

Ettan IPGphor Cup Loading Strip Holder

Ettan IPGphor isoelectric focusing system



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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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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 fonctionnement ou de maintenance.

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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.

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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.

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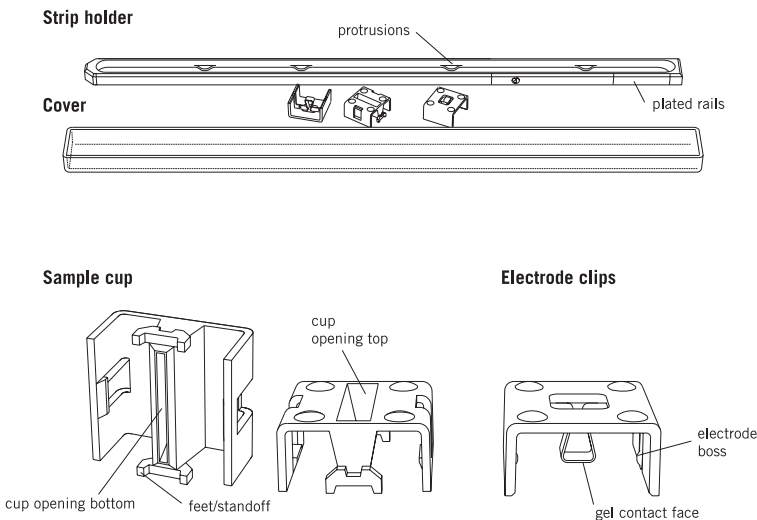
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Ettan IPGphor Cup Loading Strip Holder

The Ettan™ IPGphor™ Cup Loading Strip Holder 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 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 faceup mode frequently yields better resolution.

Fig 1. Components of the Ettan IPGphor Cup Loading Strip Holder system.



Strip holder

The strip holders are made of aluminum oxide ceramic for efficient heat transfer and temperature control during IEF. Two areas of metal plating on the bottom extend up the sides of the strip holder. These plated areas make contact with the power supply contact pads when placed on the IPGphor separation platform.

Protrusions along the channel inside the strip holder align the rehydrated IPG strip, keeping it straight and centered when placed inside the strip holder. The anodic end of the strip holder is somewhat pointed to indicate the direction of placement of the pointed IPG strip.

The strip holders have a coating to minimize protein adsorption. Because some cleaning agents can remove this coating, clean the strip holders only with the Ettan IPGphor Strip Holder Cleaner as directed. The strip holders are very fragile and should be handled with care.

Electrodes

The movable electrode clips can be placed anywhere along the strip holder where the electrode bosses make electrical contact with the conducting rails on the sides of the strip holder. IPG strips from 7 cm to 24 cm long can be used.

Sample cups

Sample cups can be placed almost anywhere along the length of the Ettan 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.

Cover

The cover is used to ensure that the electrodes stay in place and that they are in good contact with the IPG strip. The cover also applies gentle pressure to ensure that the strip holder makes good contact with the IPGphor separation platform and power supply contact pads when the safety lid is closed.

Required but not included:

Immobiline DryStrip Cover Fluid

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

Immobiline DryStrip Reswelling Tray

The Immobiline DryStrip Reswelling Tray is required for proper strip rehydration. The channel in the Cup Loading Strip Holder is too wide for 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.

Optional:

IEF electrode strips

If desired, small rectangular pads cut from the IEF electrode strips can be placed at the ends of the rehydrated Immobiline DryStrips just under the electrodes. Although electrode pads are not required for making electrical contact between the IPG strip and the electrode, they can improve the quality of results. The pads absorb excess water as well as proteins with pIs that are outside the pH range of the IPG strip.



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

Instructions

1

Rehydrate the Immobiline DryStrip gels with the gel side down in the appropriate volume of rehydration solution using the Immobiline DryStrip Reswelling Tray. If the tray is not available, strips can be rehydrated in a standard Ettan IPGphor Strip Holder. Rehydration in the Cup Loading Strip Holder is not recommended: The channel is too wide for the recommended rehydration volume. Any unused rehydration solution can be frozen overnight and used to check sample cup placement later.

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

2

Allow the strips to rehydrate overnight.

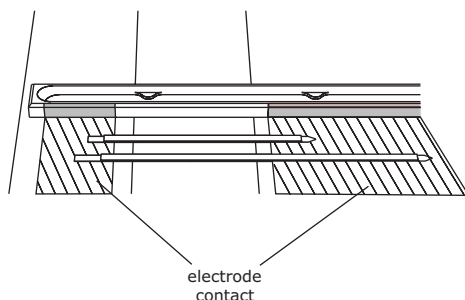
3

Position the strip holder on the Ettan IPGphor platform. The pointed end of the strip holder should contact the anodic electrode area (+), and the blunt end should contact the cathodic electrode area (–) of the Ettan IPGphor separation platform.

4

Transfer the strips to the Ettan IPGphor Cup Loading Strip Holder. The IPG strips should be placed faceup in the tray with the anodic (+, pointed) end of the IPG strip toward the pointed end of the strip holder. The strip must be positioned so that the gel extends into both plated contact regions of the strip holder. For the 7 cm and 11 cm IPG strips, the cathodic end must be approximately 1.5 cm from the end of the channel and about 1 cm from the end of the cathodic rails (see Fig 2). Center the strip down the length of the strip holder channel. Protrusions along the sides guide the strip approximately straight, although some manual adjustment of the strip may be necessary.

Fig 2. Positioning 7 cm and 11 cm IPG strips.



Overlay Immobiline DryStrip Cover Fluid across the surface of the IPG strip. It is important to distribute the oil *evenly* across the IPG strip and *down the entire length of the holder*. Use only enough fluid to completely cover the strip without overfilling (3–5 ml).

5

Optional: Cut electrode pads about 5 mm long from IEF electrode strips. Two pads per strip holder are required. Wet the pads with deionized water and blot them almost completely dry. Longer pads can be used if desired. If using longer pads, one end of the pad should overlap the end of the gel on the IPG strip. The electrode must contact the other end of the pad.

6

Place pads on both ends of the IPG strip (Fig 3). Slide an electrode down on top of each pad (Fig 4). Depending on the thickness of the IEF wick, the electrode may not feel solid on top of the filter paper. Pressure applied by the cover, however, will ensure complete contact when the lid of the Ettan IPGphor unit is closed.

Fig 3. Placing a filter paper pad at the end of the gel strip.

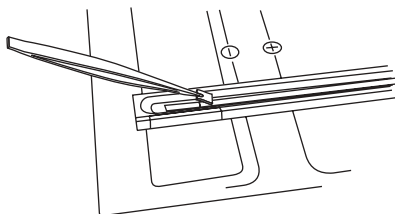
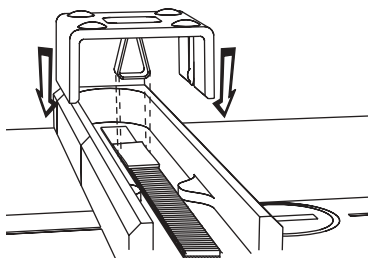


Fig 4. Seat the electrode on the pad at the end of the gel strip.

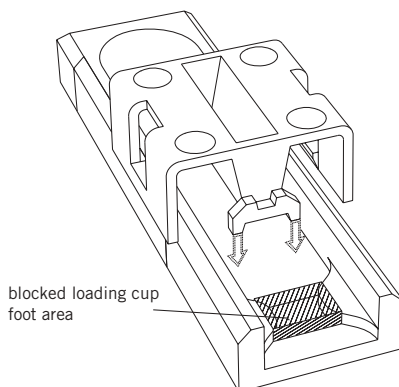


7

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.

Place the sample cup over the IPG strip. Do not place the cup with the feet over an alignment protrusion: The cup will not seat squarely and seal on the IPG strip. The sample cup can straddle these protrusions, however. Press the cup down until fully seated against the bottom of the strip holder. The feet on the sample cup stop the cup at a height above the floor of the strip holder sufficient to seal against the strip without cutting into it (Fig 5). Make sure the cup and electrodes are positioned properly prior to loading the sample in the cup.

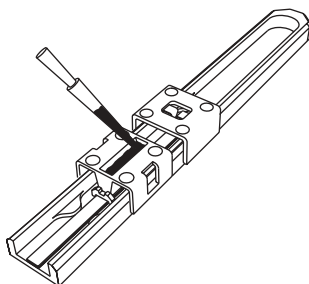
Fig 5. Side protrusions prevent feet from seating properly at certain points.



8

To confirm that the sample cup has sealed, pipette a small amount of rehydration buffer (without sample) into the cup (Fig 6). Check the seal. Remove the solution prior to loading the sample.

Fig 6. Pipette sample into the sample cup.



Common causes of leaking include:

- A foot of the cup is resting on top of one of the protrusions. Remove the cup and gently remove any excess solution from the top of the gel strip. Reposition the cup and recheck for leakage.
- The gel strip is not centered in the channel. Pipette up excess liquid from the strip holder, being careful not to damage the IPG strip. Remove the cup, center the IPG strip, reposition the cup, and check again.
- The strip may not have rehydrated properly. Examine the strip closely for a thin region in which the cup may not be able to seal properly. Be sure to use the proper volume of rehydration solution for a sufficient time to allow complete rehydration.

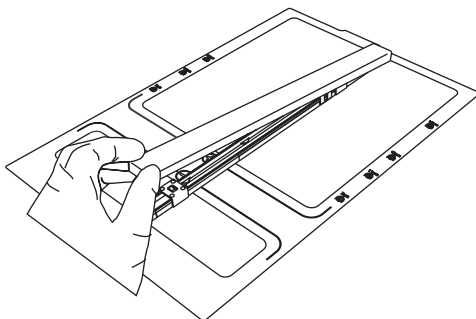
9

Briefly centrifuge the protein sample prior to loading to remove insoluble material. Particulates and insoluble material will impede sample entry and result in vertical streaks in the second-dimension gel. Pipette up to 100 μ l of sample into the sample cup.

Fig 7. Place the cover over the assembled strip holder.

10

Place the cover over the strip holder (Fig 7).



11

Close the Ettan IPGphor cover.

12

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 Vh (volt-hours) totals must be determined empirically for each sample type. Focusing after sample cup application frequently requires fewer Vh than in-gel sample rehydration loading methods, particularly on basic pH-range strips.

Suggested run conditions

50 μ A per strip, 20 $^{\circ}$ C

	voltage	voltage gradient type	step duration (h:min)	step duration (Vh)
1	500	Gradient	0:01	
2	4 000	Gradient	1:30*	
3	8 000	Step-n-hold		as per below
7 cm	3–10 L, 3–10 NL, 4–7 L, 6–11		(0.5–1 h) [†]	3 700 Vh
11 cm	3–10 L, 4–7 L		(1.3–3 h) [†]	10 600 Vh
11 cm	6–11		(1–2.5 h) [†]	8 500 Vh
13 cm	3–10 L, 3–10 NL, 4–7		(1.5–4 h) [†]	13 000 Vh
13 cm	6–11		(1.5–3 h) [†]	11 600 Vh
18 cm	3–10 L, 3–10 NL, 4–7		(3–6 h) [†]	24 600 Vh
18 cm	6–11		(2.5–5 h) [†]	20 600 Vh
18 cm	6–9		(5–11 h) [†]	41 600 Vh
18 cm	3.5–4.5, 4–5, 4.5–5.5, 5–6, 5.5–6.7		(7–14h) [†]	56 600 Vh
24 cm	3–10 L, 3–10 NL, 4–7 L, 3–7 NL		(6–13 h) [†]	51 600 Vh
24 cm	3.5–4.5, 4–5, 4.5–5.5, 5–6, 5.5–6.7		(12–20 h) [†]	95 600 Vh
24 cm	6–9		(7–14 h) [†]	56 500 Vh

*The sample entry phase should be extended for high-protein loads or, for convenience, if the strips are to be run overnight.

[†]Times are estimates for completion of the volt-hours required for focusing. Depending on the conductivity of the rehydration solution, and the length and pH range of the IPG strips, the focusing run may not reach the final desired voltages. It is more important that IPG strips are focused for the required volt-hour for complete focusing rather than achieving 8 000 V. Programming the IPGphor for the desired volt-hour in the final phase will help ensure focusing.

Using preparative protein loads and high salt concentrations in the cup loading strip holder may cause cover oil to redistribute and flow out of the stripholder. See Table 1 below.

Table 1. Troubleshooting:

To counteract oil leakage from Ettan IPGphor Cup Loading Strip Holder at high salt concentrations use the following running conditions (current limit 50 μ A per strip).

	voltage	step duration (h:min)	step duration (Vh)	voltage gradient type
1	300	3:00	900	Step-n-hold
3	1 000	6:00	3 900	Gradient
4	8 000	3:00	13 500	Gradient
5	8 000	*	*	Step-n-hold

*Total step duration and Vh as recommended for individual strip lengths.

Troubleshooting

Sample application at the anodic or cathodic end of the IPG strip usually gives the best results, but the placement must be determined empirically for each sample type. As a general rule, anodic application is more successful on basic IPG strips.

problem	possible cause	remedy
No electrical contact.	Plated ends of strip holder are not in proper contact with the Ettan IPGphor unit.	Check that the anode and cathode ends of the strip holder are in proper contact with the electrode contact areas on the Ettan IPGphor unit.
	Electrode contacts are not placed correctly.	Check that the bosses on the inside of the electrode contacts are in contact with the plated side rails.
Sample leaks from cup.	Incorrect cup placement.	Check that the foot of the cup is not resting on a protrusion on the inside of the strip holder.
	Incorrect strip alignment.	Check that the strip is centered inside the channel.
Cover fluid wicks out of the strip holder.	Excess cover fluid.	Do not add more than the recommended volume of cover fluid to cover the strip. Excess oil may capillary wick out of the holder, resulting in strips drying out during the run.
	High salt in sample	Use slower ramping up of voltage (see Table 1).
Strips turn white and opaque after focusing.	Water evaporation.	If the strips are not covered properly with fluid, the strips will lose moisture and urea will crystallize. Make sure the recommended volume of cover fluid is spread across the entire length of the IPG strip.
Strips do not achieve 8000 V.	Higher conductivity of the rehydration solution.	With higher concentration of IPG buffers or carrier ampholytes, the IPG strips are more conductive than are strips with lower concentrations. This may increase the time to reach 8000 V and complete focusing because the current is limited to 50 μ A per strip in the Ettan IPGphor unit. Using 0.5% IPG buffer is recommended for higher voltages. 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 of the sample.	Salts and ionic impurities in the sample can raise the conductivity of the strip.
	Short length IPG strips.	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 μ A current limit is reached at a lower overall voltage.



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.

Care and cleaning

Ceramic strip holders

Gently clean the strip holders after each use: Wash the holder with the Ettan IPGphor Strip Holder Cleaner. Rinse thoroughly with water and then deionized water and allow the strip holders to air-dry. Do not use abrasive cleaning agents or pads, as they may damage the metal conducting rails. Take care to avoid chipping the strip holders.

Electrodes

Rinse well with deionized water after use and allow to air-dry.

Covers

Wash with standard lab detergents, rinse, and 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

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Ordering information

Replacement parts

	code no.
Ettan IPGphor Cup Loading Strip Holder, 3 pk, complete	80-6459-43
Sample cups, pack of 50	80-6459-81
Electrodes, pack of 6	80-6464-94
Cover, Cup Loading Strip Holder	80-6465-13
Ceramic Base, Cup Loading Strip Holder (without cover, electrode, and cups)	80-6459-62

Accessory products

	quantity	code no.
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
IEF electrode strips	100	18-1004-40
Ettan IPGphor Strip Holder Cleaner	950 ml	80-6452-78

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