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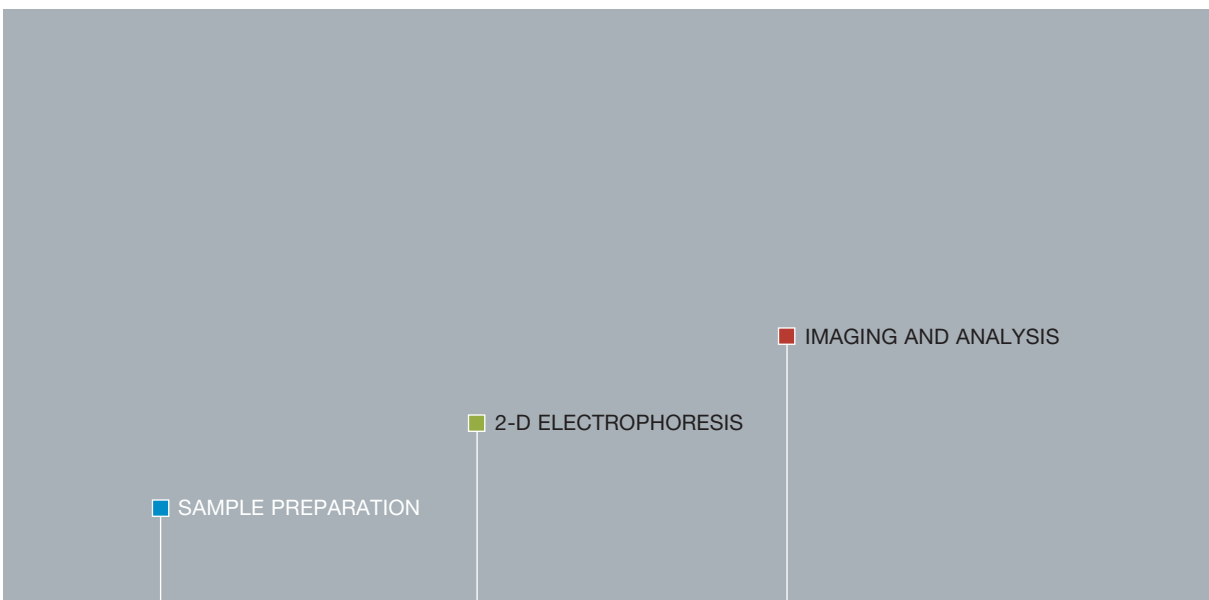
## Sample Preparation

Tools for Protein Sample Extraction,  
Cleanup, Fractionation, and Depletion

**BIO-RAD**

SAMPLE  
PREPARATION

BIO-RAD  
EXPRESSION PROTEOMICS



Expression proteomics defines patterns of proteins expressed in different biological samples. Bio-Rad's approach to expression proteomics focuses on three technologies: sample preparation, two-dimensional (2-D) electrophoresis, and imaging and analysis.

Good sample preparation prior to 2-D electrophoresis is critical for producing meaningful, reproducible results. From reagent kits that facilitate protein extraction and cleanup, to robust systems for sample fractionation and enrichment, Bio-Rad has developed a number of products to suit your sample preparation needs.

### Chemical Reagents — A Robust, Cost-Effective Approach

Proteins exhibit different solubility characteristics in different chemical environments. Combining detergents and other chemicals at appropriate temperatures provides solubility conditions that facilitate protein extraction, precipitation of a protein of interest, or selective precipitation of unwanted proteins or contaminants.

The ReadyPrep™ and MicroRotofor™ kits and reagents for sample preparation require little hardware or instrumentation, making them an easy approach to try with minimal time or material investment.

### Chromatography — Convenient One-Step Methods

Chromatographic methods separate, enrich, and purify proteins by exploiting the differential binding properties of proteins to solid supports. Ion exchange, affinity, and combinatorial chemistry methods may be used alone or in combination to remove contaminants and reduce complexity of a protein sample. Aurum™ and ProteoMiner™ kits provide a means to fast, convenient protein sample preparation using column chromatography.

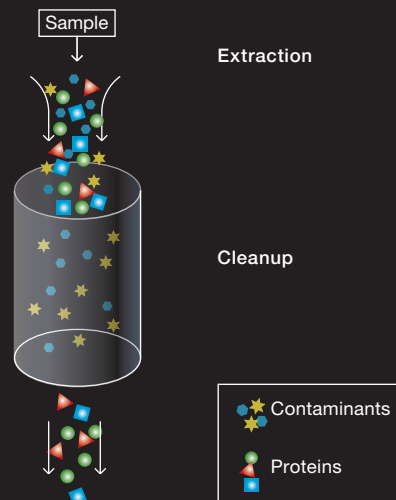
### Electrophoresis — Effective Tools for Enrichment of Low-Abundance Proteins

Electrophoretic tools fractionate proteins according to their isoelectric point (pI) or molecular weight. The Rotofor® family of cells separate and concentrate proteins according to pI by liquid-phase isoelectric focusing (IEF). The Model 491 prep cell and mini prep cell separate proteins based on their molecular weight through preparative continuous-elution electrophoresis. The large sample capacities of the Rotofor and Model 491 prep cells make them particularly effective for the enrichment of low-abundance proteins.

Bio-Rad has applied its expertise in solution chemistry, chromatography, and electrophoresis to develop the most comprehensive suite of products available for protein sample extraction, cleanup, fractionation, and depletion.

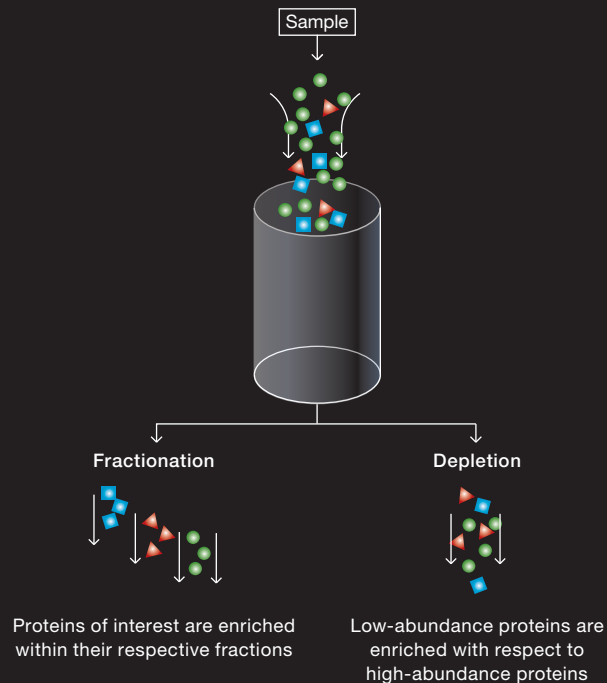
## Extraction/Cleanup

Success depends on sample purity. Contaminants, such as salts, detergents, and ionic compounds can ruin a 2-D experiment, so it is crucial to eliminate these contaminants prior to analysis. Bio-Rad provides a variety of effective extraction and cleanup options to ensure high-quality results.



## Fractionation/Depletion

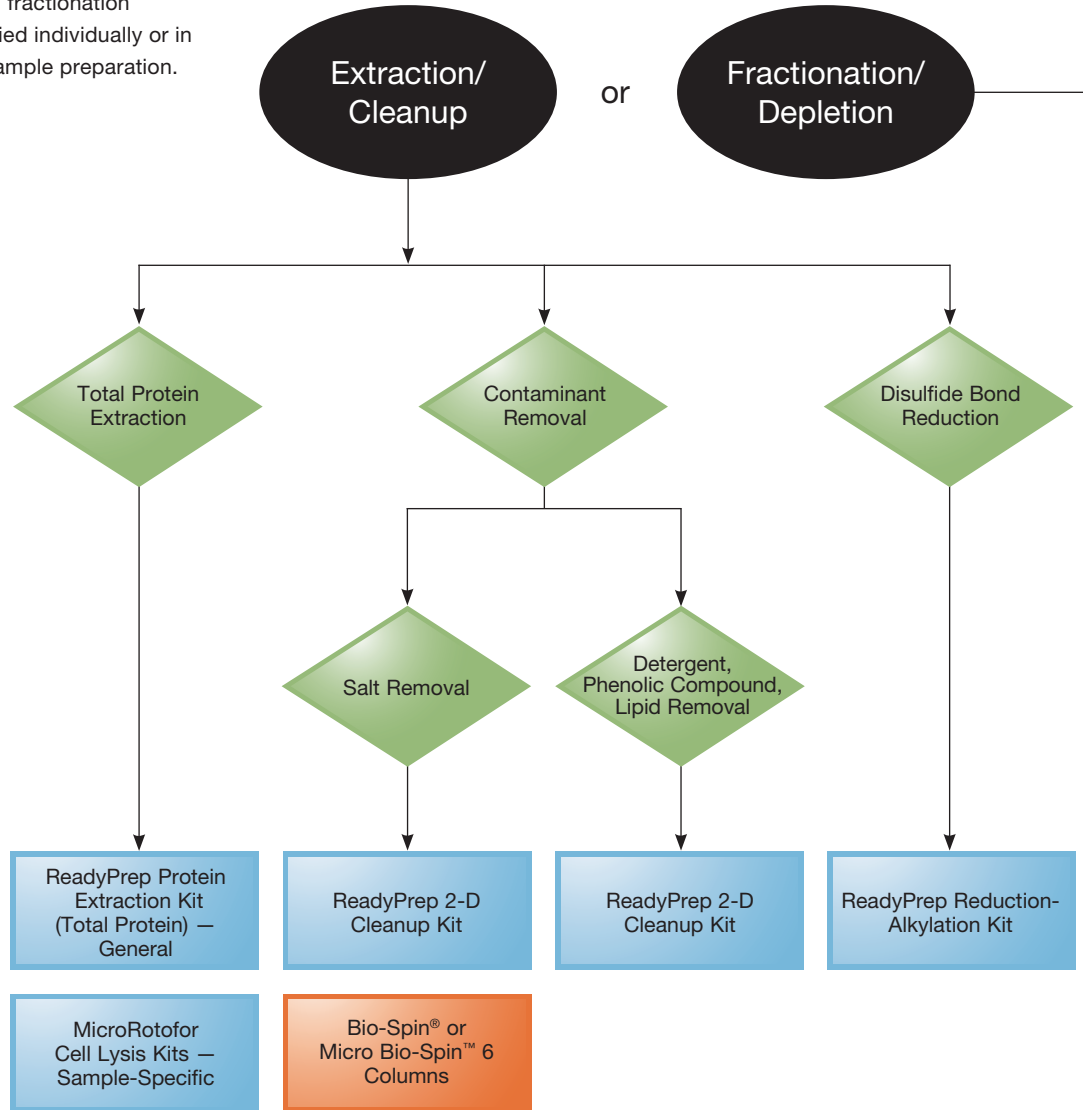
Fractionation and depletion reduce sample complexity and enrich samples for proteins of interest. This can greatly improve visualization of low-abundance proteins by removing other proteins that mask their detection. Bio-Rad offers a wide range of approaches for fractionating complex protein mixtures and depleting high-abundance proteins.



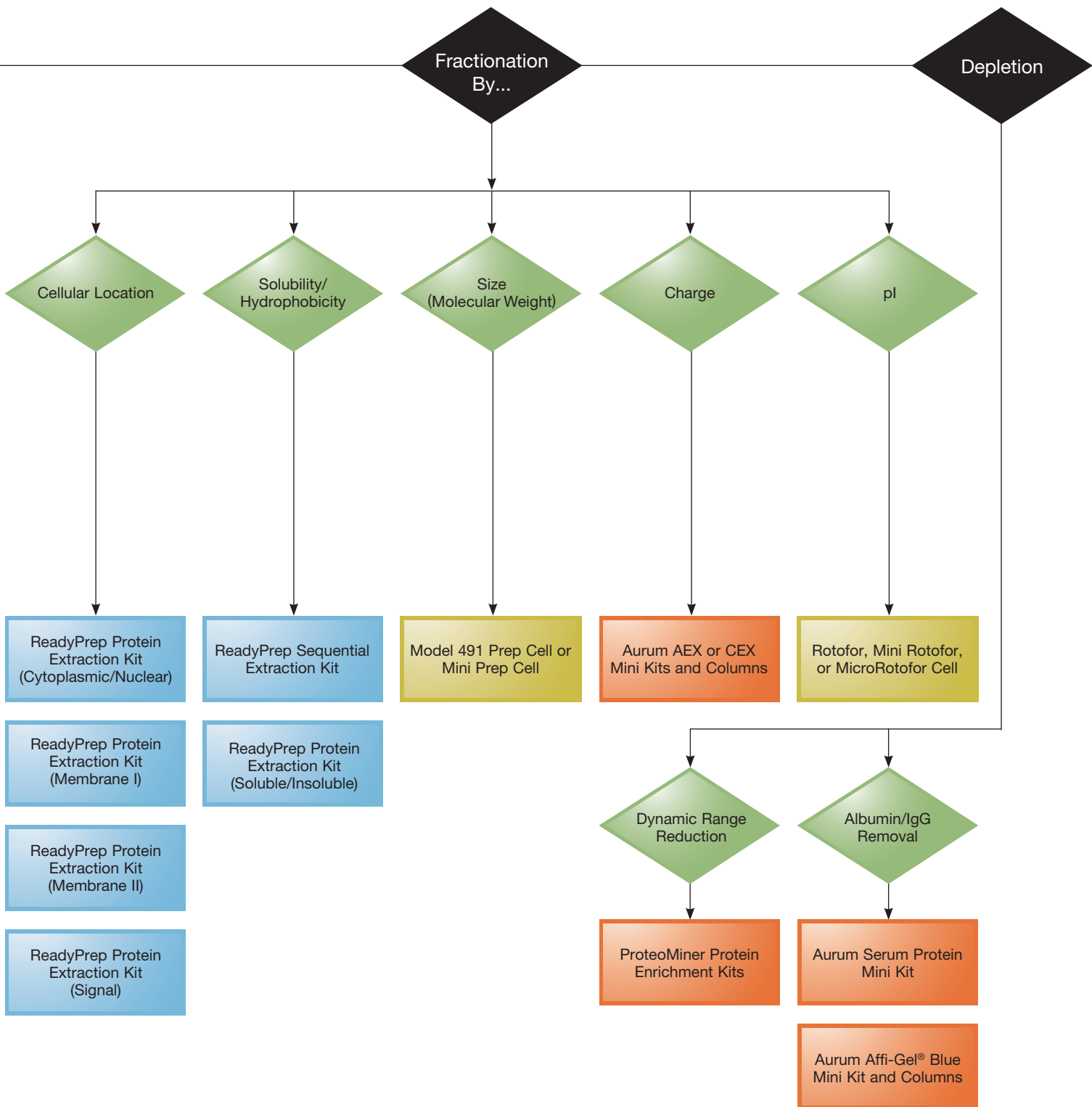
## Product Selection Guide

### Bio-Rad's sample preparation products.

These products are based on well-understood purification, extraction, and fractionation principles and may be applied individually or in combination for effective sample preparation.



- ◆ Applications
- Chemical reagent products
- Chromatography products
- Electrophoresis products

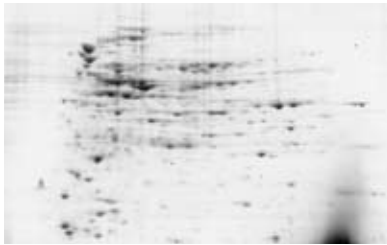


## Extraction/Cleanup

Before

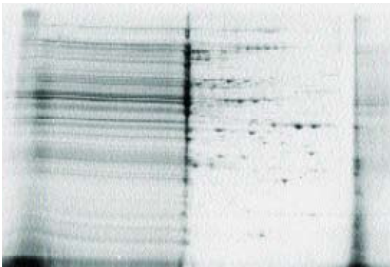


After

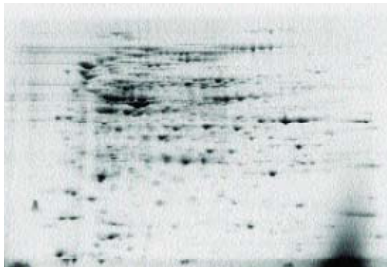


**Salt removal using the ReadyPrep 2-D cleanup kit.** *E. coli* extracts containing 1 M NaCl were electrophoresed before and after treatment with the 2-D cleanup kit. The samples were focused using 11 cm ReadyStrip™ pH 3–10 IPG strips, then run on Criterion™ 8–16% Tris-HCl precast gels for the second dimension.

Before



After



**Detergent removal using the ReadyPrep 2-D cleanup kit.** *E. coli* extracts containing 1% SDS were electrophoresed before and after treatment with the ReadyPrep 2-D cleanup kit. The samples were focused using 11 cm ReadyStrip pH 3–10 IPG strips, then run on Criterion 8–16% Tris-HCl precast gels for the second dimension.

### Enhanced Resolution and Reproducibility

Protein extraction and cleanup may be the only sample preparation steps you need to ensure good resolution and limit variability on your 2-D gels.

### Total Protein Extraction — General

Obtaining a sample of total cellular protein will sometimes be your only goal.

#### ReadyPrep Protein Extraction Kit (Total Protein)

- Uses a quick and efficient extraction protocol
- Incorporates the zwitterionic detergent ASB-14 to help solubilize proteins

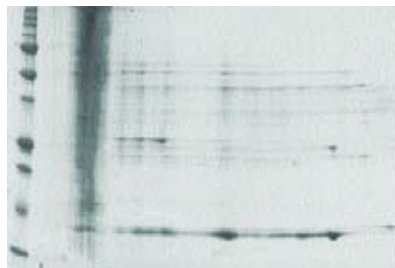
### Total Protein Extraction — Sample-Specific

Some sample types may require tailored extraction protocols.

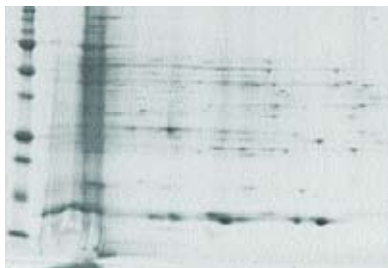
#### MicroRotofor Cell Lysis Kits

- Provide tailored protocols for cell lysis and total protein extraction based on sample type (mammalian tissues and cells, bacteria, yeast, or plants)
- Incorporate a chaotropic protein solubilization buffer (PSB)
- May be applied directly to numerous downstream applications, such as 2-D gel electrophoresis, Rotofor fractionation, etc.

Reduced Using DTT



Reduced Using ReadyPrep Kit



**Disulfide bond removal using the ReadyPrep reduction-alkylation kit.**

Protein samples were reduced using either 50 mM DTT in rehydration/sample buffer or the reduction-alkylation kit. Both samples were applied by cup loading onto 11 cm ReadyStrip pH 7–10 IPG strips and focused for the first dimension, then run on Criterion 8–16% Tris-HCl precast gels for the second dimension.

**Salt Removal**

Removal of salts reduces streaking and improves reproducibility of 2-D gels.

**ReadyPrep 2-D Cleanup Kit**

- Uses TCA-like precipitation for salt removal
- Can concentrate protein samples

**Bio-Spin and Micro Bio-Spin 6 Columns**

- Provide fast salt removal in an easy-to-use spin-column format
- Remove compounds <6 kD by size exclusion chromatography
- Accommodate up to 100  $\mu$ l of sample

**Detergent, Phenolic Compound, and Lipid Removal**

Ionic contaminants such as detergents, lipids, and phenolic compounds interfere with 2-D resolution and reproducibility.

**ReadyPrep 2-D Cleanup Kit**

- Uses TCA-like precipitation to wash away contaminants
- Can concentrate protein samples

**Disulfide Bond Reduction**

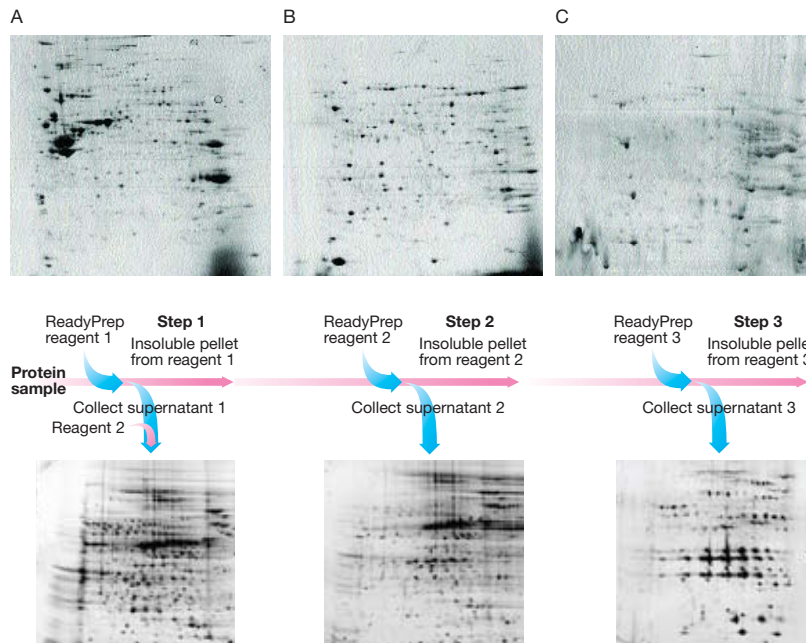
Disulfide bond reduction ensures proper protein migration, better-resolved spots, fewer streaks, and greater reproducibility.

**ReadyPrep Reduction-Alkylation Kit**

- Is essential for 2-D analysis of basic proteins
- Permanently eliminates disulfide bonds prior to IEF
- Reduces proteins using TBP, then alkylates proteins with iodoacetamide



# Fractionation



**Differences in 2-D patterns obtained using ReadyPrep signal (A), membrane I (B), and membrane II (C) kits.** Mouse liver samples were extracted using the recommended protocols for each kit. Purified proteins (~450 µg) were focused on 17 cm pH 3–10 nonlinear ReadyStrip IPG strips and run on 8–16% PROTEAN® II precast gels for the second dimension. Overall spot patterns differ for A, B, and C even though all three kits isolate membrane proteins, indicating each kit isolates different types of membrane proteins.

**Distribution of proteins based on differential solubility using the ReadyPrep sequential extraction kit.** The generation of three fractions provides increased resolution of proteins on 2-D gels.

## The Quest for Low-Abundance Proteins

Select from a number of fractionation tools to reduce sample complexity and enrich samples for low-abundance proteins — screen all fractions or only those of interest.

## Fractionation by Cellular Location

Bio-Rad offers choices for fractionation by cellular location.

### ReadyPrep Protein Extraction Kits

#### Cytoplasmic/Nuclear

- Enriches cytoplasmic and nuclear proteins
- Isolates nuclei, then extracts with a strongly chaotropic buffer

#### Membrane I

- Uses a quick and effective protocol that does not require density gradients or ultracentrifugation

#### Membrane II

- Isolates more complex membrane proteins than the membrane I kit

#### Signal

- Isolates membrane proteins involved in membrane trafficking and signaling

## Fractionation by Solubility/Hydrophobicity

Bio-Rad provides two options to reduce sample complexity using differential solubilization.

### ReadyPrep Sequential Extraction Kit

- Divides protein samples into 3 fractions by differential solubilization
- Uses the detergent SB 3-10 in a strong solubilizing solution

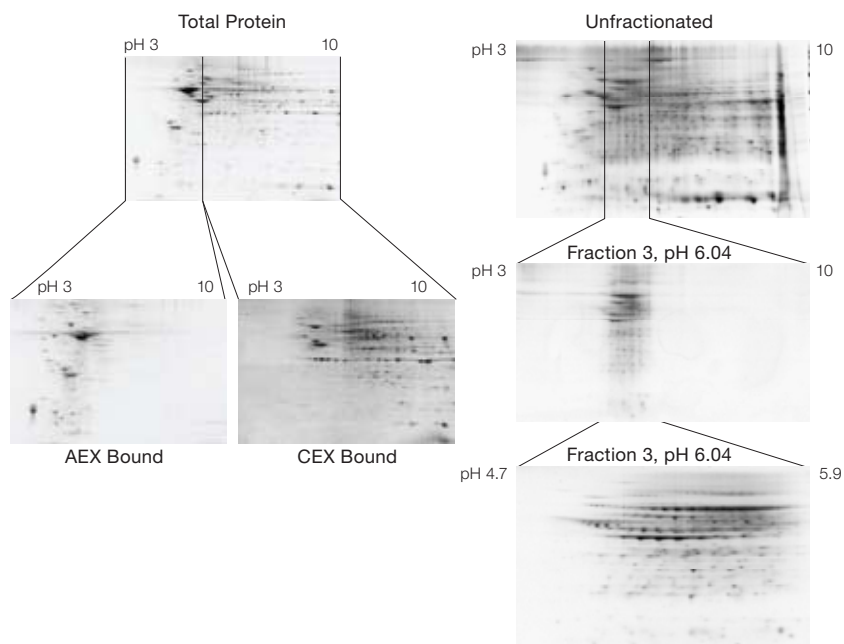
### ReadyPrep Protein Extraction Kit (Soluble/Insoluble)

- Divides protein samples into 2 fractions
- Uses the detergent ASB-14 (a stronger detergent than SB 3-10) in a strong solubilizing solution



**Fractionation of rat brain tissue using Aurum ion exchange mini columns (left image).** Rat brain total protein extracts (3 ml) were loaded onto an Aurum AEX or CEX mini column and eluted. The unfractionated and fractionated samples were then treated with the ReadyPrep reduction-alkylation and 2-D cleanup kits and separated by 2-D gel electrophoresis.

**Clean fractionation by pI (right image).** Mouse liver extract was fractionated using the MicroRotor cell. 2-D separations of the unfractionated sample (120 µg protein) and fraction 3 (20 µg protein) are shown. Prior to 2-D separation, samples were treated with the ReadyPrep 2-D cleanup kit. Note the clean pH boundaries of fraction 3 and the enrichment of proteins in the pH region it covers.



### Fractionation by Size

Fractionation by size (molecular weight) is an effective enrichment strategy for studies of protein families and posttranslational modifications because the sizes of these proteins tend to be similar.

#### Model 491 Prep Cell and Mini Prep Cell

- Fractionate by preparative continuous-elution electrophoresis
- Perform high-resolution separations
- Separate up to 500 mg of total protein
- Can be used as a complementary separation strategy to 2-D gels and for downstream protein purification

### Fractionation by Charge

Fractionation by charge allows separation of acidic and basic proteins.

#### Aurum AEX and CEX Mini Kits and Columns

- Fractionate acidic and basic proteins, respectively, by ion exchange chromatography
- Use a patented polymerization technology to achieve ultrahigh protein binding capacity
- Accommodate up to 1 ml of sample
- Use a quick, easy spin-column format

### Fractionation by pI

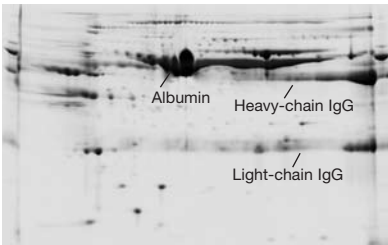
Fractionation by pI improves downstream sample separation on narrow- and micro-range IPG strips by eliminating proteins outside the pH region of interest.

#### Rotofor, Mini Rotofor, and MicroRotor Cells

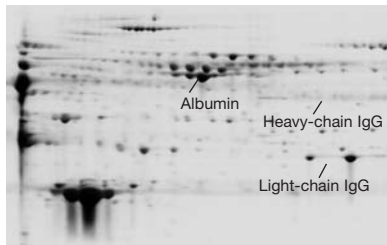
- Fractionate proteins by preparative liquid-phase isoelectric focusing
- Separate and concentrate proteins from 2.5–60 ml samples into 10–20 discrete fractions
- Separate up to 1 g of total protein
- Can be used as a complementary separation strategy to 2-D gels and for downstream protein purification
- Accommodate customizable pH gradients for enhanced resolution

## Depletion

Before

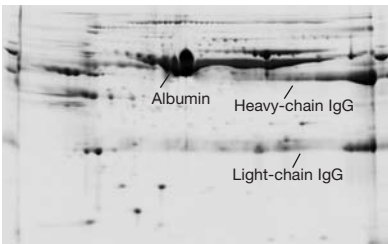


After

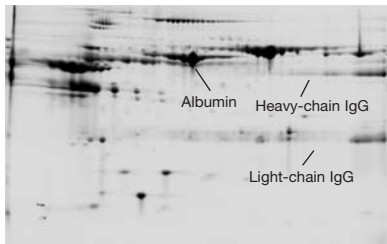


**Enrichment of low-abundance serum proteins with the ProteoMiner protein enrichment kit.** Resolution is dramatically improved on a gel with an equal amount of total protein from sample treated with ProteoMiner beads. The high-abundance proteins are greatly reduced and a greater number of protein spots are visualized. Samples (100 µg protein) were focused on 11 cm ReadyStrip pH 5–8 IPG strips, then run on Criterion 8–16% Tris-HCl precast gels for the second dimension.

Before



After



**Albumin and IgG removal from serum using the Aurum serum protein mini kit.** Serum proteins were electrophoresed before and after treatment with an Aurum serum protein mini column. Albumin and IgG are removed following treatment with an Aurum serum protein column, improving resolution of other protein spots. Samples (100 µg protein) were focused on 11 cm ReadyStrip pH 5–8 IPG strips, then run on Criterion 8–16% Tris-HCl precast gels for the second dimension.

### The Quest for Low-Abundance Proteins (continued)

In complex samples such as serum, high-abundance proteins (albumin, IgG, and others) obscure detection of less abundant ones. Removing high-abundance proteins leads to enrichment of proteins of lower abundance.

### Dynamic Range Reduction

ProteoMiner technology is based on the interaction of proteins with a large, highly diverse library of hexapeptides bound to chromatographic supports, which reduces all high-abundance proteins, while simultaneously capturing low-abundance proteins. This results in a dramatic level of low-abundance protein enrichment. ProteoMiner kits can be used with virtually any sample type.

#### ProteoMiner Protein Enrichment Kits

- Enrich medium- and low-abundance proteins
- Decrease the amount of all high-abundance proteins
- Are not dependent on a predefined set of antibodies, unlike immunodepletion products
- Minimize the potential co-depletion of proteins bound to high-abundance proteins

### Albumin/IgG Removal

The Aurum serum kits use affinity chromatography to reduce albumin and IgG, which improves resolution on 2-D gels.

#### Aurum Affi-Gel Blue and Aurum Serum Protein Mini Kits and Columns

- Easily and effectively remove albumin, or both albumin and IgG, by affinity chromatography
- Use a quick and easy spin-column format
- Provide eluted proteins ready for IEF analysis

## Related Products



Bio-Rad Protein Assay



Quick Start™ Bradford Protein Assay



DC™ Protein Assay



RC DC™ Protein Assay



SmartSpec™ Plus Spectrophotometer



trUView™ Cuvettes

## Protein Quantitation of IEF Samples

### Bio-Rad Protein Assay

This modified Bradford assay is recommended for determining the protein content in typical sample solutions used to load IPG strips. It can be performed in tubes, microtubes, or microtiter plates.

### Quick Start Bradford Protein Assay Protein Assay\*

This ready-to-use assay provides prediluted standards and 1x dye reagent for maximum convenience and ease-of-use.

### DC Protein Assay

The DC (detergent compatible) protein assay is a colorimetric assay for protein determination following detergent solubilization. This modified Lowry assay requires only a single 15 min incubation, and the absorbance readings are stable for about 2 hours.

### RC DC Protein Assay

The RC DC (reducing agent and detergent compatible) protein assay is a colorimetric assay for protein determination in the presence of reducing agents and detergents. The RC DC protein assay has all the unique features of the original DC protein assay, but improved to cover a broader range of reagents. Its expanded capabilities offer a simple, proven protocol for protein quantitation directly in complex reagent mixtures, such as Laemmli buffer and ReadyPrep reagents.

### SmartSpec Plus Spectrophotometer

This UV/visible spectrophotometer features a working wavelength range of 200–800 nm. It has preprogrammed methods for protein quantitation using the Bradford, Lowry, and BCA methods.

### trUView Cuvettes

These disposable cuvettes are suitable for most UV and visible spectroscopic assays. They feature a low volume requirement ( $\geq 50 \mu\text{l}$ ) that conserves limited samples.

\* Like the Bio-Rad protein assay, this assay is based on the classic Bradford method.

## Support



### Application Support

Bio-Rad's expression proteomics experts offer field support to customers worldwide. Each specialist has a solid understanding of the technology and research experience that will help you find solutions to your experimental needs.

### Sales Support

Bio-Rad's trained, knowledgeable customer support staff operates worldwide. They can help you choose the best system to fit your particular needs.

For more information, contact your local Bio-Rad sales representative or visit us on the Web at [www.expressionproteomics.com](http://www.expressionproteomics.com)

### Global Technical Support

Bio-Rad has over 30 years of experience in 2-D technology. Our worldwide technical support staff is highly trained and can advise you on how to obtain good results. They can help with troubleshooting or with advice on suitable tools for sample preparation or other expression proteomics technologies.

### Research and Development

Bio-Rad's expression proteomics R&D team develops ideas into reliable research tools. By continuing to make 2-D electrophoresis a more reproducible and robust technology, R&D helps customers to focus on research, rather than perfecting techniques.

**BIO-RAD**

**Bio-Rad  
Laboratories, Inc.**

Life Science  
Group

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**Spain** 34 91 590 5200 **Sweden** 08 555 12700 **Switzerland** 061 717 95 55 **Taiwan** 886 2 2578 7189 **United Kingdom** 020 8328 2000