Cup Loading Tray for the PROTEAN® IEF Cell

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Introduction

1.1 Overview

The Cup Loading Tray for the PROTEAN IEF cell is designed for the optimal resolution and separation of proteins with isoelectric points (pI) at either extreme of the pH range. The Cup Loading Tray can accommodate up to 12 IPG strips from 7 cm to 24 cm in length.

The Cup Loading Tray includes a tray base, a pair of movable electrode assemblies, and one bag each (120 count) of 100 and 150 µl sample cups. No modifications to the PROTEAN IEF cell are needed for compatibility with the Cup Loading Tray.

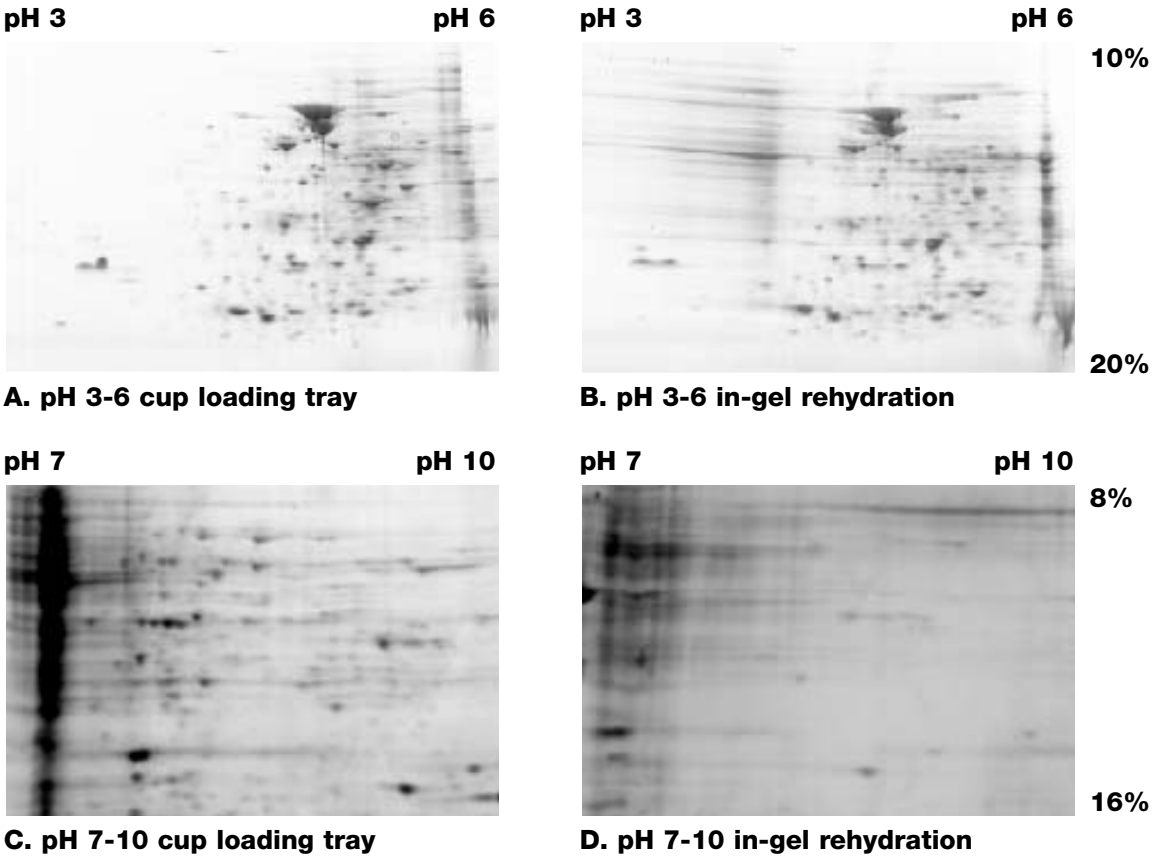
The Cup Loading Tray offers the following benefits:

- Enhanced resolution and separation of proteins with pIs at extreme pH ranges
- Optimal focusing for users of ReadyStrip™ IPG strips pH 3-6, pH 7-10, and Micro-range ReadyStrip IPG strips pH 6.3–8.3
- Tray runs up to 12 IPG strips per run for high throughput
- Tray accommodates 7 cm to 24 cm IPG strips with movable electrode assemblies
- Two sample cup sizes, 100 and 150 µl, are available to allow greater flexibility
- The Cup Loading Tray is compatible with all PROTEAN IEF cells, so no modification to existing PROTEAN IEF cell is necessary.

1.2 Introduction

Isoelectric focusing of proteins in the acidic and basic pH ranges is often associated with streaking due, in part, to protein precipitation when utilizing standard in-gel rehydration of IPG strips. Protein resolution can be markedly improved when the proteins are applied to the IPG strips by means of a sample cup positioned in a discreet area of a rehydrated IPG strip.

The Cup Loading Tray allows for sample application via cup loading after rehydration of the IPG strips. Certain conditions make cup loading the preferred method of sample loading. The examples below compare in gel rehydration resolution with cup loading resolution of an E. coli sample on pH 3-6 and pH 7-10 IPG strips.



A. pH 3-6 cup loading tray

B. pH 3-6 in-gel rehydration

C. pH 7-10 cup loading tray

D. pH 7-10 in-gel rehydration

Figure 1.
In-gel rehydration: 400 µg E.coli lysate in 8M Urea, 4% Chaps, 50 mM DTT, 0.2% Bio-Lytes.
Cup loading: 400 µg E.coli lysate in 100 µl 8M Urea, 4% Chaps, 50 mM DTT, 0.2% Bio-Lytes.
A. pH 3-6 cup loading at cathode (-), B. pH 3-6 in-gel rehydration
C. pH 7-10 cup loading at anode (+), D. pH 7-10 in-gel rehydration

1.3 Description

The Cup Loading Tray for use with the PROTEAN IEF cell consists of the following components:

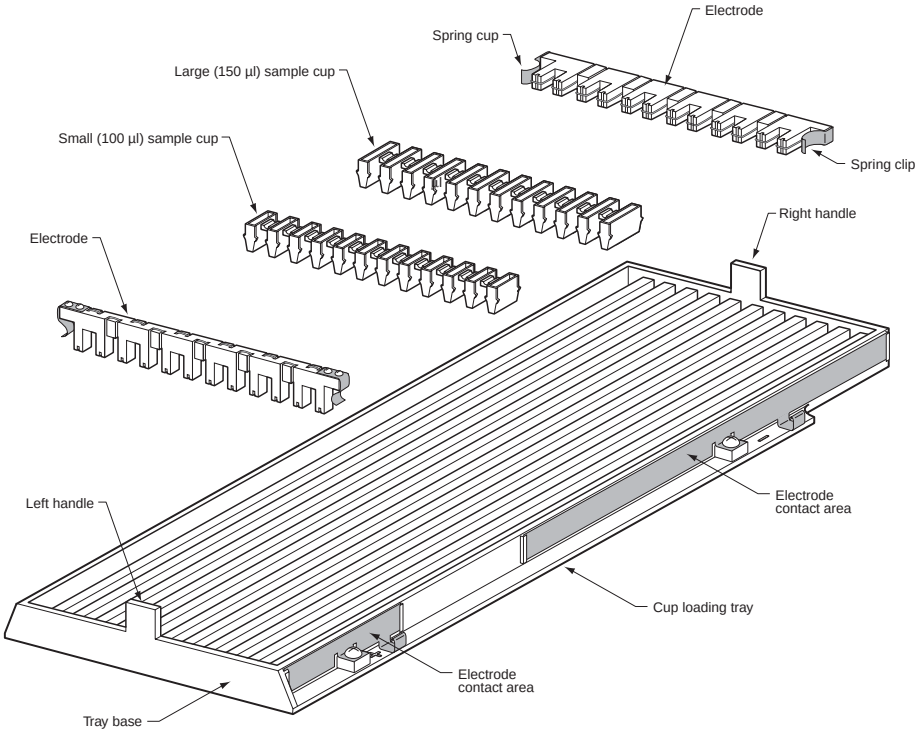


Figure 2. Diagram of Cup Loading Tray components.

1. A twelve-cup strip that holds up to 100 µl of sample per cup and a twelve-cup strip that holds up to 150 µl of sample per cup.

   Note: For precise mechanical fit, a minimum of two cups is required. A single sample cup provides an incomplete “stop” on the channel walls and the gel could be punctured.

2. A twelve channel focusing tray that accommodates IPG strips from 7cm to 24 cm.

3. Two movable electrode assemblies, each having 12 individual teeth with cavities which allow mineral oil to flow through the entire channel of the focusing tray.

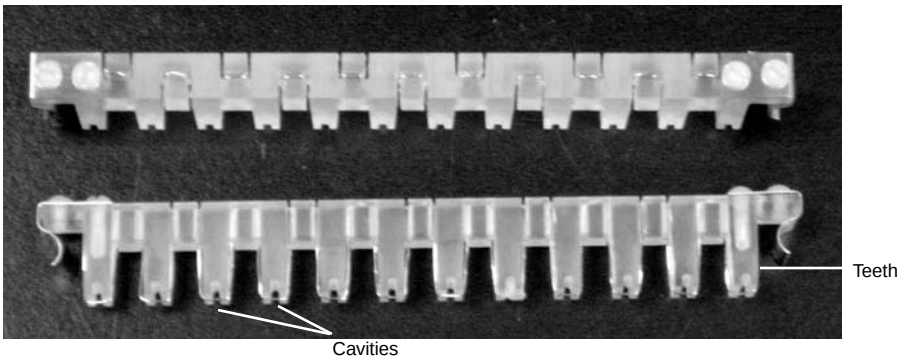


Figure 3. Movable electrode assemblies.

1.4 Specifications

Material	Polycarbonate
IPG strip size capacity	7 cm–24 cm
Tray Capacity	Up to 12 IPG strips per run
Electrodes	Platinum wire on movable electrode assembly
Inter-electrode distance	User defined. Dependent on IPG strip length
IPG strip rehydration	Rehydration/equilibration tray specific to IPG strip length

Section 2
Instructions

2.1 General Overview

Isoelectric focusing with the Cup Loading Tray includes the following steps:

1. Prepare rehydration solutions and rehydrate IPG strips overnight in rehydration tray with appropriate volume of rehydration buffer.

   Note: The Cup Loading Tray is not intended for passive or active IPG strip rehydration. Please use appropriate rehydration/equilibration tray.
2. Prepare sample.
3. Transfer the rehydrated IPG strips from the rehydration tray and place the strips gel side up into the focusing tray's channels.

   Note: Reduced rehydration times result in incomplete rehydration of the IPG Strips causing an imperfect seal between the sample cup and the IPG strip.
4. Optional. Place electrode wicks at anode and cathode.
5. Position the movable electrode assemblies on top of the strips at the anode and cathode ends. Ensure the spring clips are in contact with the electrode contact areas.
6. Place the sample loading cups on the rehydrated IPG strips near the anode for basic pH range and near the cathode for acidic pH range IPG strips.
7. Load sample in sample cup and overlay with mineral oil.
8. Overlay IPG strips in each channel with mineral oil.
9. Transfer tray to cooling platform in PROTEAN IEF cell and start isoelectric focusing.

2.2 IPG Strip Rehydration Solution Preparation and Strip Rehydration

1. Prepare rehydration sample buffer and rehydrate IPG strips just prior to sample cup-loading and isoelectric focusing. Add the indicated volume of rehydration buffer to each of the required channels in the rehydration tray to rehydrate to 0.53 mm thickness.

   Note: Rehydration to 0.53 mm thickness ensures a complete seal between the cup and the IPG strip. The following volumes are 10% greater than those recommended in the ReadyStrip IPG Strip instruction manual.

IPG Length	7 cm	11 cm	17 cm	18 cm	24 cm
Rehydration volume for cuploading	135 µl	200 µl	330 µl	345 µl	450 µl

2. Carefully remove the IPG strip cover sheet and place the IPG strip gel side down in the rehydration trays. To ensure even rehydration of the IPG strips, the entire surface to the IPG strips must be completely wetted.

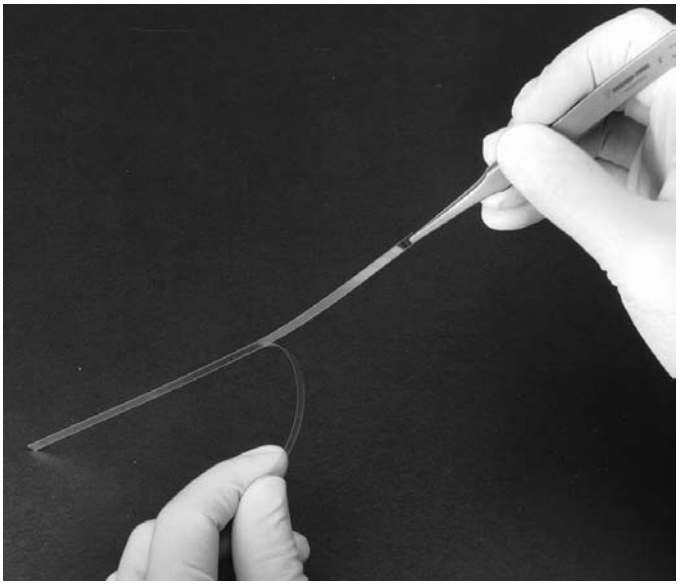


Figure 4. Removal of IPG strip cover sheet.

3. Overlay IPG strips with mineral oil and rehydrate strips ~ 12 hours (or overnight). To maintain a constant temperature during rehydration, place the rehydration tray on the cooling platform of the PROTEAN IEF cell and set-up the rehydration program. (See PROTEAN IEF cell manual for details).

2.3 Transfer and Placement of Rehydrated IPG Strips in the Focusing Tray

1. Remove the rehydrated IPG strips from the rehydration tray. Remove excess mineral oil by carefully blotting the IPG strip on filter paper wetted with DI water.

2. Place the rehydrated IPG strip, gel side up, in the focusing tray. Place the anode side (marked with + on ReadyStrip IPG Strips) of the 17 cm, 18 cm, and 24 cm strips flush against the anode (left) side of the focusing tray. Place the anode side of the 7 cm and 11 cm approximately 4 cm from the anode side of the tray to ensure proper placement and contact of the cathode electrode assembly with the electrode contact area.

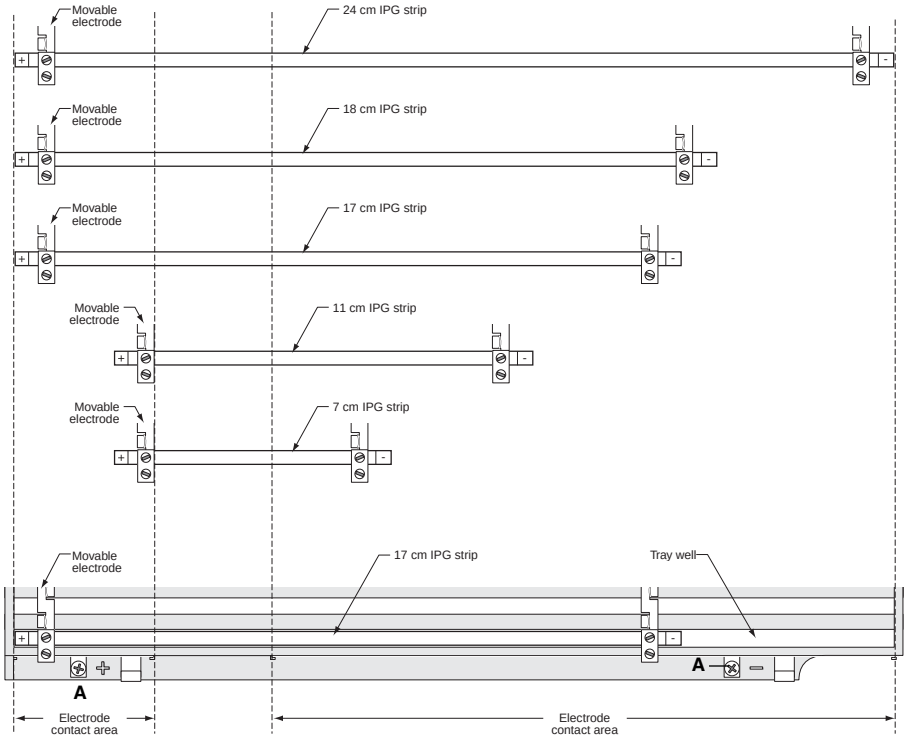


Figure 5. Correct placement regions for various IPG strip lengths.

2.4 Placement of Movable Electrode Assemblies

1. Place a movable electrode assembly at the cathode and anode end of the IPG strip. Make sure the electrodes make contact with the electrode contact areas on the front of the focusing tray.

   Note: If the electrode screws, indicated by the letter “A” in Figure 5, on the front of the focusing tray interfere with the placement of the moveable electrode assembly, reposition the IPG strips until the electrode assembly clears the electrode screws.

   Optional: Electrode wicks can be inserted at this point. Carefully place damp electrode wicks on the gel surface prior to positioning the anode and cathode electrode assemblies.

2.5 Positioning Sample Loading Cups and Loading Sample

1. Determine which sample cup size will be used and cut the required number of sample cups from a twelve-cup strip with scissors. A minimum of two sample cups is needed for a tight, leak proof fit in the focusing tray.

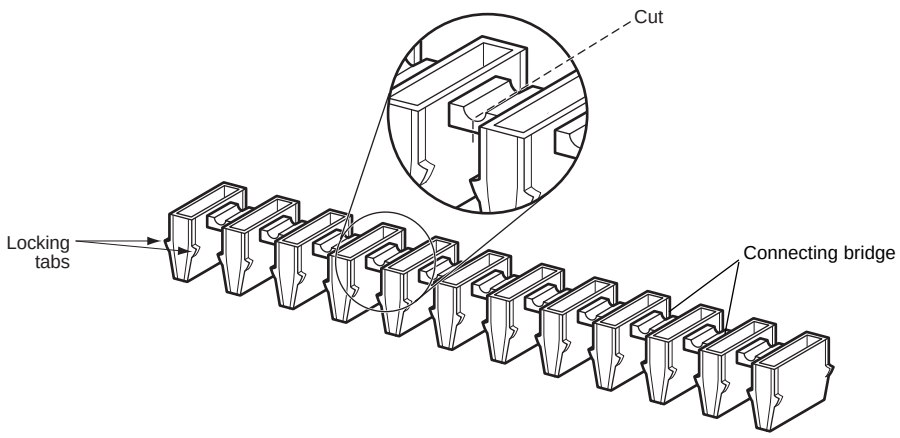


Figure 6. Twelve-cup strip.

Note: When cutting the indent of the bridge between two cups, ensure that no burrs remain in the cut that would prevent the bridge from sitting directly on the wall that divides two channels in the focusing tray.

2. Place the sample cups on the gel surface near the anode for basic pH range IPG strips and near the cathode for acidic pH range IPG strips. To position the sample cups properly, make sure that the bridges connecting two cups are pushed down onto each wall that divides the focusing tray channels.

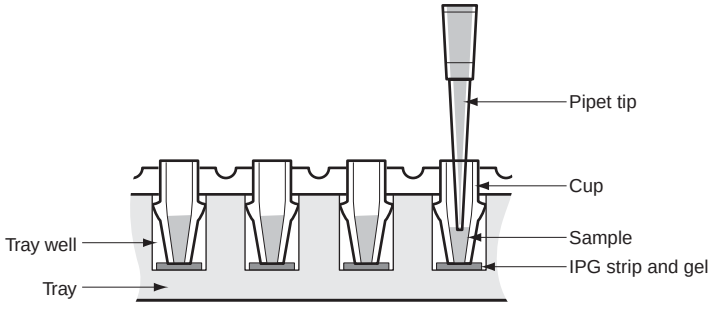


Figure 7. Side view of Focusing tray with IPG strips, cups, and sample.

Note: Improper placement of the sample cup can result in sample leaking.

3. When the cups are securely positioned, add the protein sample to the sample cups. We recommend a maximum of 100 µl for the smaller cups and a maximum of 150 µl for the large volume cups. We have observed improved resolution with smaller volumes containing a higher protein concentration, and recommend using the maximum protein concentration possible in which proteins do not precipitate.

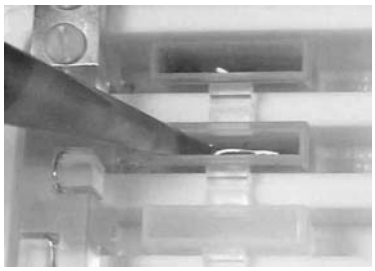


Figure 8. Loading sample into cup with pipet.

Optional: The integrity of the seal between the gel surface and the cup can be verified by adding some rehydration buffer to the cup prior to loading the sample. When a leak proof seal is confirmed, carefully remove the rehydration buffer and replace with the sample.

4. Overlay each sample cup with mineral oil to prevent evaporation.

5. Completely overlay each strip with mineral oil.

6. Carefully place the focusing tray with the properly positioned electrodes and sample cups on the temperature-controlling platform of the PROTEAN IEF cell.

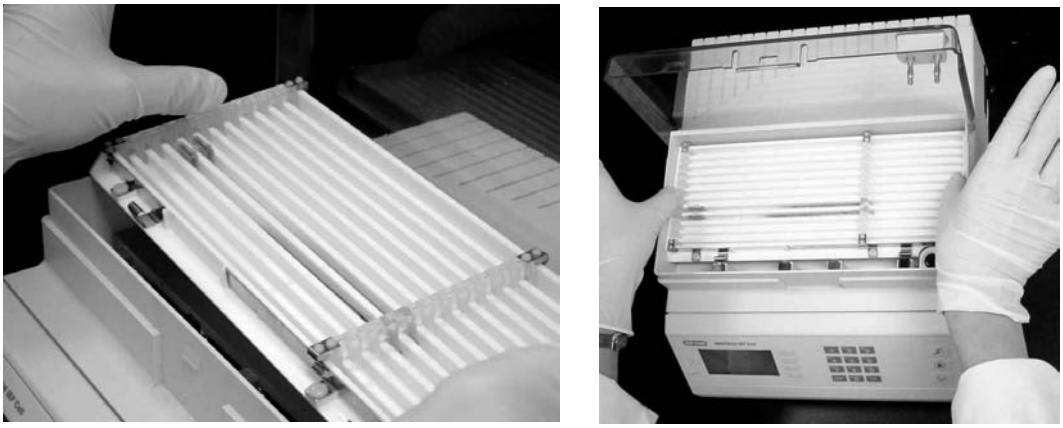


Figure 9. Insertion of Focusing tray into PROTEAN IEF cell.

2.6 Focusing Conditions

Focusing conditions will vary with sample composition, sample complexity, IPG pH range and strip length. The following parameters are recommended guidelines. We recommend maintaining a current limit of 50 µA per strip to minimize excessive heat generation and subsequent increase in the possibility of burned regions in the IPG strips.

Recommended Focusing Parameters

	7 cm	11 cm	17 & 18 cm	24 cm
Step 1: 250 V Rapid Voltage Ramping	30 min.	30 min.	30 min.	30 min.
Step 2*:				
4,000 V	60 min.	—	—	—
8,000 V	—	60 min.	—	—
10,000 V Slow Voltage Ramping	—	—	60 min.	60 min.
Step 3*:				
4,000 V	8–10 kV hrs.	—	—	—
8,000 V	—	15–20 kV hrs.	—	—
10,000 V Rapid Voltage Ramping	—	—	30–40 kV hrs.	40–60 kV hrs.

* The maximum attainable voltage is determined by the combined final conductivity of the IPG strip, sample, and rehydration buffer. A lower final voltage does not adversely affect resolution provided that the set volt-hours are reached.

2.7 After Focusing Run is Complete

1. Remove the focusing tray from the PROTEAN IEF cell when the focusing run is complete by lifting the tray by the handle with your thumbs.

Figure 10. Removal of Focusing tray from PROTEAN IEF cell.

2. Remove the cups from the focusing tray by inserting the enclosed forceps underneath the connecting bridge, or by pinching the wall of the cup with forceps and lifting upwards. If a full strip of cups (12) is used, you may have to lift the cups in a few places along the strip.

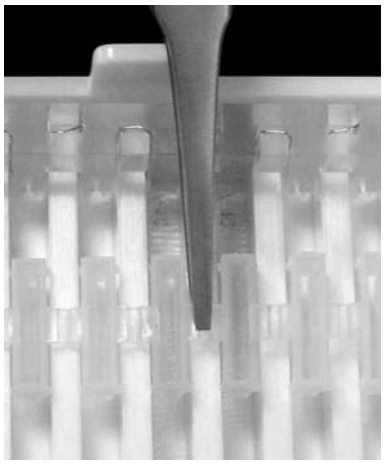


Figure 11. Connecting bridge method.

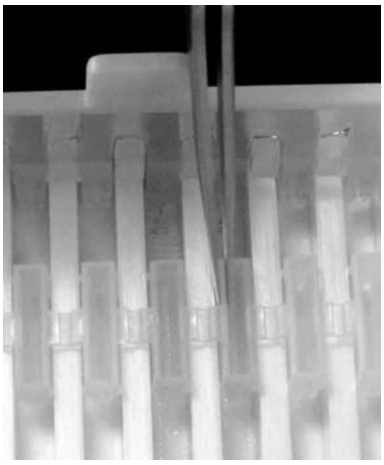


Figure 12. Pinching cup walls method.

3. Remove the IPG strips from the focusing tray. Carefully blot the IPG strip on clean, wetted filter paper to remove excess oil and sample buffer. Equilibrate the IPG strips as required prior to the 2nd dimension. See the ReadyStrip IPG Strip instruction manual for details.

Section 3
Cleaning, Maintenance, and Chemical Compatibility

Proper cleaning and maintenance of the Cup Loading Tray components will ensure the best results while providing the longest life for the product.

3.1 Focusing tray

The focusing tray is made of polycarbonate. Several chemicals are not compatible with polycarbonate: acetone, acetonitrile, benzene, chloroform, hexane, hydrazine, methylene chloride, and acetaldehyde. This list is representative, but not exhaustive. The IEF sample preparation reagents at the concentrations normally used for IEF, such as ampholytes, various detergents, and reducing agents, including tri-butyl phosphine, have no effect on the trays.

Clean the focusing tray after each use. Use the nylon cleaning brushes included with the PROTEAN IEF cell or a soft-bristled brush with hot water and an Alconox® type of detergent called Liqui-Nox®, which is specifically designed for removing greasy substances, to remove all residual mineral oil and sample solution. Rinse with DI water and air dry. Do not use ethanol to wash or dry the focusing tray. Use of ethanol will craze or crack the tray and voids the warranty.

Note: Do not try to remove the mineral oil with any solvent, as most solvents will likely damage the focusing tray and void the warranty.

3.2 Movable Electrode Assemblies

Use the nylon cleaning brushes included with the PROTEAN IEF cell or a soft-bristled brush to clean the movable electrode assemblies with hot water and Liqui-Nox. Take care to ensure the platinum wire does not break. Rinse with DI water and air dry. Do not use ethanol to wash or dry the movable electrode assemblies.

3.3 Cups

The Cup Loading Tray's cups are designed to be disposable and are not recommended for reuse.

*Alconox and Liqui-Nox are registered trademarks of Alconox.

Section 4
General Guidelines for Sample Preparation

See ReadyStrip IPG Strip instruction manual for general guidelines for sample preparation. For additional information on sample preparation, go to www.ProteomeWorksSystem.com

Section 5
Troubleshooting

Problem	Cause	Solution
1. Sample is leaking from the sample cups.	Cup positioning is incorrect.	When positioning the cups, press down on the connecting bridge until it stops on the channel wall of the focusing tray. (See illustration on page 7 .)
	The cut edge from the connecting bridge contains a burr that prevents the connecting bridge from proper positioning on the channel wall.	Make sure to use a sharp cutting device such as scissors to evenly cut the connecting bridge. Inspect cut to ensure that it does not contain any burrs.
	The cup is positioned in an IPG area that is not completely rehydrated.	Make sure the IPG strips are rehydrated evenly and thoroughly. See ReadyStrip IPG Strip Manual for correct rehydration directions. Make sure the correct volume is used for the selected IPG strip. See table in Section 2.2.
	The cup itself is malfunctioning.	Replace cup.
2. Initial low or zero current (no electrical contact)	Incorrect positioning of the electrode assemblies.	Make sure the electrodes are positioned directly on the IPG strips. See page 6 for correct positioning. When using electrode wicks make sure they are wetted properly and are placed on the IPG strips and the electrode assemblies are in contact with the electrode wicks. Make sure the movable electrode assemblies make contact with the electrode contact area on the focusing tray.
	Platinum wire on electrode holder has been compromised.	Inspect the platinum electrode wire to make sure it is intact. If in doubt, use a voltmeter to check for continuity.

Problem	Cause	Solution
3. Sample wicks out of sample cups.	The sample cups are disposable. When re-using the sample cups, salts can build up in the corners and provide a capillary path for the sample to travel.	For optimum performance use new sample cups for each focusing run.
4. Programmed max. voltage is not reached with a current limit of 50 µA per strip.	Final combined conductivity of the IPG strip, sample, and rehydration buffer determines the maximum attainable voltage.	Run at maximum attainable voltage for longer time to arrive at the required number of volt-hours. If possible, reduce the conductivity of the rehydration buffer by reducing the Bio-Lyte® concentration. Increase the current limit per strip. However, higher current limits generate more heat and increase the possibility of burned regions in the IPG strips.

Section 6
Warranty

The Cup Loading Tray is warranted for 1 year against defects in materials and workmanship. If any defects should occur during this warranty period, Bio-Rad Laboratories will replace the defective parts without charge. However, the following defects are specifically excluded:

- Defects caused by improper operation
- Repairs or modifications performed by anyone other than Bio-Rad Laboratories or their authorized agent
- Damage caused by accidental misuse
- Damage caused by disaster
- Common replacement parts including platinum wire
- Damage caused by use of organic solvents or basic detergents

For inquiries or to request repair service, contact your local Bio-Rad office.

Warranty information: _____
Model: _____
Catalog number: _____
Date of delivery: _____
Lot number: _____
Invoice number: _____
Purchase order number: _____

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