

IPGbox

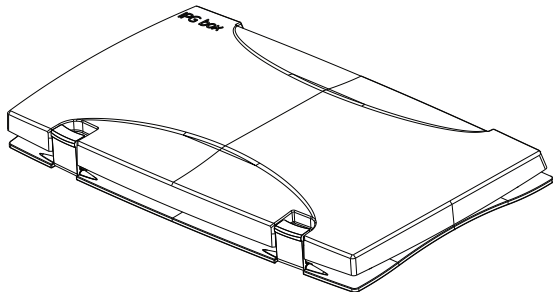
IPGbox Kit

Instructions

IPGbox™ is used together with the IPGbox Kit to rehydrate Immobiline™ DryStrip gels in a convenient way. The IPGbox Kit contains Reswell Trays and an IPGbox Insert, both disposable.

This instruction contains information about how to:

- Assemble IPGbox
- Clean IPGbox
- Change IPGbox Insert
- Select rehydration solution
- Prepare rehydration solution
- Rehydrate Immobiline DryStrip gels



Intended use

The IPGbox, Reswell Trays and IPGbox Insert are intended for research use only, and shall not be used in any clinical or in vitro procedures for diagnostic purposes.

1 Introduction

Immobiline DryStrip gels require rehydration before use. IPGbox is a convenient product for rehydration of Immobiline DryStrip gels. A Reswell Tray is placed in the IPGbox, rehydration solution is filled into the slots in the Reswell Tray, and the Immobiline DryStrip gels are immersed into the rehydration solution. After 10 hours (or over night) of reswelling the Immobiline DryStrip gels are ready to use.

The IPGbox Insert keeps the conditions optimal for rehydration and is positioned inside the lid of IPGbox. It can be used in ten reswelling cycles before disposal. Immobiline DryStrip Cover Fluid is no longer required.

The rehydration solution is selected to fit the research experiment. There are three options, one with DeStreak reagent - the DeStreak Rehydration Solution, and two that you mix yourself. The pH is adjusted by addition of desired IPG Buffer.

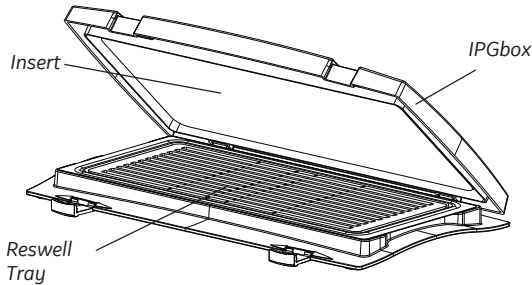


Figure 1.1: IPGbox parts

2 Maintenance

Assemble IPGbox and change IPGbox Insert

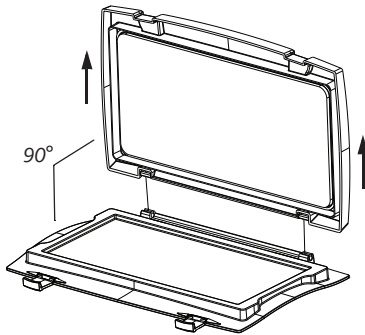
To assemble IPGbox for the first time start at step 3. To change IPGbox Insert follow all steps. IPGbox Insert should be changed for every 10th rehydration.

Note: Do not touch the flat surface of the IPGbox Insert. It is important to keep the flat surface clean and free from heavy pressure.

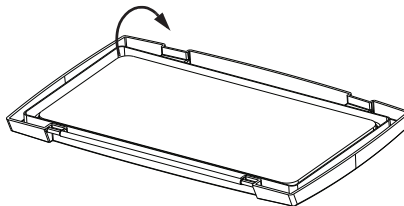
Note: A convenient way to keep track of the number of rehydrations is to change IPGbox Insert every time a new package (10 Reswell Trays, 1 IPGbox Insert) is opened.

| Step | Action |
|------|--------|
|------|--------|

- | | |
|---|---|
| 1 | Remove the lid by opening the IPGbox 90° and lift up the lid. |
|---|---|



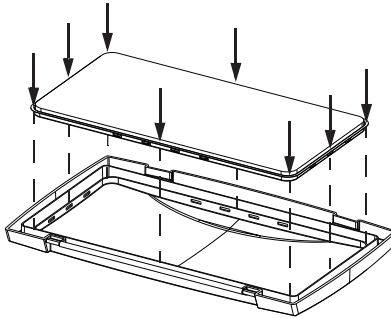
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|---|--|
| 2 | Pull out the old IPGbox Insert from the lid by pulling in the plastic corners of the IPGbox Insert. Discard the old IPGbox Insert. |
|---|--|



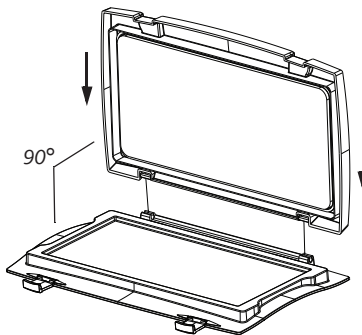
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|---|---|
| 3 | Take out a new IPGbox Insert from its protective plastic bag. Hold only on the plastic edges to keep the flat surface intact. |
|---|---|

| Step | Action |
|-------------|---------------|
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| 4 | Carefully push on the edges of the IPGbox Insert to snap the IPGbox Insert into the lid. |
|---|--|



- | | |
|---|---|
| 5 | Mount the lid by lowering the lid at 90° angle to the lower part. |
|---|---|



Clean IPGbox Insert

When necessary, wipe the surface of IPGbox Insert carefully with a soft tissue moistened with 70% ethanol.

Clean IPGbox

Clean IPGbox when necessary by:

| Step | Action |
|-------------|---------------|
|-------------|---------------|

- | | |
|---|---|
| 1 | Open IPGbox and remove IPGbox Insert if present. |
| 2 | Handwash IPGbox with <i>IPGphor™ Strip Holder Cleaning Solution</i> and rinse with water. |

| Step | Action |
|-------------|---------------|
|-------------|---------------|

| | |
|---|---|
| 3 | Check that IPGbox is dry before IPGbox Insert is mounted. |
|---|---|

3 Rehydration protocol

Protocol for rehydration of Immobiline DryStrip gels using IPGbox and the Reswell Tray:

- | Step | Action |
|------|---|
| 1 | Select and prepare a rehydration solution, see Selection of rehydration solution in Chapter 4 and Recipes in Chapter 5. |
| 2 | Change the IPGbox Insert if required, see Maintenance in Chapter 2. |
| 3 | Place the IPGbox on a levelled table and place a Reswell Tray in the IPGbox. Ensure that the Reswell Tray and the IPGbox Insert are clean and dry. If required wipe the IPGbox Insert with a soft tissue moistened with 70% ethanol. |
| 4 | Pipette the appropriate volume of selected rehydration solution (and sample if applied) evenly over the slots corresponding to the length of the DryStrip gels. See the table below for rehydration solution volumes. Strip lengths are measured from the straight ended part of the wells of the Reswell Tray and marked with lines on the top of the Reswell Tray. |

Note:

For complete sample uptake, do not apply excess rehydration solution.

Table 3.1: Rehydration solution volume per Immobiline DryStrip gel

| Immobiline DryStrip gel length (cm) | 7 | 11 | 13 | 18 | 24 |
|--|-----|-----|-----|-----|-----|
| Total volume per strip ¹ (µl) | 125 | 200 | 250 | 340 | 450 |

¹ Including sample, if applied

| Step | Action |
|-------------|---------------|
|-------------|---------------|

- | | |
|---|--|
| 5 | Carefully pull off the cover film from Immobiline DryStrip gel and place Immobiline DryStrip gel into the slot, gel-side down. Distribute the rehydration solution evenly under the strip. Gently lift and lower the strip and slide it back and forth along the surface of the solution to get complete and even wetting of the entire gel. |
|---|--|

Note:

Ensure that no bubbles are trapped under the Immobiline DryStrip gel.

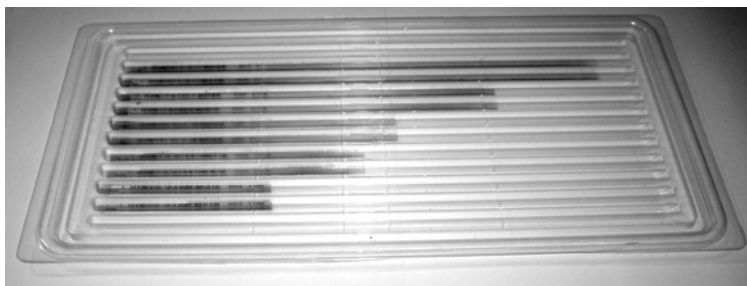


Figure 3.1: Example of IPG strips in a Reswell Tray.

- | | |
|---|--|
| 6 | Gently close the lid of the IPGbox and allow the Immobiline DryStrip gels to rehydrate at room temperature for 10 to 24 hours. Overnight is recommended. |
| 7 | Use the Immobiline DryStrip gels within 20 minutes of opening the IPGbox. Discard the used Reswell Tray. |

Note:

Do not store the Reswell Tray in IPGbox. Long time pressure of a Reswell Tray can reduce the life span of the IPGbox Insert.

4 Selection of rehydration solution

Depending on sample and strip, select the appropriate rehydration solution. Three options are available:

- DeStreak Rehydration Solution (17600319), with DeStreak reagent, urea, thiourea and CHAPS
- 2× Sample buffer/rehydration solution stock 1, with urea, thiourea and CHAPS (see Recipes in Chapter 5)
- 2× Sample buffer/rehydration solution stock 2, with urea and CHAPS (see Recipes in Chapter 5)

DeStreak Rehydration Solution contains DeStreak reagent and optimized concentrations of urea, thiourea and CHAPS. DeStreak in the rehydration solution is recommended for strips with a pH above 7. If sample is added in the rehydration solution (when performing In-Gel rehydration), DeStreak Rehydration Solution is not recommended.

The pH is adjusted by addition of IPG Buffer with same pH interval as the IPG strip used. Detailed information on selection and making of rehydration solution can be found in the Appendix of the *Technical Handbook 80642960* or in *User Manual 18117317*.

5 Recipes

Stock solutions

Table 5.1: 2× Sample buffer/rehydration solution stock 1

| Reagent | Quantity | Final concentration |
|---------------------------------|------------------|---------------------|
| Urea (M _r 60.06) | 10.5 g | 7 M |
| Thiourea (M _r 76.12) | 3.8 g | 2 M |
| CHAPS (M _r 614.89) | 0.5 g | 2% (w/v) |
| Distilled water | Make up to 25 ml | |

Small aliquots (e.g. 2.5 ml) can be stored at -20°C. Stable for 6 months.

Table 5.2: 2× Sample buffer/rehydration solution stock 1

| Reagent | Quantity | Final concentration |
|-------------------------------|------------------|---------------------|
| Urea (M _r 60.06) | 12 g | 8 M |
| CHAPS (M _r 614.89) | 0.5 g | 2% (w/v) |
| Distilled water | Make up to 25 ml | |

Small aliquots (e.g. 2.5 ml) can be stored at -20°C. Stable for 6 months.

Working solutions

Table 5.3: DeStreak Rehydration Solution

| Reagent | Quantity | Final concentration |
|--|--------------------|---------------------|
| DeStreak Rehydration Solution (17600319) | 3 ml | |
| IPG Buffer, same pH interval as the Immobiline DryStrip gels being used. | 60 µl ¹ | 2% ¹ |

¹ If *IPGphor standard Strip Holder* or *Immobiline DryStrip 7-11NL* or *3-11NL* is used, use 0.5% IPG Buffer instead of 2% IPG Buffer as final concentration.

Table 5.4: Rehydration solution made from stock

| Reagent | Quantity | Final concentration |
|---|----------|---------------------|
| 2× Sample buffer/ rehydration solution stock 1 or 2 | 2.5 ml | |

| Reagent | Quantity | Final concentration |
|--|-------------------------|---------------------|
| IPG Buffer, same pH interval as the Immobiline DryStrip gels being used. | 50 μ l ¹ | 2% ¹ |
| DTT | 7 mg | |

¹ If *IPGphor standard Strip Holder* or *Immobiline DryStrip 7-11NL* or *3-11NL* is used, use 0.5% IPG Buffer instead of 2% IPG Buffer as final concentration.

6 Ordering information

| Product | Pack size | Product code |
|-----------------|------------------------------------|--------------|
| IPGbox complete | 1 IPGbox + 1 IPGbox Kit | 28933465 |
| IPGbox Kit | 10 Reswell Trays + 1 IPGbox Insert | 28933492 |

| Related product | Pack size | Product code |
|--|-----------|--------------|
| DeStreak Rehydration Solution | 5 × 3 ml | 17600319 |
| Urea | 500 g | 17131901 |
| Thiourea | 100 g | RPN6301 |
| CHAPS | 1 g | 17131401 |
| Ettan™ IPGphor 3 Isoelectric Focusing Unit | 1 | 11003364 |
| IPGphor Manifold Ceramic Tray | 1 | 80649857 |
| Electrode set | 1 | 80649876 |
| Sample cups | 120 | 80649895 |
| IPGphor Strip Holder Cleaning Solution | 950 ml | 80645278 |
| IPG Buffer pH 3.5 to 5.0 | 1 ml | 17600202 |
| IPG Buffer pH 4.5 to 5.5 | 1 ml | 17600204 |
| IPG Buffer pH 5.0 to 6.0 | 1 ml | 17600205 |
| IPG Buffer pH 5.5 to 6.7 | 1 ml | 17600206 |
| IPG Buffer pH 4 to 7 | 1 ml | 17600086 |
| IPG Buffer pH 6 to 11 | 1 ml | 17600178 |
| IPG Buffer pH 7 to 11 NL (Non-Linear) | 1 ml | 17600439 |
| IPG Buffer pH 3 to 10 NL | 1 ml | 17600088 |
| IPG Buffer pH 3 to 10 | 1 ml | 17600087 |
| IPG Buffer pH 3 to 11 NL | 1 ml | 17600440 |

| Related product | 7 cm | 11 cm | 13 cm | 18 cm | 24 cm |
|---------------------------------------|----------|----------|----------|----------|----------|
| Immobiline DryStrip pH 3 to 10 NL | 17600112 | - | 17600115 | 17123501 | 17600245 |
| Immobiline DryStrip pH 3 to 10 | 17600111 | 18101661 | 17600114 | 17123401 | 17600244 |
| Immobiline DryStrip pH 3 to 11 NL | 17600373 | 17600374 | 17600375 | 17600376 | 17600377 |
| Immobiline DryStrip pH 3 to 7 NL | - | - | - | - | 17600243 |
| Immobiline DryStrip pH 7 to 7 | 17600110 | 18101660 | 17600113 | 17123301 | 17600246 |
| Immobiline DryStrip pH 6 to 9 | - | - | - | 17600188 | 17600247 |
| Immobiline DryStrip pH 6 to 11 | 17600194 | 17600195 | 17600196 | 17600197 | - |
| Immobiline DryStrip pH 3 to 5.6 NL | 17600353 | 17600354 | 17600355 | 17600356 | 17600357 |
| Immobiline DryStrip pH 5.3 to 6.5 | 17600358 | 17600359 | 17600360 | 17600361 | 17600362 |
| Immobiline DryStrip pH 6.2 to 7.5 | 17600363 | 17600364 | 17600365 | 17600366 | 17600367 |
| Immobiline DryStrip pH 7 to 11 NL | 17600368 | 17600369 | 17600370 | 17600371 | 17600372 |

| Related literature | Product code |
|--|--------------|
| 2-D Electrophoresis Principles and Methods, Appendix | 80642960 |
| Ettan DIGE System User Manual | 18117317 |

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