

The Rotofor® Cell in the Public Domain

Selected references demonstrating the versatility of the Rotofor system

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Protein Fractionation for Enhanced MS Identification

Liquid-phase isoelectric focusing (IEF) on the Rotofor provides a unique method of sample fractionation and enrichment of low-abundance proteins as well as a robust separation strategy complementary to conventional 2-D gel electrophoresis/MS and LC/MS.

Fractionation Prior to 2-D Gel Electrophoresis

These articles demonstrate that a prefractionation step on the Rotofor prior to conventional 2-D gel electrophoresis (2-DGE) enables higher sample loads and greater resolution for the pI fractions of interest. This, coupled with the high sample capacity of the Rotofor, results in the enrichment of low-abundance proteins.

Puchades M, Hansson SF, Nilsson CL, Andreasen N, Blennow K, Davidsson P
Proteomic studies of potential cerebrospinal fluid protein markers for Alzheimer's disease
Brain Res Mol Brain Res 118, 140–146 (2003)

Davidsson P, Folkesson S, Christiansson M, Lindbjör M, Dellheden B, Blennow K, Westman-Brinkmalm A
Identification of proteins in human cerebrospinal fluid using liquid-phase isoelectric focusing as a prefractionation step followed by two-dimensional gel electrophoresis and matrix-assisted laser desorption/ionisation mass spectrometry
Rapid Commun Mass Spectrom 16, 2083–2088 (2002)

Westman-Brinkmalm A, Davidsson P
Comparison of preparative and analytical two-dimensional electrophoresis for isolation and matrix-assisted laser desorption/ionization-time-of-flight mass spectrometric analysis of transthyretin in cerebrospinal fluid
Anal Biochem 301, 161–167 (2002)

Hochstrasser AC, James RW, Pometta D, Hochstrasser D
Preparative isoelectrofocusing and high resolution 2-dimensional gel electrophoresis for concentration and purification of proteins
Appl Theor Electrophor 1, 333–337 (1991)

Fractionation Prior to SDS-PAGE

The Rotofor is often used as an alternative first-dimension separation step, particularly when native separations are preferred, large sample loads are required for the enrichment of low-abundance proteins, or when proteins of interest are insoluble in gel-based IEF media. Rotofor fractions may be further separated by SDS-PAGE in either analytical or preparative workflows. Protein-containing bands may then be excised from analytical PAGE gels, eluted from preparative gels with the whole gel eluter, or eluted off the bottom of the preparative Model 491 prep cell.

Such electrophoretic separations can increase sequence coverage and provide proteins of high purity and in yields sufficient for characterization by MS.

Analytical PAGE to MS

Peirce MJ, Wait R, Begum S, Saklatvala J, Cope AP
Expression profiling of lymphocyte plasma membrane proteins
Mol Cell Proteomics 3, 56–65 (2004)

Preparative PAGE to MS

Rotofor to PAGE to Whole Gel Eluter to MS

These articles describe a preparative 2-D system wherein protein samples are fractionated on a Rotofor, separated on SDS-PAGE slab gels and eluted from the gels with the whole gel eluter prior to MS analysis.

¹Thoren K, Gustafsson E, Clevnert A, Larsson T, Bergstrom J, Nilsson CL
Proteomic study of non-typable *Haemophilus influenzae*
J Chromatogr B Analyt Technol Biomed Life Sci 782, 219–226 (2002)

Westman-Brinkmalm A, Davidsson P
Comparison of preparative and analytical two-dimensional electrophoresis for isolation and matrix-assisted laser desorption/ionization-time-of-flight mass spectrometric analysis of transthyretin in cerebrospinal fluid
Anal Biochem 301, 161–167 (2002)

Covert BA, Spencer JS, Orme IM, Belisle JT
The application of proteomics in defining the T cell antigens of *Mycobacterium tuberculosis*
Proteomics 1, 574–586 (2001)

Davidsson P, Paulson L, Hesse C, Blennow K, Nilsson CL
Proteome studies of human cerebrospinal fluid and brain tissue using a preparative two-dimensional electrophoresis approach prior to mass spectrometry
Proteomics 1, 444–452 (2001)

Gustafsson E, Thoren K, Larsson T, Davidsson P, Karlsson KA, Nilsson CL

¹ Available as reprint # RP0025 in the Rotofor Technical Folder

Identification of proteins from *Escherichia coli* using two-dimensional semi-preparative electrophoresis and mass spectrometry
Rapid Commun Mass Spectrom 15, 428–432 (2001)

Hesse C, Nilsson CL, Blennow K, Davidsson P
Identification of the apolipoprotein E4 isoform in cerebrospinal fluid with preparative two-dimensional electrophoresis and matrix assisted laser desorption/ionization-time of flight-mass spectrometry
Electrophoresis 22, 1834–1837 (2001)

²Nilsson CL, Larsson T, Gustafsson E, Karlsson KA, Davidsson P
Identification of protein vaccine candidates from *Helicobacter pylori* using a preparative two-dimensional electrophoretic procedure and mass spectrometry
Anal Chem 72, 2148–2153 (2000)

Rotofor to Prep Cell to MS

These articles describe the use of preparative 2-D electrophoresis on the Rotofor and Model 491 prep cell prior to MS analysis. Using this approach, larger volumes/amounts of samples can be loaded, yielding sufficient amounts of low-abundance proteins for further characterization. Since proteins remain in liquid phase during the entire procedure, extra steps such as electroelution, extraction, or transfer to membranes from the gels prior to mass spectrometric analysis are obviated. This method is applicable to a wide range of sample types, such as cerebrospinal fluid, serum, tissue extracts, cell media, whole cells, and bacterial lysates.

Davidsson P, Westman A, Puchades M, Nilsson CL, Blennow K
Characterization of proteins from human cerebrospinal fluid by a combination of preparative two-dimensional liquid-phase electrophoresis and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
Anal Chem 71, 642–647 (1999)

Nilsson CL, Puchades M, Westman A, Blennow K, Davidsson P
Identification of proteins in a human pleural exudate using two-dimensional preparative liquid-phase electrophoresis and matrix-assisted laser desorption/ionization mass spectrometry
Electrophoresis 20, 860–865 (1999)

Puchades M, Westman A, Blennow K, Davidsson P
Analysis of intact proteins from cerebrospinal fluid by matrix-assisted laser desorption/ionization mass spectrometry after two-dimensional liquid-phase electrophoresis
Rapid Commun Mass Spectrom 13, 2450–2455 (1999)

² Available as reprint # RP0015 in the Rotofor Technical Folder

Fractionation Prior to Chromatographic Separations

These articles describe multidimensional liquid phase separations of proteins and/or peptides coupling liquid isoelectric focusing on the Rotofor as the first phase and reversed-phase HPLC or size-exclusion chromatography as the second phase of separation. Peptides and proteins remain in liquid phase throughout the separation, thus making the entire procedure highly amenable to automation and high throughput.

Hamler RL, Zhu K, Buchanan NS, Kreunin P, Kachman MT, Miller FR, Lubman DM
A two-dimensional liquid-phase separation method coupled with mass spectrometry for proteomic studies of breast cancer and biomarker identification
Proteomics 4, 562–577 (2004)

Kachman MT, Wang H, Schwartz DR, Cho KR, Lubman DM
A 2-D liquid separations/mass mapping method for interlysate comparison of ovarian cancers
Anal Chem 74, 1779–1791 (2002)

Lubman DM, Kachman MT, Wang H, Gong S, Yan F, Hamler RL, O'Neil KA, Zhu K, Buchanan NS, Barder TJ
Two-dimensional liquid separations-mass mapping of proteins from human cancer lysates
J Chromatogr B Analyt Technol Biomed Life Sci 782, 183–196 (2002)

Wall DB, Parus SJ, Lubman DM
Three-dimensional protein map according to pI, hydrophobicity and molecular mass
J Chromatogr B Analyt Technol Biomed Life Sci 774, 53–58 (2002)

Wang H, Kachman MT, Schwartz DR, Cho KR, Lubman DM
A protein molecular weight map of ES2 clear cell ovarian carcinoma cells using a two-dimensional liquid separations/mass mapping technique
Electrophoresis 23, 3168–3181 (2002)

Wall DB, Kachman MT, Gong SS, Parus SJ, Long MW, Lubman DM
Isoelectric focusing nonporous silica reversed-phase high-performance liquid chromatography/electrospray ionization time-of-flight mass spectrometry: a three-dimensional liquid-phase protein separation method as applied to the human erythroleukemia cell-line
Rapid Commun Mass Spectrom 15, 1649–1661 (2001)

Wall DB, Parus SJ, Lubman DM
Comparison of the capabilities of liquid isoelectric focusing-one-dimensional nonporous silica reversed-phase liquid chromatography-electrospray ionization time-of-flight mass spectrometry and liquid isoelectric focusing-one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis mass mapping for the analysis of intact protein molecular masses
J Chromatogr B Biomed Sci Appl 763, 139–148 (2001)

³Wall DB, Kachman MT, Gong S, Hinderer R, Parus S, Misek DE, Hanash SM, Lubman DM
Isoelectric focusing nonporous RP HPLC: a two-dimensional liquid-phase separation method for mapping of cellular proteins with identification using MALDI-TOF mass spectrometry
Anal Chem 72, 1099–1111 (2000)

³ Available as reprint# RP0014 in the Rotofor Technical Folder

This article describes a multidimensional liquid-phase separation of proteins coupling liquid isoelectric focusing on the Rotofor as the first phase and size-exclusion chromatography as the second phase of separation.

Lecchi P, Gupte AR, Perez RE, Stockert LV, Abramson FP
Size-exclusion chromatography in multidimensional separation schemes for proteome analysis
J Biochem Biophys Methods 56, 141–152 (2003)

Fractionation Prior to MALDI-TOF MS

These articles describe direct analysis of Rotofor fractions by MALDI-TOF MS.

Wang MZ, Howard B, Campa MJ, Patz, EF, Fitzgerald MC
Analysis of human serum proteins by liquid phase isoelectric focusing and matrix-assisted laser desorption/ionization-mass spectrometry
Proteomics 3, 1661–1666 (2003)

Howard BA, Wang MZ, Campa MJ, Corro C, Fitzgerald MC, Patz EF Jr
Identification and validation of a potential lung cancer serum biomarker detected by matrix-assisted laser desorption/ionization-time of flight spectra analysis
Proteomics 3, 1720–1724 (2003)

Fractionation Prior to LC-MS/MS

These articles describe direct analysis of Rotofor fractions by LC-MS/MS.

Harper RG, Workman SR, Schuetzner S, Timperman AT, Sutton JN
Low-molecular-weight human serum proteome using ultrafiltration, isoelectric focusing, and mass spectrometry
Electrophoresis 25, 1299–1306 (2004)

Xiao Z, Conrads TP, Lucas DA, Janini GM, Schaefer CF, Buetow KH, Issaq HJ, Veenstra TD
Direct ampholyte-free liquid-phase isoelectric peptide focusing: application to the human serum proteome
Electrophoresis 25, 128–133 (2004)

Janini GM, Conrads TP, Veenstra TD, Issaq HJ
Development of a two-dimensional protein-peptide separation protocol for comprehensive proteome measurements
J Chromatogr B Analyt Technol Biomed Life Sci 787, 43–51 (2003)

Protein Purification

Liquid-phase IEF on the Rotofor is a powerful protein purification technique. As an initial purification step, the Rotofor separates proteins of interest from bulk contaminants in crude samples. Selected fractions from an initial run may be collected, pooled and refractionated, resulting in up to 1000-fold purifications. In addition, Rotofor fractions may be easily subjected to further purification by gel electrophoresis, chromatography, and other methods. As a final purification step, the Rotofor eliminates specific contaminants that might be difficult to remove by other means.

General Methods

Whether alone or in combination with other techniques — polyacrylamide gel electrophoresis (PAGE), electroelution, or chromatography, for example — the Rotofor system integrates into any purification scheme. The following articles illustrate the use of the Rotofor in a variety of different protein purification and enrichment methods.

Madoz-Gurpide J, Wang H, Misek DE, Brichory F, Hanash SM
Protein based microarrays: a tool for probing the proteome of cancer cells and tissues
Proteomics 1, 1279–1287 (2001)

Ayala A, Parrado J, Machado A
Use of Rotofor preparative isoelectrofocusing cell in protein purification procedure
Appl Biochem Biotechnol 69, 11–16 (1998)

Masuoka J, Glee PM, Hazen KC
Preparative isoelectric focusing and preparative electrophoresis of hydrophobic *Candida albicans* cell wall proteins with in-line transfer to polyvinylidene difluoride membranes for sequencing
Electrophoresis 19, 675–678 (1998)

Weldingh K, Rosenkrands I, Jacobsen S, Rasmussen PB, Elhay MJ, Andersen P
Two-dimensional electrophoresis for analysis of *Mycobacterium tuberculosis* culture filtrate and purification and characterization of six novel proteins
Infect Immun 66, 3492–500 (1998)

Yvon S, Rolland D, Charrier JP, Jolivet M
An alternative for purification of low soluble recombinant hepatitis C virus core protein: preparative two-dimensional electrophoresis
Electrophoresis 19, 1300–1305 (1998)

Lucietto P, Fossati G, Ball HL, Giuliani P, Mascagni P
Mycobacterium tuberculosis chaperonin 10 and N-truncated fragments. Their synthesis and purification by the isoelectric focusing technique carried out in solution
J Pept Res 49, 308–323 (1997)

Goldfarb MF
Use of Rotofor in two-dimensional electrophoretic analysis: identification of a 100 kDa monoclonal IgG heavy chain in myeloma serum
Electrophoresis 14, 1379–1381 (1993)

Shimazaki K, Kawaguchi A, Sato T, Ueda Y, Tomimura T, Shimamura S

Analysis of human and bovine milk lactoferrins by Rotofor and chromatofocusing
Int J Biochem 25, 1653–1658 (1993)

Caslavska J, Gebauer P, Odermatt A, Thormann W
Recycling and screen-segmented column isotachopheresis, two free-fluid approaches for
fractionation of proteins
J Chromatogr 545, 315–329 (1991)

Hochstrasser AC, James RW, Pometta D, Hochstrasser D
Preparative isoelectrofocusing and high resolution 2-dimensional gel electrophoresis for
concentration and purification of proteins
Appl Theor Electrophor 1, 333–337 (1991)

Shelton KR, Klann E, Nixon G, Egle PM
A procedure for purifying low-abundance protein components from the brain cytoskeleton-nuclear
matrix fraction
J Neurosci Methods 37, 257–266 (1991)

Evans CH, Wilson AC, Gelleri BA
Preparative isoelectric focusing in ampholine electrofocusing columns versus immobiline
polyacrylamide gel for the purification of biologically active leukoregulin
Anal Biochem 177, 358–363 (1989)

Preparative 2-D Applications

Microgram to milligram quantities of protein may be separated using Bio-Rad's unique two-step preparative electrophoresis system. In this system, first-dimension preparative isoelectric focusing on Bio-Rad's Rotofor cell is followed by preparative electrophoresis on the Model 491 prep cell.

Yvon S, Rolland D, Charrier JP, Jolivet M

An alternative for purification of low soluble recombinant hepatitis C virus core protein:
preparative two-dimensional electrophoresis
Electrophoresis 19, 1300–1305 (1998)

Austin PR, Hovde CJ

Purification of recombinant shiga-like toxin type I B subunit
Protein Expr Purif 6, 771–779 (1995)

Schletter J, Kruger C, Lottspeich F, Schutt C

Improved method for preparation of lipopolysaccharide-binding protein from human serum by
electrophoretic and chromatographic separation techniques
J Chromatogr B Biomed Appl 654, 25–34 (1994)

Hochstrasser AC, James RW, Pometta D, Hochstrasser D

Preparative isoelectrofocusing and high resolution 2-dimensional gel electrophoresis for
concentration and purification of proteins
Appl Theor Electrophor 1, 333–337 (1991)

Isoform Resolution

Liquid-phase isoelectric focusing can be used as a method for effective resolution of protein isoforms, which may have similar molecular mass, but different pIs.

Stephenson K, Jensen CL, Jorgensen ST, Lakey JH, Harwood CR
The influence of secretory-protein charge on late stages of secretion from the Gram-positive bacterium *Bacillus subtilis*
Biochem J 350, (Pt 1), 31–39 (2000)

Kabir S
The isolation and characterisation of jacalin [*Artocarpus heterophyllus* (jackfruit) lectin] based on its charge properties
Int J Biochem Cell Biol 27, 147–156 (1995)

Chang YM, Lin S, Liao TH
Bovine pancreatic deoxyribonuclease F: isoelectric focusing, peptide mapping and primary structure.
Biotechnol Appl Biochem 19 (Pt 1), 129–140 (1994)

Park YH, Lee SS
Identification and characterization of capsaicin-hydrolyzing enzymes purified from rat liver microsomes
Biochem Mol Biol Int 34, 351–360 (1994)

Wang G, Bhattacharyya N, Wilkerson C, Ramsammy RA, Eatman E, Anderson WA
Estrogen induced peroxidase secretion from the endometrial epithelium: a possible function for the luminal enzyme
J Submicrosc Cytol Pathol 26, 405–414 (1994)

Malle E, Hess H, Munscher G, Knipping G, Steinmetz A
Purification of serum amyloid A and its isoforms from human plasma by hydrophobic interaction chromatography and preparative isoelectric focusing
Electrophoresis 13, 422–428 (1992)

Paliwal R, Costa G, Diwan JJ
Purification and patch clamp analysis of a 40-pS channel from rat liver mitochondria
Biochemistry 31, 2223–2229 (1992)

Petrash JM, DeLucas LJ, Bowling E, Egen N
Resolving isoforms of aldose reductase by preparative isoelectric focusing in the Rotofor
Electrophoresis 12, 84–90 (1991)

Jimenez J, Dufresne M, Poirot S, Vaysse N, Fourmy D
Electric properties of photoaffinity-labelled pancreatic A-subtype cholecystokinin
J Chromatogr 511, 333–339 (1990)

Use of Denaturants/Detergents to Improve Solubility

The Rotofor system provides a gentle separation technique that does not alter the native state of the protein. Occasionally, however, denaturing agents or detergents may be added to enhance the solubility of some proteins during focusing.

Kachman MT, Wang H, Schwartz DR, Cho KR, Lubman DM

A 2-D liquid separations/mass mapping method for interlysate comparison of ovarian cancers
Anal Chem 74, 1779–1791 (2002)

Rosenkrands I, Weldingh K, Jacobsen S, Hansen CV, Florio W, Gianetri I, Andersen P

Mapping and identification of *Mycobacterium tuberculosis* proteins by two-dimensional gel electrophoresis, microsequencing and immunodetection
Electrophoresis 21, 935–948 (2000)

Brightbill HD, Libraty DH, Krutzik SR, Yang RB, Belisle JT, Bleharski JR, Maitland M, Norgard

MV, Plevy SE, Smale ST, Brennan PJ, Bloom BR, Godowski PJ, Modlin RL
Host defense mechanisms triggered by microbial lipoproteins through toll-like receptors
Science 285, 732–736 (1999)

Burns JM Jr, Adeeku EK, Dunn PD

Protective immunization with a novel membrane protein of *Plasmodium yoelii*-infected erythrocytes
Infect Immun 67, 675–680 (1999)

Dobbs LG, Gonzalez RF, Allen L, Froh DK

HTI56, an integral membrane protein specific to human alveolar type I cells
J Histochem Cytochem 47, 129–137 (1999)

Stan RV, Ghitescu L, Jacobson BS, Palade GE

Isolation, cloning, and localization of rat PV-1, a novel endothelial caveolar protein
J Cell Biol 145, 1189–1198 (1999)

Weldingh K, Rosenkrands I, Jacobsen S, Rasmussen PB, Elhay MJ, Andersen P

Two-dimensional electrophoresis for analysis of *Mycobacterium tuberculosis* culture filtrate and purification and characterization of six novel proteins
Infect Immun 66, 3492–3500 (1998)

Lucietto P, Fossati G, Ball HL, Giuliani P, Mascagni P

Mycobacterium tuberculosis chaperonin 10 and N-truncated fragments. Their synthesis and purification by the isoelectric focusing technique carried out in solution
J Pept Res 49, 308–323 (1997)

Nore BF, Harrison MA, Keen JN, Allen JF

Partial purification of a cyanobacterial membrane protein with amino terminal sequence similarity to the N-methylphenylalanine pilins
Acta Chem Scand 48, 578–581 (1994)

Ni J, Karpas A

Isolation of a novel cytotoxic lymphokine (factor 2) from a human B-cell line (Karpas 160b) by preparative isoelectric focusing in the Rotofor cell and chromatofocusing
Cytokine 5, 31–37 (1993)

Purification of Animal Proteins

The Rotofor system has been used as a primary purification tool for proteins from a variety of animal sources.

Zeng Y, Graner MW, Feng H, Li G, Katsanis E

Imatinib mesylate effectively combines with chaperone-rich cell lysate-loaded dendritic cells to treat *bcr-abl*+ murine leukemia

Int J Cancer 110, 251–259 (2004)

Graner MW, Zeng Y, Feng H, Katsanis E

Tumor-derived chaperone-rich cell lysates are effective therapeutic vaccines against a variety of cancers

Cancer Immunol Immunother 52, 226–234 (2003)

Lavagna C, Poiree JC, Fournel S, Rampal P

Purification of a new intestinal anti-proliferative factor from normal human small intestine

Eur J Biochem 259, 821–828 (1999)

Maesaka JK, Palaia T, Chowdhury SA, Shimamura T, Fishbane S, Reichman W, Coyne A, O'Rear JJ, El-Sabban ME

Partial characterization of apoptotic factor in Alzheimer plasma

Am J Physiol 276 (Pt 2), F521–F527 (1999)

Aguiar AS, Melgarejo AR, Alves CR, Giovanni-De-Simone S

Single-step purification of crotafotin and crotafotine from *Crotalus durissus terrificus* venom using preparative isoelectric focusing

Braz J Med Biol Res 30, 25–28 (1997)

Feng L, Prestwich GD

Expression and characterization of a lepidopteran general odorant binding protein

Insect Biochem Mol Biol 27, 405–412 (1997)

Furster C, Zhang J, Toll A

Purification of a 3 β -hydroxy- Δ^5 -C27-steroid dehydrogenase from pig liver microsomes active in major and alternative pathways of bile acid biosynthesis

J Biol Chem 271, 20903–20907 (1996)

Aslam M, Jimenez-Flores R, Kim HY, Hurley WL

Two-dimensional electrophoretic analysis of proteins of bovine mammary gland secretions collected during the dry period

J Dairy Sci 77, 1529–1536 (1994)

Ohshima Y, Morita M, Hirashima M, Mori KJ, Akutagawa H, Katamura K, Mayumi M, Mikawa H
Characterization of an eosinophilic leukemia cell differentiation factor (ELDF) produced by a human T cell leukemia cell line, HIL-3

Exp Hematol 21, 749–754 (1993)

Prestwich GD

Bacterial expression and photoaffinity labeling of a pheromone binding protein

Protein Sci 2, 420–428 (1993)

Thurston RJ, Korn N, Froman DP, Bodine AB

Proteolytic enzymes in seminal plasma of domestic turkey (*Meleagris gallopavo*)
Biol Reprod 48, 393–402 (1993)

Valaitis AP, Bowers DF
Purification and properties of the soluble midgut trehalase from the gypsy moth, *Lymantria dispar*
Insect Biochem Mol Biol 23, 599–606 (1993)

Yui S, Yang D, Mikami M, Yamazaki M
Characterization of cell growth-inhibitory factor in inflammatory peritoneal exudate cells of rats
Microbiol Immunol 37, 961–969 (1993)

Mirowski M, Sherman U, Hanausek M
Purification and characterization of a 65-kDa tumor-associated phosphoprotein from rat
transplantable hepatocellular carcinoma 1682C cell line
Protein Expr Purif 3, 196–203 (1992)

Peritt D, Flechner I, Okunev E, Yanai P, Halperin T, Treves AJ, Barak V
The M20 IL-1 inhibitor. I. Purification by preparative isoelectric focusing in free solution
J Immunol Methods 155, 159–165 (1992)

Wellstein A, Fang WJ, Khatri A, Lu Y, Swain SS, Dickson RB, Sasse J, Riegel AT, Lippman ME
A heparin-binding growth factor secreted from breast cancer cells homologous to a
developmentally regulated cytokine
J Biol Chem 267, 2582–2587 (1992)

Egen NB, Bliss M, Mayersohn M, Owens SM, Arnold L, Bier M
Isolation of monoclonal antibodies to phencyclidine from ascites fluid by preparative isoelectric
focusing in the Rotofor
Anal Biochem 172, 488–494 (1988)

Purification From Non-Animal Sources

The Rotofor system has been used as a primary purification tool for proteins from a wide range of non-animal systems.

Di Matteo A, Federici L, Mattei B, Salvi G, Johnson KA, Savino C, De Lorenzo G, Tsernoglou D, Cervone F

The crystal structure of polygalacturonase-inhibiting protein (PGIP), a leucine-rich repeat protein involved in plant defense

Proc Natl Acad Sci USA 100, 10124–10128 (2003)

Bond CS, Blankenship RE, Freeman HC, Guss JM, Maher MJ, Selvaraj FM, Wilce MC, Willingham KM

Crystal structure of auracyanin, a "blue" copper protein from the green thermophilic photosynthetic bacterium *Chloroflexus aurantiacus*

J Mol Biol 306, 47–67 (2001)

Krappmann S, Helmstaedt K, Gerstberger T, Eckert S, Hoffmann B, Hoppert M, Schnappauf G, Braus GH

The *aroC* gene of *Aspergillus nidulans* codes for a monofunctional, allosterically regulated chorismate mutase

J Biol Chem 274, 22275–22282 (1999)

Nishimura A, Ozaki Y, Oyama H, Shin T, Murao S

Purification and characterization of a novel 5-oxoprolinase (without ATP-hydrolyzing activity) from *Alcaligenes faecalis* N-38A

Appl Environ Microbiol 65, 712–717 (1999)

Braig HR, Zhou W, Dobson SL, O'Neill SL

Cloning and characterization of a gene encoding the major surface protein of the bacterial endosymbiont *Wolbachia pipientis*

J Bacteriol 180, 2373–2378 (1998)

Green G, Dicks LM, Bruggeman G, Vandamme EJ, Chikindas ML

Pediocin PD-1, a bactericidal antimicrobial peptide from *Pediococcus damnosus* NCFB 1832

J Appl Microbiol 83, 127–132 (1997)

Osuji GO, Madu WC

Regulation of peanut glutamate dehydrogenase by methionine sulphoximine

Phytochemistry 46, 817–825 (1997)

Bilbrey RE, Penheiter AR, Gathman AC, Lilly, WW

Characterization of a novel phenylalanine-specific aminopeptidase from *Schizophyllum commune*

Mycol Res 100, 462–466 (1996)

Bono JL, Legendre AM, Scalarone GM

Detection of antibodies and delayed hypersensitivity with Rotofor preparative IEF fractions of *Blastomyces dermatitidis* yeast phase lysate antigen

J Med Vet Mycol 33, 209–214 (1995)

Fisher MA, Bono JL, Abuodeh RO, Legendre AM, Scalarone GM

Sensitivity and specificity of an isoelectric focusing fraction of *Blastomyces dermatitidis* yeast lysate antigen for the detection of canine blastomycosis

Mycoses 38, 177–182 (1995)

Dhugga KS, Ray PM

Purification of 1,3-beta-D-glucan synthase activity from pea tissue. Two polypeptides of 55 kDa and 70 kDa copurify with enzyme activity
Eur J Biochem 220, 943–953 (1994)

Segers R, Butt TM, Kerry BR, Peberdy JF

The nematophagous fungus *Verticillium chlamydosporium* produces a chymoelastase-like protease which hydrolyses host nematode proteins in situ
Microbiology 140 (Pt 10), 2715–2723 (1994)

Kum WW, Laupland KB, See RH, Chow AW

Improved purification and biologic activities of staphylococcal toxic shock syndrome toxin 1
J Clin Microbiol 31, 2654–2660 (1993)

Tosado-Acevedo R, Toranzos GA, Alsina A

Extraction and purification of a catalase from *Candida albicans*
P R Health Sci J 11, 77–80 (1992)

St Clair NL, Sax M

Free-solution isoelectric focusing for the purification of *Staphylococcus aureus* enterotoxin C1
Protein Expr Purif 1, 97–103 (1990)



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France 01 47 95 69 65 **Germany** 089 318 84-177 **Hong Kong** 852-2789-3300 **Hungary** 36 1 455 8800 **India** (91-124)-6398112/113/114, 6450092/93
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Russia 7 095 721 1404 **Singapore** 65-6415 3188 **South Africa** 00 27 11 4428508 **Spain** 34 91 590 5200 **Sweden** 08 555 12700
Switzerland 061 717-9555 **Taiwan** (8862) 2578-7189/2578-7241 **United Kingdom** 020 8328 2000