



Rotofor[®] System Starter Kit Manual

**Catalog Number
170-2910**

BIO-RAD

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Section 1

Introduction

This kit was designed to familiarize you with your new Rotofor cell. The steps outlined on the following pages will guide you through assembly of the Rotofor cell and allow you to complete a fractionation run before running your own sample.

The unique Rotofor system fractionates complex protein samples in free solution using preparative isoelectric focusing. The Rotofor system is used for the initial clean up of crude samples and in purification schemes for the elimination of specific contaminants from proteins of interest that might be difficult to remove by other means.

The Rotofor cell provides up to 500-fold purification for a particular molecule in less than 4 hours. Because isoelectric focusing is carried out in free solution, fractions from an initial run can be collected, pooled, and refractionated, resulting in 1,000-fold enrichment for a particular molecule. Purification using isoelectric focusing is especially advantageous when protein activity must be maintained. Bioactivity is maintained because the proteins remain in solution in their native conformation.

The main component of the Rotofor cell is the cylindrical focusing chamber with an internal ceramic cooling finger. The membrane core, with nineteen parallel, monofilament polyester membranes divides the focusing chambers into 20 compartments, each holding one fraction. Rotation of the chamber at 1 rpm stabilizes against convective and gravitational disturbances. After focusing, the solution in each compartment is rapidly collected without mixing using the harvesting apparatus supplied with the unit.

The use of interchangeable focusing chambers allows the Rotofor system to accommodate a range of sample volumes. The Mini Rotofor chamber is used for sample volumes of 18 milliliters containing micrograms to milligrams of total protein. The Standard Rotofor chamber is used for samples of 35 to 60 milliliters containing milligrams to grams of total protein.

*Patent No. 4,588,492

Section 2

Starter Kit Check List

This kit contains:

1. **Bio-Lyte® Ampholytes**

10 ml, pH range 3/10

2. **Protein Sample (in 1 ml vial)–Catalog Number 170-2919**

Phycocyanin, 2 mg Color: Blue (pI range of visible subunits is 4.5-5.5)

Hemoglobin, 2 mg Color: Red (pI range of visible subunits 6.0-7.5)

Cytochrome c, 2 mg Color: Orange (pI range of visible subunits is 8.0-9.0)

3. **Ion Exchange Membranes**

Anion Exchange Membrane, 1, in cathode electrolyte solution 0.1 M NaOH,
33 ml

Cation Exchange Membrane, 1, in anode electrolyte solution 0.1 M H₃PO₄,
33 ml

4. **Vent Buttons, 2**

5. **Sample Loading Syringe and Needle, 50 ml**

Required Equipment and Reagents:

Rotofor cell

3,000 volt power supply

Water recirculation chiller

Vacuum source

Approximately 60 ml dH₂O

Beaker, 100 ml

Pipettes 1 ml, for preparing the sample

Section 3

Setting Up for a Run

Assemble the electrodes first. Each electrode assembly consists of an inner and outer component, two grey silicone rubber gaskets, one ion exchange membrane, two small O-rings, one large O-ring, and a vent button. To seal the electrolyte within the chamber, one small O-ring is placed around the central shaft of each component. To seal the sample within the focusing chamber, one large O-ring is placed on the side of the inner component facing the sample.

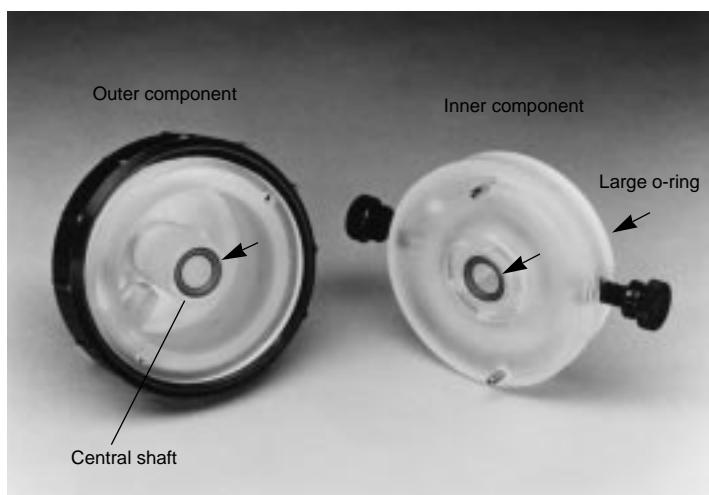


Fig. 1. Outer (left) and inner components of the electrode assemblies. Arrows indicate the O-rings. Electrolyte solutions should just cover the central shaft when filled.

Note that alignment pins prevent misassembly of the two chambers. The four grey rubber gaskets can be used in either electrode assembly.

Ion exchange membranes are used in the electrode assemblies to separate the electrolytes from the sample while allowing current to pass through and set up the pH gradient. There are two types of ion exchange membranes. The anion exchange membranes are notched to fit only the cathode electrode assembly (black button) and the cation exchange membranes will fit only the anode electrode assembly (red button). For your convenience, the membranes are pre-equilibrated in anode and cathode electrolyte solutions.

Anion Exchange Membranes are equilibrated in 0.1 M NaOH. Use this solution in the cathode chamber.

Cation Exchange Membranes are equilibrated in 0.1 M H_3PO_4 . Use this solution in the anode chamber.

Step 1: Assemble the Anode and Cathode Electrode Chambers

1. Place one grey gasket over the two alignment pins of the inner component of the anode electrode. The three oblong holes in the gaskets should not obstruct the six holes of the electrolyte chamber.
2. Remove the cation exchange membrane equilibrated in the acidic electrolyte solution. **DO NOT DISCARD THE BUFFER.** Rinse the membrane with water to remove residual acid.
3. Place the cation exchange membrane on the grey gasket by aligning the notches in the membrane with the pins of the inner electrode compartment. Cover the membrane with another grey gasket. Make sure that there is a small O-ring inset in the central shaft of the outer portion of the electrode assembly and fasten the halves of the electrode assembly together by finger tightening the captive, threaded sleeve.

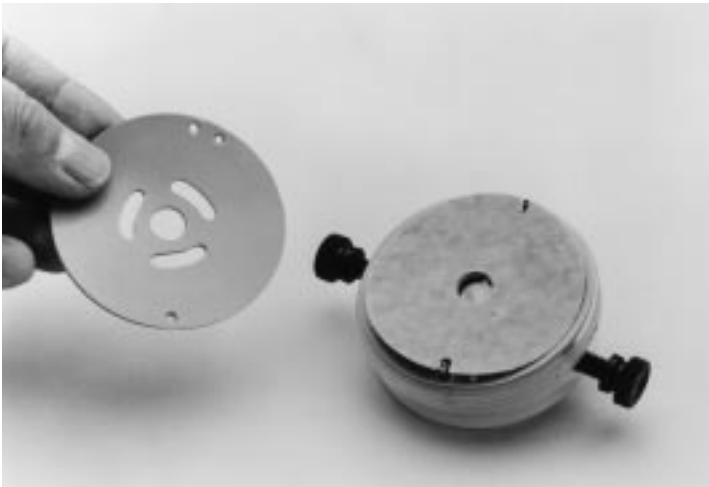


Fig. 2. The “Ion exchange membrane and silicone gasket sandwich” assembled on the inner component of electrode assembly.

4. Repeat the assembly process for the cathode electrode.
5. Fill each electrode chamber with electrolyte immediately after assembly to prevent the membranes from drying. Add 33 ml of the appropriate electrolyte to each chamber and replace the vent buttons. The acidic electrolyte goes into the anode electrode (red dot) and basic electrolyte goes into the cathode electrode (black dot).

Step 2: Assemble the Focusing Chamber

1. Slide the assembled anode electrode assembly over the ceramic cooling finger so that the two protruding screw heads fit into the holes in the black plastic base of the cooling finger support assembly.

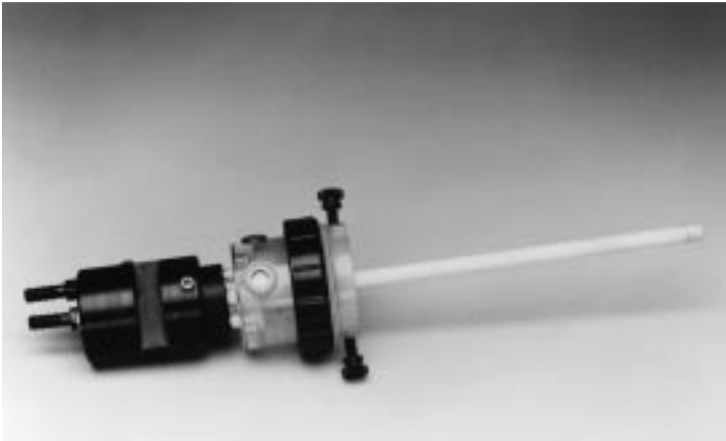


Fig. 3. Anode electrode assembled on the cooling finger.

2. Slide the membrane core onto the ceramic cooling finger, making sure the core abuts the acrylic ridge on the anode chamber.
3. Slide the focusing chamber over the membrane core, inserting the metal pin into the small hole in the anode chamber. Remove one of the black port covers to visually check the position the focusing chamber. Each membrane screen should lie between two adjacent ports. These ports must not be blocked by the membrane screens. If the ports are blocked, remove the focusing chamber, and slide it once more over the membrane core. Tighten the black, nylon retaining screws. Check again to make sure the membrane screens do not block the ports of the chamber.



Fig. 4. Slide the focusing chamber over the membrane core.

4. Slide the assembled cathode compartment over the cooling finger, aligning the metal pin and hole in the cathode chamber, and tighten the nylon retaining screws.

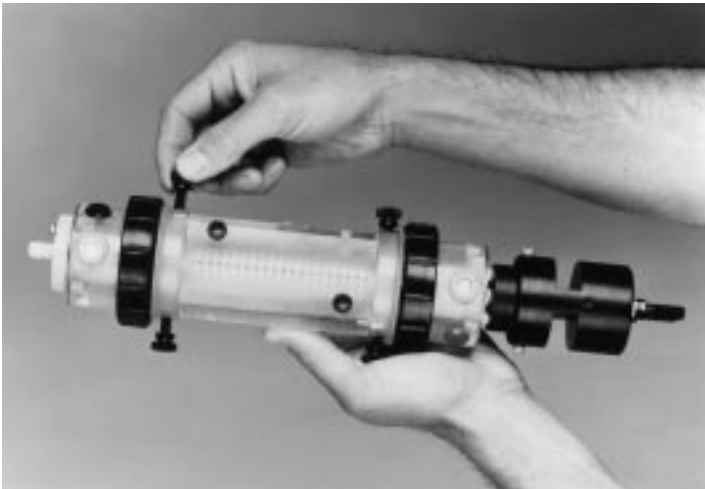


Fig. 5. Assembled focusing chamber.

5. Mount the assembled focusing chamber in the stand. The gear on the cathode electrode assembly should be fully engaged with the gear on the base unit. If the focusing chamber does not slide in easily, remove it to check that all parts are properly assembled.
6. Attach the power cord to the back of the unit and connect it to an electrical outlet.

Step 3: Prepare the Focusing Chamber

1. With the cell mounted on the stand, rotate the focusing chamber so the collection ports, identified by the two metal alignment pins, are facing up.
2. Cover the ports with a piece of the sealing tape (10.5 cm) provided with the cell.
3. Reinforce the taped ports with one of the two black acrylic cell-cover blocks, and finger tighten the screws.

Step 4: Load the Sample

1. Turn the toggle switches to ON and HARVEST. In the harvest mode the focusing chamber will automatically stop with the filling ports facing up and the collection ports facing down.
2. Prepare the sample:
 - A. For the standard focusing chamber combine the following in a beaker:
 - 58 ml dH₂O
 - 1 ml sample
 - 3 ml Bio-Lyte ampholytes (pH range 3/10)
 - B. For the mini focusing chamber combine the following in a beaker:
 - 18.5 ml dH₂O
 - 0.5 ml sample
 - 1.0 ml Bio-Lyte ampholytes (pH range 3/10)
3. Fill the cell with sample through the loading ports using the syringe provided in this kit. Typically, every other port is filled, and the sample spreads into the adjoining compartments.

For the standard focusing chamber, the minimum sample volume must be sufficient to cover the cooling finger (33 ml minimum; 58 ml maximum). For the mini focusing chamber always load the maximum sample volume of 18 ml.



Fig. 6. Loading the sample.

Step 5: Seal the Loading Ports

- A. Mini Rotofor chamber: Place the grey silicone gasket in the slot covering the loading ports then seal the ports with the second cell cover block (tape is unnecessary), and the Rotofor cell is ready for operation.
- B. Standard Rotofor chamber: Seal the loading ports with the second cell cover block (tape is unnecessary), and the Rotofor cell is ready for operation.

Step 6: Remove Air Bubbles

1. During filling of the sample, air bubbles can become trapped in the 6 ports between the sample chamber and the ion exchange membranes. If the bubbles are not removed, they will produce fluctuations in the voltage due to the discontinuity they create in the electrical field. Some power supplies, such as Bio-Rad's PowerPac 3000, have safety sensors that may trip and turn off the voltage in response to the resistance change that occurs when a bubble rotates into the electrical circuit. Thus, bubbles must be eliminated prior to commencing electrophoresis. To remove air bubbles, lift the assembled, loaded cell from the stand and turn it vertically. With the palm of your hand, tap the electrode chamber to dislodge the bubbles. Then turn the cell 180° and tap the other chamber. When all the bubbles are eliminated from the electrode ports, return the cell to the stand and start the fractionation.

Step 7: Starting the Fractionation Run

1. Excessive heating may denature proteins and damage the Rotofor cell. Connect the ports of the cooling finger to a source of recirculating coolant and begin coolant flow. The ports are interchangeable, so either one may be connected to the coolant inlet. It is usually sufficient to set the chiller at 4 °C. For more critical temperature control, the chiller can be adjusted accordingly.

When focusing power is applied to the jacks without the cover in place, several high voltage elements become exposed. To avoid personal injury due to accidental contact with these elements, always operate the cell with the cover in place.

2. Place the lid on the cell. Attach the high voltage leads to the power supply, and the Rotofor cell is ready for use. To begin rotation, flip the toggle switches to On and Run.
3. Set power supply conditions:

When using the Standard Rotofor chamber, set the supply to 15 W constant power and begin the run.

When using the Mini Rotofor chamber, set the supply to 10 W constant power and begin the run.

4. The progress of a run is easily monitored by observing the voltage increase over time. The run is complete when the voltage stabilizes. Once the voltage stabilizes, allow the run to continue for 30 minutes before harvesting fractions. The run time for either the mini chamber or the standard chamber is approximately 2 hours.

Step 8: Fraction Collection

1. Load the test tube rack with twenty 12 x 75 mm culture tubes and place it inside the harvest box. Place the lid on the box, making certain that each stainless steel collecting tube is inside a test tube. Connect a vacuum source to the vacuum port on the box. A vacuum pump or house vacuum of 10-50 mm Hg is recommended.
2. When focusing is completed, move the black toggle switch to the harvest position. This stops the cell rotation with the cell properly aligned for sample collection, i.e., with the alignment pins and taped collection ports on the bottom of the focusing chamber.
3. Turn the power supply off, disconnect the power supply, remove the cover, and move the Rotofor cell and the harvesting box next to one another. Remove both the upper and lower focusing chamber cell cover blocks.
4. Apply a vacuum to the collection box.

5. Mount the needle array on the two alignment pins on the bottom of the chamber. Grasp the needle array with the fingers of both hands while placing the thumbs on the top of the focusing chamber. Take care not to block any of the uppermost ports. Quickly push the needles firmly and uniformly all the way through the sealing tape into the chamber. This will cause all 20 fractions to be simultaneously aspirated from the cell and delivered to the collection tubes.

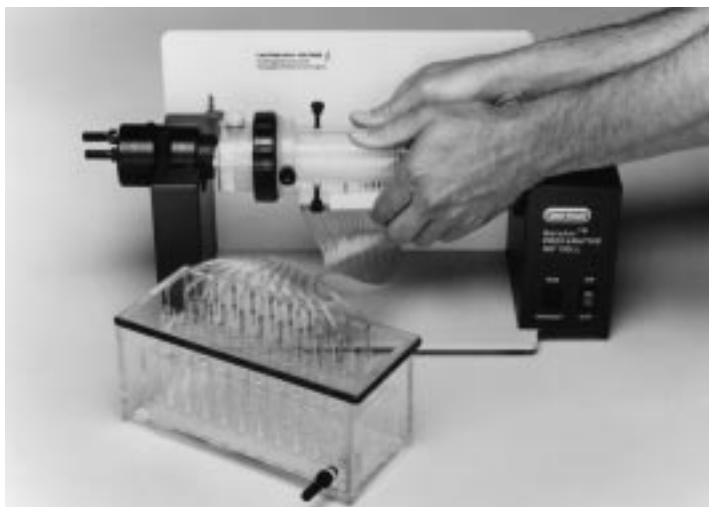


Fig. 7. Harvesting samples after focusing is complete. Make sure thumbs do not cover the uppermost ports.

6. Turn off the vacuum source and remove the test tube rack. Note that all the odd numbered fractions are in one row and the even numbered fractions are in the other row of the rack.

Step 9: Expected Results

1. The sample separated was a mixture of three naturally colored proteins in distilled water containing 2.0% pH 3-10 Bio-Lyte ampholytes.
 - A. Phycocyanin consists of three blue protein subunits of pI 4.5, 4.7, and 5.0. These should focus nearest the anode in 1-2 fractions.
 - B. Hemoglobin A and Hemoglobin C are two red colored proteins of pI 7.1 and 7.4, respectively. These should focus near the middle of the focusing chamber in 2-3 fractions.
 - C. Cytochrome c is an orange protein with a pI of 9.6. This protein should focus near the cathode in 1 to 2 fractions.

2. After about 1 hour, the proteins began to concentrate near their respective isoelectric points during initial formation of the pH gradient.
3. After the voltage stabilized, the proteins focused at their isoelectric points. The total run took approximately 2 hours.
4. Following harvesting, test tube fractions may be measured for pH to confirm the linearity of the pH gradient.

Step 10: Disassembly and Cleaning

1. Rinse the needle array and its associated tubing with water as soon as possible after use.
2. Take the focusing chamber from the stand. Loosen the nylon screws and remove the cathode chamber.
3. Leave the cathode and anode chambers intact. The ion exchange membranes must be stored wet. Remove the electrolyte and fill the electrode chambers with distilled water. Before starting a new run, the electrolytes must be replaced with fresh solutions.
4. Loosen the nylon screws on the anode chamber and remove the focusing chamber and membrane core. Rinse all chamber components with water and air dry.

Section 4 Product Information

Catalog Number	Product Description
170-2919	Protein Sample for Rotofor System Starter Kit , 1 ml

Bio-Lyte Ampholytes

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

Catalog Number	Product Description
163-1153	Bio-Lyte 5/7 Ampholyte , 40%, 25 ml
163-1193	Bio-Lyte 5/8 Ampholyte , 40%, 25 ml
163-1163	Bio-Lyte 6/8 Ampholyte , 40%, 25 ml

Replacement Accessories

170-2956	Ion Exchange Membranes , 5 pair
170-2957	Vent Buttons , 8
170-2991	Mini Membrane Cores , 2
170-2952	Membrane Cores , 2



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**U.S. (800) 4BIORAD California (510) 741-1000 Australia 02-9914-2800 Austria
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