

## The Model 491 Prep Cell in the Public Domain

Selected references demonstrating the versatility of the Model 491 prep cell and mini prep cell

	<u>Page</u>
Protein Fractionation for Enhanced MS Identification	
Prep Cell Fractions to 2-D Gels	1
Preparative 2-D Separations Prior to MS	2
Prep Cell Fractions to RP-HPLC to MS	2
Protein Purification	
Preparative 2-D Applications	3
Native Gels	4
Medium to Large Proteins (>50 kD)	5
Small Proteins	7
Other Applications	9
Nucleic Acid and Lipopolysaccharide Purification	10

# Protein Fractionation for Enhanced MS Identification

Continuous elution gel electrophoresis on the Model 491 prep cell is an effective means of fractionating complex protein mixtures according to their molecular mass. Size-dependent fractionation is an important strategy in studies focused on a particular protein or protein family and all related posttranslational modifications as these proteins tend to possess similar molecular mass. Moreover, large sample volumes and microgram to milligram quantities of protein may be separated on the Model 491 prep cell, thus yielding sufficient amounts of low-abundance proteins for detection on conventional 2-D gels, for further separation by chromatographic or other means, or for characterization by mass spectroscopy.

## Prep Cell Fractions to 2-D Gels

The Model 491 prep cell is an effective means of enriching for low-abundance proteins according to their MW range. The resulting liquid fractions may be pooled and proteins precipitated prior to loading onto IPG strips for 2-D gel electrophoretic separations. This technique is particularly powerful for studies aimed at understanding the presence of various isoforms and posttranslational states of a given protein.

Fountoulakis M, Juranville JF, Tsangaris G, Suter L  
Fractionation of liver proteins by preparative electrophoresis  
*Amino Acids* 26, 27–36 (2004)

<sup>1</sup>Fountoulakis M, Juranville JF  
Enrichment of low-abundance brain proteins by preparative electrophoresis  
*Anal Biochem* 313, 267–282 (2003)

Zugaro LM, Reid GE, Ji H, Eddes JS, Murphy AC, Burgess AW, Simpson RJ  
Characterization of rat brain stathmin isoforms by two-dimensional gel electrophoresis-matrix assisted laser desorption/ionization and electrospray ionization-ion trap mass spectrometry  
*Electrophoresis* 19, 867–876 (1998)

---

<sup>1</sup> Available as reprint #RP0026 in the Prep Cell Technical Folder

## Preparative 2-D Separations Prior 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 or 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)

## Prep Cell Fractions to RP-HPLC to MS

This technique was developed for high-throughput tandem MS (MS/MS) of whole, intact proteins. The protocol describes use of an acid-labile detergent (ALS) in the prep cell gel to facilitate desalting and further fractionation by RP-HPLC.

Meng F, Du Y, Miller LM, Patrie SM, Robinson DE, Kelleher NL  
Molecular-level description of proteins from *Saccharomyces cerevisiae* using quadrupole FT hybrid mass spectrometry for top down proteomics  
Anal Chem 76, 2852–2858 (2004)

Meng F, Cargile BJ, Patrie SM, Johnson JR, McLoughlin SM, Kelleher NL  
Processing complex mixtures of intact proteins for direct analysis by mass spectrometry  
Anal Chem 74, 2923–2929 (2002)

## Protein Purification

The Model 491 prep cell and mini prep cell — using native or SDS-PAGE — separate and purify proteins into discrete liquid fractions for sequencing, antibody production, crystallography, and other downstream applications. The prep cell system offers high-resolution separations and high sample capacities, enabling efficient size-dependent purification, whether used alone or in combination with other separation techniques.

## 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, preparative electrophoresis on the Model 491 prep cell follows first-dimension preparative isoelectric focusing on Bio-Rad's Rotofor 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)

## Native Gels

The Model 491 prep cell is frequently used under non-denaturing conditions, providing purified proteins or multienzyme complexes in their active forms.

Madhavarao CN, Chinopoulos C, Chandrasekaran K, Namboodiri MA  
Characterization of the N-acetylaspartate biosynthetic enzyme from rat brain  
J Neurochem 86, 824–835 (2003)

Lamparter T, Esteban B, Hughes J  
Phytochrome Cph1 from the cyanobacterium *Synechocystis* PCC6803. Purification, assembly and quaternary structure  
Eur J Biochem 268, 4720–4730 (2001)

Galland-Irmouli AV, Pons L, Lucon M, Villaume C, Mrabet NT, Gueant JL, Fleurence J  
One-step purification of R-phycoerythrin from the red macroalga *Palmaria palmata* using preparative polyacrylamide gel electrophoresis  
J Chromatogr B Biomed Sci Appl 739, 117–123 (2000)

D'Silva I, Heath MC  
Purification and characterization of two novel hypersensitive response-inducing specific elicitors produced by the cowpea rust fungus  
J Biol Chem 272, 3924–3927 (1997)

Zhao Q, Gottschalk I, Carlsson J, Arvidsson LE, Oscarsson S, Medin A, Ersson B, Janson JC  
Preparation and purification of an end to end coupled mEGF-dextran conjugate  
Bioconj Chem 8, 927–934 (1997)

Dostmann WR, Koep N, Endres R  
The catalytic domain of the cGMP-dependent protein kinase I $\alpha$  modulates the cGMP-binding characteristics of its regulatory domain  
FEBS Lett 398, 206–210 (1996)

Tikkanen K, Haataja S, Francois-Gerard C, Finne J  
Purification of a galactosyl- $\alpha$  1-4-galactose-binding adhesin from the gram-positive meningitis-associated bacterium *Streptococcus suis*  
J Biol Chem 270, 28874–28878 (1995)

Fountoulakis M, Takacs-di Lorenzo E, Juranville JF, Manneberg M  
Purification of interferon gamma-interferon gamma receptor complexes by preparative electrophoresis on native gels  
Anal Biochem 208, 270–276 (1993)

vanderSpek JC, Mindell JA, Finkelstein A, Murphy JR  
Structure/function analysis of the transmembrane domain of DAB389-interleukin-2, an interleukin-2 receptor-targeted fusion toxin. The amphipathic helical region of the transmembrane domain is essential for the efficient delivery of the catalytic domain to the cytosol of target cells  
J Biol Chem 268, 12077–12082 (1993)

## Medium to Large Proteins (>50 kD)

If you can resolve a protein in an analytical slab gel, you can purify that same protein with the Model 491 prep cell or mini prep cell. The following articles describe purifications of medium to large proteins.

Mulvey C, Ohlendieck K

Use of continuous-elution gel electrophoresis as a preparative tool for blot overlay analysis  
Anal Biochem 319, 122–130 (2003)

Viard M, Blumenthal R, Raviv Y

Improved separation of integral membrane proteins by continuous elution electrophoresis with simultaneous detergent exchange: application to the purification of the fusion protein of the human immunodeficiency virus type 1  
Electrophoresis 23, 1659–1666 (2002)

Cooper KW, Baneyx F

*Escherichia coli* FtsH (HflB) degrades a membrane-associated TolAII-beta-lactamase fusion protein under highly denaturing conditions  
Protein Expr Purif 21, 323–332 (2001)

Griffiths S, Cook M, Mallory B, Ritchie R

Characterisation of ISAV proteins from cell culture  
Dis Aquat Organ 45, 19–24 (2001)

Monier F, Surla A, Guillot M, Morel F

Gelatinase isoforms in urine from bladder cancer patients  
Clin Chim Acta 299, 11–23 (2000)

Saviana B, Pons L, Namour F, Quilliot D, Ziegler O, Gueant JL

Sodium dodecyl sulphate gel electrophoretic preparation of protein standard human apolipoprotein B-48  
J Chromatogr B Biomed Sci Appl 742, 421–426 (2000)

Feldman DA, Weinhold PA

Cytidylyltransferase-binding protein is identical to transcytosis-associated protein (TAP/p115) and enhances the lipid activation of cytidylyltransferase  
J Biol Chem 273, 102–109 (1998)

Charlton MR, Balagopal P, Nair KS

Skeletal muscle myosin heavy chain synthesis in type 1 diabetes  
Diabetes 46, 1336–1340 (1997)

Dubois B, Deloron P

Purification of Pf155/RESA antigen from supernatants of in vitro *Plasmodium falciparum* cultures by continuous elution electrophoresis  
J Immunol Methods 208, 29–34 (1997)

Brown WC, Logan KS, Zhao S, Bergman DK, Rice-Ficht AC

Identification of *Babesia bovis* merozoite antigens separated by continuous-flow electrophoresis that stimulate proliferation of helper T-cell clones derived from *B. bovis*-immune cattle  
Infect Immun 63, 3106–3116 (1995)

Ko YG, Thompson GA Jr

Purification of glycosylphosphatidylinositol-anchored proteins by modified triton X-114 partitioning and preparative gel electrophoresis  
Anal Biochem 224, 166–172 (1995)

Remacle AG, Baramova EN, Weidle UH, Krell HW, Foidart JM  
Purification of progelatinases A and B by continuous-elution electrophoresis  
Protein Expr Purif 6, 417–422 (1995)

Balagopal P, Nair KS, Stirewalt WS  
Isolation of myosin heavy chain from small skeletal muscle samples by preparative continuous elution gel electrophoresis: application to measurement of synthesis rate in human and animal tissue  
Anal Biochem 221, 72–77 (1994)

Nyormoi O, Schneider T, Smith RG  
A large scale preparative gel electrophoresis separation of alpha 1 and alpha 2 subunits of the voltage-gated  $\text{Ca}^{2+}$  channel from rabbit skeletal muscle  
Electrophoresis 15, 1186–1190 (1994)

Treuheit MJ, Ataei A, Wallick ET, Kirley TL  
Purification of the alpha and beta subunits of (Na,K)-ATPase by continuous elution electrophoresis  
Prep Biochem 23, 375–387 (1993)

Li Z, Smith CD, Smolley JR, Bridge JH, Frank JS, Philipson KD  
Expression of the cardiac  $\text{Na}^{+}$ - $\text{Ca}^{2+}$  exchanger in insect cells using a baculovirus vector  
J Biol Chem 267, 7828–7833 (1992)

## Small Proteins

The following articles describe purifications of small proteins (molecular weights less than 50 kD) with the Model 491 prep cell or mini prep cell.

Hamilton CA, Good AG, Taylor GJ  
Induction of vacuolar ATPase and mitochondrial ATP synthase by aluminum in an aluminum-resistant cultivar of wheat  
Plant Physiol 125, 2068–2077 (2001)

Moese S, Selbach M, Zimny-Arndt U, Jungblut PR, Meyer TF, Backert S  
Identification of a tyrosine-phosphorylated 35 kDa carboxy-terminal fragment (p35CagA) of the *Helicobacter pylori* CagA protein in phagocytic cells: processing or breakage?  
Proteomics 1, 618–629 (2001)

Perrier AL, Cousin X, Boschetti N, Haas R, Chatel JM, Bon S, Roberts WL, Pickett SR, Massoulie J, Rosenberry TL, Krejci E  
Two distinct proteins are associated with tetrameric acetylcholinesterase on the cell surface  
J Biol Chem 275, 34260–34265 (2000)

Zhao M, Schlame M, Rua D, Greenberg ML  
Cardiolipin synthase is associated with a large complex in yeast mitochondria  
J Biol Chem 273, 2402–2408 (1998)

Zugaro LM, Reid GE, Ji H, Eddes JS, Murphy AC, Burgess AW, Simpson RJ  
Characterization of rat brain stathmin isoforms by two-dimensional gel electrophoresis-matrix assisted laser desorption/ionization and electrospray ionization-ion trap mass spectrometry  
Electrophoresis 19, 867–876 (1998)

Kromer WJ, Carafoli E, Bailey JE  
Purification of the cardiac sarcoplasmic reticulum membrane protein phospholamban from recombinant *Escherichia coli*  
Eur J Biochem 248, 814–819 (1997)

Schmidt HH, Genschel J, Haas R, Manns MP  
Preparative electrophoresis: an improved method for the isolation of human recombinant apolipoprotein A-I  
Biotechniques 23, 778–779 (1997)

Srivastava OP, Srivastava K  
Characterization of three isoforms of a 9 kDa gamma D-crystallin fragment isolated from human lenses  
Exp Eye Res 62, 593–604 (1996)

Yao Q, Bevan JL, Weaver RF, Bigelow DJ  
Purification of porcine phospholamban expressed in *Escherichia coli*  
Protein Expr Purif 8, 463–468 (1996)

Bhattacharyya AK, Chavan AJ, Haley BE, Taylor MF, Collins DC  
Identification of the NADP(H) binding site of rat liver microsomal 5 alpha-reductase (isozyme-1): purification of a photolabeled peptide corresponding to the adenine binding domain  
Biochemistry 34, 3663–3669 (1995)

Brown WC, Logan KS, Zhao S, Bergman DK, Rice-Ficht AC  
Identification of *Babesia bovis* merozoite antigens separated by continuous-flow electrophoresis that stimulate proliferation of helper T-cell clones derived from *B. bovis*-



immune cattle

Infect Immun 63, 3106–3116 (1995)

Chiao JH, Yang CH, Roy K, Pain J, Sirotnak FM

Ligand-directed immunoaffinity purification and properties of the one-carbon, reduced folate transporter. Interspecies immuno-cross-reactivity and expression of the native transporter in murine and human tumor cells and their transport-altered variants

J Biol Chem 270, 29698–29704 (1995)

Ko YG, Thompson GA Jr

Purification of glycosylphosphatidylinositol-anchored proteins by modified triton X-114 partitioning and preparative gel electrophoresis

Anal Biochem 224, 166–172 (1995)

Kyd JM, Dunkley ML, Cripps AW

Enhanced respiratory clearance of nontypeable *Haemophilus influenzae* following mucosal immunization with P6 in a rat model

Infect Immun 63, 2931–2940 (1995)

van den Berg CW, Harrison RA, Morgan BP

A rapid method for the isolation of analogues of human CD59 by preparative SDS-PAGE: application to pig CD59

J Immunol Methods 179, 223–231 (1995)

Kyd JM, Taylor D, Cripps AW

Conservation of immune responses to proteins isolated by preparative polyacrylamide gel electrophoresis from the outer membrane of nontypeable *Haemophilus influenzae*

Infect Immun 62, 5652–5658 (1994)

Harwig SS, Chen NP, Park AS, Lehrer RI

Purification of cysteine-rich bioactive peptides from leukocytes by continuous acid-urea-polyacrylamide gel electrophoresis

Anal Biochem 208, 382–386 (1993)

## **Other Applications**

This article describes use of the Model 491 prep cell as a preparative alternative to isolate sufficient amounts of a homogenous protein sample to be used as a peroxidase-labeled probe in blot overlays.

Mulvey C, Ohlendieck K

Use of continuous-elution gel electrophoresis as a preparative tool for blot overlay analysis  
Anal Biochem 319, 122–130 (2003)

## Nucleic Acid and Lipopolysaccharide Purification

The Model 491 prep cell and mini prep cell are versatile tools for preparative continuous elution electrophoresis. Any molecules that can be resolved by electrophoresis — including proteins, nucleic acids, and lipopolysaccharides — can be purified with these devices.

Prieto DA, Harvey LK, Nelson EL  
Separation and purification of plasmid mixtures by continuous elution electrophoresis  
Biotechniques 29, 1204–1206 (2000)

<sup>2</sup>Cunningham L, Kittikamron K, Lu Y  
Preparative-scale purification of RNA using an efficient method which combines gel electrophoresis and column chromatography  
Nucleic Acids Res 24, 3647–3648 (1996)

<sup>3</sup>Kim JS, Reuhs BL, Rahman MM, Ridley B, Carlson RW  
Separation of bacterial capsular and lipopolysaccharides by preparative electrophoresis  
Glycobiology 6, 433–437 (1996)

<sup>4</sup>Schlax PE Jr, Capp MW, Record MT Jr  
Preparative-scale purification of DNA restriction fragments by continuous-flow gel electrophoresis  
Biotechniques 18, 94–100 (1995)

---

<sup>2</sup> Available as reprint #RP0003 in the Prep Cell Technical Folder

<sup>3</sup> Available as reprint #RP0001 in the Prep Cell Technical Folder

<sup>4</sup> Available as reprint #RP0002 in the Prep Cell Technical Folder



**Bio-Rad  
Laboratories, Inc.**

*Life Science  
Group*

**Web site** [www.bio-rad.com](http://www.bio-rad.com) **USA** (800) 4BIORAD **Australia** 02 9914 2800 **Austria** (01)-877 89 01 **Belgium** 09-385 55 11 **Brazil** 55 21 2527 3454  
**Canada** (905) 712-2771 **China** (86-21) 63052255 **Czech Republic** + 420 2 41 43 05 32 **Denmark** 44 52 10 00 **Finland** 09 804 22 00  
**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  
**Israel** 03 951 4127 **Italy** 39 02 216091 **Japan** 03-5811-6270 **Korea** 82-2-3473-4460 **Latin America** 305-894-5950 **Mexico** 55-52-00-05-20  
**The Netherlands** 0318-540666 **New Zealand** 64 9 415 2280 **Norway** 23 38 41 30 **Poland** + 48 22 331 99 99 **Portugal** 351-21-472-7700  
**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