

Ettan IPGphor II

isoelectric focusing system



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Protocol worksheet

English



Safety warnings and precautions

Important user information

Please read this entire manual to fully understand the safe and effective use of this product. The lightning symbol within an equilateral triangle is intended to alert the user to the risk of exposure to high voltages. Should you have any comments on this manual, we will be pleased to receive them via email at ts-ep@am.amershambiosciences.com or at:

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Français



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Español



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Deutsch



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Italiano



Informazioni importanti per l'operatore

Per un utilizzo sicuro del prodotto, leggere attentamente l'intero contenuto del presente manuale. Il simbolo del fulmine all'interno di un triangolo equilatero indica all'utente la presenza di un rischio di esposizione ad alte tensioni. Si prega di inviare eventuali commenti al presente manuale a:

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1. Ettan IPGphor II Isoelectric Focusing System function and description

The Ettan™ IPGphor™ II Isoelectric Focusing System performs first dimension isoelectric focusing (IEF) in 2-dimensional (2-D) electrophoresis. The system comprises Immobiline™ DryStrip gel strips, which contain an immobilized pH gradient (IPG); strip holders, which serve as both IEF chambers for the IPG strips; and the Ettan IPGphor II unit, which includes a high-voltage DC power supply, solid state temperature control, and can be programmed with up to ten user-defined IEF protocols.

Programmable functions include:

- rehydration time,
- platform temperature,
- maximum current limit,
- voltage change pattern,
- voltage for each step in the isoelectric focusing program, and
- step duration.

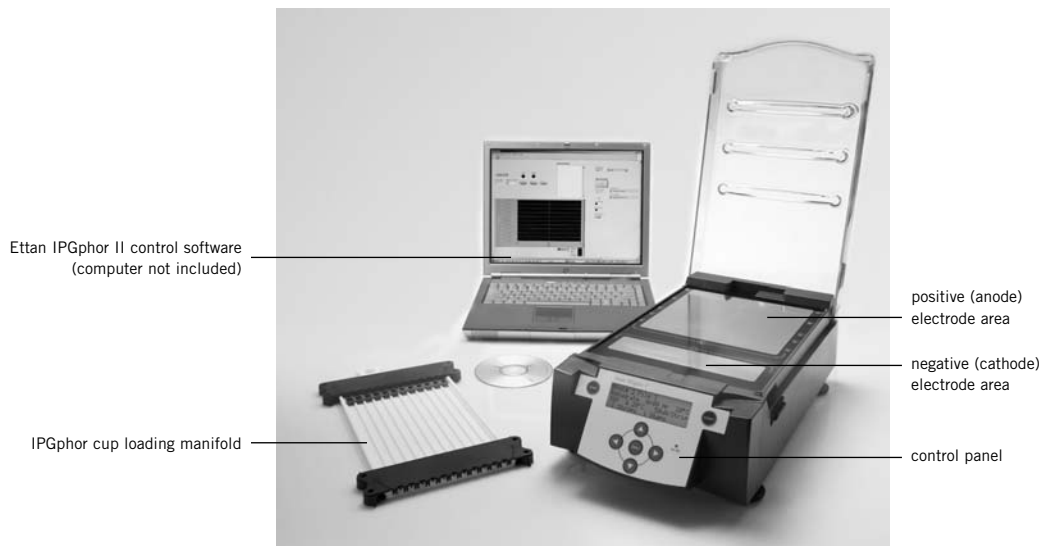
IPG strips are available in five lengths—7, 11, 13, 18, and 24 cm—and a number of pH ranges, several linear and nonlinear (listed in section 6.2, Ordering information). Two types of strip holders are available, a fixed-length strip holder and a variable-length cup loading strip holder. Once the IPG strip is in place in a fixed-length strip holder, no further handling is required. The holder is placed on the Ettan IPGphor II electrode platform for isoelectric focusing and the sample is introduced either during or after the rehydration step. Up to 12 strip holders of the same size can be accommodated for any one protocol.

The cup loading manifold accommodates 3 mm wide strips from 7 to 24 cm long. After rehydration, strips are placed in the strip holder and the sample cup positioned as desired for loading in a particular zone. Up to 9 cup loading strip holders can be accommodated at one time.

1.1. Unpacking

Unwrap all packages carefully and compare contents with the packing list, making sure all items arrived. If any part is missing, contact your local Amersham Biosciences sales office. Inspect all components for damage that may have occurred while the unit was in transit. If any part appears damaged, contact the carrier immediately. Be sure to keep all packing material for damage claims or to use should it become necessary to return the unit.

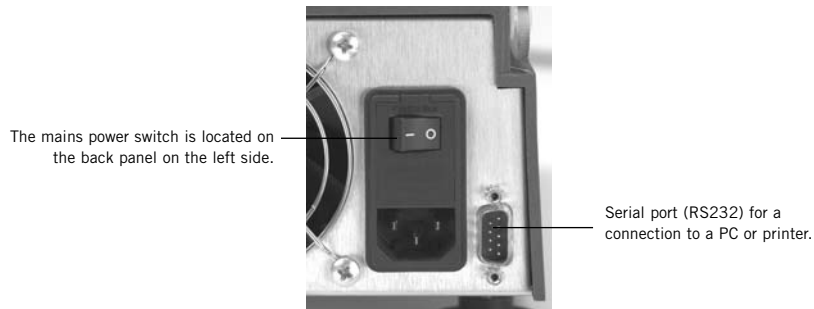
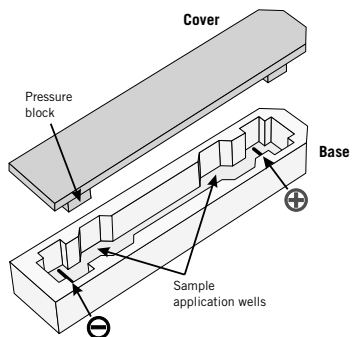
1.2. Instrument components



Five strip holder lengths are available—7, 11, 13, 18, and 24 cm—one for each IPG strip length.

Note: all strips (and holders) used at any one time must be of the same length.

A cup loading strip holder that accommodates all strip lengths is also available.



Control panel	The Ettan IPGphor II is controlled by a 7-key membrane keypad. Instrument status is indicated on the LCD screen.
LCD screen	The main screen is "Protocol #1, Name." The default location of the cursor is always under the protocol number "1." Select any protocol by pressing the up and down arrow keys to display the number of the desired protocol. Once the selected protocol is started, the LCD screen indicates the parameters of the step currently being carried out. In "edit mode" the LCD displays the programmable parameters. Place 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.
COMMAND keys	The EDIT key switches the display to "edit mode," in which protocol steps can be programmed. (See section 3.2.) Pressing the EDIT key in edit mode switches the display back to the identification screen for the protocol being edited. 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. At the bottom row the cursor is repositioned at the left side of the top row. 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. At the bottom row the cursor is repositioned at the left side of the top row. The up arrow key increases the value of the selected field or digit, and the down arrow key decreases the value of the selected field or digit. The START key begins the selected protocol. The STOP key pauses the protocol. Pressing the STOP key a second time cancels the protocol in progress.
HV ON indicator lamp	The HV ON lamp indicates that the high voltage power supply is on and isoelectric focusing is in progress. This lamp is not illuminated during the rehydration phase of a protocol.
Platform	Up to 12 fixed-length strip holders of the same length or 9 variable-length cup loading strip holders can be placed on the platform for rehydration and IEF. The embedded electrode areas provide the electrical connection between strip holder electrodes and the integral power supply. Platform temperature is controlled by Peltier thermoelectric modules.
Electrode areas	Both electrode areas are gold-plated copper. Placing a strip holder so that it bridges both electrode areas completes the electrical circuit (once the safety lid is in place). Each holder must be oriented properly: Negative (cathode) area: Place the flat end of the holder in the shorter electrode area, visually verifying that there is metal-to-metal contact between the electrode on the bottom of the holder and the electrode area for the fixed length strip holders. Positive (anode) area: The pointed end of the holder points toward the back of the unit, and the electrode must contact the longer electrode area; the position of the tip should correspond to the guidemarks on the edge of the platform (7, 11, 13, 18, and 24 cm) for the fixed length strip holders.
Safety lid	The safety lid must be properly latched before power can be applied. The pressure pads in the lid secure the strip holders. A minimum of two pads should contact the cover of each strip holder to ensure an electrical connection.
Strip holders	The strip holders and manifold are made of aluminum oxide ceramic of minimal thickness for efficient heat transfer. This material 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 IPGphor Cleaning Solution supplied to retain that coating.
Fixed-length strip holders	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. The holders, like IPG strips, have a flat and a pointed end. The pointed end ("point is positive") designates the anode, and thus the location of the acidic end of the immobilized gradient. Embedded electrodes provide electrical connection between electrode areas and the gel.
Sample application wells	Each of the two wells can accept up to 15 μ l of sample (7.5 μ l on either side of the strip) in excess of the rehydration volume (i.e. 30 μ l maximum for both wells). Introduce the sample through the wells if it is not included in the rehydration solution.
Cover	The cover protects the IPG strip during rehydration, and the shape of the underside of the cover ensures electrical continuity during IEF by pressing strip against the electrode while allowing electrolysis gases to escape. Rehydration and separation progress can be monitored visually through the clear cover.
Cup loading manifold	The cup loading manifold holds up to 12 IPG strips. The manifold allows for cup loading or bridge loading. The manifold allows the user to run strips that have been rehydrated into the IPG strip using the reswelling tray. The manifold itself is not used for rehydration.
Electrodes	The cup loading manifold has contact pins on the side of the electrodes. These contact pins make contact with the power supply electrode areas when placed on the IPGphor separation platform.
Sample cups	Sample cups can be placed almost anywhere along the length of the IPGphor strip holder that is not blocked by a protrusion. For proper sealing of the cup to the gel, the feet of the sample cup must all rest on the bottom of the channel.

English



1.3. Important information

Instrument operation

- **Caution!** Ettan IPGphor II is a high voltage instrument that can cause fatal electrical shock if the safety features are disabled. The safety lid must be properly latched before starting a protocol.
- Exceeding the recommended current limit of 50 μ A per IPG strip can cause the strip to burn and may damage the instrument.
- 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.
- Plug the instrument into a properly grounded outlet.
- Always disconnect the power cord before servicing.
- Ensure that the cooling vents at the front, back, and bottom of the instrument are not obstructed.
- Operate Ettan IPGphor II on a level surface.
- Avoid spilling liquids on the body of the instrument.
- Avoid scratching the electrode areas.

IPG strips, strip holders, and reagents

- The strip holders and manifold trays are very brittle and fragile. Handle carefully: Do not bump or drop.
- 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 the silver stained 2-D patterns.
- 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.
- Use the appropriate rehydration volume for the IPG strip length.
- Solutions containing urea may be warmed briefly. Do not heat any solutions containing urea above 30–40 °C as isocyanate, a urea degradation product, will carbamylate the proteins in the sample, thus changing their isoelectric points.
- All chemicals should be of the highest purity. Double distilled water should be used.

Français



Informations importantes

Précautions d'emploi

- **Attention!** l'Ettan IPGphor II est un instrument à très haut voltage pouvant causer un choc électrique mortel si les conditions de sécurité ne sont pas respectées. Le couvercle de sécurité doit être convenablement verrouillé avant de démarrer un protocole.
- Dépasser les limites de courant recommandées, 50 μ A un bande IPG, peut amener les bandes à brûler et endommager l'instrument!
- Durant l'isoélectrofocalisation, ne pas s'appuyer sur le couvercle de sécurité, ne pas exercer de pression excessive ni de poids inutile sur le couvercle, et ne pas placer d'objets sur le couvercle. Une telle pression pourrait provoquer un arc électrique entre les électrodes des cassettes et la plate-forme de migration porte-électrodes, abîmant l'instrument.
- Brancher l'instrument à une prise de terre appropriée.
- Toujours déconnecter le cordon d'alimentation avant la maintenance.
- S'assurer que les ventilations devant et à l'arrière de l'appareil ne sont pas obstruées.
- Utiliser l'Ettan IPGphor II sur une surface plane.
- Éviter de renverser des liquides sur l'instrument.
- Éviter de rayer la plate-forme de migration porte-électrodes..

Mèches IPG, cassettes et réactifs

- Les cassettes sont très fragiles. Manipulez-les avec précaution: évitez de les heurter ou de les laisser tomber.
- Toujours mettre des gants pour manipuler les bandes IPG ainsi que l'équipement en contact avec celles-ci. Ceci aide à minimiser la contamination protéique qui peut occasionner des spots artefactuels sur le gel 2D après la coloration à l'argent.
- Nettoyer les sarcophages avec la solution dédiée à cet effet. Nettoyez tous les composants qui rentrent en contact avec les bandes IPG ou l'échantillon, avec un détergent pour matériel en verre. Bien rincer avec de l'eau distillée.
- Utilisez le volume approprié de réhydratation.
- Les solutions contenant de l'urée peuvent être chauffées brièvement. Ne pas chauffer de solution contenant de l'urée à plus de 30–40 °C car l'isocyanate, un produit de dégradation de l'urée, produira une carbamylation des protéines de l'échantillon, changeant ainsi leurs points isoélectriques.
- Tous les produits chimiques doivent être de la plus grande pureté. Veillez à utiliser de l'eau doublement distillée.

This declaration of conformity is only valid for the instrument when it is:

- used in laboratory locations, and
- used as delivered from Amersham Biosciences except for alterations described in the User Manual.

*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.

1.4. Specifications

Working surface

Electrode areas	Gold-plated copper
Strip holder capacity	Up to 12 fixed-length, up one manifold tray
Platform temperature	15–30 °C ± 2 °C

Strip holders

Material	Aluminum oxide ceramic base, acrylic cover, polycarbonate, platinum on titanium
Holders for IPG strip lengths:	7, 11, 13, 18, and 24 cm
Cup loading manifold	For all strip lengths, cup and bridge loading

User interface

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, step voltage, step voltage change pattern, and step duration.
Protocol capacity	10, with up to 9 steps each
Serial port	RS232C, 1200 or 9600 baud, 8 data bits 1 stop bit, no parity, no flow control

Operating environment

Temperature	15–35 °C
Relative humidity	0–70%*

Power requirements

Line voltage	115/230 V~
Frequency	50/60 Hz
Power consumption	100 W

Power outputs

Voltage	0–8000 V $\overline{=}$, resolution: 10 V
Current	0–1.5 mA, resolution: 1 μ A
Power	12 W max.

Physical parameters

Dimensions (h × w × d)	14 × 25 × 46 cm
Weight	6.8 kg (15 lbs)

Safety

Safety features	Automatic voltage cutoff when safety lid is opened
Safety certifications	CE, UL, CSA

2. Instrument set up

2.1. Placing the instrument

1

Important! The vents at the front, back, and bottom of the instrument must not be obstructed. Air must be free to circulate for the cooling apparatus to function properly.

2

Place the instrument on a flat surface with the bubble level in the center. Turn the leveling feet as necessary to make the instrument level and stable.

2.2. Power connection

Two power cords are supplied: one cord has a “North American” style (NEMA 5-15-P) 3-pronged plug for 115 V~ power outlets and the other has a “Central European” style (CEE 7/7) plug for 230 V~ power outlets.

1

Select the cord with the appropriate plug for the power outlet in your laboratory. If neither of these cords are suitable for your power outlets, obtain a detachable cord with an IEC-320-C13 input.

2

Plug the connectors into the power cord receptacle on the rear panel and into a properly grounded power outlet.

2.3. Optional: Serial port connector for a printer or computer connection

The RS232C serial port for a printer or computer connection is at the left rear of the unit. The type of cable required (straight serial or null modem) depends on the type of device (DTE or DCE) that it is connected to: If the printer or computer is configured to receive data on pin 2 and transmit on pin 3, a regular straight serial cable is required. For any other configuration, a null modem cable is required.

Pin number assignments and device set up details follow:

Ettan IPGphor II RS232C signal and pin number assignments:

Pin 2	Transmit
Pin 3	Receive
Pin 5	Ground
Other pins	Not connected

The Ettan IPGphor II requires these settings in the device receiving data:

Baud rate	1200 or 9600
Data bits	8
Stop bit	1
Start bit	1
Parity	None
Flow control	None

To set the baud rate Press the Up and Down arrows together to access the Baud menu: Use the Up or Down arrows to set the baud rate and the Right arrow to exit to the main menu.

```
Serial Port Setup
Baud Rate: 1200
Set Up or Dn      Exit>
```

3. Operating instructions

The following sections will guide you through each stage of the isoelectric focusing process using the Ettan IPGphor II.

3.1. Power up

Turn on the mains power switch (I) located on the right side of the rear panel. This activates a self diagnostic program that runs for approximately 10 seconds. A series of screens indicates progress. If any component fails, the diagnostic program will stop and a message will indicate the source of the fault; note the fault and press the START key to skip to the next test. If any failure is detected, call the technical service department.

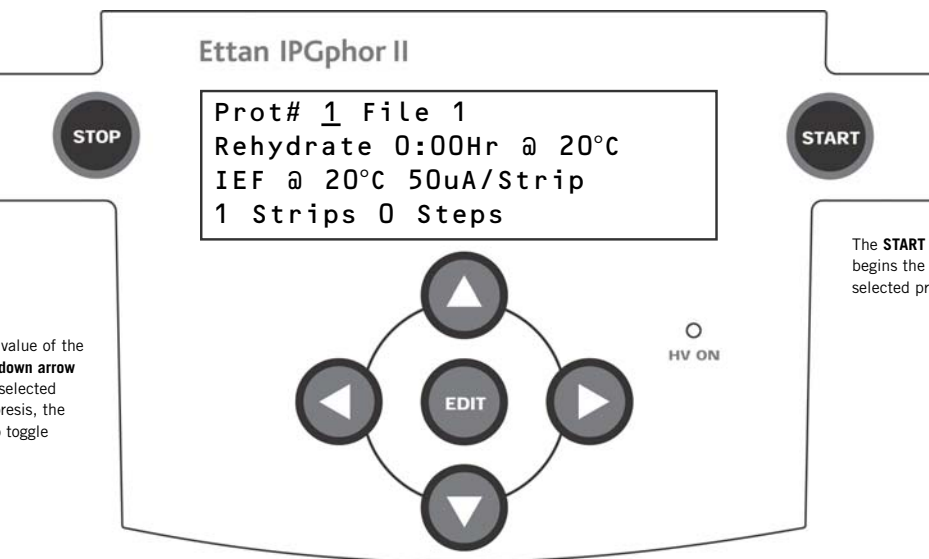
Once the diagnostic program is successfully completed, the main screen indicates that the instrument is ready, as shown below.

Fig 1. Ettan IPGphor II control panel.

The **main screen** is "Protocol #1, Name." The default location of the cursor is always under the protocol number "1." Select any protocol by pressing the up and down arrow keys to display the number of the desired protocol.

The **STOP key** pauses the protocol. Pressing the STOP key a second time cancels the protocol in progress, and pressing it a third time resets the instrument to start another protocol.

The **up arrow key** increases the value of the selected field or digit, and the **down arrow key** decreases the value of the selected field or digit. During electrophoresis, the up/down arrow keys are used to toggle between VhS, VhT, and Hrs.



The **START key** begins the selected protocol.

The **left arrow key** moves the cursor to the left. Moving the cursor off the screen to the left wraps the cursor either to the left end of the next line or to the left end of the top row.

The **EDIT key** switches the display to "edit mode," in which protocol steps can be programmed. Pressing the EDIT key in edit mode switches the display back to the identification screen for the protocol being edited. The key also allows the estimated run time remaining to be viewed while the unit is operating.

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 or wraps the cursor to the left end of the top row.

3.2. Creating and editing protocol programs

3.2.1. Protocol parameters

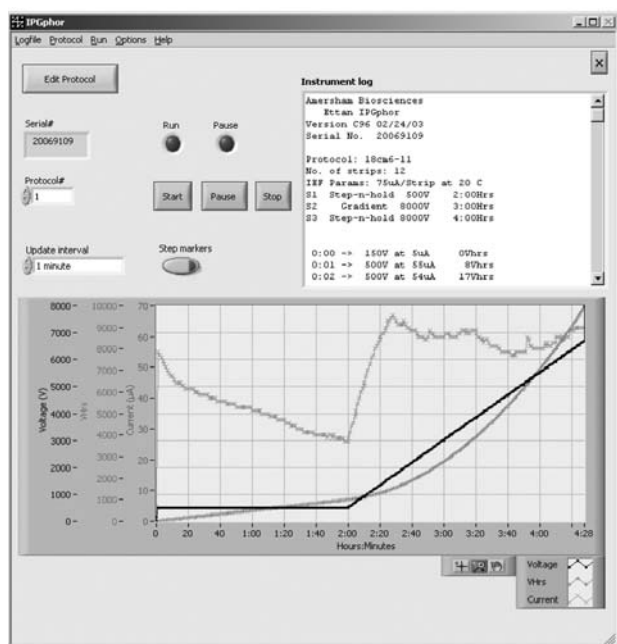
The Ettan IPGphor II can store up to ten protocols. Programmable protocol parameters include: duration and temperature of the rehydration phase; and duration, temperature, and power parameters for up to nine IEF steps for each protocol. Programmable power parameters include maximum current, voltage, and voltage change pattern.

Please refer to the *2-D Electrophoresis, principles and methods* guide for sample handling recommendations and protocol guidelines. Protocol worksheets (pages 27–28) can aid in mapping out and documenting each protocol. The allowable range of values for each programmable parameter is listed below.

Parameter	operational range	recommended values
Steps	1–9	
Step duration (hrs)	rehydration: 0–99:59 focusing: 0–99:59	10:00–14:00 2:00–10:00
Volts (V)	0–8000	100–8000
Max. current	1500 μ A total for 12 strips max.	50 μ A per strip
Platform temperature ($^{\circ}$ C)	rehydration: 15–30 focusing: 15–30	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 neighboring 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 ten’s 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. The Ettan IPGphor II can be programmed in volt-hours (Vhr) or hours and minutes in step-n-hold or in gradient mode. Any values within the operating limits listed above can be used

Fig 2. Ettan IPGphor II Control Software



3.2.2 Ettan IPGphor II Control Software

Ettan IPGphor II Control Software (80-6506-55) is available which allows the user to control the instrument and capture data from the serial port with a Windows™ based computer and serial cable. The following functions are provided:

- Editing of the protocols, loading to and retrieving them from the instrument.
- Starting, stopping and pausing of IEF runs on the instrument. Real time capture of a log file and graphing of the voltage, current and volthours at user selected intervals from 1–30 minutes.
- Printing and saving of the data to a text or spreadsheet file.

For detailed instructions on installation and usage of this software, please refer to the Ettan IPGphor II Control Software User Manual (80-6500-47).

3.2.3. Editing protocol parameters

All protocol parameters are set in the edit mode. Pressing the EDIT key toggles between the first editable screen and the protocol identification screen. For each protocol, the four different screens accessible in edit mode are:

- Screen 1** 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.
- Screen 2–4** the nine steps available for IEF conditions (voltage change pattern, voltage, and step duration) in groups of 3

Screen 1. Rehydration and IEF parameters.

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

1

Select the protocol to be created or edited from the main screen: Place the cursor under the protocol number field using the right and left arrow keys and then move to the desired protocol number using the up and down arrow keys.

```

Prot# 1 File 1
Rehydrate 0:00Hr @ 20°C
IEF @ 20°C 50uA/Strip
1 Strips 0 Steps

```

The file name may be edited by using the right arrow 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 3–10L,” requires scrolling through the alpha-numeric options for each of the 11 digits in the name.

2

Edit the Rehydration parameters, time and temperature and the IEF parameters, temperature, uA/Strip and number of strips as necessary.

```

Prot# 1 File 1
Rehydrate 10:00Hr @ 20°C
IEF @ 20°C 50uA/Strip
1 Strips 0 Steps

```

Note that the cursor stops only in the fields that can be edited. Once the cursor is in place, press the up or down arrow keys to adjust the value.

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 h 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.

Note: Maintaining the actual platform temperature at <18 °C or >25 °C may require placement of the Ettan IPGphor II unit 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 at greater than 7 °C below ambient temperature.

Screen 1. rehydration and IEF parameters.

To adjust the **rehydration temperature**, use the left and right arrow keys to place the cursor and the up and down arrow keys to select the value within the range of 15–30 °C. The original factory programmed value is 20 °C.

```
Prot# 1 File 1
Rehydrate 10:00Hr @ 20°C
IEF @ 20°C 50uA/Strip
1 Strips 0 Steps
```

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

The original factory programmed IEF temperature is 20 °C. To adjust the temperature, use the left and right arrow keys to place the cursor in the active °C field, and use the up and down arrow keys to select the value within the range of 15–30 °C.

```
Prot# 1 File 1
Rehydrate 10:00Hr @ 20°C
IEF @ 20°C 50uA/Strip
1 Strips 0 Steps
```

The original factory programmed value is 50 µA per IPG strip. This is the maximum recommended setting to prevent overheating and possible damage to the strip or the unit.

To adjust the current limit, use the left and right arrow keys to place 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 0–200 µA per strip.

Note: The total deliverable amperage is 1500 µA.

```
Prot# 1 File 1 Rehydrate
10:00Hr @ 20°C
IEF @ 20°C 50uA/Strip
1 Strips 0 Steps
```

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

To advance to the next edit screen, press the EDIT key.

3

Screens 2–4. IEF protocol steps.

The second screen encountered 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.

Each step is identified by “S” and the step number and can be accessed by placing the cursor under the step number using the right and left arrow keys. 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.

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; and “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 programmed option is “Step” for each step. To toggle to “Gradient,” move the cursor to the Step field using the right and left arrow keys and toggle with the up or down arrow key:

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

IEF voltage is programmable from 0–8000 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 1000V 4:00 Hrs
S3 Step 8000V 80000 Vhr
```

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 volt-hours (0 to 300000 Vhr) are programmable for each step.

To select, set the cursor under the currently selected field and use the up or down arrow key to toggle between Hrs and Vhrs. To set the digits, 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.

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 1–300000 Vhr.

```
Prot#1 3 Steps
S1 Grad 500V 1:00 Hrs ————— hours step
S2 Grad 1000V 4:00 Hrs
S3 Step 8000V 80000 Vhr ————— volt-hours step
```

Programming example

Simplified 4-step protocol and resulting voltage profile

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.

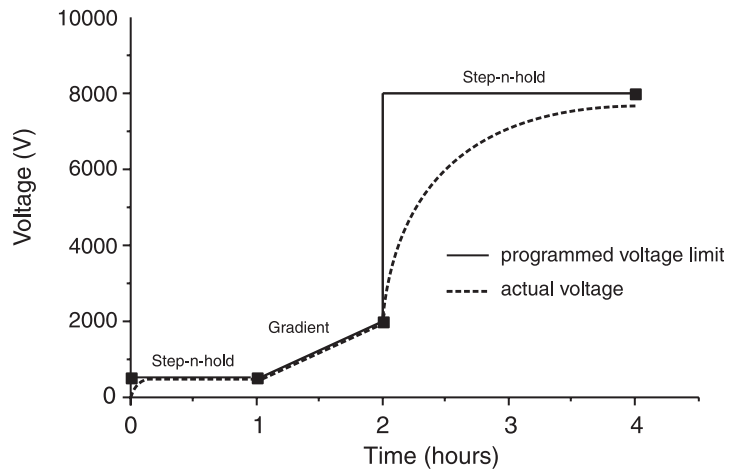
For recommended IEF protocols, see the 2-D Electrophoresis Principles and Methods guide.

```

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

Fig 3. Voltage profile.

The set voltage is the voltage limit. Under conditions of limiting current, it may take some time for the maximum voltage to be reached, and under certain power conditions, it may not be reached at all.



A lower voltage step may be entered at the end of the Ettan IPGphor II protocol. Band sharpness will decrease however.

4

To verify the protocol, scroll through each step: place the cursor in the step number field using the right and left arrow keys and then advance through the steps using the up and down arrow keys.

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. The Protocol Worksheets at the back of the manual can be useful in this step.

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.

5

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

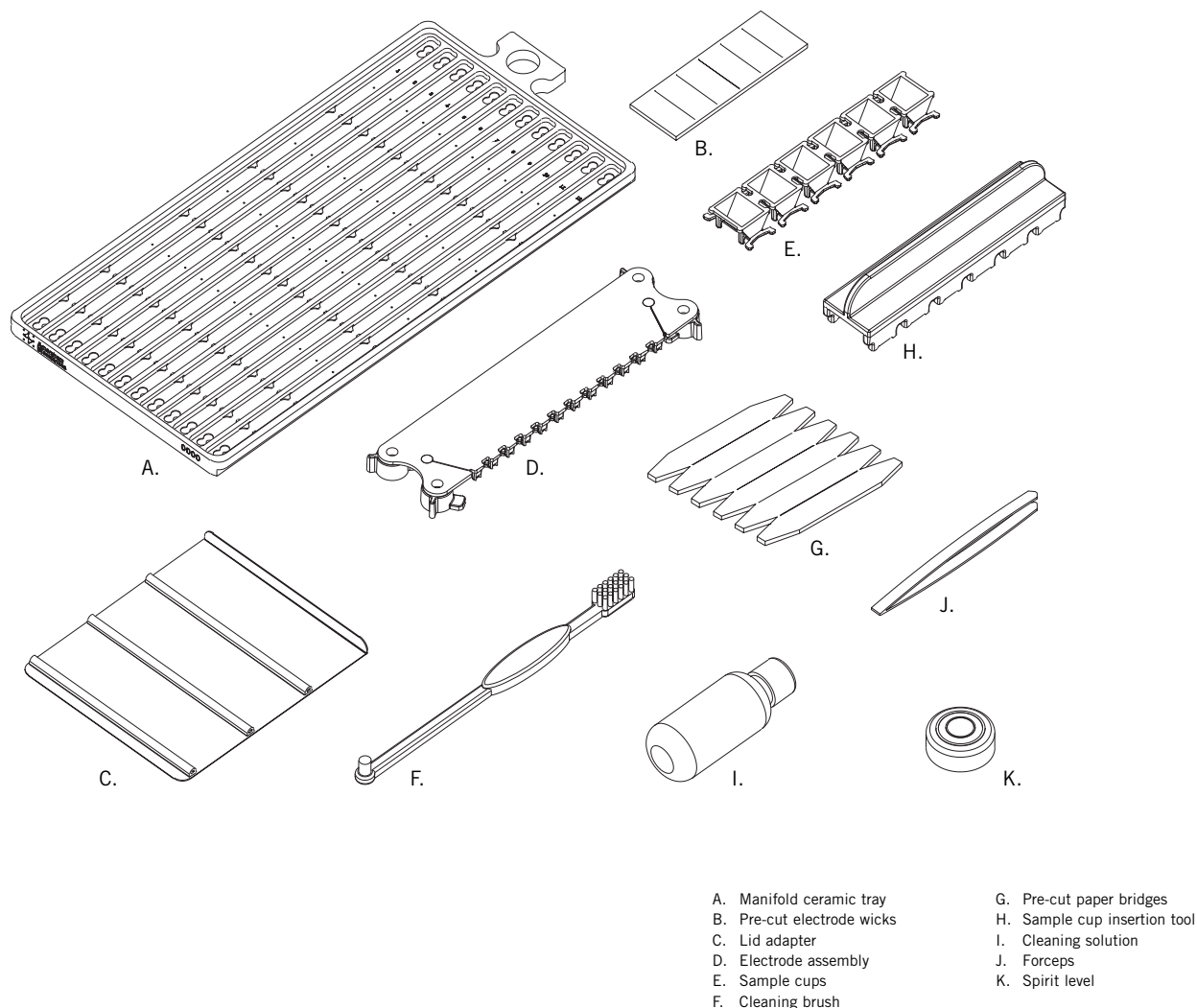
Note: Pressing the up arrow key in screen 4 wraps back to the rehydration screen.

3.3. IPG strip rehydration and sample application: Manifold Cup Loading Tray

Ettan IPGphor II Cup Loading Manifold

The Ettan IPGphor II Cup Loading Manifold is suitable for first-dimension isoelectric focusing (IEF) of proteins on Immobiline DryStrip immobilized pH gradient (IPG) gel strips 3 mm wide, from 7 cm to 24 cm long, in the Amersham Biosciences Ettan IPGphor II Electrophoresis unit. IEF is performed with the gel side facing up. Samples are applied in a localized region through an open bottom loading cup. Cup loading has been found to improve protein focusing patterns, particularly on basic IPG strips (pH 6–9 and 6–11). Under conditions where substantial water transport (electroendosmosis) accompanies focusing, such as with protein loads in excess of 1 mg, the face up mode frequently yields better resolution. The Manifold can accommodate anodic and cathodic loading.

Fig 4. Components of the Ettan IPGphor II Cup Loading Manifold system.



3.4. IPG strip rehydration and sample application: Fixed-length IPGphor Strip Holders

IPGphor 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. To rehydrate:

1

Prepare the strip holder(s).

Select the strip holder(s) corresponding to the IPG strip length chosen for the experiment (7, 11, 13, 18, or 24 cm). Wash each holder with strip holder cleaning solution supplied to remove residual protein. Rinse thoroughly with double distilled water. 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.

2

Prepare the rehydration solution

The 2-D Electrophoresis, Principles and Methods guide contains commonly used recipes. Required volumes per strip are itemized in the table below:

IPG strip length (cm)	Total volume per strip holder (µl, including any sample)
7	125
11	200
13	250
18	350
24	450

3

Pipet the appropriate volume of rehydration solution into each holder.

Deliver the solution slowly at a central point in the strip holder channel away from the sample application wells. Remove any larger bubbles.



4

Place the IPG strip

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



Finally, lower the cathodic (square) end of the strip into the channel, making sure that the IPG gel contacts 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.



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

Important! To ensure complete sample uptake, do not apply excess rehydration solution.

5

Apply Immobiline DryStrip Cover Fluid to minimize evaporation and urea crystallization. Pipet the fluid dropwise into one end of the strip holder until one half of the strip is covered. Then pipet the fluid dropwise into the other end of the strip holder, adding fluid until the entire IPG strip is covered.

6

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

7

Rehydration can proceed on the bench top or on the Ettan IPGphor II unit 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 II protocol. This is especially convenient if temperature control during rehydration is a concern, or if a low voltage is applied during rehydration.

Optional: Sample 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. To perform rehydration under voltage, set the rehydration time to 0:00 and program Step 1 of the protocol for low voltage (30–100 V) for 10–12 h. Program additional steps as desired to achieve complete focusing.

Optional: Apply electrode pads just prior to IEF

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.

1

To apply, cut two 3 mm wide electrode pads from a paper IEF electrode strip (18-1004-40). Place on a clean, flat surface such as a glass plate and soak with deionized water. Remove excess water by blotting with tissue paper.

Important! Electrode pads must be damp, not wet.

2

Using forceps or tweezers, lift one end of the rehydrated IPG strip. Position an electrode pad over the electrode, then lower the strip back in place. Repeat at the other end.

Optional: Apply sample after rehydration

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

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 µl of sample solution can be added to each side (i.e. 15 µl per well or 30 µl max. if both sides of both wells are used).



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

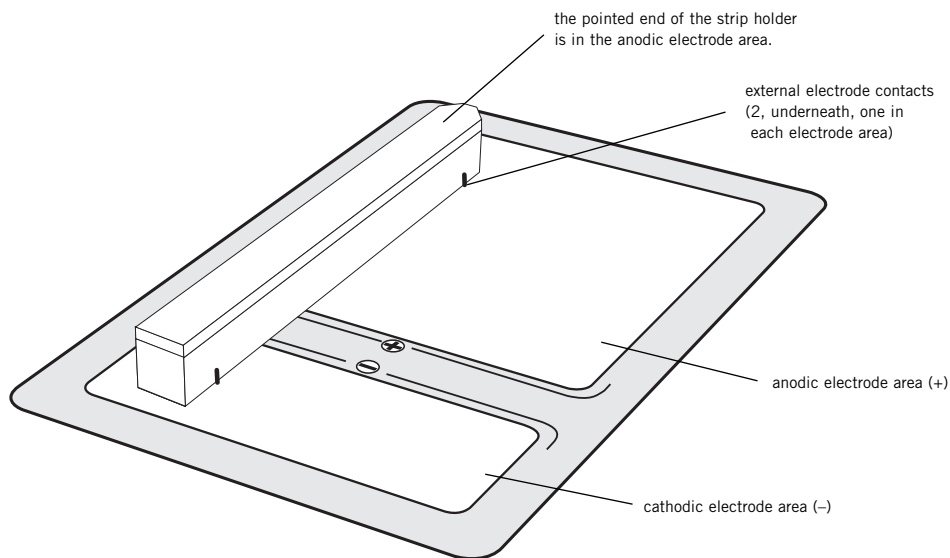
3.5. Running a protocol

3.5.1. Final assembly

①

Place the strip holders

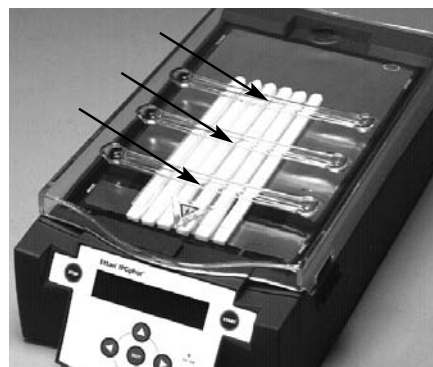
The Ettan IPGphor II platform has two electrode areas: the larger area is the positive electrode (anode) and the smaller area is the negative electrode (cathode). Position the strip holder on the platform as shown below: The pointed end of the holder is over the anode (pointing to the back of the unit) 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). Check that each of the two external electrode contacts makes metal to metal contact.



②

Close the safety lid

If using fixed length strip holders place the Lid adapter (80-6499-71) on top of the strip holders with the bent edge down at the front of the unit.



3.5.2. Starting isoelectric focusing

1

Advance to the protocol identification screen by placing the cursor in the protocol number field using the right and left arrow keys and selecting the protocol number using the up and down arrow keys.

```
Prot# 1 MY PROTOCOL
Rehydrate 10:00Hr @ 20°C
IEF @ 20°C 200uA/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):
Press the START key.

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

```
Number of strips :12
Press START to continue
```

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

```
Lid open step 1
Close lid to continue
```

Important! During IEF:

- 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.

Important! Pressure on the safety lid could cause arcing between the strip holder electrodes and the electrode areas, and may damage the instrument.

After securing the safety lid, press the START key again.

Once the protocol begins, each step is carried out automatically, and instrument status for each step is indicated by 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.

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
```


To change rehydration duration or other parameters, press the EDIT key to display the set screen:

The first line indicates the protocol is running. Pressing Edit again will access the step setting screen. Pressing Edit again will return to the Running screen. Any changes made while a protocol is running are applied to the current run only, the stored protocol is not affected.

```
Running Prot# 1
Rehydrate 10:00Hr @ 20°C
IEF @ 20°C 200uA/Strip
12 Strips 3 Steps
```

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. The temperature field can be changed directly (without pressing the EDIT key): use the right and left arrow keys to place 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 40uA/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 μ A settings.

The actual platform temperature is indicated.

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

The Ettan IPGphor II 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. 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 and change the display to reflect VhT, VhS and Hrs using the up and down keys.

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 buttons.

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

Pressing the Edit key will access the step parameter screen. Pressing Edit again will return to the Running screen. Use the up arrow in the S1 field 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:** 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 will display:

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

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

3.5.3. Pausing or stopping a protocol

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.

3.5.4. Printing a log of instrument conditions

The RS232C serial port is programmed to report instrument status every 5 minutes during isoelectric focusing. The rehydration period is not logged. Indicated are the time elapsed, voltage, current, and cumulative volt-hours. Also indicated are any interruptions in power, such as when the lid is opened or when the end of the programmed run is reached.

An abbreviated log illustrates the format.

Ettan IPGphor II Control Software (80-6506-55) may also be used to log and graph and print the output.

Additionally the output may be export to a text or spreadsheet file.

Amersham Biosciences
Ettan IPGphor II
Version A 05/25/03
Serial No. 20076543

Protocol: NAME
No. of strips: 1
IEF Params: 50 µA/ strip at 20 C
S1 Step-n-hold 500V 3:00Hrs

```
0:00 50V at 1 µA 0Vhr
0:05 500V at 9 µA 37Vhrs
0:10 500V at 10 µA 78Vhrs
0:15 500V at 10 µA 120Vhrs
0:20 500V at 10 µA 162Vhrs
0:25 500V at 9 µA 204Vhrs
0:26 ***End of run ***
```

4. Care and maintenance

4.1. Important!

- Strip holders are made of ceramic and thus are very brittle and fragile. Handle carefully. Do not bump or drop. When cleaning, take care not to bend or break the strip holder electrodes.
- Take care to avoid scratching the electrode areas.
- Wipe the exterior surfaces with a damp cloth after each use. Never use abrasive cleansers or solvents.
- Always turn the mains power switch off and unplug the power cord before doing any maintenance.

4.2. Cleaning

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. Never use ketones, hot strong acids, or hydrocarbon solvents.

IPGphor 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 withstand temperatures of up to 300 °C, however it can be damaged by prolonged exposure to extremes of pH.

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 (80-6452-78) can be ordered in 950-ml bottles from Amersham Biosciences.

Cleaning procedure

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

1

First rinse off the strip holder. Use a mild liquid soap to remove any residual IPG cover fluid.

2

Squeeze a few drops of Ettan IPGphor II Strip Holder Cleaning Solution into the strip holder slot. Use a toothbrush and vigorous agitation to clean the strip holder.

3

Rinse well with distilled or deionized water.

Thoroughly air dry the strip holders or dry well with a lint-free tissue prior to use.

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.

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

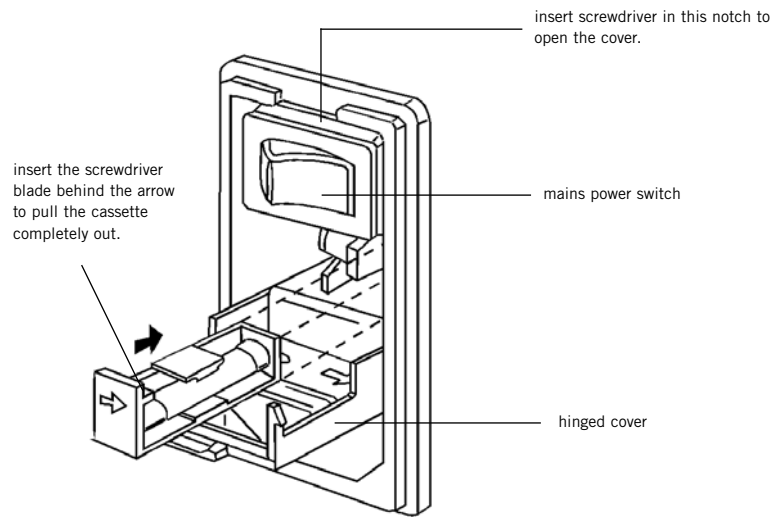
Important! Fixed length strip holders may be baked, boiled or autoclaved. DO NOT EXPOSE THEM TO STRONG ACIDS OR BASES, INCLUDING ALKALINE DETERGENTS. THE CUP LOADING MANIFOLD SHOULD NOT BE BAKED, BOILED OR AUTOCLAVED!



Important! 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 fuses which are the same for all models: T 1.6A 250V, 5 × 20 mm.

Fig 4. The mains power module is located on the back panel.

4.3. Replacing fuses



1

Caution! Turn the mains power supply switch off and detach the power cord before replacing input fuses!

2

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.

3

Insert the screwdriver above the arrow on one fuse cassette, catch the cassette end, and slowly slide it completely out of the module.

4

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.)

5

After placing a good 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).

6

Repeat steps 3 to 5 for second cassette.

7

Close the fuse compartment cover and gently press it into the power module until it snaps shut.

8

Plug the power cord into the unit and turn the mains power switch on.

5. Troubleshooting

symptom	possible solutions
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. HV 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 START key to continue through the diagnostic program. Call your local Amersham Biosciences sales office for further information on how to remedy the failure.
Power delivery	
Current too low or zero	<p>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.</p> <p>The gel must be evenly and completely rehydrated 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.</p>
Voltage limit not reached	<p>The ionic strength of the rehydration solution is too high; reduce the IPG buffer concentration; use a mixed-bed ion-exchange resin to remove ionic breakdown products of urea of other additives.</p> <p>Desalt the sample or prepare the sample so that the salt concentration is less than 10 mM.</p>
Sparks or burning in strips	<p>Reduce the current limit. Do not exceed 50 μA per strip.</p> <p>Prevent the IPG strip from drying out by always applying Immobiline DryStrip Cover Fluid during rehydration.</p> <p>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.</p> <p>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 electroendosmosis which will dry out the middle of the strip, possibly leading to arcing and burning there.</p>

Important! Request a copy of the Amersham Biosciences “Health and Safety Declaration” form before returning the item. No items can be accepted for servicing or return unless this form is properly completed.

6. Customer service information

6.1. Technical service and repair

Amersham Biosciences offers complete technical support for all our products. If you have any questions about how to use this product, or would like to arrange to repair it, please call or fax your local Amersham Biosciences representative.

6.2. Ordering information

product	quantity	code number
Ettan IPGphor II Isoelectric Focusing Unit		
Basic unit. Order strip holders separately	1	80-6505-03
Fuses, 1.6 A, 250V, 5 × 20mm	5	80-6108-69
Cable Serial 9 Pin F/F with Null Modem Adapter	1	80-6506-36
Ettan IPGphor II Control software	1	80-6506-55
Strip Holder Covers		
7 cm	2	80-6455-44
11 cm	2	80-6455-63
13 cm	2	80-6455-82
18 cm	2	80-6456-01
24 cm	2	80-6479-76
Strip Holders for use with Immobiline DryStrip gels		
7 cm, complete	6	80-6416-11
11 cm, complete	6	80-6416-30
13 cm, complete	6	80-6416-49
18 cm, complete	6	80-6416-68
24 cm, complete	6	80-6469-88
7 cm, complete	1	80-6416-87
11 cm, complete	1	80-6417-06
13 cm, complete	1	80-6417-25
18 cm, complete	1	80-6417-44
24 cm, complete	1	80-6470-07
Manifold Kit	1	80-6498-38
Immobiline DryStrip Reswelling Trays		
7–18 cm	1	80-6371-84
7–24 cm	1	80-6465-32
IPG Buffer		
pH 4–7	1 ml	17-6000-86
pH 3–10 L	1 ml	17-6000-87
pH 3–10 NL	1 ml	17-6000-88
pH 3.5–5.0	1 ml	17-6002-02
pH 4.5–5.5	1 ml	17-6002-04
pH 5.0–6.7	1 ml	17-6002-05
pH 5.5–6.7	1 ml	17-6002-06
pH 6–11	1 ml	17-6001-78
Rehydration Solutions		
DeStreak Rehydration Solution	1 ml	17-6003-19
DeStreak Reagent	5 × 3 ml	17-6003-18

product	quantity	code number
Ettan preparation kits and reagents		
2-D Fractionation Kit	10 preps	80-6501-04
Albumin and IgG Removal Kit	10 preps	RPN6300
Protease Inhibitor	1 ml	80-6501-23
Nuclease Mix	0.5 ml	80-6501-42
Thiourea	100 g	RPN6301
Iodoacetamide	25 g	RPN6302
Sample Grinding Kit	50 samples	80-6483-37
2-D Clean-Up Kit	50 samples	80-6484-51
SDS-PAGE Clean-Up Kit	50 samples	80-6484-70
2-D Quant Kit	500 assays	80-6483-56
Mini Dialysis Kit, 1 kDa cut-off, 250 µl	1	80-6483-75
Mini Dialysis Kit, 1 kDa cut-off, 2 ml	1	80-6483-94
Mini Dialysis Kit, 8 kDa cut-off, 250 µl	1	80-6484-13
Mini Dialysis Kit, 8 kDa cut-off, 2 ml	1	80-6484-32
PlusOne™ reagents		
Urea	500 g	17-1319-01
CHAPS	1 g	17-1314-01
Triton™ X-100	500 ml	17-1315-01
Dithiothreitol (DTT)	1 g	17-1318-01
Bromophenol Blue	10 g	17-1329-01
Immobiline DryStrip Cover Fluid	1000 ml	17-1335-01
Immobiline DryStrip gels		
7 cm		
pH 4–7	12	17-6001-10
pH 3–10	12	17-6001-11
pH 3–10NL	12	17-6001-12
pH 6–11	12	17-6001-94
11 cm		
pH 4–7	12	18-1016-60
pH 3–10	12	18-1016-61
pH 6–11	12	17-6001-95
13 cm		
pH 4–7	12	17-6001-13
pH 3–10	12	17-6001-14
pH 3–10NL	12	17-6001-15
pH 6–11	12	17-6001-96
18 cm		
pH 4–7	12	17-1233-01
pH 3–10	12	17-1234-01
pH 3–10NL	12	17-1235-01
pH 3.5–4.5	12	17-6001-83
pH 4.0–5.0	12	17-6001-84
pH 4.5–5.5	12	17-6001-85
pH 5.0–6.0	12	17-6001-86
pH 5.5–6.7	12	17-6001-87
pH 6–9	12	17-6001-88
pH 6–11	12	17-6001-97

product	quantity	code number
24 cm		
pH 3.5–4.5	12	17-6002-38
pH 4.0–5.0	12	17-6002-39
pH 4.5–5.5	12	17-6002-40
pH 5.0–6.0	12	17-6002-41
pH 5.5–6.7	12	17-6002-42
pH 3–7 NL	12	17-6002-43
pH 3–10	12	17-6002-44
pH 3–10 NL	12	17-6002-45
pH 4–7	12	17-6002-46
pH 6–9	12	17-6002-47
Cleaning solution, IPGphor Strip Holder	950 ml	80-6452-78
2-D Electrophoresis Principles and Methods	1	80-6429-60
Equilibration tube set for 24 cm IPG Strips	12	80-6467-79
IEF electrode strips	100	18-1004-40

Ettan IPGphor II Isoelectric Focusing System

First Dimension Protocol Worksheet

Program steps

Prot# _____
 Rehydrate ___:___Hr @ ___°C
 IEF @ ___°C ___uA/Strip
 ___ Strips ? Steps

Prot#? ? Steps
 S1 _____V _____
 S2 _____V _____ S3
 _____V _____

Prot#? ? Steps
 S4 _____V _____
 S5 _____V _____ S6
 _____V _____

Prot#? ? Steps
 S7 _____V _____
 S8 _____V _____ S9
 _____V _____

Step of Grad

_: _ Hrs or ___Vhrs

IPG strip preparation

Rehydration volume (circle one)

IPG strip length (cm)	rehydration volume per strip (µl)
7	125
11	200
13	250
18	350
24	450

Rehydration solution

	reagent final conc.	volume or weight
Urea		
Detergent		
Reductant		
IPG buffer pH		
Dye		
Sample		
Water		
Total volume		

Notes:

Date _____
 Researcher _____
 Sample _____



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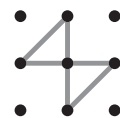
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