MicroRotofor™ Cell Starter Kit Manual

Catalog Number 170-2804

Table of Contents

Section 1
Introduction

This kit was designed to familiarize you with the MicroRotofor™ Cell before running your own sample. The setup and operation guides you through the assembly and a complete fractionation and harvesting of a mixture of three naturally colored proteins.

Note: For a detailed description of the MicroRotofor™ components, the setup, and analysis of the results, please refer to the MicroRotofor™ instruction manual.

Section 2
Starter Kit Components

This kit contains:

- Bio-Lyte® Ampholytes. 10 ml, pH range 3-10 – Catalog #163-1112
- Protein Sample, 1 ml – Catalog Number 170-2919

  Phycocyanin, 2 mg: Blue protein, subunits pI range is 4.5–5.5

  Hemoglobin, 2 mg: Red protein, subunits pI range is 6.0–7.5

  Cytochrome c, 2 mg: Orange protein, subunits pI range is 8.0–9.0
- Focusing Chamber (1)
- Anode Membranes (Cation Exchange Membranes): 2 membranes equilibrated in 15 ml electrolyte solution 0.1 M H_3PO_4
- Cathode Membranes (Anion Exchange Membranes): 2 membranes equilibrated in 15 ml electrolyte solution 0.1 M NaOH
- Harvesting Tray (1)
- Sample loading syringe, 3 ml (1)
- Electrolyte loading syringe, 10 ml (2)

Section 3
Required Equipment and Reagents

- MicroRotofor™ Cell with Instruction manual
- Power supply capable of 1 Watt constant power control or multistep constant Voltage control, e.g. the PowerPac™ HV Power Supply (Catalog N#164-5056)

- Vacuum source and vacuum trap. (Vacuum in 22–28 mm Hg range)
- Deionized water
- Pipettes (100 µl – 2.75 ml volumes)
- Beaker or equivalent to hold the 3 ml sample volume.

Section 4
Setup and Operation

Note: For a detailed description of the MicroRotofor™ components, the setup, and analysis of the results, please refer to the MicroRotofor™ instruction manual.

4.1 Overview

- Prepare the Focusing assembly: Electrode chambers and focusing chamber with electrode membranes.
- Prepare and load the starter kit protein sample and seal the loading ports.
- Add electrolyte solutions to the electrode chambers.
- Position the focusing assembly in the chassis and start the IEF run.
- Stop the run, remove the lid, and connect the system to a vacuum source.
- Remove the loading port sealing tape and position the focusing chamber into the harvesting station.
- Aspirate the fractions into the harvesting tray.

4.2 Prepare the Focusing Assembly

The focusing assembly consists of the electrode assemblies, the electrode membranes and the focusing chamber.

Fig. 1. MicroRotofor components and accessories. MicroRotofor chassis and lid (1), harvesting tray (2), focusing chamber (3), anode assembly (4a), cathode 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).

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

Fig. 2. Inserting an anion exchange membrane into the focusing chamber.

3. Attach the electrode assemblies to the focusing chamber.
    a. The anode assembly (red) is attached to the focusing chamber end containing the anode membrane (red casing).

b. The cathode assembly (black) is attached to the focusing chamber end containing the cathode membrane (black casing).

4. Align one row of focusing chamber ports with the vents on the electrode assemblies. These are the sample loading ports. (Figure 3)

Fig. 3. Alignment of the sample loading ports with the vents on the anode (left) and cathode (right) assemblies.

4.3 Prepare and Load the Protein Sample

Prepare the protein sample

The protein sample included in the starter kit contains three naturally colored proteins in deionized water, Phycocyanin (pI range 4.5–5.5), Hemoglobin (pI range 6.0–7.5), and Cytochrome c (pI range 8.0–9.0). Each protein is present at a concentration of 2 mg/ml for a total protein concentration of 6 mg/ml.

Note: Vortex sample vial to homogenize material.

- To 100 µl of protein sample (600 µg total protein), add 150 µl Bio-lytes pH 3-10, and 2.75 ml deionized water for a total volume of 3.0 ml.

Load the Protein 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 (Figure 4). Position the tape across the template covering all three cutting grooves. Cut the tape with a cutting blade at all three grooves. Each strip of tape can be picked up through the cutouts between two grooves.

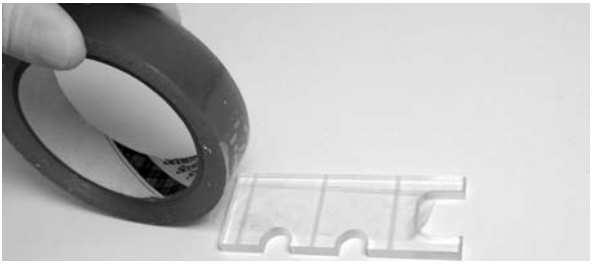

Fig. 4. Using the assembly tool to measure the appropriate length of sealing tape.

2. Seal the lower row of ports (harvesting ports) with a strip of tape. Make sure to cover all of the ports, and to not extend the tape beyond the focusing chamber (Figure 5).

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

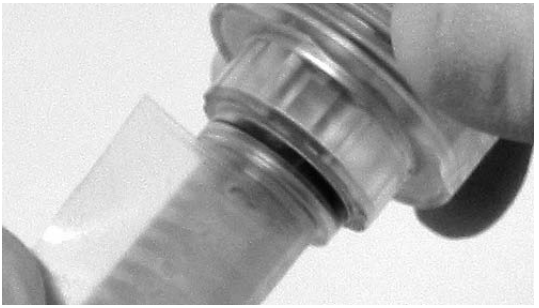

Fig. 5. Applying sealing tape to cover the harvesting ports on the focusing chamber.

3. Fill the 3 ml syringe with sample. Slowly load the sample through the centermost loading port of the focusing chamber (Figure 6). As this channel fills, the sample will slowly spread to and fill all of the adjacent channels. Continue adding sample slowly to this channel until all of the channels are filled. Alternatively, fill every other channel and wait for the sample to spread to the adjacent chambers.

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

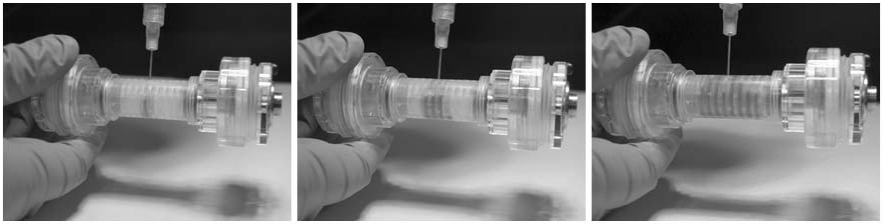

Fig. 6. Loading sample into the focusing chamber.

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

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

4.4 Perform the Focusing Run

1. Open the cooling block cover by unscrewing the block screw.
2. With the sealed loading ports and vents on the electrode assemblies facing up, place the focusing assembly into the focusing station of the chassis. Make sure the anode end (red) is to the left and the cathode end (black) is to the right.
    I. Gently push the anode end (red) of the focusing assembly into the anode connection on the chassis until it is completely retracted (Figure 7).
    II. Lower the focusing assembly into the cooling block and slide the cathode end of the assembly into the notch on the cathode end of the chassis (Figure 8). If necessary, rotate the focusing assembly until the slots on the cathode assembly align with the notch on the chassis. Alternatively, turn power on to the oscillating motor and wait until the notch is in a better position to connect with the focusing assembly.

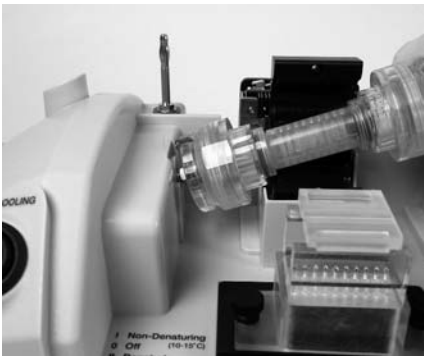

Fig. 7. Placing the focusing assembly onto the anode of the chassis.

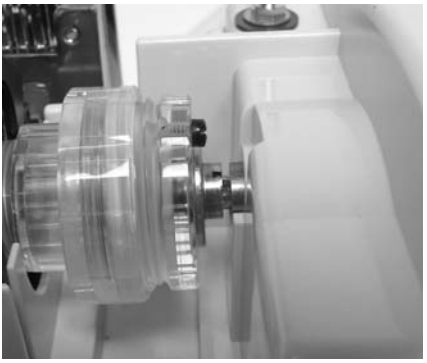

Fig. 8. Fitting the cathode assembly into the cathode of the chassis.

3. Using a 10 ml syringe, add electrolyte solutions to the electrode assemblies (Figure 9).

(**Note**: The electrode membrane storage solution from the starter kit can be used. Alternatively fresh electrolyte solutions can be prepared)

I. Add 6 ml 0.1 M H_3PO_4 through the vent hole of the anode assembly (red).

II. Add 6 ml 0.1 M NaOH through the vent hole of the cathode assembly (black).

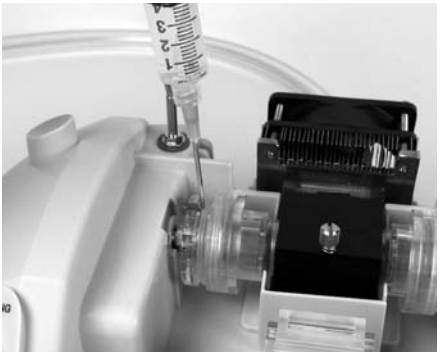

Fig. 9. Adding electrolyte to the anode assembly.

4. Close the cooling block cover and tighten the screw.
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 II (20°C).

   (**Note**: See Table 3.2 in the MicroRotofor instruction manual, for detailed cooling setting information.)
10. 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 stepwise constant voltage mode with limiting current (see Section 4.5 for detailed power conditions.)
11. A typical run is completed in less than 3 hours. To monitor the focusing progress, observe the voltage increase over time. The run is complete when the voltage stabilizes. At that point, allow the run to continue for 30 minutes before harvesting. Longer run times do not improve focusing and may result in a collapse of the pH gradient.
12. The progress of the run can also be monitored by following the migration of the colored proteins in the sample.

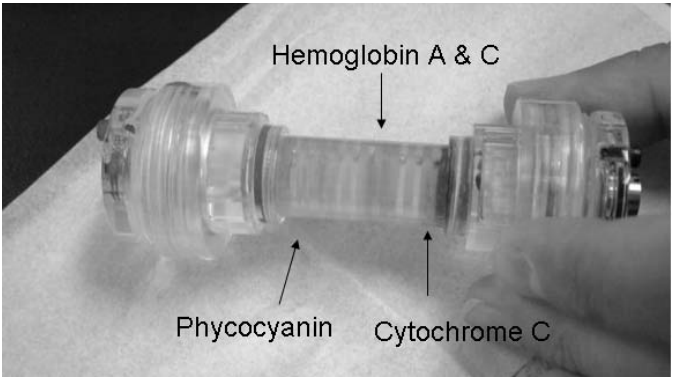

Fig. 10. Migration of colored proteins.

- Phycocyanin has three blue subunits of pI 4.5, 4.7 and 5.0 that migrate towards the anode and are expected to focus in fraction # 3.
- Hemoglobin A and Hemoglobin C, two red colored proteins of pI 7.1 and 7.4, respectively, migrate towards the center of the focusing chamber and are expected to focus in fraction # 6 and/or #7.
- Cytochrome c, an orange protein of pI 9.6 migrates toward the cathode and is expected to focus in fraction # 9 and/or 10.

4.5 Power Conditions

Table 4.1 lists the recommended power conditions.

Power conditions are listed for power supplies capable of maintaining 1 Watt constant power and for power supplies which cannot run under constant power, but are programmable for stepwise constant voltage with limiting current.

Note: Refer to the MicroRotofor instruction manual, Tables 3.2, 3.3, & 3.4 for detailed power conditions and cooling setting information.

Table 4.1: Power conditions

Cooling Setting II: Internal Temperature of 20 ± 2°C at ambient temperature range of 19–26°C		
Constant Power 1 Watt	**Step Method: Constant Voltage**	
Voltage range	**Step#. V / time**	**Current Range**
100–500 V	1. 150V / 10 min.	8-3 mA
	2. 200V / 10 min.	4-2 mA
	3. 300V / 60+ min.	4-3 mA
	A 20mA current limit and a 2W power limit are recommended for the Step Method.	

4.6 Harvest the Fractions

Once the IEF run is complete, i.e., the colored proteins have focused in the expected fractions, harvesting should be completed 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 running station. First, push focusing assembly towards anode to dislodge the connector from the cathode notch in the chassis. Then lift up gently on the cathode end and remove the anode end (red).

7. With the row of sample loading ports facing up (**sealing tape on loading ports removed**), position the focusing assembly in the harvesting station (Figure 10). 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 of the focusing chamber. DO NOT puncture the sealing tape covering the harvesting ports.

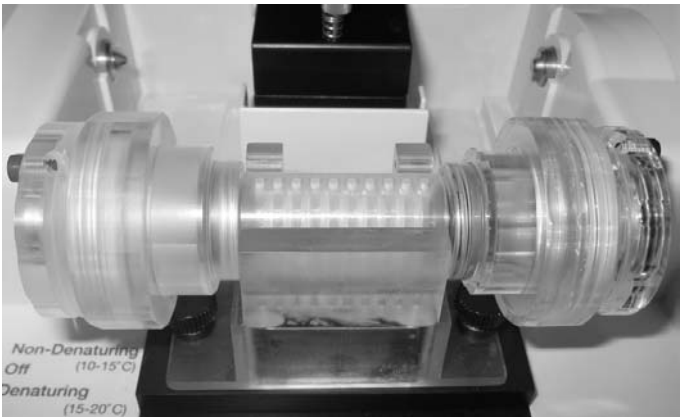

Fig. 11. Placement of the focusing assembly into the harvesting station.

8. Taking care to not cover any of the loading ports with your fingers, and using both hands, press down evenly and firmly on the electrode assemblies so that all the needles penetrate the sealing tape and harvesting ports simultaneously. At the same time and using the thumbs of both hands, press the harvesting tray against the seal of the vacuum assembly (Figure 12).

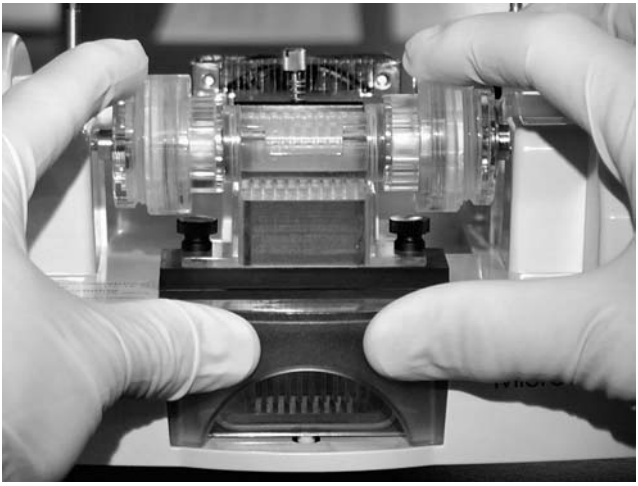

Fig. 12. Harvesting the fractions.

9. Continue to press down on the focusing chamber for several seconds to aspirate the ten fractions into the harvesting tray.
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 13).

Fig. 13. Transferring the fractions.

Section 5
Disassembly and Cleaning

5.1 Focusing Assembly

1. Using the assembly tool, loosen and remove the electrode assemblies from the focusing chamber (Figure 14).

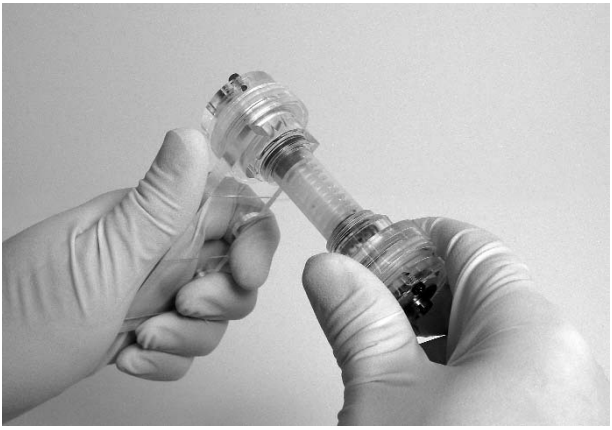

Fig. 14. Using the assembly tool to remove the anode assembly from the focusing assembly.

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.

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 and cause leakage of electrolyte into the focusing chamber. Equilibrated membranes, if stored properly, can be reused for 4–5 runs.

3. Rinse the electrode assemblies with deionized water.

4. Wash the focusing chamber. Place the focusing chamber in 2% SDS solution overnight. Thoroughly rinse the focusing chamber with deionized H_2O to remove the SDS.

5.2 Harvesting Station

To maintain optimal 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 and remove the positioning block, needle array, and needle array holder (Figure 15).

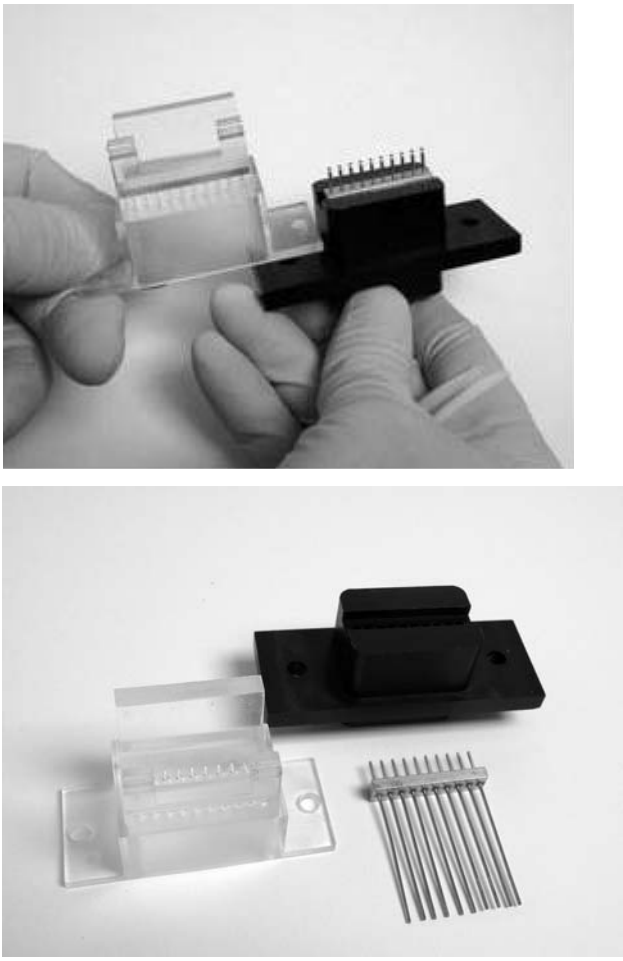

Fig. 15. Disassembling the harvesting station (top) and harvesting station components (bottom).

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

Note: For additional information on analysis of results, optimizing protein separation, and troubleshooting, please refer to the MicroRotofor instruction manual.

Appendix A
Product information

Catalog #	Description
170-2800	**MicroRotofor Cell Kit,** 100/120 V
170-2801	**MicroRotofor Cell Kit,** 220/240 V
170-2802	**MicroRotofor System,** 100/120 V, includes kit with PowerPac HV power supply
170-2803	**MicroRotofor System,** 220/240 V, includes kit with PowerPac HV power supply
170-2804	**MicroRotofor Starter Kit**
170-2810	**MicroRotofor Harvesting Trays,** 3
170-2820	**MicroRotofor Sealing Film,** harvesting tray sealing film, 10 sheets
170-2960	**MicroRotofor Sealing Film,** focusing chamber port sealing tape, 1 roll
170-2821	**MicroRotofor Focusing Chambers,** 3
170-2822	**MicroRotofor Cathode Assembly,** 1
170-2826	**MicroRotofor Electrode Assembly Gasket Kit,** (electrode buffer chamber o-ring and gaskets)
170-2829	**MicroRotofor Anode Assembly,** 1
170-2832	**MicroRotofor Assembly Tool**
170-2833	**MicroRotofor Ion Exchange Membrane Assemblies**
170-2835	**MicroRotofor Cleaning Brush**
170-2836	**MicroRotofor Syringes,** 3 x 3 ml and 3 x 10 ml
170-2850	**MicroRotofor Harvesting Station,** alignment station, needle assembly and needle holder
170-2851	**MicroRotofor Needle Assembly**
170-2852	**MicroRotofor Vacuum Block O-Ring**
170-2855	**MicroRotofor Lid**
165-5056	**PowerPac HV Power Supply and Accessories,** 100–120 V/220–240 V

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