step.

As isoelectric focusing proceeds, the bromophenol blue tracking dye will migrate toward the anode and leave the IPG strip. This generally occurs well before focusing is complete

#### Note:

7

8

A colorless strip is no indication that IEF is complete.

If the tracking dye does not move, then no current is flowing across the strip. Check that the strip holder electrodes are in contact with the electrode areas. See the Troubleshooting section for additional suggestions.

When the protocol ends, audible beeps will sound and the following screen is displayed:

```
Run ended at
8000V 12000 Vhrs Total
50µA 8:00 Hr Total
Press Stop to reset
```

The final instrument values are displayed. Press **STOP**, as indicated, to reset the instrument.

# 5.3.5 Pause or stop a protocol

# Start and stop keys

To pause or stop a protocol in progress, press the **STOP** key:

```
Paused in step 1
Press START to continue
```

To continue the protocol, press the **START** key. To cancel the protocol, press the **STOP** key again. This will cause the instrument to respond as though the end of an uninterrupted protocol had been reached as described above.
## 5.4 Run a pre-programmed protocol from the PC

## In this section

Section		See page
5.4.1	Setting up a run	74
5.4.2	Perform a run	79

## 5.4.1 Setting up a run

## **Precautions**



#### WARNING

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

## Main screen and buttons

Ef Ettan IPOr or 3 File Protoco Communication Help Instri Instr2 Instr4 Instr4 Fast Run setting & details Session log	···· FI	<b>()</b> C		or 3
Strip length pH range	Number of strips		Jun 07, 2022	
1- 0.8- 0.4- 0.4- 0.2- 0.4- 0.	00 Tme	200- 180- 140- 120- 100- 100- 40- 40- 20- 0-	1 - Instr1 Step 0/0 Measured Setpoint 0 V 0 μA 0 μA Ο Vh 0:00:00:00 -00:00 (estimated)	
Measured current [µA]  V Setpoint voltage	[V]		Total time Total Vh 00:00 0	<b>n</b> =
Measured current [µA]	M •		Total time Total Vh 00:00 0	

Part	Function
1	Instrument selection buttons
2	Protocol mode buttons
3	Instrument control buttons
4	Info button
5	Protocol view button

## **Connect instrument**

Step	Action
1	Start the computer and log on to Windows.
2	Right-click on Ettan IPGphor 3 control software and choose <b>Run as admin-</b> istrator.
3	Select the desired Ettan IPGphor 3 by clicking one of the four instrument selection buttons ( <i>Instr1 - Instr4</i> ).



The identity of the instrument should now be shown below the buttons. Up to four instruments can be run simultaneously with the software.

4 Select menu **Communication/Instrument (1-4)** to open the instrument communication settings.

E Settings for in:	strument 1	l		×
Instrument 1	Active	Com port	Name	Connected
				Done

- 5 Check Active.
- 6 Select **COM** port in the **COM port** list box.
- 7 Enter instrument name in the *Name* field.
- 8 Click Done.

A green lamp is lit in the corresponding instrument selection button.



## Select protocol mode options

For setting the protocol for the run there are two main options:

- Fast mode
- Advanced mode

The selection is made by the *protocol mode buttons* and the selected mode is indicated below the buttons. In the fast mode option the software selects an optimized protocol based on the tray type, strip length and pH range to be used. Further, by switching to advanced mode, this protocol can be edited and saved to a file.

## Use the fast mode protocol

2

#### Step Action

1 In Ettan IPGphor main window, click on the appropriate instrument selection button (*Instr1 - Instr4*) to select the desired instrument.



Select *Fast*. Fast should be shown below the buttons.



3 Select the **Run settings & details** tab by clicking the tab.

Run settings & details	Session log	
Strip length pH	range	Number of strips

- 4 Set experimental conditions, that is *Strip length*, *pH range* and *Number of strips*, by clicking the corresponding button.
- 5 The software now selects an optimized Cytiva protocol for the specified experimental parameters.

### Edit the selected protocol

Select the *Protocol view* in the *Run settings & details* tab by clicking the right of the two buttons at the bottom right hand corner of the tab window.



The protocol selected by the software is now shown in detail. It is now possible to edit any values in the protocol by clicking an editable field and enter desired changes.

### 5 Operation 5.4 Run a pre-programmed protocol from the PC 5.4.1 Setting up a run

Proto	ocol name	Cyt_13	_3-11NL	
Rehydr. time		00:00	Tem	p 15 °C
IEF:	I/strip	50	μA <b>Tem</b>	p 20 °C
Step		U [V]	Time o	or Vh
1	Step $\bigtriangledown$	300	01:00	HH:mm 🔽
2	Step 💎	1000	01:00	HH:mm 🔽
3	Step 💎	8000	02:30	HH:mm 🔽
4	Step 💎	8000	00:30	HH:mm 🔽
5	Step 💎	0	00:00	HH:mm 🔽
6	Step 💎	0	00:00	HH:mm 🔽
7	Step 💎	0	00:00	HH:mm 🔽
8	Step 💎	0	00:00	HH:mm 🔽
9	Step 💎	0	00:00	HH:mm 🔽
	<b>Total time</b> 05:00		<b>Total Vh</b> 25300	0 =

## Save protocol

Step	Action		
1	Check that the desired pr	otocol name is entered in	the <b>Protocol name</b> box.
	Tip:		
	Name the protocol accorc limit 16 characters).	ling to user name_strip le	ngth_pH range (upper
2	Click the middle of the thr dialog is opened. Change desired.	ee buttons in order to sav protocol file name and pr	ve the protocol. A <b>Save as</b> otocol directory folder, if
	Run settings & details	Session log	
	Protocol file		Number of strips
			8  \[ \[ \]

#### Note:

If the name of an edited protocol will be overwritten it will be lost.

## Download protocols to a specific position

Ettan IPGphor 3 can store up to ten protocols.

Step	Action	
1	To download a protocol to Ettan IPGphor 3 a 1, select menu <b>Protocol → Transfer protoc</b> ment position (1-10).	t another position than position col. and select desired Instru-
	Transfer protocol to instrument	×
	Instrument position 1	
	ОК	Cancel
2	Click <b>OK</b> .	

The current protocol is now downloaded to the selected position in Ettan IPGphor 3. Any existing protocol at that position is overwritten.

## **Delete protocols**

Click on the right button of the three buttons in the top of the **Protocol** view.

Run settings & details	Session log	
Protocol file		Number of strips
		8

The current protocol is now deleted.

## 5.4.2 Perform a run

### Start a run

Follow the instructions below to start the run after transferring the protocol.



Follow the instructions below to pause a run.

Step	Action
------	--------

1

Pause a run

Click **Pause** button to pause an ongoing run.



The run is now paused which is indicted by the word **Paused** below the instrument control buttons.

Step	Action
2	Click <b>Start</b> button to proceed the run. The run is started from the same position in the protocol from where it was paused.

## Stop a run

Follow the instructions below to stop an ongoing run.

Step	Action
1	Press <b>Stop</b> . The run is now stopped. To indicate that the run has stopped, <b>Idle</b> is displayed below the instrument control buttons.
2	The run is now stopped which is indicted by the word Idle below the instru-

- ment control buttons.
- **Note:** While using **Stop** it is not possible to start the protocol again except from the beginning of the protocol.

## **Display graphics and data**

Ettan IPGphor 3 control software provides graphics of the data (Voltage, Volt-hours and Current) as it is retrieved from Ettan IPGphor 3.

The user can monitor and display the progress of any individual run from any one of four different instruments being run in parallel.

## Monitor a run

Follow the instructions below to monitor the run.

Step	Action
1	Select the correct instrument selection button ( <i>Instr1 - Instr4</i> ) to select the desired instrument. It is possible to monitor and display the progress of any individual run from any one of four different instruments being run in parallel. The identity of the selected instrument is shown below the instrument control buttons.

2 Open the *Run settings & details* tab by clicking the tab heading.

#### Step Action

3

5

In the **Protocol details** graph two of three possible parameters (setpoint voltage, measured voltage and measured current) are displayed to give an overview of the protocol and the run.



4 Open any of the combo boxes below the graph and then click on the desired parameter to be displayed in the **Protocol details** graph.

#### Note:

If measured voltage and measured current are selected, the time scale changes and only the elapsed time is shown.

Click the left of the two buttons at the lower right corner of the tab window to select the Info and data view.



The main running parameters are now displayed. *Vh* or *Time* is indicated depending on what is selected to define the length of the current step.

5 Operation 5.4 Run a pre-programmed protocol from the PC 5.4.2 Perform a run

Step	Action				
	1 - In	str1			
	Step Measured 500 V 1 µA	<b>5/5</b> Setpoint 500 V 75 μA (limit)			
	6750	Vh			
	04:41 -00:00 (estim	L:50			
	Total time 04:42	Total Vh 6750	0 =		

6

Open the **Session log** tab by clicking the tab heading to view the running data collected in the log file.

n settings & details Sessio	on log	
Session log:		
The second secon		
Ettan iPGpnor 3		^
Serial Number: 0000003	20/22	
FILMWALE VEISION. AI4 03/	30722	
Date: Nov 29, 2022		
09.36 User Crispo logged	on	
09.36		
Protocol: RC2_Verificatio	n	
No. of strips: 1		
IEF Params: 75uA/Strip at	: 20 C	
Step 1: Step 300V 200	Vhr	
Step 2: Grad 1000V 300	Vhr	
Step 3: Grad 5000V 4000	Vhr	
Step 4: Step 5000V 1250	Vnr	
step 5. Step 5000 2.00	nr.	
09.36 Step 1 started		
10.15 Step 2 started		
10.43 Step 3 started		
12.03 Step 4 started		
12.18 Step 5 started		
14.17 Run finished		
Nov 29, 2022		

The session log file stores instrument identity, User ID, current protocol, start time and many other parameters.

7 Click another instrument selection button for monitoring runs on other instruments controlled from the software. This way it is possible to monitor up to four parallel runs.

## Capture run data

Run data is acquired from the Ettan IPGphor 3 Isoelectric Focusing Unit automatically every minute during a run, and the data is presented in a graph and in numerical displays.

## Store data

All run data and the session log can be exported as a Microsoft Excel, .jpg or a .csv file. Follow the instructions below to export data.

Step	Action
1	Select <b>Export:File</b> .
2	Select file. The default file name is the date and time (e.g. Apr 11, 2017 10.56.45).
3	Select location. The default location for the saved file is C:\IPGphor3\RunData.

## PC settings while running the protocol

The list below shows PC settings that are important to have while running the protocol.

- Keep the PC on the never sleep mode.
- The .csv file can be opened in any version of Excel.
- Run as administrator is the preferred way to open the control software.

## 6 Maintenance

## In this chapter

Section		Seepage
6.1	Cleaning before planned service	86
6.2	Cleaning Ettan IPGphor 3	87
6.3	Replace fuses	90

## **Precautions**



### WARNING

The customer must make sure that all installation, maintenance, operation and inspection is carried out by qualified personnel who are adequately trained, understand and adhere to local regulations and the operating instructions, and have a thorough knowledge of the product and the entire process.



### WARNING

Ettan IPGphor 3 covers must not be opened by the user. It contains electrical circuits which can give a lethal electric shock. Service and planned maintenance should be performed by personnel authorized by Cytiva.



### WARNING

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



## WARNING

Do not remove the main cover. There are no user serviceable components inside, and you can be exposed to high voltage.



## 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.

## 6.1 Cleaning before planned service

## Cleaning before planned maintenance/service

To ensure the protection and safety of service personnel, all equipment and work areas must be clean and free of any hazardous contaminants before a Service Engineer starts maintenance work.

Complete the checklist in the On Site Service Health and Safety Declaration Form or the Health and Safety Declaration Form for Product Return or Servicing, depending on whether the instrument is going to be serviced on site or returned for service, respectively.

## Health and safety declaration forms

Health and safety declaration forms are available for copying or printing in the *Reference information* chapter of this manual, or on digital media supplied with the user documentation.

## 6.2 Cleaning Ettan IPGphor 3

## Precautions



#### WARNING

If large volumes of liquid have penetrated the casing of the instrument and come into contact with the electrical components, immediately switch off the instrument and contact an authorized service technician.



### 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.



### NOTICE

Clean strip holders and manifold with the strip holder cleaning solution provided. Clean all other components that come in contact with the IPG strip or the sample with a detergent designed for glassware. Rinse well with distilled water.

## **Clean Ettan IPGphor 3**

The parts of the instrument that come in contact with liquid reagents are resistant to chemicals typically used for IEF. Before introducing any other chemicals into the system, first test the affected parts.

## **Cleaning procedures**

IPGphor Strip Holder Cleaning Solution has been specifically formulated for removing protein deposits. It will not damage the strip holder or manifold. IPGphor Strip Holder Cleaning Solution (29011927) can be ordered in 1 liters bottles from Cytiva.

## Clean ceramic strip holders/manifold



## NOTICE

The ceramic strip holders and manifold are very brittle and fragile. Take care in handling and do not subject to impacts. The Ettan IPGphor 3 strip holders and manifold are made from a thermally conductive aluminum oxide ceramic. They have been surface-treated to minimize adsorption and interaction with proteins. The surface coating is mechanically robust and can with-stand temperatures of up to 300°C, however it can be damaged by prolonged exposure to extremes of pH.

Clean strip holders/manifold after each first-dimension IEF run. Do not let solutions dry in the strip holder/manifold. Cleaning may be more effective if the strip holder/manifold is first soaked a few hours or overnight in a solution of 2% to 5% IPGphor Strip Holder Cleaning Solution in water. Strip holders and manifold may also be immersed in boiling 1% SDS.



#### NOTICE

You must use a neutral pH detergent, such as the IPGphor Strip Holder Cleaning Solution, to remove residual protein from the strip holders/manifold.



#### NOTICE

Fixed length strip holders and manifold may be baked, boiled or autoclaved. DO NOT EXPOSE THEM TO STRONG ACIDS OR BASES, INCLUDING ALKALINE DETERGENTS.

## Instruction

Step	Action
1	First rinse off the strip holder/manifold. Use a mild liquid soap to remove any residual IPG cover fluid.
2	Squeeze a few drops of IPGphor Strip Holder Cleaning Solution into the strip holder/manifold slot. Use a toothbrush and vigorous agitation to clean the strip holder/manifold.
3	Rinse well with distilled or deionized water.
4	Thoroughly air dry the strip holders/manifold or dry well with a lint-free tissue prior to use.
5	Wipe the platform and plate electrodes with a lab tissue or paper towel after each use. Other exterior surfaces can be cleaned with a damp cloth. Never use abrasive cleanser or solvents.

## **Clean Manifold Light**

Clean Ettan IPGphor Manifold Light with water, no warmer than 40°C, and with IPGphor Strip Holder Cleaning Solution.



#### CAUTION

Do not use any organic solvents during cleaning.

## **Clean electrodes**



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

## 6.3 Replace fuses

## Precautions



#### WARNING

**Disconnect power.** The power must be disconnected before replacing fuses.

### **Fuses**

Fuses protect equipment by disconnecting loads too large for the instrument's circuit design, so it is imperative that fuses are replaced only with fuses of identical rating. The mains power module, located at the back of the instrument, contains two input mains fuses which are the same for all models: T2.5 AL 250V, 5 × 20 mm.

## **Fuse compartment**



Part	Function
1	Mains power switch
2	Hinged cover
3	Slot
4	Casette end

## Instruction for replacing fuses

Step	Action
1	Open the fuse compartment by inserting a small flat-blade screwdriver into the slot at the top of the power module. Twist the screwdriver 1/8 – turn to release the cover, then pull out the hinged compartment, which opens out.
2	Insert the screwdriver above the arrow on one fuse cassette, catch the cassette end, and slowly slide it completely out of the module.
3	Pull the fuse out of its cassette and inspect. If the fuse element is burned or broken, replace the fuse with an identical type. If the fuse appears to be intact, check it with a multi-meter. (A reading of 1 ohm or less indicates the fuse is still usable.)
4	After placing a new fuse into the cassette, slide it into the power module, making sure the arrow on the cassette points to the right (in the same direction as the guide arrows on the inside of the compartment door).
5	Repeat steps 2 to 4 for the second cassette.
6	Close the fuse compartment cover and gently press it into the power module until it snaps shut.
7	Plug the power cord into the unit and turn the mains power switch on.
8	If the instrument does not start when power is switched on after replace- ment of mains fuse(s), call Cytiva service.

## 7 Troubleshooting

## **Troubleshooting guide**

The following table specifies the troubleshooting situations that you might encounter while using Ettan IPGphor 3.

Symptom	Possible solution	
Problems indicated by LCD messages		
Lid open step 1, close to continue	The safety lid is not properly closed. When the safety lid is open, the system has an automatic voltage cutoff safety feature. In order for the protocol to proceed, the safety lid must be closed.	
Locked screen in edit mode	Turn off the mains power switch to reset the instrument.	
Blank display	If no electrical components are functioning (e.g. <b>HV ON</b> lamp does not light and the cooling fans are motionless), check the fuses in the mains power module.	
Diagnostic program indicates component failure	Note the component that failed and press the <b>START</b> key to continue through the diagnostic program. Call your local Cytiva service for further information on how to remedy the failure.	
Arc	An electric arc has been detected. The instru- ment will reduce the voltage setting 500 V auto- matically. The run continues, and there is no need for further action.	

Symptom	Possible solution		
Power delivery			
Current too low or zero	At least two of three pressure pads under the safety lid should press gently against the strip holders to ensure electrical continuity between the strip holder electrodes and the electrode areas on the platform.		

Symptom	Possible solution
	The gel must be evenly and completely rehy- drated to conduct current. Make sure the proper amount of rehydration solution is applied to the IPG strip holder and allow a minimum of 10 hours for rehydration.
	Check that both connecting points of the strip holder or the connecting pins of the manifold electrodes are in contact with the gold surface.
Voltage limit not reached	The ionic strength of the rehydration solution is too high. The instrument has reached the current limit; reduce the IPG buffer concentra- tion; use a mixed-bed ion-exchange resin to remove ionic breakdown products of urea of other additives.
	Desalt the sample or prepare the sample so that the salt concentration is less than 10 mM.
Sparks or burning in strips	Reduce the current limit. Do not exceed 50 µA per strip.
	Prevent the IPG strip from drying out by always applying cover fluid during rehydration.
	Ensure that the IPG strip is fully rehydrated along the entire length of the strip. The IPG strip should be in complete contact with the correct volume of rehydration solution. Remove any air bubbles trapped under the IPG strip.
	Desalt the sample or prepare the sample so that the salt concentration is less than 10 mM. De- ionize additives to the rehydration solution. Excessive charged material in the sample or rehydration buffer lead to electro-endosmosis which will dry out the middle of the strip, possibly leading to arcing and burning at these points.

## 8 Reference information

## About this chapter

This chapter lists the technical specifications of Ettan IPGphor. The chapter also includes ordering information, and Health and Safety Declaration form for service.

## In this chapter

Section		See page
8.1	Technical specifications	95
8.2	Recycling information	97
8.3	Regulatory information	98
8.4	Ordering information	108
8.5	Health and Safety Declaration Form	109

## 8.1 Technical specifications

## **Power specifications**

Parameter	Specification
Mains supply voltage	100-240 V AC ±10%
Frequency	50/60 Hz
Power consumption	230 VA
Transient overvoltages	Overvoltage category II

## Electrophoresis

Parameter	Specification
High voltage	Up to 10 kV
Current	Up to 1.5 mA

## Working surface

Parameter	Specification
Electrode areas	Gold-plated copper.
Capacity	Up to 12 fixed-length strip holders or one manifold.
Platform temperature	15°C to 30°C ±2°C.

## **User interface**

Item/Parameter	Specification
Control panel	7-key membrane keypad.
Liquid crystal display (LCD)	4 lines, 24 characters per line.
Programmable parameters	Rehydration time, platform temperature, maximum current limit per strip, voltage limit in each step, voltage step or gradient, and step duration.
Protocol capacity	10 protocols, with up to 9 steps each.

Item/Parameter	Specification
USB Type-C port	1200 or 9600 baud, 8 data bits 1 stop bit, no parity, no flow control. 9600 baud is manda- tory for connection with Ettan IPGphor 3 control software.

## 8.2 Recycling information

## Introduction

This section contains information about the decommissioning of the product.



## CAUTION

Always use appropriate personal protective equipment when decommissioning the equipment.

## Decontamination

The product must be decontaminated before decommissioning. All local regulations must be followed with regard to scrapping of the equipment.

## **Disposal of the product**

When taking the product out of service, the different materials must be separated and recycled according to national and local environmental regulations.

## **Recycling of hazardous substances**

The product contains hazardous substances. Detailed information is available from your Cytiva representative.

## **Disposal of electrical components**



Waste electrical and electronic equipment must not be disposed of as unsorted municipal waste and must be collected separately. Contact an authorized representative of the manufacturer for information concerning the decommissioning of the equipment.

## 8.3 Regulatory information

## Introduction

This section lists the regulations and standards that apply to the product. Your system is marked or listed according to the applicable regulatory requirements for your region. Local language translations are only provided according to regulatory requirements.

## In this section

Section		See page
8.3.1	Contact information	99
8.3.2	European Union and European Economic Area	100
8.3.3	Great Britain	101
8.3.4	Eurasian Economic Union (Евразийский экономический союз)	102
8.3.5	North America	104
8.3.6	Regulatory statements	105
8.3.7	Declaration of Hazardous Substances (DoHS)	106

8 Reference information 8.3 Regulatory information 8.3.1 Contact information

## 8.3.1 Contact information

## Introduction

This section shows the contact information for support and manufacturing information.

## **Contact information for support**

To find local contact information for support and sending troubleshooting reports, visit *cytiva.com/contact*.

## **Manufacturing information**

The table below summarizes the required manufacturing information.

Requirement	Information
Name and address of manufacturer	Cytiva Sweden AB
	Björkgatan 30
	SE 751 84 Uppsala
	Sweden
Telephone number of manufacturer	+ 46 771 400 600

## 8.3.2 European Union and European Economic Area

## Introduction

This section describes regulatory information for the European Union and European Economic Area that applies to the product.

## **Conformity with EU Directives**

See the EU Declaration of Conformity for the directives and regulations that apply for the CE marking.

If not included with the product, a copy of the EU Declaration of Conformity is available on request.

## **CE** marking

CE

The CE marking and the corresponding EU Declaration of Conformity is valid for the product when it is:

- used according to the Operating Instructions or user manuals, and
- used in the same state as it was delivered, except for alterations described in the *Operating Instructions* or user manuals.

## 8.3.3 Great Britain

## Introduction

This section describes regulatory information for Great Britain that applies to the product.

## **Conformity with UK Regulations**

See the UK Declaration of Conformity for the regulations that apply for the UKCA marking.

If not included with the product, a copy of the UK Declaration of Conformity is available on request.

## **UKCA** marking

## UK CA

The UKCA marking and the corresponding UK Declaration of Conformity is valid for the product when it is:

- used according to the Operating Instructions or user manuals, and
- used in the same state as it was delivered, except for alterations described in the *Operating Instructions* or user manuals.

# 8.3.4 Eurasian Economic Union (Евразийский экономический союз)

This section describes the information that applies to the product in the Eurasian Economic Union (the Russian Federation, the Republic of Armenia, the Republic of Belarus, the Republic of Kazakhstan, and the Kyrgyz Republic).

## Introduction

This section provides information in accordance with the requirements of the Technical Regulations of the Customs Union and (or) the Eurasian Economic Union.

## Введение

В данном разделе приведена информация согласно требованиям Технических регламентов Таможенного союза и (или) Евразийского экономического союза.

## Manufacturer and importer information

The following table provides summary information about the manufacturer and importer, in accordance with the requirements of the Technical Regulations of the Customs Union and (or) the Eurasian Economic Union.

Requirement	Information
Name, address and telephone number of manufacturer	See Manufacturing information
Importer and/or company for obtaining information about	Cytiva RUS LLC
	109004, Moscow
	internal city area Tagansky municipal district
	Stanislavsky str., 21, building 5, premises I, offices 24,25,29
	Russian Federation
	Telephone: +7 985 192 75 37
	E-mail: rucis@cytiva.com

## Информация о производителе и импортере

В следующей таблице приводится сводная информация о производителе и импортере, согласно требованиям Технических регламентов Таможенного союза и (или) Евразийского экономического союза.

#### 8 Reference information

8.3 Regulatory information

8.3.4 Eurasian Economic Union (Евразийский экономический союз)

Требование	Информация
Наименование, адрес и номер телефона производителя	См. Информацию об изготовлении
Импортер и/или лицо для	ООО "Цитива РУС"
импортере	109004, г. Москва
	вн. тер. г. муниципальный округ Таганский
	ул. Станиславского, д. 21 стр. 5, помещ. I, ком. 24,25,29
	Российская Федерация
	Телефон: +7 985 192 75 37
	Адрес электронной почты: rucis@cytiva.com

Description of symbol on the system label Описание обозначения на этикетке системы



This Eurasian compliance mark indicates that the product is approved for use on the markets of the Member States of the Customs Union of the Eurasian Economic Union

Данный знак о Евразийском соответствии указывает, что изделие одобрено для использования на рынках государств-членов Таможенного союза Евразийского экономического союза

## 8.3.5 North America

## Introduction

This section describes the information that applies to the product in the USA and Canada.

## **FCC** compliance

This device complies with part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

# **Note:** The user is cautioned that any changes or modifications not expressly approved by Cytiva could void the user's authority to operate the equipment.

This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

8 Reference information 8.3 Regulatory information 8.3.6 Regulatory statements

## 8.3.6 Regulatory statements

## Introduction

This section shows regulatory statements that apply to regional requirements.

## EMC emission, CISPR 11: Group 1, Class A statement



## South Korea

Regulatory information to comply with the Korean technical regulations.





A급 기기 (업무용 방송통신 기자재)

이 기기는 업무용환경에서 사용할 목적으로 적합성평가를 받 은 기기

로서 가정용 환경에서 사용하는 경우 전파간섭의 우려가 있습 니다.

## 8.3.7 Declaration of Hazardous Substances (DoHS)

This section describes the information that applies to the product in China.

根据 SJ/T11364-2014《电子电气产品有害物质限制使用标识要求》特提供如下 有关污染控制方面的信息。

The following product pollution control information is provided according to SJ/ T11364-2014 Marking for Restriction of Hazardous Substances caused by electrical and electronic products.

## 电子信息产品污染控制标志说明 Explanation of Pollution Control Label



该标志表明本产品含有超过中国标准 GB/T 26572《电子电气产品中限用物质的 限量要求》中限量的有害物质。标志中的数字为本产品的环保使用期,表明本 产品在正常使用的条件下,有毒有害物质不会发生外泄或突变,用户使用本产 品不会对环境造成严重污染或对其人身、财产造成严重损害的期限。单位为 年。

为保证所申明的环保使用期限,应按产品手册中所规定的环境条件和方法进行 正常使用,并严格遵守产品维修手册中规定的定期维修和保养要求。

产品中的消耗件和某些零部件可能有其单独的环保使用期限标志,并且其环保 使用期限有可能比整个产品本身的环保使用期限短。应到期按产品维修程序更 换那些消耗件和零部件,以保证所申明的整个产品的环保使用期限。

本产品在使用寿命结束时不可作为普通生活垃圾处理,应被单独收集妥善处 理。

This symbol indicates the product contains hazardous materials in excess of the limits established by the Chinese standard GB/T 26572 Requirements of concentration limits for certain restricted substances in electrical and electronic products. The number in the symbol is the Environment-friendly Use Period (EFUP), which indicates the period during which the hazardous substances contained in electrical and electronic products will not leak or mutate under normal operating conditions so that the use of such electrical and electronic products will not result in any severe environmental pollution, any bodily injury or damage to any assets. The unit of the period is "Year".

In order to maintain the declared EFUP, the product shall be operated normally according to the instructions and environmental conditions as defined in the product manual, and periodic maintenance schedules specified in Product Maintenance Procedures shall be followed strictly.

Consumables or certain parts may have their own label with an EFUP value less than the product. Periodic replacement of those consumables or parts to maintain the declared EFUP shall be done in accordance with the Product Maintenance Procedures.

This product must not be disposed of as unsorted municipal waste, and must be collected separately and handled properly after decommissioning.

## 有害物质的名称及含量 Name and Concentration of Hazardous Substances

## 产品中有害物质的名称及含量

Table of Hazardous Substances' Name and Concentration

部件名称 Component name	有害物质 Hazardous substance					
	铅 (Pb)	汞 (Hg)	镉 (Cd)	六价铬 (Cr(VI))	多溴联苯 (PBB)	多溴二苯醚 (PBDE)
28926930	Х	0	0	0	0	0

- 0: 表示该有害物质在该部件所有均质材料中的含量均在 GB/T 26572 规定的 限量要求以下。
- X: 表示该有害物质至少在该部件的某一均质材料中的含量超出 GB/T 26572 规定的限量要求。
- 此表所列数据为发布时所能获得的最佳信息.
- **0:** Indicates that this hazardous substance contained in all of the homogeneous materials for this part is below the limit requirement in GB/T 26572.
- X: Indicates that this hazardous substance contained in at least one of the homogeneous materials used for this part is above the limit requirement in GB/T 26572
- Data listed in the table represents best information available at the time of publication.

## 8.4 Ordering information

This section lists accessories and user replaceable spare parts that are available for Ettan IPGphor.

## Replacements

Replacement part	Code number
Ettan IPGphor Cup Loading Manifold, ceramic	80649857
Ettan IPGphor Cup Loading Manifold Light	11002580
Sample cups, pack of 20 (6x)	80649895
Paper electrode wicks, pack of 40 (6x)	80649914
Paper bridge pads, pack of 20 (6x)	80649933
Electrode set	80649876

## Accessories

Accessory part	Quan- tity	Code number
IPGphor box	1	28933465
IPGphor box kit	1	28933492
IPGphor Strip Holder Cleaning Solution	1 liter	29011927

**Note:** Cover fluid can be ordered using CAS number 8012-95-1.

## **More information**

For more details on ordering information and for information about spare parts and accessories, visit cytiva.com or contact your local Cytiva representative.
#### Health and Safety Declaration Form 8.5

## On site service



## **On Site Service Health & Safety Declaration Form**

Service Ticket #:

To make the mutual protection and safety of Cytiva service personnel and our customers, all equipment and work areas must be clean and free of any hazardous contaminants before a Service Engineer starts a repair. To avoid delays in the servicing of your equipment, complete this checklist and present it to the Service Engineer upon arrival. Equipment and/or work areas not sufficiently cleaned, accessible and safe for an engineer may lead to delays in servicing the equipment and could be subject to additional charges.

Yes	No	Review the ac Provide expla	Review the actions below and answer "Yes" or "No". Provide explanation for any "No" answers in box below.						
0	С	) Instrument has Rinse tubing of Make sure the suitable survey	Instrument has been cleaned of hazardous substances. Rinse tubing or piping, wipe down scanner surfaces, or otherwise make sure removal of any dangerous residue. Make sure the area around the instrument is clean. If radioactivity has been used, perform a wipe test or other suitable survey.						
0	С	Adequate spa installation. In prior to Cytiva	Adequate space and clearance is provided to allow safe access for instrument service, repair or installation. In some cases this may require customer to move equipment from normal operating location prior to Cytiva arrival.						
0	С	) Consumables any area that	Consumables, such as columns or gels, have been removed or isolated from the instrument and from any area that may impede access to the instrument.						
0	С	All buffer / wa Excess contai	All buffer / waste vessels are labeled. Excess containers have been removed from the area to provide access.						
Provide explanation for any "No" answers here:									
Equipm	nent t	ype / Product No:		Serial No:					
I hereby confirm that the equipment specified above has been cleaned to remove any hazardous substances and that the area has been made safe and accessible.									
Name:				Company or institution:					
Position or job title:				Date (YYYY/MM/DD):					
Signed	:								
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## **Product return or servicing**



## **Health & Safety Declaration Form** for Product Return or Servicing

Return authorization	and/or	
number:	Service Ticket/Request:	

To make sure the mutual protection and safety of Cytiva personnel, our customers, transportation personnel and our environment, all equipment must be clean and free of any hazardous contaminants before shipping to Cytiva. To avoid delays in the processing of your equipment, complete this checklist and include it with your return.

- 1. Note that items will NOT be accepted for servicing or return without this form
- 2. Equipment which is not sufficiently cleaned prior to return to Cytiva may lead to delays in servicing the equipment and could be subject to additional charges
- 3. Visible contamination will be assumed hazardous and additional cleaning and decontamination charges will be applied

Yes	No	Specify if the equipment has been in contact with any of the following:								
$\bigcirc$	$\bigcirc$	Radioactivity (specify)								
$\bigcirc$	$\bigcirc$	Infectious or haza	rdous biological	substances (sp	ecify)					
$\bigcirc$	$\bigcirc$	Other Hazardous	Chemicals (spec	ify)						
Equipm you for	Equipment must be decontaminated prior to service / return. Provide a telephone number where Cytiva can contact you for additional information concerning the system / equipment.									
Telepho	one No:									
Liquid a	and/or ga	as in equipment is	:	Water						
				Ethanol	Ethanol					
				None, emp	None, empty					
				Argon, Hel	Argon, Helium, Nitrogen					
				Liquid Nitr	Liquid Nitrogen					
			Other, specify							
Equipm	nent type	/ Product No:	·		Serial No:					
I hereby confirm that the equipment specified above has been cleaned to remove any hazardous substances and that the area has been made safe and accessible.										
Name:					Company or institution:					
Positio	n or job t	itle:			Date (YYYY/MM/DD)					
Signed	:									
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or service number, call local technical support or customer service.

9 ZUDU Cycle Synaw Angood Synaw S

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