MicroRotofor™ Liquid-Phase IEF Cell

Instruction Manual

Catalog Numbers
170-2800 (100/120 V)
170-2801 (220/240 V)

For Technical Support Call Your Local Bio-Rad Office or in the US Call **1-800-4BIORAD** (1-800-424-6723)

BIO-RAD

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Safety Information

This instrument is intended for laboratory use only. This product conforms to the "Class A" standard for electromagnetic emissions intended for laboratory equipment applications. Emissions from this product may interfere with some sensitive appliances when placed near or in the same circuit as those appliances. The user should take appropriate measures to avoid such interference.

Power to the MicroRotofor cell is to be supplied by an external DC voltage power supply. This power supply must employ a safety isolation transformer to isolate the DC voltage output with respect to ground. All of Bio-Rad's power supplies meet this important safety requirement. Regardless of which power supply is used, the maximum specified operating parameters for the cell are:

1,000 VDC	maximum voltage limit
2 W	maximum power limit
40°C	maximum ambient temperature limit

Current to the MicroRotofor cell, provided by the external power supply, enters the unit through the lid assembly, which also provides a safety interlock. Current to the cell is broken when the lid is removed. Do not attempt to circumvent this safety interlock, and always turn the power supply off before removing the lid or when working with the MicroRotofor cell in any way.

Important: This MicroRotofor cell is designed and certified to meet International Electrotechnical Commission (IEC) 61010-1 safety standards. Certified products are safe to use when operated in accordance with the instruction manual. This instrument should not be modified or altered in any way. Alteration of this instrument will:

- Void the manufacturer's warranty
- Void the IEC61010-1 safety certification
- Create a potential safety hazard

Bio-Rad is not responsible for any injury or damage caused by the use of this instrument for purposes other than for which it is intended or by modifications of the instrument not performed by Bio-Rad or any authorized agent.

*IEC61010-1 is an internationally accepted electrical safety standard for laboratory instruments.

Section 1
Introduction

1.1 Overview

The MicroRotofor cell is a preparative isoelectric focusing (IEF) device that fractionates complex protein samples in free solution. Whether used alone or as part of a broader separation or purification scheme, the MicroRotofor cell offers rapid, simple, and effective fractionation and enrichment of proteins from complex protein mixtures.

With the MicroRotofor cell, IEF is carried out entirely in free solution. As a result, fractions from a run can be easily collected, pooled, and even refractionated for further purification. Fractionation by liquid-phase IEF is particularly beneficial for those proteins that are insoluble or otherwise do not separate well in other, gel-based IEF media. Moreover, IEF in solution enables fractionation of proteins in their native state by isoelectric point (pI). Lastly, because ampholytes are used to generate the pH gradient employed for separation, a continuous and, if desired, customized pH gradient may be formed, which permits true screening of a protein sample by pI.

The technology behind the design of the MicroRotofor cell was pioneered by Bier et al. (1979) and was originally commercialized as the Rotofor® and mini Rotofor cells, which perform liquid IEF-based fractionation of 60 ml and 18 ml samples, respectively. The MicroRotofor cell accommodates 2.5 ml samples, enabling application of the powerful Rotofor technology to smaller samples, the development of separation protocols, and the refractionation of fractions obtained from runs on the Rotofor or mini Rotofor cells. The cell uses a cylindrical focusing chamber divided into ten compartments (each containing a single fraction) by nine parallel, monofilament polyester screens. As temperature regulation is critical for reproducibility and maintenance of protein integrity during IEF, the MicroRotofor cell features an integrated Peltier-driven cooling block that provides external cooling to the sample in the focusing chamber during a run. In addition, oscillation, or rocking, of the focusing chamber within the cooling block and around the focusing axis stabilizes the sample against convective and gravitational disturbances. After focusing, the solution in each compartment is rapidly collected without mixing using the vacuum-assisted harvesting station that is integrated within the cell. Use of the MicroRotofor cell can enrich low-abundance proteins to enhance the results obtained from downstream applications as varied as one- and two-dimensional gel electrophoresis, chromatography, and mass spectrometry.

The Rotofor and mini Rotofor cells have been manufactured and in use in laboratories around the world for over 20 years. Hundreds of publications demonstrate their use for protein fractionation and purification. For a list of selected publications and for more information about the MicroRotofor cell and other sample preparation tools of Bio-Rad's expression proteomics program, visit us on the Web at www.expressionproteomics.com

1.2 Features

The MicroRotofor cell has the following features:

- Accommodates 2.5 ml samples (microgram to milligram amounts total protein)
- Separates samples into ten 200–250 µl fractions
- Provides external cooling to the focusing chamber through an integrated Peltier-driven cooling block
- Offers two cooling settings:
    - 20°C — for applications that do not require nondenatured proteins
    - 10°C — for applications, such as activity and structural assays, that require nondenatured proteins
- Enables use of customized pH gradients for enhanced resolution of proteins of interest
- Facilitates fraction collection with an integrated, regulated vacuum-controlled harvesting station
- Uses preassembled, disposable focusing chambers

1.3 Specifications

Construction

Focusing chamber	Molded polypropylene
Screens	Molded polycarbonate with polyester
Electrode assemblies	Acrylic
Ion exchange membranes	Molded polypropylene with Ionac
Chassis	Molded polycarbonate/acrylonitrile butadiene styrene (ABS)
Lid	Molded polycarbonate
Cooling block	Aluminum
Harvesting station:	
Needle array	Stainless steel
Needle array holder	Acrylic
Positioning block	Polycarbonate
Harvesting tray	Acetal
Vacuum chamber	

Chemical compatibility

The MicroRotofor components are not compatible with chlorinated hydrocarbons, aromatic hydrocarbons, or acetone. Use of organic solvents voids all warranties.

Shipping weight	10.25 lb
Dimensions (W x D x H)	29.5 cm x 18.8 cm x 16 cm (11.5" x 7.4" x 6.3")
Cell voltage limit	1,000 V
Cell power limit	2 W
Cooling	To prevent excessive overheating and damage to the unit, the MicroRotofor cell must be run with the cooling block operational (set to either setting I or II).
Input power requirements	100–120 VAC 50/60 Hz or 220–240 VAC 50/60 Hz
Fuses	1.0 A Type T 5 x 20 (100–120 VAC) or 0.63 A Type T (220–240 VAC)

1.4 Unpacking

Upon receipt of the MicroRotofor cell, carefully inspect the container for any damage that may have occurred during shipping. Severe damage to the container may indicate damage to the unit itself. If you suspect damage to the unit, contact your local Bio-Rad representative, or in the US, call Bio-Rad technical support at 1-800-4-BIORAD.

The contents include:

- The MicroRotofor IEF cell, which includes the following components:
  - Chassis and lid
  - Harvesting trays (2)
  - Focusing chambers (2)
  - Cathode assembly
  - Anode assembly
  - Anode membranes (cation exchange membranes) (5)
  - Cathode membranes (anion exchange membranes) (5)
- Power cord
- Instruction manual
- Warranty card
- Declaration of conformity
- Sealing tape
- Sealing film (10)
- Assembly tool
- Cleaning brush
- Forceps
- 3 ml syringe
- 10 ml syringes (2)

If any part is missing or damaged, contact Bio-Rad laboratories immediately.

Section 2
Description of Components

2.1 Major Components

The MicroRotofor cell consists of a number of major components (Figure 1). The features and functions of each are described below.

Fig. 1. MicroRotofor components and accessories. MicroRotofor chassis and lid (1), harvesting tray (2), focusing chamber (3), cathode assembly (4a), anode assembly (4b), cathode membrane, (5a), anode membrane, (5b), 10 ml syringes (6), 3 ml syringe (7), forceps (8), assembly tool (9), sealing tape (10), cleaning brush (11), vacuum hose (12), vacuum chamber (13).

MicroRotofor chassis and lid

The chassis is the base of the cell and features two major components (Figure 2):

The focusing station is used during separation. It is comprised of the oscillating motor, electrical contacts that apply power to the focusing assembly during fractionation, and the Peltier-driven cooling block.

The harvesting station is used for harvesting the fractions from the focusing chamber following fractionation. Its components are described in more detail below.

There are two switches on the front of the chassis (Figure 2). Located to the right, the power switch controls power to the oscillating motor and to the left, the cooling switch controls the settings of the cooling block. The cooling switch regulates three cooling settings:

Setting I (20°C) is used when downstream applications, such as IEF on IPG strips, do not require nondenatured proteins.

Setting II (10°C) is used when downstream applications, such as activity and structural assays, may require nondenatured proteins.

Setting O shuts off power to the cooling block. Operating the MicroRotofor cell using this setting is not recommended because excessive overheating and damage to the unit may result.

On the back of the chassis, there is a connector for the power cord. In addition, a piece of vacuum tubing, which is connected to the vacuum chamber, extends from the back of the cahssis and is used to connect the cell to an external vacuum source (see "Additional Requirements", below).

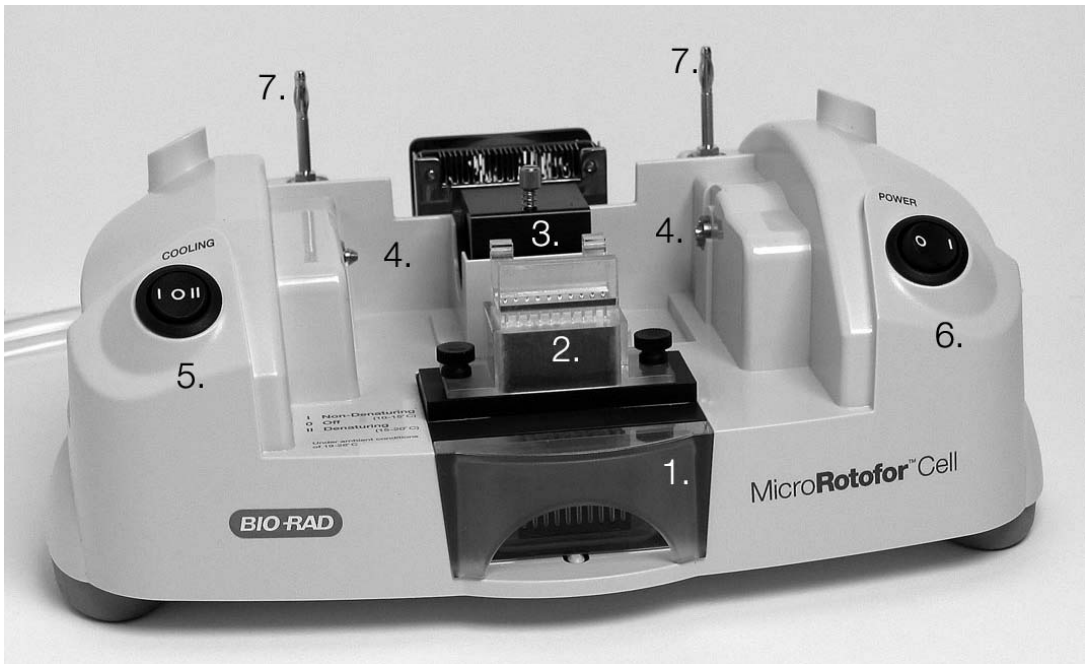

Fig. 2. Components of the MicroRotofor chassis. Harvesting tray positioned in vacuum chamber (1), harvesting station (2), cooling block (3), electrode contacts (4), cooling switch (5), power switch (6), banana plug contacts (7).

The lid fits on top of the chassis and contains the leads that are used to connect the MicroRotofor cell to an external power supply. The lid also provides a safety interlock; current to the cell is broken when the lid is removed.

Note: Do not attempt to circumvent this safety interlock. Always turn the power supply off before removing the lid or when working with the MicroRotofor cell in any way.

Focusing assembly

Fractionation of the sample occurs within the focusing assembly (Figure 3), which consists of a focusing chamber that is attached at either end to an electrode assembly. Ion exchange membranes are inserted between the focusing chamber and electrode assemblies to separate the electrolytes from the sample while allowing current to pass through.

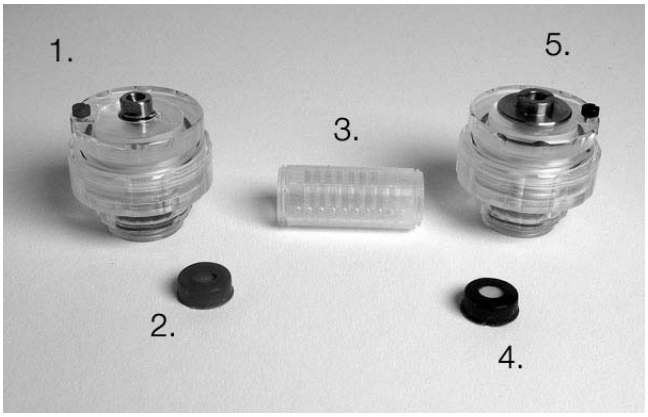

Fig. 3. Components of the focusing assembly. Anode assembly (1), anode membrane (2), focusing chamber (3), cathode membrane (4), cathode assembly (5).

Focusing chamber

The focusing chamber is designed to hold a 2.5 ml sample for fractionation. Internally, a set of nine evenly spaced monofilament polyester screens separates the chamber into ten 250 µl compartments. The screens have a 10 µm nominal pore size to allow free movement of proteins during fractionation. They are inserted into the focusing chamber to stabilize the zones of fractionated proteins during harvesting.

Two sets of ten holes, or ports, are bored into opposite sides of the focusing chamber. One set of ports is used for sample loading and the other, for harvesting. These ports are indistinguishable in the unassembled chamber. During the run, both sets of ports are sealed with sealing tape. Additionally, the focusing chamber is flattened on two sides, which helps to secure and align the focusing assembly in the positioning block of the harvesting station during harvesting.

Electrode assemblies

The cathode and anode assemblies hold the cathode and anode electrolyte solutions, respectively, and provide electrical contact between the focusing chamber and the chassis. These assemblies are color-coded to prevent incorrect assembly (Figure 4).

The anode assembly is color-coded with a red button.

The cathode assembly is color-coded with a black button.

Each assembly consists of two components that are joined together with a threaded sleeve.

The electrode component maintains electrical contact with the chassis. It also features a vent hole that allows the escape of gases that build up during a run. **The vent hole is also used to add electrolyte to the electrode assemblies.** The electrode components are not interchangeable.

The electrolyte component features a threaded sleeve for attachment to the electrode component.

Fig. 4. Electrode assemblies. Left, components of the cathode assembly (black button); right, anode assembly (red button). The threaded sleaves are shown attached to the electrolyte components.

Ion exchange membranes

Positioned between the focusing chamber and each electrode assembly, ion exchange membranes separate the electrolytes from the sample while allowing current to pass through. The ion exchange membranes also create a concentration gradient of ions at the respective ends of the focusing chamber, which helps to establish the pH gradient. There are two types of ion exchange membranes **and they are not interchangeable**:

The cathode membrane (anion exchange membrane, in black casing) is used with the cathode assembly. This membrane is positively charged and repels positive ions, preventing them from contaminating the electrolyte in the cathode assembly.

The anode membrane (cation exchange membrane, in red casing) is used with the anode assembly. This membrane is negatively charged and repels negative ions.

Prior to use, the ion exchange membranes must be equilibrated overnight in an appropriate electrolyte solution. See Section 3.2 for details about equilibration.

Harvesting station

The harvesting station is integrated within the MicroRotofor chassis, toward the front of the unit (Figure 2). With the harvesting station, fractions are aspirated from the focusing chamber into the harvesting tray through of a series of needles. The harvesting station is connected to an external vacuum source (see "Additional Requirements", below) and vacuum is regulated through a 0.75 mm fixed opening in the vacuum chamber to provide uniform aspiration.

The harvesting station is made up of four parts (Figure 5):

The positioning block serves to secure the focusing chamber and align the harvesting ports with the needle array.

The needle array consists of ten needles that are used to aspirate fractions from the focusing chamber to the harvesting tray.

The needle array holder positions the needle array within the harvesting station.

The vacuum chamber (Figure 1) is connected to the vacuum source and holds the harvesting tray (Figure 2).

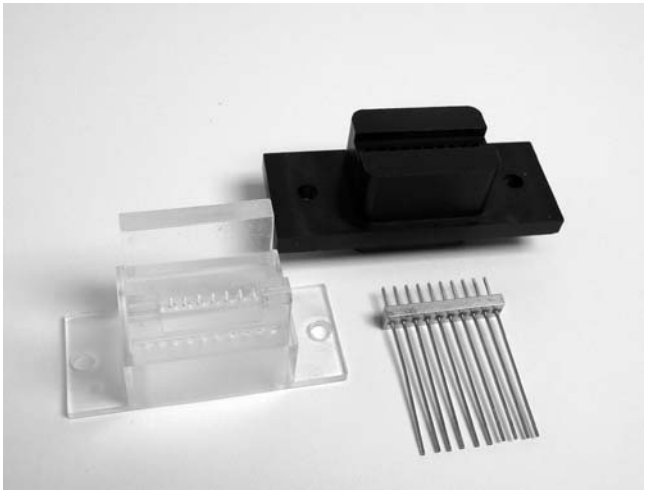

Fig. 5. Components of the harvesting station. Clockwise, from top: needle array holder, needle array, and positioning block.

Harvesting tray

The removable, disposable tray fits beneath the harvesting station. It features ten wells that collect and hold the ten fractions. It can also be sealed and used for temporary storage of the collected fractions.

2.2 Accessories

Sealing tape

The sealing tape is used to seal both the loading and harvesting ports during a run.

Sealing film

The sealing film is used to seal the storage tray after the fractions are harvested.

Assembly tool

This tool features a template used to cut the precise length of sealing tape required to seal the ports of the focusing chamber. It is also used to tighten the focusing chamber to the cathode and anode assemblies.

10 ml syringe (2)

These syringes are used for adding electrolyte solution to the electrode assemblies.

3 ml syringe

This syringe is used for adding the sample to the focusing chamber.

Forceps

These forceps are used to remove the sealing tape covering the loading ports prior harvesting. They may also be used to remove the ion exchange membranes from the focusing assemblies.

Cleaning brush

The cleaning brush is used to clean the harvesting tray.

2.3 Additional Requirements

The following components will also be required to run the MicroRotofor cell.

High voltage power supply

Power to the MicroRotofor cell is to be supplied by an external DC voltage power supply. This power supply must employ a safety isolation transformer to isolate the DC voltage output with respect to ground. All of Bio-Rad's power supplies meet this safety requirement. The power supply must also be capable of:

- Power control at 1 W constant power or, if constant power mode is not available, the ability to program multiple-step constant voltage methods.
- Supplying up to 500 V
- Operation at low currents of 1–15 mA

Note: Power supplies employing an automatic "power down" feature at very low currents must have this feature disabled before starting a run.

A high voltage power supply, such as the PowerPac™ HV (catalog #164-5056) or PowerPac Universal (catalog #164-5070) power supply, can be used with the MicroRotofor cell.

Vacuum source

A house vacuum or vacuum pump capable of 22–27” Hg is required for optimum fraction collection with the harvesting apparatus.

Vacuum trap and tubing

Use of a vacuum trap between the MicroRotofor cell and the vacuum source is strongly recommended. An extra piece of vacuum tubing will be required to connect the vacuum source with the vacuum trap.

Electrolyte solutions

Electrolyte solutions are required for equilibrating the ion exchange membranes prior to use and for filling the electrode assemblies. Generally, 6 ml each of 0.1 M H_3PO_4 and 0.1 M NaOH is sufficient for both applications. Alternative electrolyte solutions may also be used and are listed in Section 6.5 of this manual.

Section 3
Operation

3.1 Overview

- Equilibrate the ion exchange membranes overnight in the appropriate electrolyte solution.
- Prepare the focusing assembly and seal the harvesting ports.
- Load the sample into the focusing chamber and seal the loading ports.
- Add the electrolyte solutions to the electrode assemblies.
- Position the focusing assembly in the MicroRotofor chassis and start the IEF run.
- Shut power off, remove the lid, and connect the MicroRotofor cell to a vacuum source.
- Remove the tape from the loading ports and place the focusing chamber into the positioning block of the harvesting station.
- Aspirate the fractions into the harvesting tray.

3.2 Equilibrate the Ion Exchange Membranes

The ion exchange membranes are shipped dry and must be equilibrated overnight in the appropriate electrolyte solution (Table 3.1) prior to first use. Equilibrated membranes that are stored in electrolyte solution or deionized water can be used for 4–5 runs.

Note: The membranes can be stored indefinitely when dry. After equilibration, the membranes must be kept moist between runs. If they dry out, the membranes may crack and cause electrolyte to leak into the focusing chamber.

Table 3.1. Ion exchange membranes and their equilibration conditions. See Section 6.5 for other electrolyte options.

Membrane	Color (casing)	Electrode Assembly	Electrolyte
Anode membrane (cation exchange membrane)	Red	Anode	0.1 M H_3PO_4
Cathode membrane (anion exchange membrane)	Black	Cathode	0.1 M NaOH

3.3 Prepare the Focusing Assembly

The focusing assembly consists of the focusing chamber, the ion exchange membranes, and electrode assemblies. Ion exchange membrane polarity is essential for the instrument to work properly. To facilitate correct assembly, all anode components are color-coded in red and cathode components, in black.

1. Rinse the equilibrated ion exchange membranes with deionized water.
2. Using forceps, insert an anode membrane (red casing) into one end of the focusing chamber and a cathode membrane (black casing) into the other end (Figure 6).

Fig. 6. Inserting an anion exchange membrane into the focusing chamber.

3. Put together both electrode assemblies (Figure 7). Place the electrode and electrolyte components of each electrode assembly together and fasten them together loosely with the threaded sleeve. Do not overtighten as some adjustment may be needed later (see Step 6, below).

Fig. 7. Unassembled components of the cathode assembly. Electrode component (1), threaded sleeve (2), electrolyte component (3).

4. Attach the anode assembly (red button) to the end of the focusing chamber containing the anode membrane (red casing) and the cathode assembly (black button) to the end containing the cathode membrane (black casing). Use the assembly tool like a wrench, to securely tighten the electrode assemblies to the focusing chamber (Figure 8).

Fig. 8. Using the assembly tool to securely attach an electrode assembly onto the focusing chamber.

5. Align one row of ports on the focusing chamber with the vents on the electrode assemblies. Rotate the electrode component of each electrode assembly within the threaded sleeve until the vents and the ports are aligned (Figure 9). These aligned ports will be the sample loading ports.

Fig. 9. Alignment of the sample loading ports with the vents on the anode (left) and cathode (right) assemblies.

6. Tighten the threaded sleeves on each electrode assembly, making sure to maintain the alignment of the loading ports and the electrolyte vents. Be careful to not overtighten.

3.4 Load the Sample

The focusing chamber features two rows of ports. The row that is aligned with the vents on the electrode assemblies will be used to load the sample (loading ports). The row on the opposite side of the focusing chamber will be used for harvesting the fractions (harvesting ports). The harvesting ports must be sealed with tape before the sample is loaded. After the sample is loaded, the loading ports will also be sealed with a piece of tape.

1. Using the assembly tool as a template, cut two pieces of sealing tape. Position the tape across the template so that it covers all three cutting grooves (Figure 10). Cut the tape with a cutting blade at all three grooves to generate two strips of tape. Each strip can be lifted off the template at the cutouts located between two grooves.

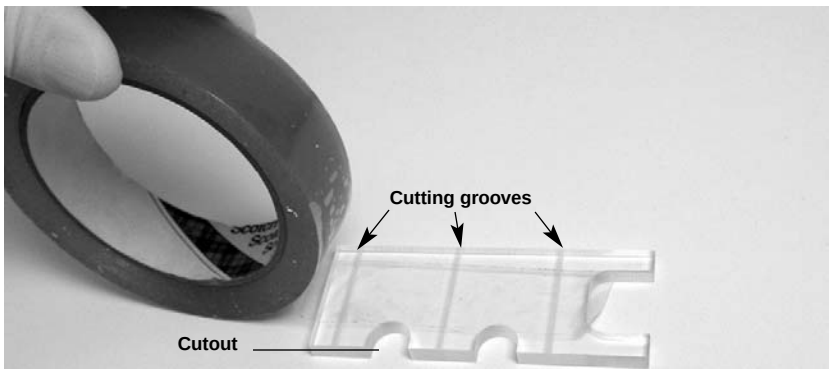

Fig. 10. Using the assembly tool to measure the appropriate length of sealing tape.

2. Use one of the strips of tape to seal the row of harvesting ports. Cover all of the ports, and do not extend the tape beyond the focusing chamber (Figure 11).

Note: Excess tape or tape that is not properly positioned will interfere with the cooling block during oscillation, which can result in leakage of sample from the focusing chamber.

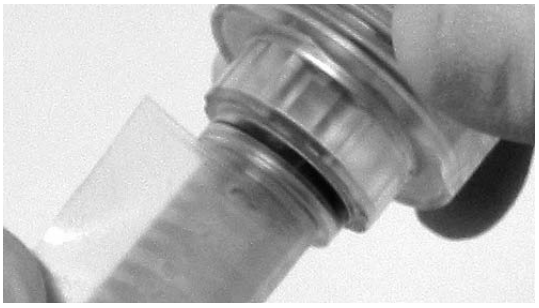

Fig. 11. Applying sealing tape to cover the harvesting ports on the focusing chamber.

3. Fill the 3 ml syringe with sample. **Slowly** load the sample through the centermost loading port of the focusing chamber (Figure 12). As this compartment fills, the sample will slowly spread to and fill all of the adjacent compartments. Continue adding sample slowly to this compartment until all of the compartments are filled. Alternatively, fill every other compartment and wait for the sample to spread to the adjacent compartments.

   Note: If air bubbles are introduced into the focusing chamber, the sample will not spread to adjacent compartments as described. Make sure to dislodge and remove all air bubbles from the chamber by aspirating the sample from a compartment and loading it again.

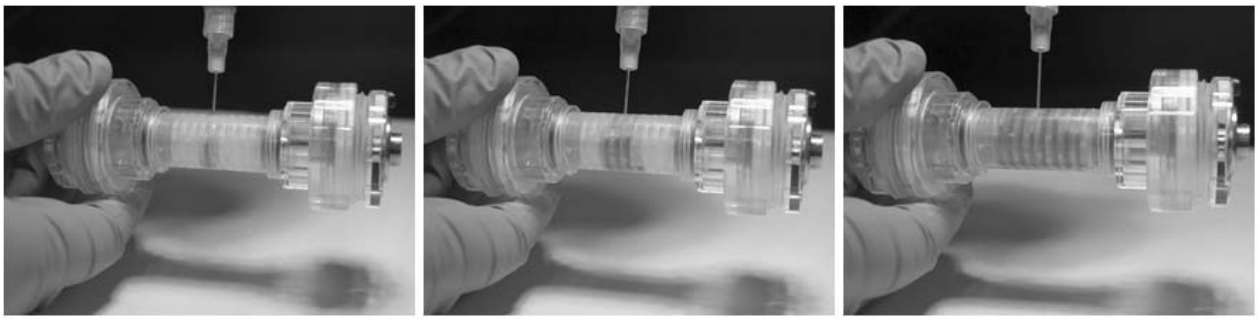

Fig. 12. Loading sample into the focusing chamber.

4. When the sample is loaded, make sure that all compartments are filled and that no bubbles remain. Air bubbles will disrupt the electric field, which can lead to poor separation. To dislodge air bubbles, aspirate the sample from a compartment and load it again.
5. Dry the outside surface of the focusing chamber and seal the row of loading ports with the other piece of sealing tape. Cover all of the ports. Do not extend the tape beyond the focusing chamber and do not overlap the tape with the strip covering the harvesting ports.

   Note: Excess tape or tape that is not properly positioned will interfere with the cooling block during oscillation, which can result in leakage of sample from the focusing chamber.

3.5 Perform the Focusing Run

1. Open the cooling block cover by unscrewing the block screw.
2. With the vents and colored buttons on the electrode assemblies facing up, place the focusing assembly into the focusing station of the chassis. Make sure the anode assembly (red button) is to the left and the cathode assembly (black end) is to the right.
   a. Gently push the anode end (red) of the focusing assembly into the anode contact (left) on the chassis until the contact is completely retracted (Figure 13).
   b. Lower the focusing assembly into the cooling block and slide the cathode end of the assembly into the notch on the chassis (Figure 14). If necessary, rotate the focusing assembly until the slots on the cathode assembly align with the notch. Alternatively, turn power on to the oscillating motor and wait until the notch is in a better position to connect with the focusing assembly.

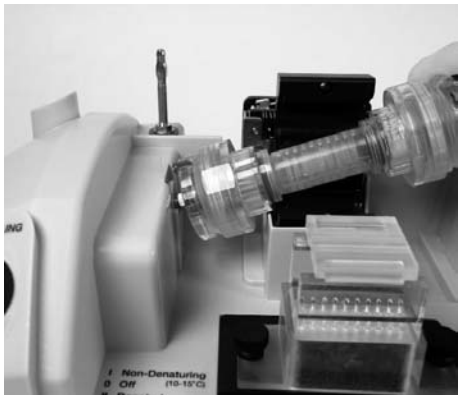

Fig. 13. Placing the focusing assembly onto the chassis.

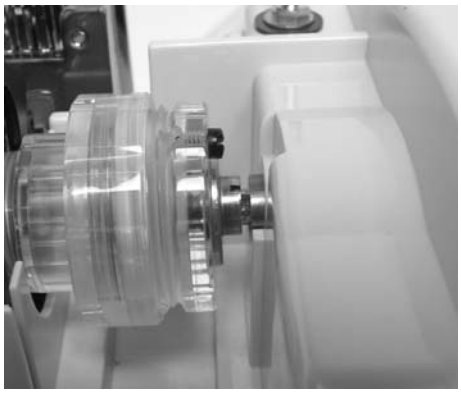

Fig. 14. Fitting the cathode assembly into the notch of the chassis.

3. Using a 10 ml syringe, add electrolyte solutions to the electrode assemblies (Figure 15).
   a. Add 6 ml 0.1 M H_3PO_4 through the vent hole of the anode assembly (red button).
   b. Add 6 ml 0.1 M NaOH through the vent hole of the cathode assembly (black button).

Fig. 15. Adding electrolyte to the anode assembly.

4. Close the cooling block and tighten the screw. Turn the power switch to the ON position and make sure that the focusing chamber rotates freely within the cooling block. If it does not, it means that the cooling block lid has been tightened too tightly, which may cause the electrolyte chambers to become loose during a run and electrolyte to leak out. To loosen the lid, turn the screw counter-clockwise until the focusing chamber can move freely.
5. Place the lid on the chassis.
6. Attach the power cord to the back of the MicroRotofor chassis and connect it to an electrical outlet.
7. Connect the MicroRotofor cell to a vacuum source. We recommend installation of a vacuum trap between the cell and the vacuum system.

   Note: Keep the vacuum valve closed until the run is completed and you are ready for harvesting the fractions.
8. Turn the power switch to the ON position to start the oscillating motor.
9. Set the cooling switch to setting I (10°C) or II (20°C), depending on your requirements for temperature regulation. See Table 3.2 below for cooling setting information.
10. For setting I (10°C), allow the system to equilibrate to the set temperature for ~15 min prior to initiating the focusing run. For setting II (20°C), system equilibration is not required.

Table 3.2. MicroRotofor cooling settings.

Ambient Temp.	Internal Temperature, Focusing Chamber	
	Cooling setting I	Cooling setting II
4°C (cold room)	6 ± 2°C	Not applicable
15°C	7 ± 2°C	17 ± 2°C
19°C	10 ± 2°C	20 ± 2°C
22°C	10 ± 2°C	20 ± 2°C
26°C	11 ± 2°C	20 ± 2°C
30°C	15 ± 2°C	21 ± 2°C
35°C	20 ± 2°C	25 ± 2°C
>35°C	Operate in a cold room to maintain an internal temperature of 6 ± 2°C	Use cooling setting I to maintain an internal temperature of 20–25°C

11. Attach the leads on the lid to a PowerPac HV power supply or other commercial power supply capable of power control at 1 W. If your power supply cannot be set to run under constant power, run the instrument in a multiple-step constant voltage mode with limiting current (see Section 3.6 for details about power conditions).
12. A typical run is completed in less than 3 H. To monitor the progress of a run under constant power, observe the voltage increase over time. The run is complete when the voltage stabilizes. At that point, allow the run to continue for 30 min before harvesting. Longer run times do not improve focusing and may result in a collapse of the pH gradient.

3.6 Power Conditions

Constant Power

The following are the recommended power conditions for power supplies capable of maintaining constant power conditions at 1 W. Refer to Table 3.2 for detailed cooling setting information. Note that the following guidelines apply to an ambient temperature of 19–26°C.

Table 3.3. Constant power settings.

	Cooling setting I	Cooling setting II
Power	1 W constant	1 W constant
Voltage range	100–350 V	100–500 V
Internal temperature	10 ± 2°C	20 ± 2°C

Multiple-Step Constant Voltage

If your power supply cannot run under constant power, use a multiple-step constant voltage with limiting current. A 20 mA current limit and 2 W power limit are recommended.

The following guidelines were developed for a sample containing 2.5 mg protein in either 2% (w/v) Bio-Lyte® ampholytes pH 3-10 (cooling setting I) or 7 M urea, 2 M thiourea, 2 mM TBP, 4% CHAPS, 2% (w/v) Bio-Lyte ampholytes pH 3-10 (cooling setting II). Samples with different protein loads, ampholyte concentrations, or pH ranges may display slightly different current ranges and may require longer run times.

Table 3.4. Multiple-step constant voltage settings.

Step	Cooling setting I		Cooling setting II	
	Volts/Time	Current	Volts/Time	Current
1	150 V/15 min	10–5 mA	150 V/10 min	8–3 mA
2	200 V/15 min	7–2 mA	200 V/10 min	4–2 mA
3	300 V/20 min	3–2 mA	300 V/60 min	4–3 mA
4	350 V/20 min	3 mA		
5	400 V/60 min	3 mA		

3.7 Harvest the Fractions

Once the IEF run is complete, fractions should be harvested as quickly as possible to avoid diffusion of the separated proteins. Throughout the following steps, minimize movement of the focusing chamber to avoid diffusion.

1. Turn the power supply off and disconnect it from the MicroRotofor cell.
2. Turn off power to the oscillating motor and cooling block on the MicroRotofor cell, and remove the lid from the chassis.
3. Make sure that the MicroRotofor chassis is connected to a vacuum source and that the harvesting tray is in place and flush against the sealing gasket.
4. Open the cooling block cover and, using forceps, remove the sealing tape from the sample loading ports.
5. Apply a vacuum to the chassis.
6. Remove the focusing assembly from the focusing station. First, lift up on the cathode end (black button) to dislodge it from the notch in the chassis. Then remove the anode end (red button).
7. With the row of sample loading ports facing up, position the focusing assembly in the harvesting station (Figure 16). Two sides of the focusing chamber are flattened to correctly orient the focusing assembly within the harvesting station and to help align the harvesting needle array with the sealed harvesting ports. DO NOT puncture the sealing tape covering the harvesting ports.

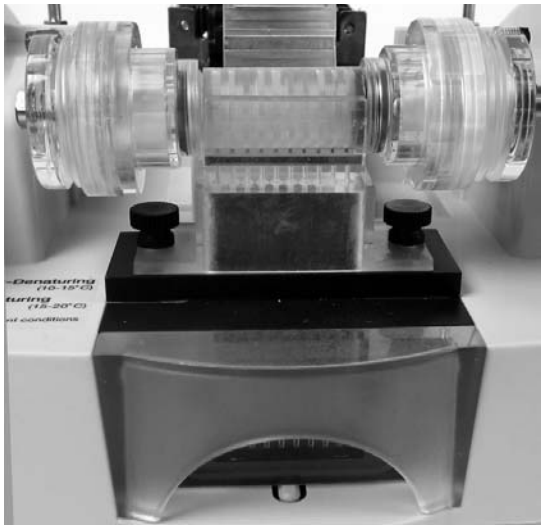

Fig. 16. Placement of the focusing assembly into the harvesting station.

8. Using both hands, press down on the focusing assembly while pressing the harvesting tray against the seal of the vacuum chamber (Figure 17). Press down evenly and firmly on the electrode assemblies so that all the needles penetrate the sealing tape and harvesting ports simultaneously. Do not cover any of the loading ports with your fingers.

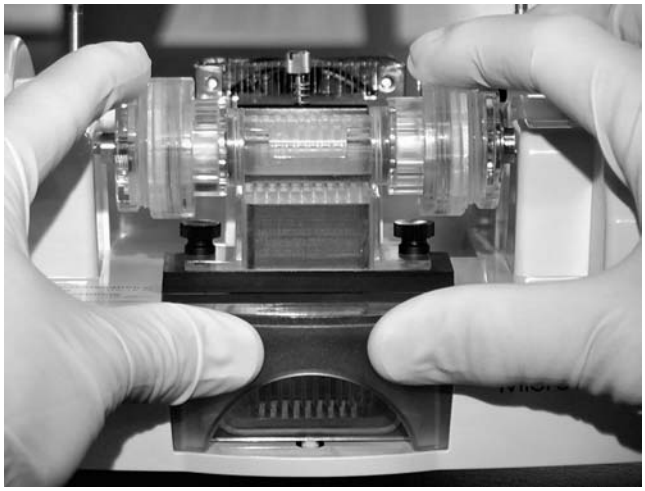

Fig. 17. Harvesting the fractions.

9. Continue to press down on the focusing chamber for several seconds to aspirate the ten fractions into the harvesting tray.

   Note: If the sample contains high-foaming detergents such as Triton, we recommend removing the harvesting tray immediately after harvesting.

10. Remove the harvesting tray and turn off the vacuum source.
11. Transfer the fractions to micro tubes or other containers with a syringe or pipet (Figure 18). For storage in the harvesting tray, seal the tray with the sealing film and store as appropriate.

Fig. 18. Transferring the fractions.

Section 4
Disassembly and Cleaning

4.1 Focusing Assembly

1. Using the assembly tool, loosen and remove the electrode assemblies from the focusing chamber (Figure 19).

Fig. 19. Using the assembly tool to remove the anode assembly from the focusing assembly.

2. Using the forceps, remove the ion exchange membranes from the focusing chamber and **immediately** store them in deionized water or in their respective electrolyte solution (Table 3.2).

   Note: The membranes cannot be allowed to dry out after they have been equilibrated. The membranes may be stored in electrolyte or in distilled water between runs. If they dry out, the membranes may crack, causing electrolyte to leak into the focusing chamber. Equilibrated membranes, if stored properly, can be used for 4–5 runs.

3. Wash the focusing chamber. Remove the ion exchange membrane and store as indicated in step 2. Place the focusing chamber in 2% SDS solution overnight. Thoroughly rinse the focusing chamber with deionized H_2O to remove the SDS.

4. After the run, turn the electrode assemblies so that the majority of the electrolyte pours out from the vents. Take apart the electrode assemblies and rinse the components with deionized water.

4.2 Harvesting Station

To maintain optimum harvesting performance, it is critical to wash the needle array immediately after harvesting is complete. The entire harvesting station and needle array may be detached from the chassis, washed with a mild detergent, and rinsed with deionized water.

1. Disconnect the chassis from the vacuum source.
2. Remove the two screws that secure the harvesting station to the vacuum block.
3. Remove the positioning block, needle array, and needle array holder (Figure 20).

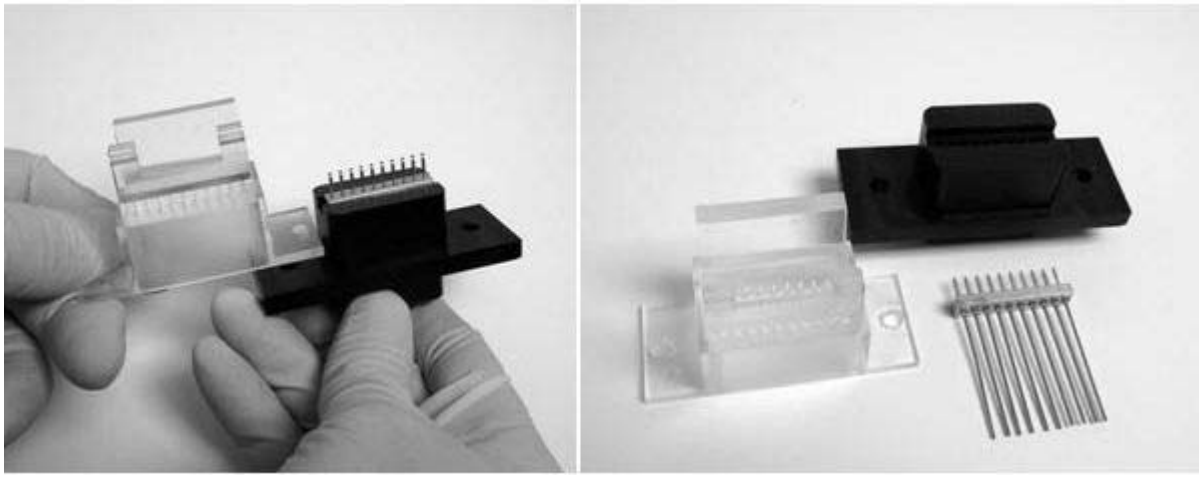

Fig. 20. Disassembling the harvesting station (left) and harvesting station components (right).

4. Clean and dry the positioning block.
5. Using a wash bottle, wash and rinse the individual needles in the needle array.
6. Clean and dry the vacuum chamber.
7. Inspect and reposition the vacuum gaskets.
8. Reassemble the harvesting station.

Section 5
Analysis of Results

After fractionation and harvesting, the ten liquid fractions are ready for further separation or analysis by a variety of techniques, including one-dimensional (1-D) or two-dimensional (2-D) electrophoresis, chromatography, or mass spectrometry. This section provides general guidelines for the initial steps of processing and analyzing fractions.

5.1 pH Measurement

One of the quickest and easiest ways to identify which fractions contain a protein of interest is to measure the pH of the fractions and select those with the pH that is closest to the pI of the protein. The pH profile of the fractions is also one of the best indicators of the efficacy of a fractionation on the MicroRotofor cell; in most cases where the cell and its components have been properly maintained and samples have been properly prepared, a linear pH gradient will be established among the fractions (Figure 21).

To measure the pH of each fraction, it is best to use a small diameter pH probe, such as the Beckman Futura refillable micro combination pH electrode (VWR catalog #BK511082). In addition, the pH range of each fraction can be estimated from the pH range of the ampholyte blend that was selected for the fractionation. For example, if pH 3–10 ampholytes were selected for fractionation, the 7 pH unit range would be separated across 10 fractions, and the estimated pH range for each fraction would be ~0.7 pH units.

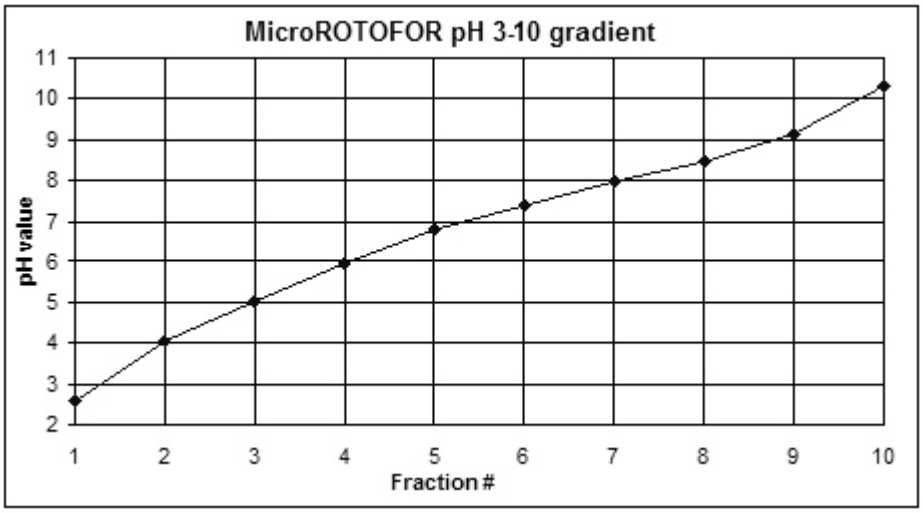

Fig. 21. pH profile of a MicroRotofor run. A sample was separated in the MicroRotofor cell using pH 3–10 Bio-Lyte ampholytes. The pH of each fraction was measured and plotted as a function of the fraction number.

5.2 Protein Quantitation

To determine the recovery or protein yield in the fractions, measure the total protein in each fraction. Because ampholytes interfere with several commonly used protein quantitation assays, such as the method developed by Bradford (1976), it is best to use a method validated for use with ampholyte-containing solutions, such as the *RC DC*™ protein assay (catalog # 500-0121 and 500-0122). Based on the method developed by Lowry (Lowry et al, 1951), this assay is compatible with a broader range of reagents and so is used for protein quantitation directly in complex sample solutions.

If ampholytes must be removed prior to downstream applications (see below), it may be best to quantitate proteins after the removal step.

5.3 Qualitative Analysis of Fractions

Though the pH of the fractions may provide sufficient information, fractions are often analyzed further to determine the extent of fractionation and to identify the fractions that contain the protein of interest. There are many methods of analysis available and the most suitable will depend on several factors, including the resources available, the protein being analyzed, and the desired downstream application. Listed here are some of the most commonly used techniques for analyzing MicroRotofor fractions:

SDS-PAGE provides both an effective view of the proteins within each fraction as well as a second-dimension separation (see below). Fractions may be loaded onto analytical gels for separation and qualitative analysis. Such analysis can indicate whether refractionation should be employed to improve protein purification (see Section 6.6 for more information about refractionation).

IEF gel separation provides an alternative approach to SDS-PAGE for qualitative analysis of the proteins in each fraction.

Immunological and activity assays assess the presence and/or activity of a known protein, respectively. Proteins can be fractionated in their native state in the MicroRotofor cell.

5.4 Downstream Applications

MicroRotofor fractions are suitable for use in an array of downstream applications.

2-D electrophoresis/ MS analysis

Fractionation with the MicroRotofor cell reduces the complexity of protein samples, thereby allowing the use of larger sample loads for 2-D electrophoresis and generating improved resolution of low-abundance proteins. Fractionation by pI complements the greater resolution afforded by narrow- and micro-range IPG strips by effectively eliminating proteins that are outside the pI range of interest.

For these applications, the pH of the fractions should be measured to determine the fractions and IPG strips to be used. In addition, it will be necessary to remove ampholytes from the fractions prior to loading on the IPG strips, especially in cases where the pH range of the IPG strip does not exactly match the range of ampholytes present in the fractions (for example, if a pH 3–5 pooled fraction is analyzed on a pH 3–10 IPG strip).

SDS-PAGE

Fractionation on the MicroRotofor cell constitutes a first-dimension IEF separation when paired with second-dimension SDS-PAGE. In such applications, MicroRotofor fractions are separated on SDS-PAGE gels. The resulting "2-D" gels may be analyzed in a number of ways, including by band excision and MS analysis, or by blotting.

Purification

Ideally, a protein of interest will be recovered in a single fraction following separation on the MicroRotofor cell. This means that a single MicroRotofor run can yield up to 10-fold concentration of a protein. In addition, the unique design of the MicroRotofor cell allows separation to occur entirely in a liquid medium, affording the user easy recovery of proteins and the possibility of entirely native separations. Though a single separation may be sufficient for a variety of purposes, refractionation of fractions of interest may also be employed to provide even greater resolution (see Section 6.6). **Electrophoresis**: Fractions of interest may be loaded onto preparative slab gels or the mini prep cell for a second dimension, preparative separation by SDS- or Native PAGE. Separation by gel electrophoresis provides the added benefit of removing residual ampholytes from the protein(s) of interest. Also, because separation on the MicroRotofor cell may be carried out under native conditions, its pairing with native PAGE provides the opportunity for entirely native 2-D separations. **Chromatography**: The liquid MicroRotofor fractions are suitable for further purification by chromatography.

Mass Spectrometry: Sample fractionation with the MicroRotofor cell reduces the complexity of protein samples, thereby improving resolution and minimizing the signal compression effects often seen in MALDI-TOF MS analysis of complex samples (Wang et al. 2003).

Immunological and activity assays

MicroRotofor fractions are often ready for use in immunological and activity assays. Some assays, however, may require the removal of ampholytes (see Section 5.5).

5.5 Ampholyte Removal

Ampholytes typically have a molecular weight of about 300–1,200 Daltons and form weak electrostatic complexes with proteins that may interfere with the measurement of protein amounts and activity. Though many applications can tolerate the presence of ampholytes in protein solutions, a number of applications, such as amino acid analysis, cannot. Several methods for separating ampholytes from focused proteins are listed below.

Precipitation of protein by addition of ammonium sulfate, alcohol, or trichloroacetic acid (TCA) is an effective, inexpensive means of removing ampholytes and other contaminants from protein samples. These methods provide the added advantage of concentrating the protein prior to further analysis. The ReadyPrep™ 2-D cleanup kit (catalog #163-2130) is a prepackaged kit that provides a TCA-like precipitation of protein from small (<100 µl) sample volumes.

Centrifugal filtration is an easy, effective means of removing ampholytes and other contaminants from protein samples. Centrifugal filtration also results in concentration of the protein sample.

Dialysis is a simple method for ampholyte removal when concentration of proteins is not required or desired. Adjust the sample to 1 M NaCl to effectively strip electrostatically bound ampholytes from proteins by ion exchange. For example, add either 10x phosphate-buffered saline (PBS) or 10 M NaCl to the sample at 1/9 the volume of the sample. The salt will compete with the ampholytes for sites on the protein. Dialyse the sample into the buffer that is appropriate to your downstream application.

Electrophoresis of MicroRotofor fractions through a polyacrylamide gel (for example, by analytical SDS-PAGE or preparative PAGE on slab gels or in the mini prep cell) separates contaminating proteins and ampholytes away from proteins of interest.

Chromatographic techniques, such as gel filtration, ion exchange, hydroxyapatite, affinity chromatography, can be used to separate proteins from ampholytes.

Section 6
Optimizing Protein Separation

6.1 Overview

For successful fractionation of your sample by liquid-phase IEF using the MicroRotofor cell, several key factors must be considered.

- Sample preparation

  Ampholyte selection — the pH range of the ampholytes should encompass the pI of the protein of interest.

  Protein solubility — a number of reagents may be added to the sample to preserve solubility during IEF.

  Sample capacity — total protein load may affect the resolution of the separation.

  Salt and buffer concentration — select a sample buffer that maintains protein solubility during IEF.

  Sample clarification — turbid sample solutions should be clarified by filtration or centrifugation to remove extraneous cellular debris.

- Power conditions
- Temperature regulation
- Electrolytes — a variety of electrolytes can be used, depending on the pH range chosen for fractionation.
- Refractionation — a second round of separation of fractions of interest can yield high-resolution fractionations.

6.2 Sample Preparation

The preparation of the protein sample prior to fractionation is one of the most critical and variable steps of the fractionation process. Many of the factors described below apply differently to different sample types and amounts, and to different research goals. Therefore, each must be determined empirically.

Ampholyte selection: The final concentration of ampholyte required in the MicroRotofor cell will depend on the protein concentration in a given sample (Table 6.1), but may be increased as required to maintain protein solubility. Up to 8% (w/v) ampholyte concentrations have been used for various applications. Low ampholyte concentrations (~1%) permit higher applied voltages and are recommended if refractionation is not required. Higher ampholyte concentrations provide better buffering, help maintain solubility, and are required if refractionation will be performed.

Table 6.1. Recommended ampholyte concentrations.

Purpose	Ampholyte concentration, % (w/v)
Fractionation (protein concentration, mg/ml)	
>2.0	2.0
1.0	1.5
0.5	1.0
0.25	0.5
Refractionation	≥2.0*
Improve solubility	Increase, up to 8%**

* Z% amphlyte is needed for the inital fractionation.

** If protein preciptation occurs during a run, sample solubility may be maintained with higher ampholyte concentrations.

Use the following formula to determine the volume (V_1) of ampholyte solution that will provide the desired final concentration (C_2) in your MicroRotofor sample.

For the equation: $(C_1)(V_1)=(C_2)(V_2)$, solve for V_1.

where:
C_1 = Starting concentration of ampholyte (20% or 40%)
V_1 = Unknown volume of ampholyte to give desired final concentration
C_2 = Final or desired concentration of ampholyte
V_2 = Final volume of the sample to be applied to the MicroRotofor cell (2.5 ml)

The proper choice of ampholyte is critical for good results. The pI of the protein(s) of interest should fall in the middle of the ampholyte range used for fractionation.

- Ampholytes with narrow pH ranges are most effective for separating the protein from the bulk of its contaminants. A narrow pH range of ampholytes that span the pI of the protein of interest should be used.
- To determine the pI of the protein of interest, run an aliquot of the sample and IEF markers (catalog #161-0310) on an analytical IEF gel, using the pH 3–10 range. Use the relative migration of the protein of interest and the IEF markers to estimate the pI of the protein.

Protein solubility: During isoelectric focusing (IEF), proteins become concentrated at their isoelectric point (pI), where they are uncharged. The lack of electrostatic repulsion may cause some proteins to precipitate by a phenomenon known as "pI fallout" that is common to all IEF methods. A number of different reagents may be added to the sample to maintain solubility of proteins during focusing (Table 6.2).

Note: Before performing the first fractionation run on the MicroRotofor cell, check the solubility of the protein of interest by diluting it in the sample buffer to be used and running it out on analytical IEF gel. If the protein shows no signs of precipitation on the analytical IEF gel, it should not precipitate in the MicroRotofor cell.

Table 6.2. Recommended solubilizing agents for the MicroRotofor cell.

Nonionic detergents (0.1–3.0%)	Zwitterionic detergents (0.1–3.0%)	Reducing agents	Chaotropic agents
Digitonin	CHAPS	DTE (5–20 mM)	Urea (1–8 M)
Octylglucoside (OGS)	CHAPSO	DTT (5–20 mM)	7 M Urea/2 M Thiourea
Triton X-114		BME (1–5 mM)	Glycine (0.1–2.0%)
		TBP (1-2mM)	Proline (0.1–2.0%)

- **Chaotropic agents**. Addition of up to 8 M urea (or 7 M urea plus 2 M thiourea) is recommended to improve protein solubility in cases where native fractionation is not required. If urea is used, be sure to deionize the urea using AG® 501-X8 mixed bed ion exchange resin (catalog #143-7424). Other chaotropes may also be added (Table 6.2).
- **Detergents**. Addition of nonionic detergents, such as CHAPS, CHAPSO, octylglucoside (OGS), digitonin, or Triton X-114 is also valuable in maintaining the solubility of focused proteins. The concentration of these detergents is generally held at 0.1–3%.
- **Reducing agents**. Reducing agents, such as β-mercaptoethanol (BME), tributylphosphate (TBP), and dithiothreitol (DTT) may be added to the protein sample to break disulfide bonds.
- **Ampholytes**. Solubility may be maintained by increasing the ampholyte concentration in the sample. Usually, 2–3% ampholyte is used for fractionation, but up to 8% may be used, if required. Ampholytes may be added during protein extraction; alternatively, the protein sample buffer may be exchanged for ampholyte-containing fractionation buffer by dialysis.
- **Glycerol**. Addition of glycerol from 5–25% (v/v) in the sample is also highly effective for maintaining the solubility and stability of some proteins. Glycerol stabilizes water structure and the hydration shell around proteins.

Sample capacity: Protein concentrations should be adjusted to accommodate the yield of protein desired after fractionation or to match the detection range of the assay(s) to be used. The maximum protein load will vary with the solubility of each sample and must be determined empirically. The minimum protein load is dependent on the final detection assay.

Salt and buffer concentration:

- Samples should be desalted (for example, by dialysis or Bio-Gel® P-6 chromatography) prior to ampholyte addition to ensure that the nominal pH range of the ampholyte will extend over the full length of the focusing chamber and that the maximum voltage can be applied.
- It is best to limit salt concentrations to 10 mM for optimum fractionation. However, since the maximum salt capacity will vary with the application, optimal running conditions must be determined empirically. Note that during focusing, all salts will migrate to the compartments adjacent to the anode and cathode, effectively desalting the sample. Additional ampholytes may sometimes be added to maintain solubility of proteins that require solutions of high ionic strength (see "Protein Solubility", above).
- Buffers should also not be added in concentrations greater than 10 mM as they add to the conductivity of a sample and decrease resolution. Also, buffering solutions may flatten the pH gradient in the region of the pKA of the buffer.

Sample clarification: Turbid samples should be clarified by filtration or centrifugation to remove cellular debris that may clog the screen core.

6.3 Power Conditions

The MicroRotofor cell should be run at 1 W constant power (see Section 3.6 for detailed power recommendations). During the initial stages of fractionation, the voltage values will vary between samples, depending on the relative concentration of proteins and salts. When a constant power is used, the voltage will gradually increase as the sample components migrate to their respective pIs. The progress of an electrofocusing run is, therefore, easily be monitored by observing the voltage increase over time. When a steady state is reached, the voltage will level off at a maximum value.

6.4 Temperature Regulation

Temperature control is required to achieve run-to-run reproducibility. Unregulated IEF runs can cause high internal temperature and increase the risk for denaturation, oxidation, carbamylation, or proteolysis.

The MicroRotofor cell has two cooling settings (see Table 3.2 and Section 3.5 for detailed temperature settings).

- **Setting I maintains a temperature of 20°C ± 2°C** inside the focusing chamber — for downstream applications, such as IEF on IPG strips, that do not require undenatured proteins.
- **Setting II maintains a temperature of 10°C ± 2°C** inside the focusing chamber — for downstream applications, such as activity and structural assays, that may require undenatured proteins.

6.5 Electrolytes

The recommended electrolyte solutions for the anode and cathode assemblies are 0.1 M H_3PO_4 and 0.1 M NaOH, respectively. Because a small amount of electrolyte may move through the ion exchange membranes during the focusing run, the channels immediately adjacent to the electrode assemblies may be more acidic or basic than expected. The result will be a concentration of the effective pH gradient to the middle fractions, which will have only a minimal effect on the final separation. Other electrolytes, which may have less effect on narrow pH ranges, may also be used (Table 6.3). The choice of these alternative electrolytes depends on the pH range being investigated.

Table 6.3 Alternative electrolytes for the MicroRotofor cell.

pH Range of Ampholyte	Anode Electrolyte	Cathode Electrolyte
3–5	0.5 M Acetic Acid	0.25 M HEPES
4–6	0.5 M Acetic Acid	0.5 M Ethanolamine
5–7	0.1M Glutamic Acid	0.5 M Ethanolamine
6–8	0.1M Glutamic Acid	0.1 M NaOH
7–9	0.25 M MES	0.1 M NaOH
8–10	0.25 M MES	0.1 M NaOH

6.6 Refractionation

Better separation may be achieved by refractionating a sample. Refractionation entails pooling the fractions that contain the protein(s) of interest, diluting the pooled sample to an appropriate volume, and fractionating it once more. These pooled samples contain a blend of ampholytes whose pI range spans the pI of the protein(s) of interest. During refractionation, this customized narrow pH range is distributed over ten fractions, resulting in a shallow pH gradient and capacity for very high resolution.

The MicroRotofor cell is ideal for refractionation because samples are minimally diluted for fractionation in its small focusing chamber. Fractions generated by a previous run on a MicroRotofor, mini Rotofor, or Rotofor cell can be refractionated by following these steps:

1. After screening the fractions collected from the first run, pool the fractions containing the protein of interest.
2. Dilute fractions to an appropriate sample volume (for example, 2.5 ml for refractionation on the MicroRotofor cell) with distilled water, up to 8 M urea, or a solution containing nonionic detergent for solubility. Make sure that this dilution maintains a minimal ampholyte concentration of 0.8%. **DO NOT ADD ADDITIONAL AMPHOLYTE**.
3. Load the sample into the cell and begin fractionation.

Since the ionic strength of the sample will be lower upon refractionation, higher voltages, which yield better separations, may be achieved. Take care to not exceed the power limit of the MicroRotofor cell.

Section 7
Troubleshooting

7.1 Precipitation of Proteins During Fractionation

By definition, a protein at its pI has no net charge. Because little electrostatic repulsion exists between focused proteins, hydrophobic interactions become predominant and cause the proteins to aggregate and precipitate. This phenomenon, known as "pI fallout", is common to all IEF techniques. Maintaining the solubility of proteins in this case requires overcoming protein-protein interactions (see Section 6.2 for detailed recommendations on preserving protein solubility).

- Several chemical reagents promote protein solubility and may be used to improve solubility during fractionation in the MicroRotofor cell (see Section 6.2).
- Because the inherent solubilities of proteins differ greatly, there is no single answer to the problem of solubility and the appropriate solubilizing conditions must be determined empirically. Compare the solubility of the protein of interest in a number of solubilizing conditions by analyzing small aliquots of sample on analytical IEF gels.
- Decreasing the protein load will also help to maintain the protein in solution.

7.2 Factors Affecting the pH Gradient

Nonlinear pH gradients are rarely observed when the sample is prepared properly and the MicroRotofor cell and its parts are carefully maintained. A nonlinear pH gradient may be caused by one or more of the following:

- **Electrolyte leakage**. Excessive leakage of electrolyte across the ion exchange membranes into the focusing chamber will decrease the number of fractions on the linear portion of the pH gradient and reduce the effective voltage across the sample. The most common cause for electrolyte leakage is a cracked, dehydrated, or worn ion exchange membrane (catalog #170-2833). If properly maintained (see Section 3.2), ion exchange membranes can be used for 4–5 runs.
- **Uneven harvesting**. Variations in the volumes of harvested fractions may affect the apparent linearity of the collected fractions (see Section 7.6 for details on how to improve harvesting).
- **Premature harvest**. If a run is terminated too early, a partially formed pH gradient and poorly focused proteins may result. For standard applications (pH 3-10), the MicroRotofor cell is normally run for 3 hr. To ensure complete focusing, continue the run for 30 minutes after the voltage stabilizes.
- **High salt concentration in sample**. Good resolution depends on both establishing a high voltage gradient and maximizing the number of fractions within the linear portion of the pH gradient. High salt concentrations will decrease the effective voltage across the focusing chamber and may reduce the number of fractions within the linear portion of the pH gradient. If a particularly high ion concentration is required to preserve the stability, solubility, or activity of a protein, Bio-Lyte ampholytes may be substituted for the salts (see Section 6.2).
- **High buffer concentration in the sample**. An excess of buffer in the protein sample may cause the pH gradient to plateau or dip. The pH gradient will be buffered at the pKA of the buffer and cause a number of fractions to have the same pH.
- **High sample temperature**. Diffusion rates of proteins are proportional to their temperature in solution. Proteins and ampholytes diffuse in and out of their focused zones; it is best to run the MicroRotofor cell at the recommended temperature setting for your application (see Table 3.2 for cooling setting recommendations).
- **Shelf life of ampholytes**. The quality of ampholytes influences the integrity and reproducibility of the pH gradient. Batches and brands of ampholytes may vary and it is important to store ampholytes at 4°C in the dark. The guaranteed shelf life of opened Bio-Lyte ampholytes is one year.

7.3 Recovery of Biological Activity

A number of factors may influence the biological activity of a focused protein.

- **Proteins are not always active at their pI**. Adjust the pH of the solution for assay.
- **Proteins may require the presence of particular ionic species (for example, Mg^{2+}, K^{+}, etc.) for activity**. Replace the ions, if necessary, for the assay.
- **Proteins may have become denatured during focusing due to a number of factors**:

  Temperature. Many proteins, especially enzymes, are temperature-labile. Make sure to set the cooling block to the setting that will maintain the activity of your protein (see Table 3.2).

  Solubilizing agents. Chaotropes, detergents, etc., which may be added to improve solubility, may also denature the protein(s) of interest. Use the smallest amount of these reagents that is possible and take steps to remove them (for example, by dialysis) immediately following fractionation.

7.4 Maximizing Resolution

Diffuse or multiband IEF patterns. Diffuse or multiband protein IEF patterns can arise from molecular interactions and conformational changes as well as from inherent isoelectric microheterogeneity. Ampholytes can reversibly bind to proteins, proteins can undergo sequential, pH-dependent conformational changes, and proteins can interact with one another. These types of reactions can alter the pH profiles of proteins. On the other hand, many proteins are inherently heterogeneous, consisting of isoelectric isomers.

To distinguish between artifactual and inherent heterogeneity, it may be necessary to run an analytical IEF gel in the presence of all constituents (for example, detergents, urea, glycerol, etc.) that are to be used during focusing in the MicroRotofor cell.

Apparent pI Shifts. Proteins may associate with reagents and other sample components and these associations may alter the pI at which the protein focuses.

a. **SDS**. Protein samples that contain charged detergents like SDS may experience an apparent pI shift and migrate to the anode end of the focusing chamber as a result of an acquired net charge. Use only nonionic or zwitterionic detergents for this reason (see Table 6.2 for a list of detergents commonly used for liquid-based IEF separations).

b. **Phospholipids, heme groups, etc**. Some proteins are inherently associated with phospholipids, heme groups, etc. that affect their electrophoretic migration in a pH gradient. The effects of these charged groups must be neutralized. The nonionic detergent digitonin is effective at removing phospholipids from integral membrane proteins while providing a suitable hydrophobic environment for maintaining the stability of these proteins during focusing.

c. **Native conformations**. Proteins in their native state may focus at a pI that is different from the predicted pI. Always validate the fractionation conditions and selected ampholyte range on an analytical IEF gel before proceeding with fractionation on the MicroRotofor cell.

7.5 Problems with Applied Power

These are problems commonly related to the application of power.

- **Voltage fluctuations** are caused by air bubbles trapped between the sample and the ion exchange membranes. Remove air bubbles from the focusing chamber prior to fractionation by aspirating and reloading the sample.
- **Voltage decreasing at the beginning of a run**. This is normal and occurs as charged molecules migrate through the chamber, creating a high initial current. Eventually, desalting will cease and the pH gradient will form. As the run proceeds, the resistance of the focusing medium will increase and the voltage will begin to increase.

7.6 Harvesting Problems

Possible causes of uneven fraction volumes include the following:

- **Harvesting technique**. Problems with harvesting technique may generate fractions of unequal volume. Review the directions outlined in Section 3.7. Be sure to not cover any of the loading ports and take care to puncture the sealing tape uniformly.
- **Clogged or dirty harvesting needles**. Particulate matter adhering to the walls of the harvesting needles may cause blockages that lead to unequal and irreproducible harvesting results. Clean the harvesting needles carefully after each use (see Section 4.2).
- **Needles that are loose, kinked or unequal in height**. Occasionally, needles may become kinked or loose in the assembly and may even be of uneven height. This may cause some of the needles to not penetrate the sealing tape covering the harvesting ports. Carefully inspect the needle array and adjust the positions of the needles so that they are all at an even height. Replace the needle array, if necessary (catalog #170-2851).
- **Osmotic effects**. As proteins become focused, the osmotic pressure in each MicroRotofor compartment may vary. If the focusing chamber is not filled with sample, the fluid within the 10 channels may become unevenly distributed in response to the differences in osmotic pressure. This effect will vary as a function of protein load and concentration of solubilizing additives. The reproducibility of results will depend on the consistency of these factors. To alleviate this osmotic effect, the cell should be run with the focusing chamber filled to capacity.
- **Weak or excessive vacuum**. Use a vacuum of 22–27” of Hg.
- **Misaligned focusing chamber**. Make sure the focusing chamber is aligned properly in the harvesting station before puncturing the harvesting ports sealing tape (see Section 3.7).
- **Loading port sealing tape not removed before harvesting**. The loading port sealing tape must be removed prior to harvesting.
- **High-foaming detergent in sample buffer**. If a high-foaming detergent such as Triton is present, remove the harvesting tray immediately after harvesting to avoid having the sample spray out the harvesting tray. Alternatively, use a nonfoaming detergent, for example, CHAPS or ASB-14.
- **Vacuum chamber gasket is damaged, misaligned, or missing**. Inspect the vacuum chamber gasket and position properly before inserting the harvesting tray.

7.7 Mechanical Problems

The cell does not oscillate.

- Make sure that the focusing assembly is properly engaged at both the cathode and anode.
- Make sure that the power switch is in the ON position and the cell is connected to a power outlet.
- Make sure that the threaded sleeve of the electrode assembly is tightened. If the threaded sleeve is loose, the focusing assembly will remain stationary while the electrode component of the electrode assembly oscillates.

The unit leaks.

- **Electrolyte solution can leak** from the electrode assembly if:
  - The focusing assembly is not aligned properly.
  - The electrode assembly is improperly assembled.
  - The electrode assembly is overfilled. See Section 3.3 for the electrode assembly alignment and filling procedure.
- **Sample can leak** from the focusing chamber if:
  - The sealing tape does not cover all of the ports.
  - A piece of misaligned sealing tape is removed by the oscillating motion during a run (see Section 3.4).

Section 8
References

Bier M et al., in Peptides: Structure and Biological Functions, (Gross E and Meienhofer J, eds), Rockford, Pierce Chemical Co. (1979)

Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal Biochem 72, 248–254 (1976)

Lowry OH et al., Protein measurement with the Folin phenol reagent, J Biol Chem 193, 265–275 (1951)

Wang MZ et al., Analysis of human serum proteins by liquid phase isoelectric focusing and matrix–assisted laser desorption/ionization–mass spectrometry, Proteomics 3, 1661–1666 (2003)

Section 9
Legal Notices

Appendix A
Rotofor System Application Notes

The Rotofor and mini Rotofor cells have been manufactured and in use in laboratories around the world for over 20 years. The following is a list of Bio-Rad technical notes describing several applications for liquid-based IEF using these cells. Because the MicroRotofor cell is based on the Rotofor technology, the techniques described may also be applied to MicroRotofor fractionation. These tech notes may be ordered or downloaded in PDF format from the Bio-Rad website at discover.Bio-Rad.com

2-D Applications

2859 Combination of 2-D Gel and Liquid-Phase Electrophoretic Separations as Proteomic Tools in Neuroscience

1773 Preparative 2-D Electrophoresis System Purifies Recombinant Nuclear Proteins From Whole Bacterial Lysates

1776 A Rapid Method for the Purification of Analytical Grade Proteins From Plant Using Preparative SDS-PAGE and Preparative Isoelectric Focusing

2043 Purification of Proteins from *Mycobacterium tuberculosis* by Simultaneous Electro-Elution of the Mini Whole Gel Eluter

1744 Preparative 2-D Purifies Proteins for Sequencing or Antibody Production

1953 Preparative SDS Gel Electrophoresis of Hydrophobic Cell Wall Proteins from *Candida albicans*

RP0014 Isoelectric Focusing Nonporous RP HPLC: A Two-Dimensional Liquid Phase Separation Method for Mapping of Cellular Proteins With Identification Using MALDI-TOF Mass Spectrometry

RP0015 Identification of Protein Vaccine Candidates From *Helicobacter pylori* Using a Preparative Two-Dimensional Electrophoretic Procedure and Mass Spectrometry

Native Proteins

1508 Isolation of a Toxic Phospholipase D from *Corynebacterium pseudotuberculosis*

1520 Isolation of an *Escherichia coli* Heat Stable Enterotoxin (STb)-Alkaline Phosphatase Fusion Protein by Preparative Isoelectric Focusing

1899 Isolation of Multiple Lipoprotein (a) Charge Forms in Human Plasma by Liquid Phase Isoelectric Focusing

1521 Isolation of Recombinant HIV1 Protease Expressed in *E. coli* and *S. cerevisiae*

1516 Separation of Secreted Immunosuppressive Proteins of the Fish Pathogen, *Renibacterium salmoninarum*, From Culture Medium and Infected Fish Tissues

1519 Isolation and Purification of a Turkey Seminal Plasma Protease

1539 Separation of Aldose-Reductase Isoelectric Forms Using the Rotofor Cell

Detergents or Denarurants Used

1518 Purification of Bacterial Eukaryotic Fusion Proteins Using the Rotofor Cell

1517 Rotofor Fractionation of Intestinal Brush Border Membrane Proteins

1514 Isolation of a Membrane Bound Immunoregulatory Molecule From Metastatic Lymphoma Cells

1515 Preparation of Spinach Cold Acclimation Proteins for Gas Phase Sequencing, Oligonucleotide Derivation and Monoclonal Antibody Production

1475 Isolation of Monoclonal Antibodies to Phencyclidine From Ascite Fluid

Appendix B
Ordering Information

MicroRotofor Cell

Catalog #	Description
170-2800	**MicroRotofor (100/120V) Cell kit**, includes chassis and lid, harvesting trays (2), focusing chambers (2), cathode assembly, anode assembly, anode (5) and cathode (5) membranes, sealing tape, sealing film (10), assembly tool, cleaning brush, forceps, syringes (3 ml (1) and 10 ml (2))
170-2801	**MicroRotofor (220/240 V) Cell kit**, includes chassis and lid, harvesting trays (2), focusing chambers (2), cathode assembly, anode assembly, anode (5) and cathode (5) membranes, sealing tape, sealing film (10), assembly tool, cleaning brush, forceps, syringes (3 ml (1) and 10 ml (2))
170-2802	**MicroRotofor System (100/120 V)**, includes MicroRotofor cell kit and PowerPac HV power supply
170-2803	**MicroRotofor System (220/240)**, includes MicroRofotor cell kit and PowerPac HV power supply
170-2804	**MicroRotofor Starter Kit**
170-2810	**MicroRotofor Harvesting Tray**, 3
170-2820	**MicroRotofor Sealing Film**, 10 sheets
170-2960	**MicroRotofor Sealing Tape**, 1 roll
170-2821	**MicroRotofor Focusing Chamber**, 3
170-2822	**MicroRotofor Cathode Assembly**
170-2829	**MicroRotofor Anode Assembly**
170-2832	**MicroRotofor Assembly Tool**
170-2833	**MicroRotofor Ion Exchange Membranes**
170-2835	**MicroRotofor Cleaning Brush**
170-2836	**MicroRotofor Syringes**, 3 x 3 ml and 3 x 10 ml
170-2850	**MicroRotofor Harvesting Station**, includes alignment station, needle assembly, needle holder
170-2851	**MicroRotofor Needle Assembly**
170-2852	**MicroRotofor Vacuum Block O-Ring**
170-2855	**MicroRotofor Lid**
170-2826	**MicroRotofor Electrode assembly O-ring/Gasket kit** (includes electrode assembly O-ring and gaskets)

Power Supplies

165-5056	**PowerPac HV Power Supply**, 100–120/220–240 V, includes power cord, instructions
165-5070	**PowerPac Universal Power Supply**100–120/220–240 V, includes power cord, instructions

Sample Preparation Kits

163-2084	**ReadyPrep™ Protein Extraction Kit (Membrane II)**, 10 preps
163-2085	**ReadyPrep Protein Extraction Kit (Soluble/Insoluble)**, 20 preps
163-2086	**ReadyPrep Protein Extraction Kit (Total Protein)**, 20 preps
163-2087	**ReadyPrep Protein Extraction Kit (Signal)**, 50 preps
163-2088	**ReadyPrep Protein Extraction Kit (Membrane I)**, 50 preps
163-2089	**ReadyPrep Protein Extraction Kit (Cytoplasmic/Nuclear)**, 50 preps
163-2090	**ReadyPrep Reduction-Alkylation Kit**, 100 preps
163-2100	**ReadyPrep Sequential Extraction Kit**, 5–15 preps, includes 1 vial reagent 1, 3 vials reagent 2, 2 vials reagent 3, 1 vial tributylphosphine (TBP), storage vial for TBP, instructions

Catalog #	Description
163-2130	**ReadyPrep 2-D Cleanup Kit**, sufficient to process 50 protein samples, 1–100 µl each, instructions
732-6701	**Aurum Serum Protein Mini Kit**, 10 purification columns, 10 clear 12 x 75 mm polystyrene tubes, 30 sample collection tubes, 10 column tips, 50 ml binding buffer, protocol overview, instructions
732-6713	**Aurum Serum Protein Mini Kit**, 2 preps, includes 2 serum protein columns, 2 clear 12 x 75 mm polystyrene tubes, 6 sample collection tubes, 10 column tips, 15 ml binding buffer, protocol overview, instructions
500-0121	**RC DC Protein Assay Kit I**, includes RC reagents package, DC protein assay reagents package, bovine gamma-globulin standard; 500 standard assays
500-0122	***RC DC*™ Protein Assay Kit II**, includes RC reagents package, DC protein assay reagents package, bovine serum albumin standard; 500 standard assays

Solubilizing Agents

161-0370	**Urea**, 250 g
161-0731	**Urea**, 1 kg
161-0460	**CHAPS**, 1 g
161-0465	**CHAPS0**, 1 g
161-0717	**Glycine**, 250 g
161-0718	**Glycine**, 1 kg

Ampholytes

163-1112	**Bio-Lyte 3/10 Ampholyte**, 40%, 10 ml
163-1113	**Bio-Lyte 3/10 Ampholyte**, 40%, 25 ml
163-1132	**Bio-Lyte 3/5 Ampholyte**, 20%, 10 ml
163-1142	**Bio-Lyte 4/6 Ampholyte**, 40%, 10 ml
163-1143	**Bio-Lyte 4/6 Ampholyte**, 40%, 25 ml
163-1152	**Bio-Lyte 5/7 Ampholyte**, 40%, 10 ml
163-1153	**Bio-Lyte 5/7 Ampholyte**, 40%, 25 ml
163-1162	**Bio-Lyte 6/8 Ampholyte**, 40%, 10 ml
163-1163	**Bio-Lyte 6/8 Ampholyte**, 40%, 25 ml
163-1172	**Bio-Lyte 7/9 Ampholyte**, 40%, 10 ml
163-1182	**Bio-Lyte 8/10 Ampholyte**, 20%, 10 ml
163-1192	**Bio-Lyte 5/8 Ampholyte**, 40%, 10 ml
163-1193	**Bio-Lyte 5/8 Ampholyte**, 40%, 25 ml

Additional Preparative Electrophoresis Instruments

170-2926	**Model 491 Prep Cell,** 100/120 V, includes buffer recirculationpump and reagent starter kit with protein standard
170-2927	**Model 491 Prep Cell**, 220/240 V
170-2908	**Mini Prep Cell Without Reagent Starter Kit**
170-2915	**Mini Prep With Reagent Starter Kit**
170-2926	**Model 491 Prep Cell**, 100/120 V, includes buffer recirculation pump, reagent starter kit with protein standard
170-2927	**Model 491 Prep Cell**, 220/240 V, includes buffer recirculation pump and reagent starter kit

Catalog #	Description

Rotofor Preparative Cell

170-2906	**Rotofor Purification System With Power Supply**, 220/240 V
170-2914	**Rotofor Purfication System With Power Supply**, 100/120 V, includes 60 ml focusing chamber, 18 ml focusing chamber, PowerPac 3000 power supply, starter kit
170-2950	**Standard Rotofor Cell**, 100/120 V, includes 60 ml focusing chamber and starter kit
170-2951	**Standard Rotofor Cell**, 220/240 V
170-2986	**Rotofor Purification System**, 100/120 V, includes 60 ml focusing chamber, 18 ml focusing chamber, and starter kit
170-2987	**Rotofor Purification System**, 220/240 V
170-2988	**Mini Rotofor Cell**, 100/120 V, includes 18 ml focusing chamber and starter kit
170-2989	**Mini Rotofor Cell**, 220/240 V

First-Dimension IEF Instrumentation and Supplies

PROTEAN® IEF System

165-4000	**PROTEAN IEF System**, complete, includes basic unit, 17, 11, and 7 cm focusing trays with lids, 1 pack each of 17, 11, and 7 cm rehydration/equilibration trays with lids, 2 pairs of forceps, pack of electrode wicks, mineral oil, cleaning brushes
165-4001	**PROTEAN IEF Cell**, 90-240 VAC, basic unit, includes cell, instructions

Cup Loading Trays

165-4050	**Cup Loading Tray**, includes 1 tray base, 1 pair movable electrodes, 1 pack each of large and small replacement cups

ReadyStrip™ IPG Strips, 7 cm

163-2000	**ReadyStrip IPG Strips**, pH 3-10, 7 cm, 12
163-2001	**ReadyStrip IPG Strips**, pH 4-7, 7 cm, 12
163-2002	**ReadyStrip IPG Strips**, pH 3-10 nonlinear, 7 cm, 12
163-2003	**ReadyStrip IPG Strips**, pH 3-6, 7 cm, 12
163-2004	**ReadyStrip IPG Strips**, pH 5-8, 7 cm, 12
163-2005	**ReadyStrip IPG Strips**, pH 7-10, 7 cm, 12
163-2028	**ReadyStrip IPG Strips**, pH 3.9-5.1, 7 cm, 12
163-2029	**ReadyStrip IPG Strips**, pH 4.7-5.9, 7 cm, 12
163-2030	**ReadyStrip IPG Strips**, pH 5.5-6.7, 7 cm, 12
163-2031	**ReadyStrip IPG Strips**, pH 6.3-8.3, 7 cm, 12

ReadyStrip IPG Strips, 11 cm

163-2014	**ReadyStrip IPG Strips**, pH 3-10, 11 cm, 12
163-2015	**ReadyStrip IPG Strips**, pH 4-7, 11 cm, 12
163-2016	**ReadyStrip IPG Strips**, pH 3-10 nonlinear, 11 cm, 12
163-2017	**ReadyStrip IPG Strips**, pH 3-6, 11 cm, 12
163-2018	**ReadyStrip IPG Strips**, pH 5-8, 11 cm, 12
163-2019	**ReadyStrip IPG Strips**, pH 7-10, 11 cm, 12
163-2024	**ReadyStrip IPG Strips**, pH 3.9-5.1, 11 cm, 12
163-2025	**ReadyStrip IPG Strips**, pH 4.7-5.9, 11 cm, 12

Catalog #	Description
163-2026	**ReadyStrip IPG Strips**, pH 5.5-6.7, 11 cm, 12
163-2027	**ReadyStrip IPG Strips**, pH 6.3-8.3, 11 cm, 12

Second-Dimension Instrumentation and Supplies

Mini-PROTEAN® 3 Cell

165-3301	**Mini-PROTEAN 3 Electrophoresis Cell**, 10-well, 0.75 mm thickness; complete system includes 2 combs, 5 sets of glass plates, casting stand, casting clamp assembly, sample loading guide, electrophoresis module (electrode assembly, clamping frame, tank, lid with power cables, mini cell buffer dam)
165-4100	**Mini-PROTEAN 3 Dodeca Cell**, includes tank with built-in cooling coil, lid with power cables, 6 electrophoresis clamping frames, 2 buffer dams, drain line, 2 gel releasers

Ready Gel® Precast Gels

161-1390	**Ready Gel Tris-HCl Gel**, 10% resolving gel, 4% stacking gel, 7 cm IPG well, 8.6 x 6.8 cm (W x L)
161-1391	**Ready Gel Tris-HCl Gel**, 12% resolving gel, 4% stacking gel, 7 cm IPG well, 8.6 x 6.8 cm (W x L)
161-1393	**Ready Gel Tris-HCl Gel**, 4-20% resolving gel, 4% stacking gel, 7 cm IPG well, 8.6 x 6.8 cm (W x L)
161-1392	**Ready Gel Tris-HCl Gel**, 4-15%, 7 cm IPG well, 8.6 x 6.8 cm (W x L)
161-1394	**Ready Gel Tris-HCl Gel**, 8-16% resolving gel, 4% stacking gel, 7 cm IPG well, 8.6 x 6.8 cm (W x L)
161-1395	**Ready Gel Tris-HCl Gel**, 10-20% resolving gel, 4% stacking gel, 7 cm IPG well, 8.6 x 6.8 cm (W x L)

Criterion™ System

165-6001	**Criterion Cell**, includes electrophoresis buffer tank, lid with power cables, 3 sample loading guides (12 2 well, 18-well, 26-well), instructions
165-4130	**Criterion™ Dodeca Cell**, includes electrophoresis buffer tank with built-in cooling coil, lid with power cables, instructions

Criterion Precast Gels

345-0013	**Criterion Tris-HCl Gel**, 10%, IPG well, 11 cm IPG strip, 13.3 x 8.7 cm (W x L)
345-0101	**Criterion Tris-HCl Gel**, 10%, IPG+1 well, 11 cm IPG strip, 13.3 x 8.7 cm (W x L)
345-0018	**Criterion Tris-HCl Gel**, 12.5%, IPG well, 11 cm IPG strip, 13.3 x 8.7 cm (W x L)
345-0102	**Criterion Tris-HCl Gel**, 12.5%, IPG1 well, 11 cm IPG strip, 13.3 x 8.7 cm (W x L)
345-0031	**Criterion Tris-HCl Gel**, 4–15%, IPG well, 11 cm IPG strip, 13.3 x 8.7 cm (W x L)
345-0103	**Criterion Tris-HCl Gel**, 4–15%, IPG+1 well, 11 cm IPG strip, 13.3 x 8.7 cm (W x L)
345-0036	**Criterion Tris-HCl Gel**, 4–20%, IPG well, 11 cm IPG strip, 13.3 x 8.7 cm (W x L)
345-0104	**Criterion Tris-HCl Gel**, 4–20%, IPG+1 well, 11 cm IPG strip, 13.3 x 8.7 cm (W x L)
345-0041	**Criterion Tris-HCl Gel**, 8–16%, IPG well, 11 cm IPG strip, 13.3 x 8.7 cm (W x L)
345-0105	**Criterion Tris-HCl Gel**, 8-–16%, IPG+1 well, 11 cm IPG strip, 13.3 x 8.7 cm (W x L)
345-0046	**Criterion Tris-HCl Gel,** 10–20%, IPG well, 11 cm IPG strip, 13.3 x 8.7 cm (W x L)
345-0107	**Criterion Tris-HCl Gel**, 10–20%, IPG+1 well, 11 cm IPG strip, 13.3 x 8.7 cm (W x L)
345-9953	**Criterion Tris-HCl Gel**, 10.5–14%, IPG well, 11 cm IPG strip, 13.3 x 8.7 cm (W x L)
345-0106	**Criterion Tris-HCl Gel**, 10.5-–14%, IPG+1 well, 11 cm IPG strip, 13.3 x 8.7 cm (W x L)
345-0115	**Criterion XT Bis-Tris Gel**, 10%, IPG+1 well, 11 cm IPG strip, 13.3 x 8.7 cm (W x L)
345-0116	**Criterion XT Bis-Tris Gel**, 10%, IPG well, 11 cm IPG strip, 13.3 x 8.7 cm (W x L)
345-0121	**Criterion XT Bis-Tris Gel**, 12%, IPG+1 well, 11 cm IPG strip, 13.3 x 8.7 cm (W x L)
345-0122	**Criterion XT Bis-Tris Gel**, 12%, IPG well, 11 cm IPG strip, 13.3 x 8.7 cm (W x L)

Catalog #	Description
345-0127	**Criterion XT Bis-Tris Gel**, 4–12%, IPG+1 well, 11 cm IPG strip, 13.3 x 8.7 cm (W x L)
345-0128	**Criterion XT Bis-Tris Gel**, 4–12%, IPG well, 11 cm IPG strip, 13.3 x 8.7 cm (W x L)
345-0133	**Criterion XT Tris-Acetate Gels**, 3–8%, IPG+1 well, 11cm IPG strip, 13.3 x 8.7 cm (W x L)
345-0134	**Criterion XT Tris-Acetate Gels**, 3–8%, IPG well, 11 cm IPG strip, 13.3 x 8.7 cm (W x L)
345-0139	**Criterion XT Tris-Acetate Gels**, 7%, IPG+1 well, 11 cm IPG strip, 13.3 x 8.7 cm (W x L)
345-0140	**Criterion XT Tris-Acetate Gels**, 7%, IPG well, 11 cm IPG strip, 13.3 x 8.7 cm (W x L)

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