

Rotofor® System

Bio-Rad Tech Note Summaries

Bulletin Title	#	Protein pI	Notes
2-D Applications			
Preparative 2-D Purifies Proteins for Sequencing or Antibody Production	1744	4.9–5.3	Two Rotofor runs contained 50 mg of total protein and yielded a 1,000-fold purification of the apolipoprotein Apo J. The Model 491 prep cell was subsequently used to isolate the 70 kD protein
Preparative 2-D Electrophoresis System Purifies Recombinant Nuclear Proteins From Whole Bacterial Lysates	1773		Liquid-phase isoelectric focusing on the Rotofor cell was the first dimension, followed by second-dimension preparative PAGE on a Model 491 prep cell for purification of recombinant proteins from bacterial contaminants
A Rapid Method for the Purification of Analytical Grade Proteins From Plants Using Preparative SDS-PAGE and Preparative Isoelectric Focusing	1776	5	A rapid technique for protein purification for both sequence analysis and antibody production. The Rotofor cell was used as a second step in purification, following preparative PAGE on a Model 491 prep cell
Preparative SDS Gel Electrophoresis of Hydrophobic Cell Wall Proteins From <i>Candida albicans</i>	1953	5.2–5.5	Very hydrophobic proteins from <i>Candida albicans</i> were purified using the mini Rotofor cell and the mini prep cell
Purification of Proteins From <i>Mycobacterium tuberculosis</i> by Simultaneous Electro-Elution on the Mini Whole Gel Eluter	2043		The Rotofor and mini whole gel eluter were used to screen, identify, and isolate antigens from short-term culture filtrates of <i>M. tuberculosis</i>
Combination of 2-D Gel and Liquid-Phase Electrophoretic Separations as Proteomic Tools in Neuroscience	2859		A summary of techniques incorporating the Rotofor, Model 491 prep cell, and whole gel eluter for prefractionation of complex protein mixtures in proteomics applications
Isoelectric Focusing Nonporous RP HPLC: A Two-Dimensional Liquid Phase Separation Method for Mapping of Cellular Proteins With Identification Using MALDI-TOF Mass Spectrometry (reprint from Anal Chem)	RP-0014	3.2–9.5	The Rotofor cell was used with nonporous reverse-phase chromatography as an alternative two-dimensional separation system; 700 bands were resolved from HEL cells
Identification of Protein Vaccine Candidates From <i>Helicobacter pylori</i> Using a Preparative Two-Dimensional Electrophoretic Procedure and Mass Spectrometry (reprint from Anal Chem)	RP-0015	4.2–8.8	The Rotofor cell and whole gel eluter were used to separate detergent-solubilized <i>H. pylori</i> membrane proteins prior to mass spec analysis. This approach was successful in identifying a high percentage of membrane proteins that are typically underrepresented in 2-D PAGE gels
Proteomic study of a non-typable <i>Haemophilus influenzae</i>	RP-0025		A preparative 2-D separation involving first-dimension separation on a Rotofor, followed by second-dimension separation by SDS-PAGE and electroelution of fractions with the whole gel eluter. Use of this strategy in proteomic studies yields proteins not seen in traditional 2-D gel electrophoresis and in amounts sufficient for characterization by MS.

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Native Proteins			
Isolation of a Toxic Phospholipase D From <i>Corynebacterium pseudotuberculosis</i>	1508	9.8	In a single, rapid step, the Rotofor cell was used to obtain protein of sufficient quantity and purity for enzymatic studies and the production of monoclonal antibodies
Separation of Secreted Immunosuppressive Proteins of the Fish Pathogen, <i>Renibacterium salmoninarum</i> , From Culture Medium and Infected Fish Tissues	1516	3.0–4.5	Two Rotofor cell runs purified antigen F (a 57–58 kD doublet protein with a pI of 3–4.5) from both infected fish kidneys and culture medium to near homogeneity
Isolation and Purification of a Turkey Seminal Plasma Protease	1519	7.0–8.5	Better purification in one Rotofor step than in two chromatography steps, requiring much less time for similar yields
Isolation of an <i>Escherichia coli</i> Heat Stable Enterotoxin (STb)-Alkaline Phosphatase Fusion Protein by Preparative Isoelectric Focusing	1520	6.4	Purified to a single band in one Rotofor run
Isolation of Recombinant HIV1 Protease Expressed in <i>E. coli</i> and <i>S. cerevisiae</i>	1521	9.95	A single Rotofor run was performed to compare protease yields from yeast (500-fold) and <i>E. coli</i> (200-fold) expression systems
Separation of Aldose Reductase Isoelectric Forms Using the Rotofor Cell	1539	4.6 and 5.0	The Rotofor cell was used as a last step to isolate two isoforms of aldose reductase
Isolation of Multiple Lipoprotein (a) Charge Forms in Human Plasma by Liquid Phase Isoelectric Focusing	1899	4.0–4.5	Large apolipoproteins (>500 kD) were easily focused in free solution with the Rotofor cell. Four charged forms of lipoprotein with multiple pIs were purified
Detergents or Denaturants Used			
Isolation of Monoclonal Antibodies to Phencyclidine From Ascites Fluid	1475	5–9	Quick and easy IgG _{2a} purification in 3 M urea, resulting in active antibody for further studies
Isolation of a Membrane-Bound Immunoregulatory Molecule From Metastatic Lymphoma Cells	1514	4.3	The detergent n-octylglucoside was used in this Rotofor purification. The resulting immunosuppressive protein was characterized
Preparation of Plant Cold Acclimation Proteins for Gas Phase Sequencing, Oligonucleotide Derivation, and Monoclonal Antibody Production	1515	5.4, 6.3, and 6.8	Urea, CHAPS, glycerol, and prefocusing helped prevent precipitation of these spinach enzymes. Three different proteins of different pIs and molecular weights were purified
Rotofor Fractionation of Intestinal Brush Border Membrane Proteins	1517	5.2	A membrane protein was solubilized under reducing conditions with the detergent CHAPS, and activity was enriched 5-fold in a single Rotofor run
Purification of Bacterial Eukaryotic Fusion Proteins Using the Rotofor Cell	1518	5.1	A 6 M urea-soluble protein was significantly purified away from a major proteolytic fragment



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