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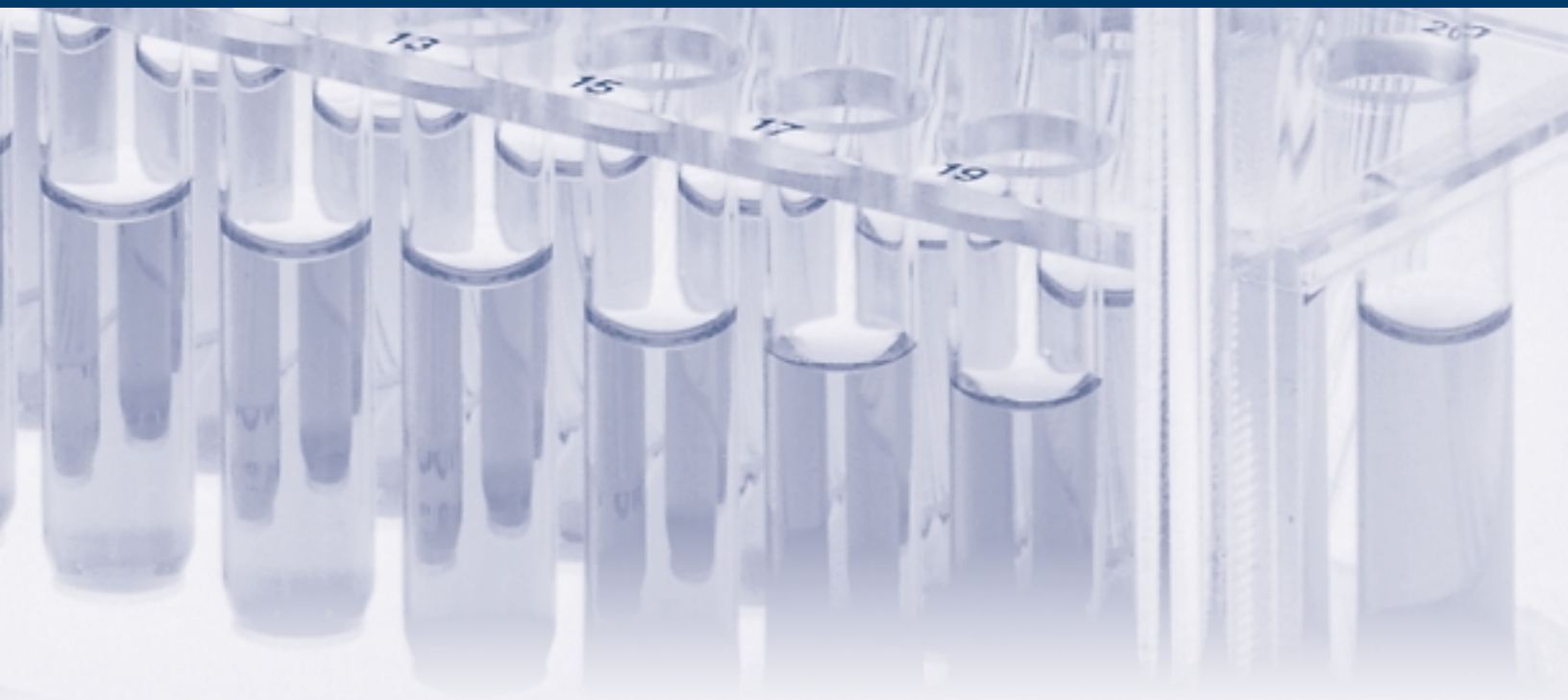
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Find the *Bio-Rad Rotofor* at our website: *Click HERE*



The **Rotofor**® Preparative IEF Cell



Protein Purification Simplified

BIO-RAD

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The Rotofor System
for Preparative Isoelectric Focusing (IEF)

Protein Purification Simplified

Protein purification has never been simpler than with the Rotofor system. This flexible system accommodates a wide range of sample volumes for analytical to preparative scale purification schemes.

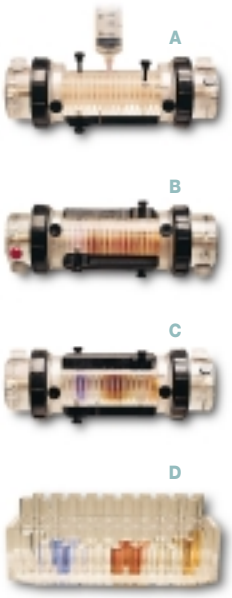
This unique purification tool performs IEF in free solution, and so is ideally suited for the isolation of uncharacterized proteins from crude or partially purified samples.

- Two interchangeable focusing chambers — mini and standard — process microgram to gram quantities of protein
- Easy setup and short run times enable 500-fold purification in <3 hours
- The patented ceramic cooling finger preserves biological activity
- The membrane core stabilizes proteins in twenty focused zones and facilitates free-solution collection without mixing
- Recovered proteins are biologically active and are suitable for sequence analysis, antibody production, and crystallography

As an initial purification step, the Rotofor system separates proteins of interest from bulk contaminants in a crude sample. The system is also useful for eliminating specific contaminants from partially purified samples. Selected fractions from an initial run can be collected, pooled, and refractionated, resulting in up to 1,000-fold purification.



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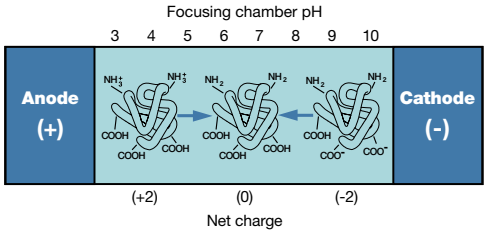


How the Rotofor System Works

To visualize the focusing process, a mixture of naturally colored proteins, including phycocyanin (blue isoforms; pI 4.5–5.0), myoglobin (red isoforms; pI 7.2–7.6), and cytochrome c (orange isoforms; pI 9.5–10.0), was combined with Bio-Lyte® ampholytes, pH range 3–10. Proteins were injected into the standard (60 ml) focusing chamber (A). The proteins were focused (B), and after 1 hour the three proteins were separated (C). Using the Rotofor system's vacuum-assisted harvesting method, fractions were simultaneously aspirated into 20 individual test tubes (D). Collection from the Rotofor is completed in seconds, eliminating diffusion or remixing of proteins.

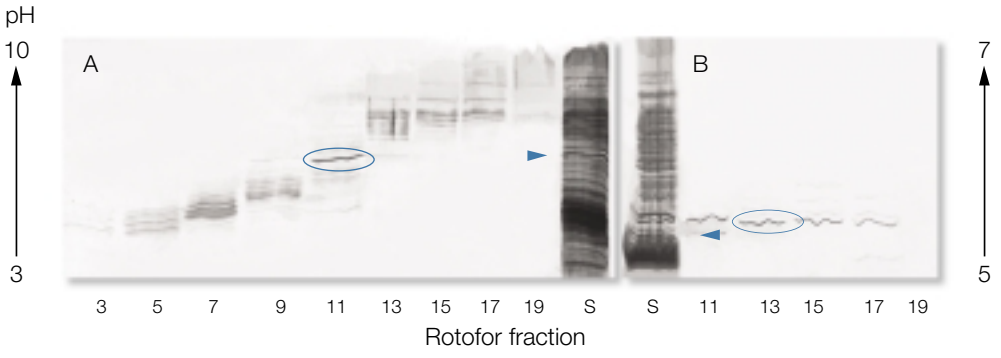
The Principle

Proteins carry net positive, negative, or neutral charges depending on the pH of their local environment. For every protein, there is a specific pH at which its net charge is zero. This pH is termed the isoelectric point (pI). A protein's pI is the property that permits fractionation of complex mixtures in a pH gradient. The Rotofor system isolates proteins by generating a linear pH gradient in the presence of an electrical field. For example, an acidic protein with a net positive charge in a particular region of the pH gradient will migrate away from the anode (+) and toward the cathode (-), giving up hydrogen ions (H^+) as it goes. When the net charge on a protein becomes zero, it ceases to migrate. If a protein should acquire a charge by diffusing in the pH gradient, the electrical field will drive it back to the pH that matches its pI. In this way, each protein in a sample becomes focused at its pI. Purified proteins are then harvested in free solution within specific pH intervals.



An Ideal Primary Purification Tool

The Rotofor system is perfectly suited for the purification of uncharacterized proteins. Because fractions are easily assayed for biological activity, purification conditions can be efficiently optimized to fit individual requirements. Purification on the Rotofor can be paired with analysis of the fractions using Bio-Rad's precast IEF gels for preliminary pI determination. The example below demonstrates how an individual protein was purified from a crude sample using the Rotofor system. Single-band purity was achieved in two steps.





Crude snake venom (60 ml) containing 150 mg total protein was fractionated in the standard Rotofor chamber in a broad-range pH gradient (pH 3–10). The fractions (10–12) containing the protein of interest (indicated by arrows) were pooled and refractionated in the mini Rotofor chamber. Fractions from both runs were analyzed for purity by IEF-PAGE and silver staining (odd lanes shown). A, initial fractionation; B, refractionation. Lanes labeled S contain crude snake venom.

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Model 491 Prep Cell

Combining the Rotofor system with the Model 491 prep cell for SDS-PAGE and native gel electrophoresis creates Bio-Rad's unique preparative 2-D electrophoresis system. This system purifies native or denatured proteins quickly and easily with the resolution of analytical 2-D PAGE.



Protocols and References

Detailed protocols describing published applications for the Rotofor system are available in the Rotofor technical folder, bulletin 1555A. To receive a free copy, contact your local Bio-Rad representative or request it online at **discover.bio-rad.com**

Specifications

	Mini Rotofor Cell	Standard Rotofor Cell
Focusing chamber inner diameter/length	19 mm/15 cm	30 mm/15 cm
Sample volume	18 ml	35–60 ml
Number of fractions	20	20
Fraction volume (approx.)	700 µl	1.75–3 ml
Sample load	micrograms to milligrams	milligrams to grams
Membrane cores	19 disks Polyester screen Pore size 10 µm	19 disks Polyester screen Pore size 10 µm
Power supply required	3,000 V with 12 W constant	3,000 V with 15 W constant

Ordering Information

Catalog #	Description
Rotofor Preparative Cells	
170-2986	Rotofor Purification System, 100/120 V, includes 60 ml focusing chamber, 18 ml mini focusing chamber, starter kit
170-2987	Rotofor Purification System, 220/240 V
170-2914	Rotofor Purification System With Power Supply, 100/120 V, includes 60 ml focusing chamber, 18 ml focusing chamber, PowerPac™ 3000 power supply, starter kit
170-2906	Rotofor Purification System With Power Supply, 220/240 V
170-2950	Standard Rotofor Cell, 100/120 V, includes 60 ml focusing chamber, starter kit
170-2951	Standard Rotofor Cell, 220/240 V
170-2988	Mini Rotofor Cell, 100/120 V, includes 18 ml focusing chamber, starter kit
170-2989	Mini Rotofor Cell, 220/240 V
170-2910	Rotofor Starter Kit, includes 10 ml Bio-Lyte ampholytes (pH range 3–10), 60 ml syringe, colored protein sample, 2 vent buttons, and one of each ion exchange membrane, hydrated
170-2919	Colored Protein Sample, 1 ml (included in Rotofor starter kit)
Rotofor System Adaptor Kits	
170-2990	Adaptor Kit, Rotofor cell to mini Rotofor cell, includes mini focusing chamber and mini membrane core, 18 ml
170-2959	Adaptor Kit, mini Rotofor cell to Rotofor cell, includes focusing chamber and membrane core, 60 ml

For information on precast IEF gels and Bio-Lyte ampholytes, please refer to Bio-Rad's Life Science Research Products catalog.

The ProteomeWorks System™

The ProteomeWorks system is the global alliance between Bio-Rad Laboratories, Inc. and Micromass-Waters, Ltd., dedicated to furthering proteomics research.

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