Ettan IPGphor Cup Loading Manifold

Ettan IPGphor Isoelectric Focusing System

um 80-6499-52/ Rev. A0/ 06-03

Amersham Biosciences

Page finder

Safety warnings and precautions

English

Important user information

Please read this entire manual to fully understand the safe and effective use of this product. The exclamation mark within an equilateral triangle is intended to alert the user to the presence of important operating and maintenance instructions in the literature accompanying the instrument. If you have any comments on this manual, please send them to us at:

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Français

Renseignements importants d'utilization

Pour une bonne compréhension et une utilisation en sécurité maximale, il convient de lire entièrement ce manuel. Dans la documentation qui accompagne l'instrument un point d'exclamation dans un triangle équilatéral a pour but d'attirer l'attention de l'utilisateur sur des instructions importantes de fonctionnment ou de maintenance. Tous vos commentaires sur ce manuel seront les bienvenus et veuillez les adresser à:

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Español

Información importante para el usuario

Para comprender el producto y utilizarlo con seguridad es necesario leer este manual en su totalidad. El signo de admiración en un triángulo equilátero en el manual, advierte al usuario sobre la presencia de instrucciones importantes de operación y mantenimiento del aparato. Si desearan hacer algún comentario sobre este manual, tengan la amabilidad de remitirlo a:

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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 punto esclamativo all'interno di un triangolo equilatero indica all'operatore la presenza di importanti istruzioni di funzionamento e manutenzione nella documentazione allegata al prodotto. Si prega di inviare eventuali commenti al presente manuale a:

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Unpacking

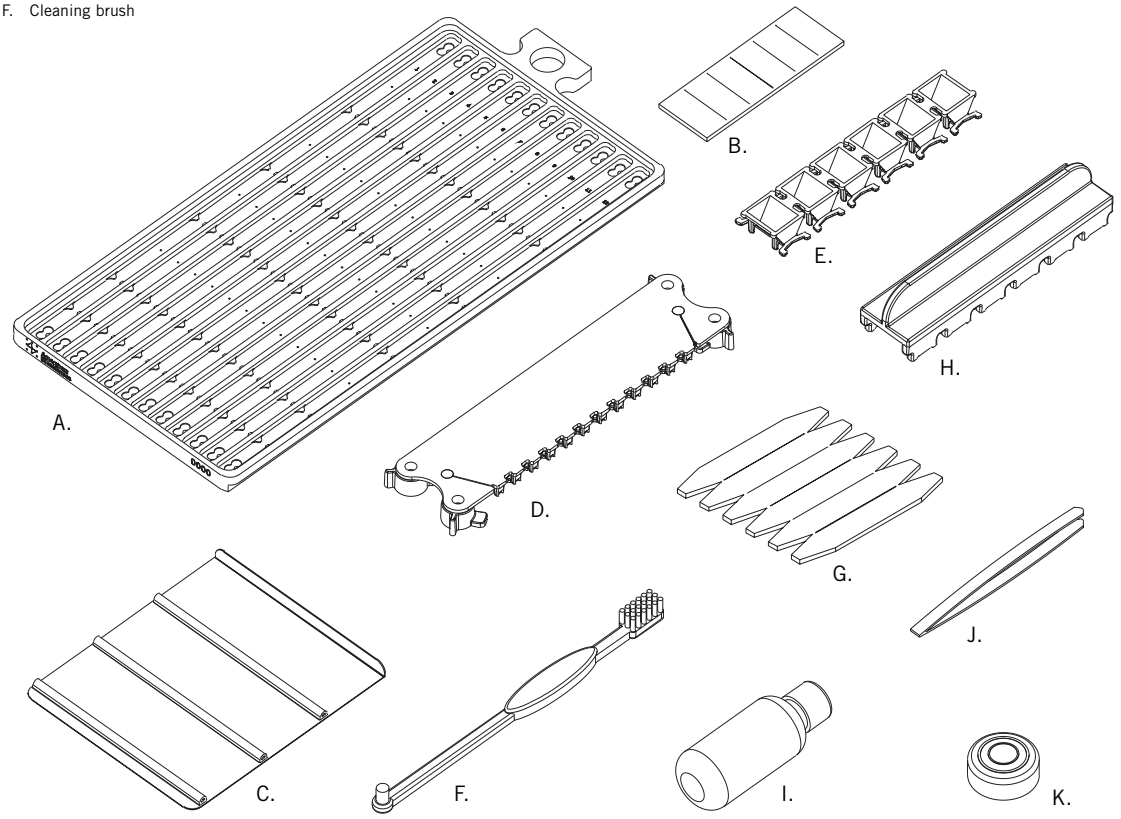
Unwrap all packages carefully and compare contents with the packing list, making sure all items arrived. If any part(s) is missing, contact your local Amersham Biosciences sales office. Inspect all components for damage that may have occurred while the unit was in transit. If any part(s) appear damaged, 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.

Ettan IPGphor Cup Loading Manifold

The Ettan™ IPGphor™ 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 and Ettan IPGphor II Isoelectric Focusing Units. 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 1. Components of the Ettan IPGphor Cup Loading Manifold system. Only one of each item shown.

A. Manifold ceramic tray
B. Pre-cut electrode wicks
C. Lid adapter
D. Electrode assembly
E. Sample cups
F. Cleaning brush
G. Pre-cut paper bridges
H. Sample cup insertion tool
I. Cleaning solution
J. Forceps
K. Spirit level



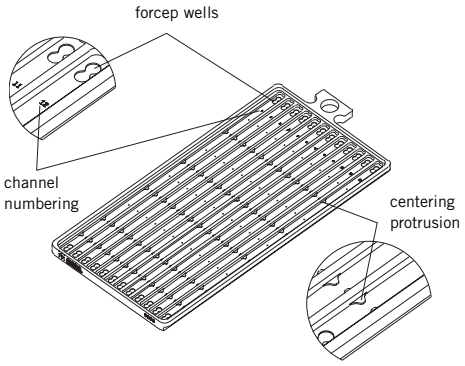


Fig 2. Ceramic manifold with details.

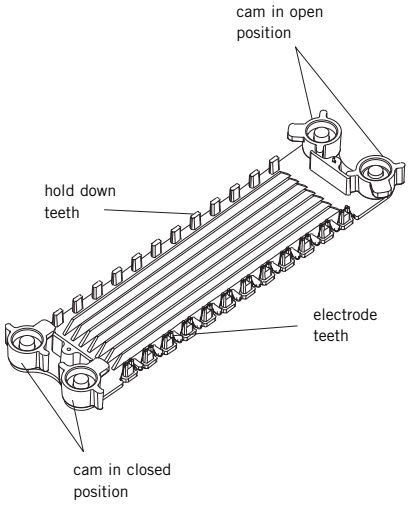


Fig 3. Electrode and details.

Manifold

The manifold (Fig 2) is made of aluminum oxide ceramic for efficient heat transfer and temperature control during IEF. Protrusions along the channel inside the manifold align the rehydrated IPG strip, keeping it straight and centered when placed inside the manifold. The manifold has a coating to minimize protein adsorption. Because some cleaning agents can remove this coating, clean the manifold only with the Ettan IPGphor Strip Holder Cleaner. The manifold is fragile and should be handled with care. IPG strips from 7 cm to 24 cm long may be used. The unit may also be used for IPG strip equilibration prior to second dimension electrophoresis. Forcep wells are located at the ends of the channels to aid strip removal after electrophoresis.

Electrode Assembly

The movable electrodes (Fig 3) can be placed anywhere along the manifold where the electrode pins will make electrical contact with the power supply pads on the bed of the Ettan IPGphor. The electrodes have a platinum wire that is strung across the bottom of the electrode teeth to provide electrical contact to the IPG strips. The hold down teeth are found along the opposite length of the electrode. They are used to apply pressure on the paper bridges when the manifold is used for bridge loading. Cams on the sides of the electrode secure the electrodes in place and must be in the closed position during IEF.

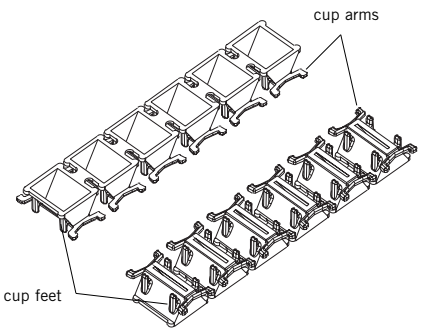


Fig 4. Sample loading cups.

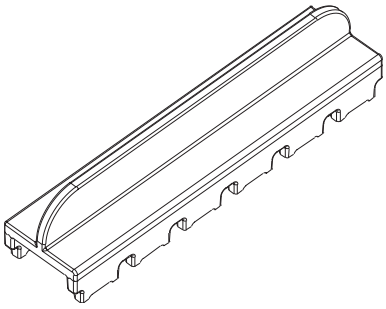


Fig 5. Sample cup insertion tool.

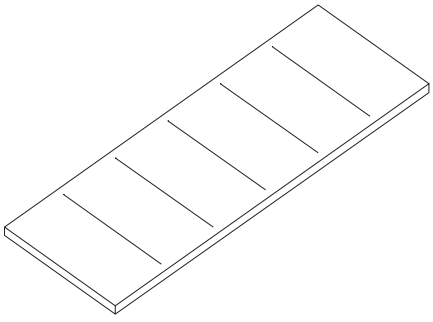


Fig 6. Paper wicks.

Sample Cups

Sample cups (Fig 4) can be placed almost anywhere along the length of the Ettan IPGphor manifold that is not blocked by a centering protrusion. The sample cups are supplied in strips of 6 for easy handling and placement. If fewer than 6 cups are required, they may be easily separated by cutting the thin plastic bridging the strip of cups together. The sample cups can accommodate sample volumes of up to 150 µl. For proper sealing of the cup to the gel, all of the feet of the sample cup must rest on the bottom of the channel and all cup arms must be fully pressed down into the channel.

Sample cup insertion tool

The sample cup insertion tool (Fig 5) is supplied with the manifold kit and refill packages of sample cups. It is used for proper placement and sealing of the cup over the IPG strips.

IEF electrode paper wicks

The small, precut rectangular wicks (Fig 6) must be placed at both the anodal and cathodal ends of the rehydrated IPG strips just under the electrodes. The wicks absorb excess water, salts, and proteins with pIs that lie outside the pH range of the IPG strip.

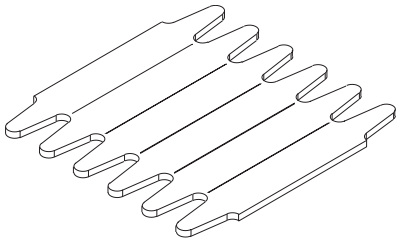


Fig 7. Paper bridges.

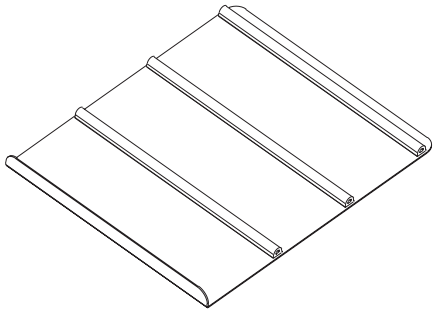


Fig 8. Standard strip holder adapter.

Paper bridge loading pads

Bridge loading pads (Fig 7) are used to load larger volumes of sample (375–500 µl) than what can be held in a sample cup. The bridges are 0.8 cm × 5.0 cm in size with pointed ends. One end of the pad contacts the IPG strip and is held in place by the hold down teeth of the electrode assembly. The other end of the bridge loading pad makes contact with a paper wick placed under the platinum wire of the electrode assembly.

Lid adaptor for standard strip holders

When using the IPGphor standard strip holders or the IPGphor cup loading strip holders a foam pad lid adapter (Fig 8) must be used to apply the correct amount of pressure to the tops of the IPGphor strip holders and keep them in contact with the IPGphor power supply pads. If running only one IPGphor strip holder a second IPGphor strip holder must be placed on the opposite side of the lid adapter to maintain the adapter in a level position.

Not included but required:

Immobiline DryStrip Cover Fluid (order separately)

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

Immobiline DryStrip Reswelling Tray (order separately)

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

IPGphor lid modifications

The IPGphor lid must be modified before using the manifold system. The three (3) foam pads on the lid must be removed (Fig 9). Once removed, any adhesive remaining on the lid must be cleaned off. The foam pads are NOT replaced on the lid. After lid modification, the lid adaptor (Fig 8) must be used when running IPGphor strip holders.

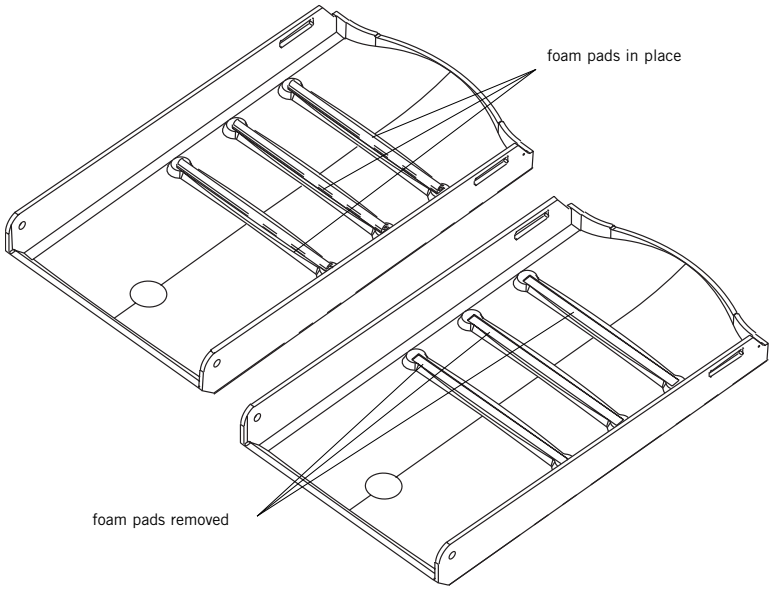


Fig 9. Lid modifications.

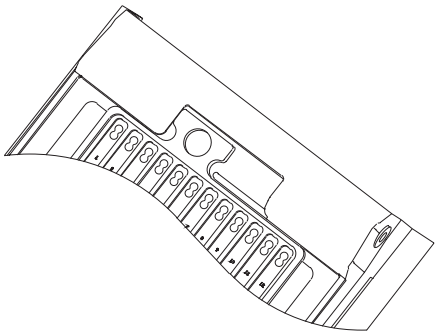


Fig 10. Manifold placement on the Ettan IPGphor.

Important! Before proceeding, make sure the Ettan IPGphor unit is placed on a level surface.

Important! Ensure that the three foam pads have been removed from the lid of the Ettan IPGphor.

Instructions

❶

Rehydrate the IPG strips with the gel side down in the appropriate volume of rehydration solution using the Immobiline DryStrip Reswelling Tray. Rehydration in the Cup Loading Manifold is not recommended: the channel is too wide to ensure proper rehydration. Follow the instructions included with the Immobiline DryStrips. The table below is provided as a reference.

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

❷

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

❸

Clean and dry the IPGphor bed before placing the manifold tray on the unit. Position the manifold on the IPGphor platform. The small T-shaped protrusion fits into a cut-out section of the IPGphor bed near the lid hinge (Fig 10). Ensure that the manifold is level by placing the round spirit level on the center of the manifold tray after it is placed on the Ettan IPGphor unit.

4

Measure out 108 ml of Immobiline DryStrip Cover Fluid (even if fewer than 12 strips will be loaded into the manifold). Add the cover fluid evenly in the 12 manifold channels. Transfer the strips to the Ettan IPGphor Cup Loading Manifold. Place the strips under the cover fluid face up in the tray with the anodic (+, pointed) end of the IPG strip resting on the appropriate mark etched into the bottom of the manifold channel (the end of the gel, not the end of the plastic, should align with the etched mark). Center the strip down the length of the manifold channel. Protrusions along the sides guide the strip approximately straight, although some manual adjustment of the strip may be necessary (Fig 11).

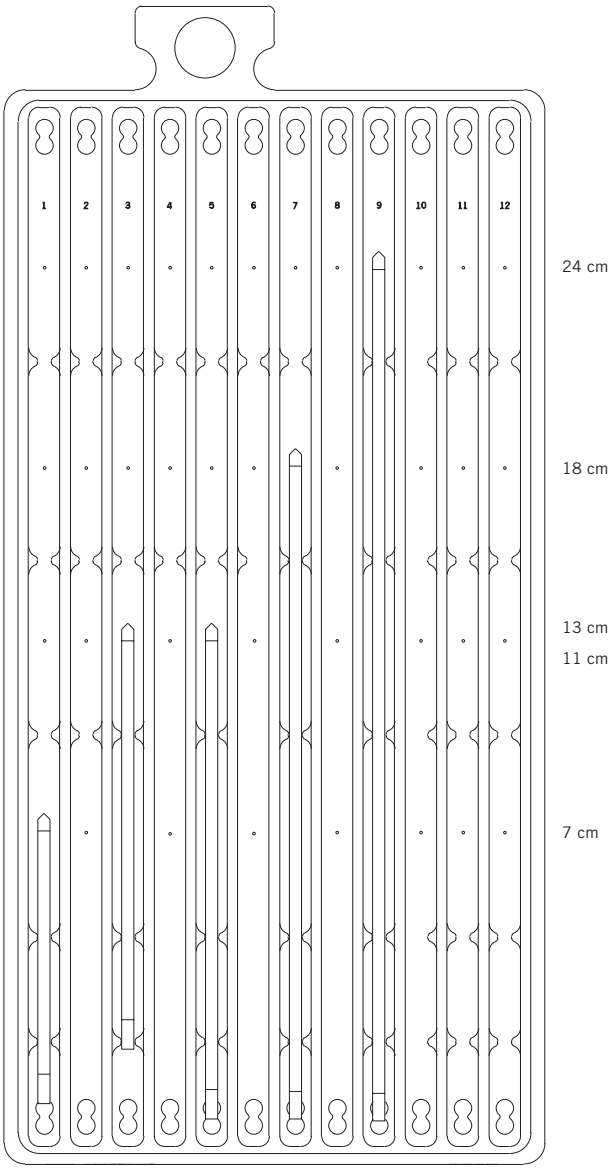


Fig 11. Placement of IPG strips in manifold channels.

Note: If cathodic cup loading is going to be used, the strips should be placed such that the anodic end of the strips is 3–4 cm beyond the etched placement mark.

❺

Place a strip of cups in the appropriate position (Fig 12), for example ~1 cm from the end of the gel portion of the IPG strip. Do NOT place the cup with the feet over a center protrusion. Push the cups into the channels with gloved fingers, starting at one end of the strip and working towards the other. Align the insertion tool over the cups and push down to ensure that the feet of the cups are properly seated at the bottom of the channel (wiggle the tool gently while pushing down in order to ensure that the cups are seated as far down as they will go). Take care not to move the cups while removing the insertion tool. Fill the cups with cover fluid to test for proper seating of the cups. Remove the cover fluid after 10 minutes.

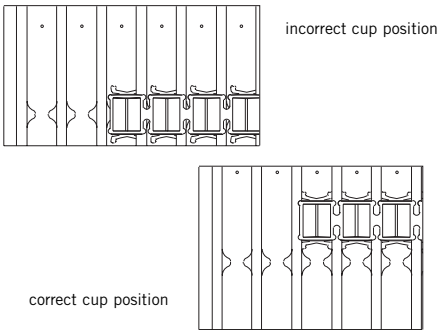


Fig 12. Sample cup positioning details.

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

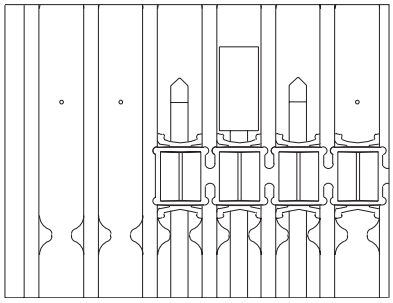


Fig 13. Correct placement of paper wicks.

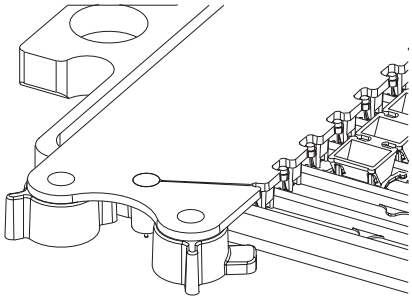


Fig 14. Placement of electrode on paper wicks. Cams are in the open position.

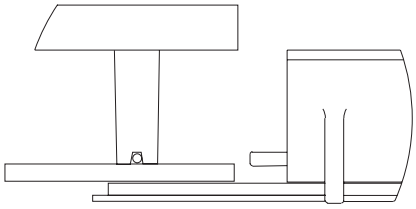


Fig 15. Placement of paper wick overlapping end of gel, electrode on paper wick, and sample cup on gel.

6

Count out the appropriate number of precut paper wicks. Two wicks per strip are required. Separate the wicks from each other. Add 150 µl distilled water to each wick. Place the wicks on the IPG strips such that one end of the wick overlaps the end of the gel on the IPG strip (Fig 13). The electrode must contact the wick. With the electrode cams in the open position, place the electrode assembly on top of all the wicks. Swivel the cams into the closed position under the external lip of the tray. The electrodes should not be moved while the cams are in the closed position (Fig 14).

7

Briefly centrifuge the protein sample prior to loading to remove insoluble material and particulate matter. These materials will impede sample entry and result in vertical streaks in the second-dimension gel. Load samples into the sample cups. A maximum of 150 µl of sample may be placed in these cups. Check to make sure that there is cover fluid over the samples. When the cups are initially placed on the manifold, cover fluid will flow into the cups as they are seated. When sample is introduced into the cups, the sample will sink to the bottom of the cup and contact the IPG strip.

Note: For basic IPG strips, superior focusing patterns are generally obtained when the sample cup is placed as close to the anodic (+) electrode as possible.

8

Close the Ettan IPGphor lid. Program the Ettan IPGphor with the desired run parameters. Ramping the voltage slowly while the sample is entering the IPG strip will improve results. Optimal ramp, voltages and times, or Vhrs (volt-hours) totals must be determined empirically for each sample type. Focusing after sample cup application frequently requires fewer Vhrs than in-gel sample rehydration loading methods, particularly on basic pH-range strips.

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

Suggested run conditions

Table 1. Guidelines for Ettan IPGphor protocols with Cup Loading Manifold for broad, medium, and narrow pH range Immobiline DryStrip gels. Running conditions: Temperature 20 °C, Current 50 µA/strip.

Immobiline DryStrip gels				Running conditions for application method			
				Cup Loading		Bridge Loading	
Length (cm)	pH range(s)	Voltage mode	Voltage (V)	Duration (h:min)	Volt-hours (kVh)	Duration (h:min)	Volt-hours (kVh)
7	3–10	1 Gradient	500	0:01	0.01	0:01	0.01
	3–10 NL	2 Gradient	4000	1:30	3.4	2:30	5.6
	4–7	3 Step and Hold	5000	0:45	3.7	0:30	2.5
	6–11	Total		2:15	7.1	3:00	8.0
11	3–10	1 Gradient	500	0:01	0.01	0:01	0.01
	4–7	2 Gradient	4000	1:30	3.4	2:30	5.6
		3 Step and Hold	8000	1:30	10.6	1:40	12.0
		Total		3:00	14.0	4:10	17.6
	6–11	1 Gradient	500	0:01	0.01	0:01	0.01
		2 Gradient	4000	1:30	3.4	2:30	5.6
		3 Step and Hold	8000	1:15	8.5	1:30	10.0
		Total		2:45	11.9	4:00	15.6
13	3–10	1 Gradient	500	0:01	0.01	0:01	0.01
	3–10 NL	2 Gradient	4000	1:30	3.4	2:30	5.6
	4–7	3 Step and Hold	8000	1:50	13.5	2:10	15.2
		Total		3:50	16.9	4:40	20.8
	6–11	1 Gradient	500	0:01	0.01	0:01	0.01
		2 Gradient	4000	1:30	3.4	2:30	5.6
		3 Step and Hold	8000	1:40	11.6	1:50	13.4
		Total		3:10	15.0	4:20	19.0
18	3–10	1 Step and Hold	300	3:00	0.9	3:00	0.9
	3–10 NL	2 Gradient	1000	6:00	3.9	6:00	3.9
	4–7	3 Gradient	8000	3:00	13.5	3:00	13.5
		4 Step and Hold	8000	1:10	9.7	1:50	14.7
		Total		13:10	28.0	13:50	33.0
	6–9	1 Step and Hold	300	3:00	0.9	3:00	0.9
		2 Gradient	1000	6:00	3.9	6:00	3.9
		3 Gradient	8000	3:00	13.5	3:00	13.5
		4 Step and Hold	8000	3:20	26.7	4:00	31.7
		Total		15:20	45.0	16:00	50.0
	6–11	1 Step and Hold	500	1:00	0.5	3:00	1.5
		2 Gradient	1000	2:00	1.5	2:00	1.5
		3 Gradient	8000	3:00	13.5	3:00	13.5
		4 Step and Hold	8000	1:05	8.5	1:30	11.5
		Total		7:05	24.0	9:30	28.0
	Narrow intervals§	1 Step and Hold	300	3:00	0.9	3:00	0.9
		2 Gradient	1000	6:00	3.9	6:00	3.9
		3 Gradient	8000	3:00	13.5	3:00	13.5
		4 Step and Hold	8000	5:10	41.7	6:00	47.7
		Total		17:20	60.0	18:00	66.0
24	3–10	1 Step and Hold	300	3:00	0.9	3:00	0.9
	3–10 NL	2 Gradient	1000	6:00	3.9	6:00	3.9
	4–7	3 Gradient	8000	3:00	13.5	3:00	13.5
	3–7	4 Step and Hold	8000	4:40	36.7	5:50	46.3
		Total		16:40	55.0	17:50	65.0
	6–9	1 Step and Hold	300	3:00	0.9	3:00	0.9
		2 Gradient	1000	6:00	3.9	6:00	3.9
		3 Gradient	8000	3:00	13.5	3:00	13.5
		4 Step and Hold	8000	5:10	41.7	6:40	53.7
		Total		17:10	60.0	18:40	72.0
	Narrow intervals§	1 Step and Hold	300	3:00	0.9	3:00	0.9
		2 Gradient	1000	6:00	3.9	6:00	3.9
		3 Gradient	8000	3:00	13.5	3:00	13.5
		4 Step and Hold	8000	9:40	77.7	11:40	93.7
		Total		21:40	96.0	23:40	112.0

§ Narrow intervals = 3.5–4.5, 4.0–5.0, 4.5–5.5, 5.0–6.0, and 5.5–6.7.

Troubleshooting first-dimension IEF:

Ettan IPGphor Isoelectric Focusing System employing the Cup Loading Manifold.

symptom	possible cause	remedy
Current is too low or zero	Electrical continuity is impeded.	Check the external electrode contacts: The electrodes at the bottom of the strip holder (one at each end) must make metal-to-metal contact with the appropriate electrode contact area.
		Check the internal electrode contacts: The gel (which becomes visible because of the dye in the rehydration solution) must contact both electrodes in the manifold through the paper wicks and/or paper bridge parts.
		Check that the IPG strip is fully rehydrated along its entire length. Electrical contact at the electrodes is reduced by incomplete rehydration.
		Check that the paper wicks are present and properly positioned.
Voltage too low or does not reach maximum set value	Ettan IPGphor protocol settings are incorrect for the experiment.	Check that the current limit is properly set.
		Check that the actual number of Immobiline DryStrip gels on the Ettan IPGphor platform is the same as the number of gels entered in the protocol.
	Conductivity/ionic strength is too high.	Prepare the sample to yield a salt concentration less than 10 mM. The recommended IPG Buffer concentration is 0.5%. A maximum of 2% is advisable only if sample solubility is a problem. High conductivity can also arise from the use of poor quality urea or other denaturants. Urea is also prone to decompose to charged breakdown products. Higher conductivity salts and ionic impurities in the sample can raise the conductivity of the strip.
		Shorter length IPG strips (e.g. 7 cm strips) will not reach 8000 V. The distance between the electrodes is shorter so that the voltage gradient (V/cm) required to reach the 50 µA current limit is reached at a lower overall voltage.
Sample leaks from cup	Incorrect cup placement.	Check that the feet of the cups are resting on the bottom of the manifold channel.
		Check for correct positioning of sample cup arms.
		Check that the feet of the cups are not resting on a centering protrusion in the channel.
	Incorrect strip placement.	Check that the strip is centered inside of the channel.
Sparking or burning in the Immobiline DryStrip gels	Current limit setting is too high.	Do not exceed the maximum recommended setting of 50 µA per Immobiline DryStrip gel.
	Immobiline DryStrip gel is not fully rehydrated.	Ensure that the Immobiline DryStrip gels are rehydrated with a sufficient volume of rehydration solution. Remove any large bubbles trapped under the Immobiline DryStrip gel after placing it on rehydration solution.
	Immobiline DryStrip gels dried out during IEF.	Always apply Immobiline DryStrip Cover Fluid to prevent dehydration of rehydrated Immobiline DryStrip gels.
Immobiline DryStrips turn white and opaque after focusing	Immobiline DryStrip gels dried out during IEF.	Always apply recommended amount of Immobiline DryStrip Cover Fluid to prevent dehydration of rehydrated Immobiline DryStrip gels.
Immobiline DryStrip Cover Fluid overflows from Cup Loading Manifold	Excess cover fluid added.	Do not add more than the recommended volume. Ensure that the outside rim of the tray does not have any oil on it.

Paper-bridge loading

Large sample volumes and large protein amounts can be applied using paper bridge loading. A paper pad (paper bridge) is soaked with sample and placed between the anodic end of the Immobiline DryStrip gel and the electrode (375–500 µl sample can be applied using the paper bridge pads supplied with the manifold). Solutions containing up to 5 mg protein have been loaded on a 18 cm-long narrow pH range Immobiline DryStrip gel. The rehydrated Immobiline DryStrip gel is first positioned in the bottom of the manifold channel. Then the paper bridge with sample is positioned, followed by a paper wick (Fig 16). With anodic application the anode electrode is positioned as far out as possible in the electrode assembly, while the cathode electrode is positioned close to the end of the Immobiline DryStrip gel to ensure good contact between the paper wick and Immobiline DryStrip gel.

Note: The application point (anodic or cathodic) is an important factor for obtaining good results.

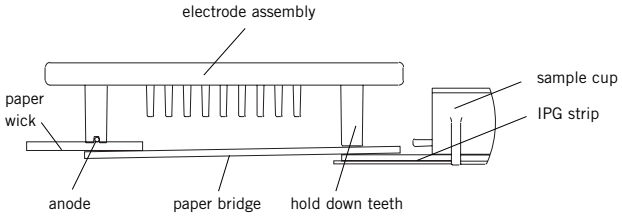


Fig 16. Electrode positioning (anodic end) on the paper bridges.

Note: A single paper bridge can be used with the 24 cm gel strip. A paper bridge can be used on both ends of all other strips at one time.

Care and cleaning

Ceramic Manifold

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

Important! 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.

Electrodes

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

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.

Customer service information

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, please call or fax your local Amersham Biosciences representative.

Ordering information

Replacement parts		Code number
Ettan IPGphor Cup Loading Manifold		80-6498-57
Sample cups, pack of 20 (6x)		80-6498-95
Paper electrode wicks, pack of 40 (6x)		80-6499-14
Paperbridge pads, pack of 20 (6x)		80-6499-33
Electrode set		80-6498-76
Lid adapter		80-6499-71
Cleaning brush		80-6505-98
Spirit level		80-6194-19
Forceps SS		80-6506-17
Accessory products		
Immobiline DryStrip Reswelling Tray, 7–18 cm	1	80-6371-84
Immobiline DryStrip Reswelling Tray, 7–24 cm	1	80-6465-32
Immobiline DryStrip Cover Fluid	1 liter	17-1335-01
Ettan IPGphor Strip Holder Cleaner	950 ml	80-6452-78

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