quick reference

A 2-D Electrophoresis Quick Reference Guide

qr IEF100-QR/Rev. A0/02-10

Hoefer®

First Dimension Separation

DAY 1

Prepare the sample

Samples are typically prepared in high concentrations of denaturants and include non-ionic detergents, carrier ampholytes, reductants, and sometimes protease inhibitors. Samples should be kept frozen or on ice until needed.

Rehydrate IPG strips

Note: Consult the strip manufacturers' recommendations for rehydration buffer (Table 1) and required rehydration volumes (Table 2). If desired, the sample can be included in the rehydration solution.

1. Select a rehydration/equilibration tray that is slightly longer in length than the strip to be rehydrated.
2. Apply the required volume of rehydration buffer to the innermost groove of each channel in the rehydration/equilibration tray.
3. Remove any protective covering from the IPG strip and, using forceps, lower the strip into the rehydration buffer gel side down.
4. Gently slide the strip back and forth to wet the entire length of the IPG gel with rehydration buffer. Lifting and lowering the IPG strip can also aid in getting the proper contact with rehydration buffer.
5. Cover the entire strip with mineral oil (Hoefer catalog #GR138-1) to prevent evaporation and urea crystallization.

Note: The rehydration tray should not be placed in the refrigerator during rehydration.

6. Allow the strip to rehydrate for 8 hours to overnight.

Table 1: Rehydration Buffer

Reagent	Concentration Range	Amount
Urea*	8 M (8 – 9 M)	4.8 gm
CHAPS	1% (1 – 4%)	10 mg
DTT	13 mM (13 – 100 mM)	20 mg
Carrier Ampholytes, 40%	0.5% (0.25 – 2%)	125 µl
Distilled de-ionized water		10 ml

*Urea can be replaced with up to 25% Thiourea.

Table 2: Rehydration of IPG Strips

IPG Strip Length	Volume per Strip (µl)
7 cm	130
18 cm	340
24 cm	450

DAY 2

Running IPG strips in the IEF100

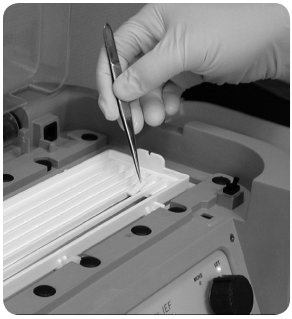
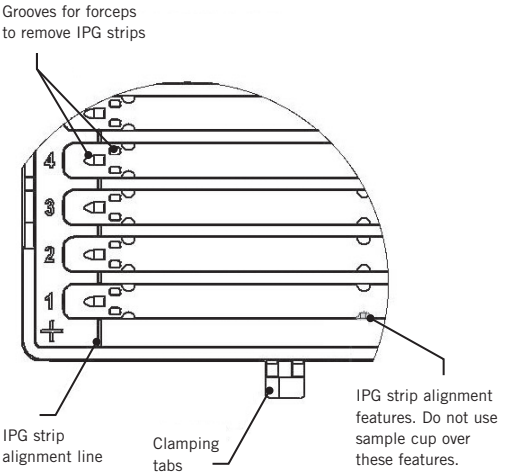


Fig 1. Loading IPG strips.

1. Place the focusing tray on the IEF platform and slide it into place.
2. Place rehydrated IPG strips gel side up in the channels of the focusing tray (Fig 1) making sure the anodic (+) end of the strip is positioned to the left (+) side of the IEF100 and aligned with the alignment mark on the tray (Fig 2).
3. From the scored sheet of wicks, cut 2 filter paper wicks for each IPG strip to be separated.
4. Moisten each wick with distilled de-ionized water and lightly blot dry with a paper towel.
5. Place a filter paper wick on each end of the IPG strip.

Fig 2. IPG strip alignment.



6. Connect the electrodes to their respective terminals.
7. Place the electrodes onto the filter paper wicks and secure by clipping the electrode in place. The platinum wire of the electrode should be centered in the area of overlap between the wicks and the IPG strip (Fig 3).
8. Fill the focusing tray with 60 milliliters of mineral oil.
9. Close the lid on the IEF100.

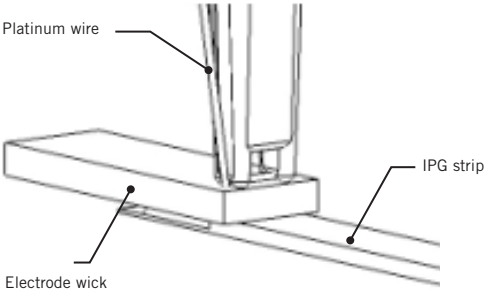


Fig 3. Electrode placement.

Note: If the electrodes are not properly contacting the strips there will be no current reading. Stop the run and correct the electrode alignment.

Start the IEF100

1. Setup and turn on the IEF100 (detailed instructions can be found on page 21 of the User Manual).
2. Highlight the desired protocol (Table 3) and press RUN.
3. Using the knob to change the value, confirm the number of IPG strips to focus.
4. Press RUN.
5. The IEF100 will beep to indicate the start of the run. The high voltage LED will light up and "Running" will flash in the top right corner of the display.

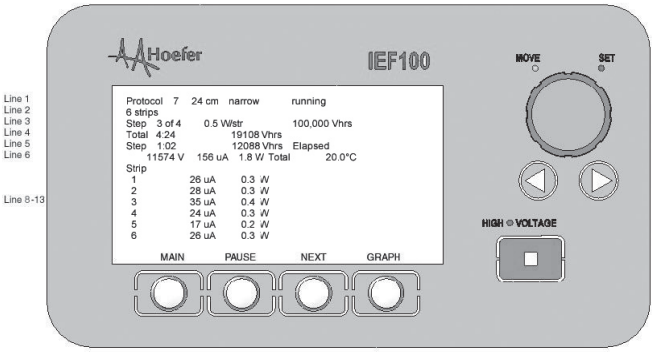
Table 3: Protocol Selection

Strip length	pH Range	Recommended Program
7 cm	Narrow	Protocol 1
18 cm	Broad	Protocol 3
18 cm	Narrow	Protocol 4
24 cm	Broad	Protocol 6
24 cm	Narrow	Protocol 7

Run Screen

The run screen displays information about the current run.

Fig 4. Run Screen.



Line 1: The protocol number and name are shown. The top right corner indicates the run status as either running, paused or finished.

Line 2: Displays the number of IPG strips being focused.

Line 3: Describes the active step; the step number, the step value (volt or watt/strip) and the step end point (Hrs or Vhrs).

Line 4: Displays the total elapsed time and the total volt-hours of focusing up to the moment.

Line 5: Displays the total elapsed time and total volt-hours of the active step.

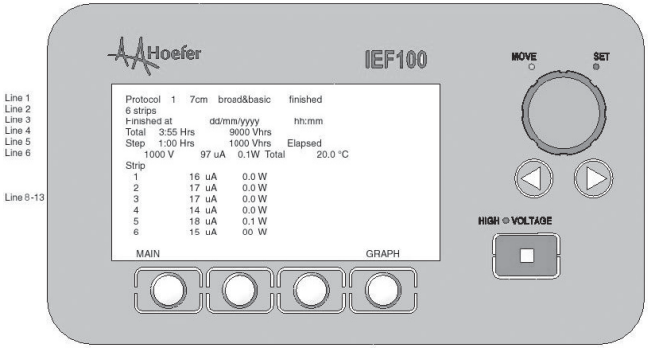
Line 6: Displays the real time output conditions of all the strips, including the current volts, the total current, the total watts and the platform temperature.

Line 8–13: Display the individual strip current and watts.

Ending the IPG strip run

When focusing is complete, the IEF100 will beep, and an end run screen will be displayed.

Fig 5. End Run Screen.



Line 1: The protocol number and name. The word finished is displayed in the upper right corner.

Line 2: The number of strips run.

Line 3: The date and time that focusing terminated.

Line 4: The total time and volt-hours of focusing.

Line 5: The last step time and volt-hours.

Line 6: The volts, and the total current and watts of all IPG strips at the end of the run.

Line 8–13: The conditions in each strip at the end of the run.

After focusing has completed

1. Open the lid.
2. Squeeze the electrodes to release the clips and remove them from the focusing tray.
3. Remove the filter paper wicks.
4. Using forceps, remove the IPG strips. Strips can be prepared for second dimension separation or stored in a freezer (-20 °C or below) until ready for use.
5. Prior to removing the running tray, remove the mineral oil overlay with a 10 ml pipette. For additional Care and Maintenance instructions see page 41 of the User Manual.

Second Dimension Separation

Prepare the overlay

Using 1X electrophoresis buffer, prepare a 1% agarose solution. Be sure the agarose is fully dissolved and keep hot until needed.

Equilibration

Note: If you would like to use a marker, insert a piece of 5 mm × 5 mm filter paper between the glass plates ensuring it makes contact with the gel. Once the filter paper is in place, add the manufacturers recommended volume of marker to the filter paper. Seal both the strip and filter paper in place using a 1% agarose overlay.

1. Select a rehydration/equilibration tray that is slightly longer in length than the strip to be rehydrated.
2. Place the IPG strips gel side up in the rehydration/equilibration tray.
3. Equilibrate the IPG strips for 10–15 minutes Equilibration Buffer I.
4. Dispose of the Equilibration Buffer I and replace with Equilibration Buffer II. Equilibrate for 10–15 minutes.
5. Using forceps, remove the IPG strip from the rehydration/equilibration tray and gently insert the strip into the top of the second dimension gel cassette.
6. Use a thin plastic strip or comb to gently seat the IPG strip directly against the surface of the second dimension gel. Avoid disrupting the gel surface or the results may be distorted.
7. To seal the strip in place, pipette a small amount of hot agarose overlay directly over the strip and allow it to solidify.
8. Run the vertical system according to the manufacturers' instructions.

Equilibration Buffer I

Note: Prepare immediately prior to use.

Reagent	Concentration Range	Amount
6 M Urea	3.6 g/10 ml	4.8 g
2% SDS	0.2 g/10 ml	10 mg
0.375 M Tris-HCl	2.5 ml 1.5 M	20 mg
20% Glycerol	2 ml/10 ml	125 µl
130 mM DTT	200 mg/10 ml	10 ml

pH 8.8 for the 10 ml mixture.

Equilibration Buffer II

Note: Prepare immediately prior to use.

Reagent	Concentration Range	Amount
6 M Urea	3.6 g/10 ml	4.8 g
2% SDS	0.2 g/10 ml	10 mg
0.375M Tris-HCl	2.5 ml 1.5 M	20 mg
20% Glycerol	2 ml/10 ml	125 µl
135 mM Iodoacetamide	250 mg/10 ml	10 ml

pH 8.8 for the 10 ml mixture.

When DTT is used a second equilibration step is required. The Iodoacetamide prevents protein re-oxidation during electrophoresis and alkylates residual DTT to minimize vertical streaking.

Equilibration Buffer Volumes

	Maximum IPG Strip Length (cm)	Buffer Volume ml)
Small tray	7	3
Medium tray	13	4.5
Large tray	24	7

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