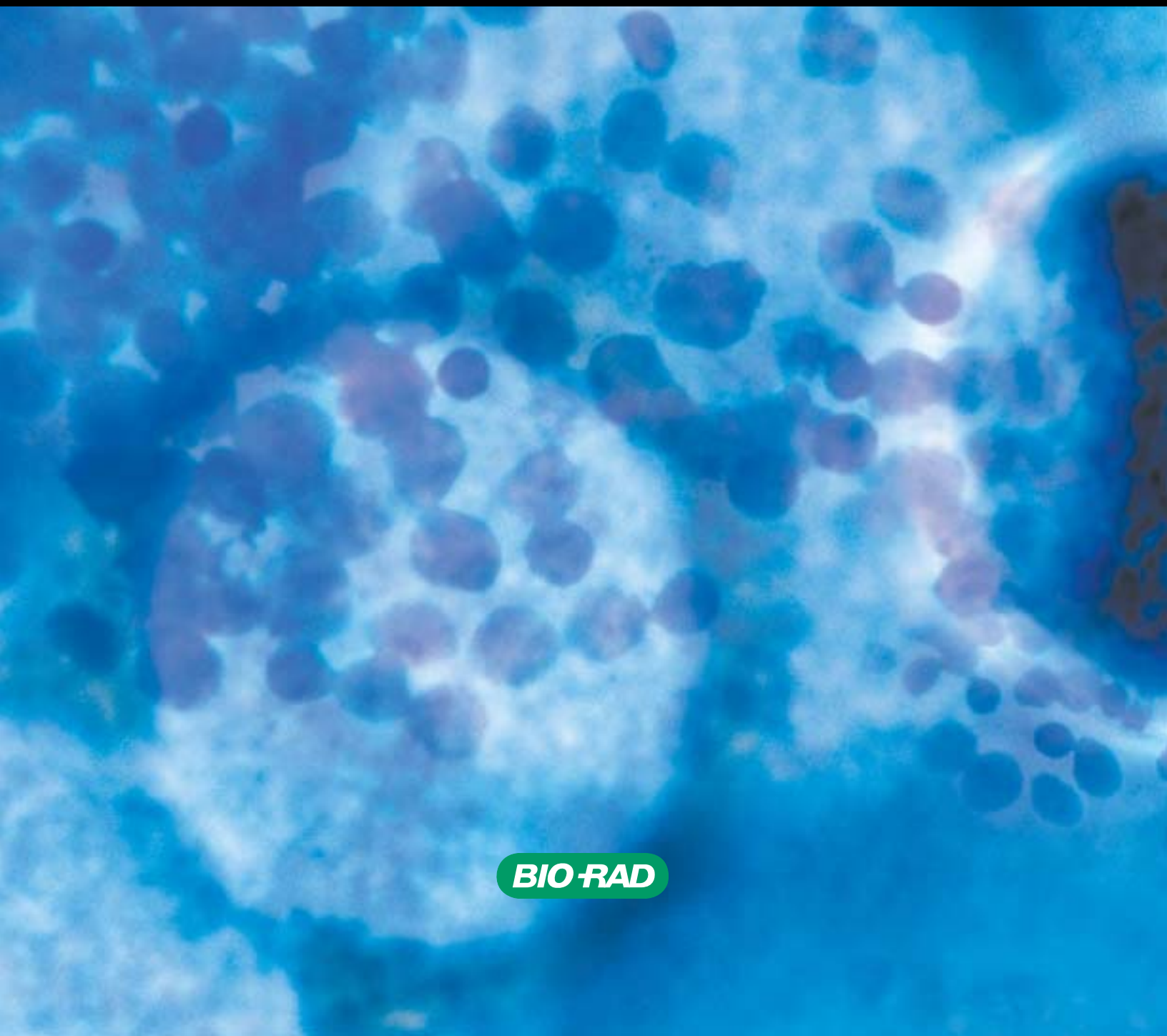
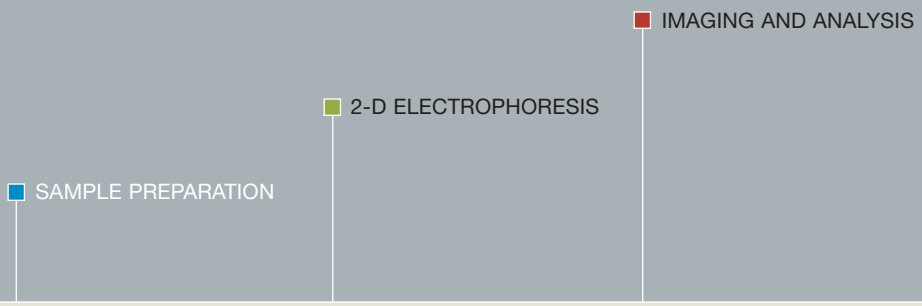
Sample Preparation

Tools for Protein Sample Cleanup and Fractionation



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. Sample preparation prior to 2-D electrophoresis is critical for producing meaningful, reproducible results. Contaminants, such as salts and detergents, must be removed from samples in order to ensure successful separation. Fractionation of a protein mixture is essential to reduce sample complexity, for example when analysis of low-abundance proteins is required. No sample preparation protocol is universally suitable; each experiment poses unique challenges due to the nature and variety of sample sources. Bio-Rad has developed a number of sample preparation products for general-purpose sample cleanup and for sample fractionation.

Chemical Reagents — A Robust, Cost-Effective Approach

Chemical reagents take advantage of differential solubility to separate protein mixtures. Combining detergents and other chemicals at appropriate temperatures provides solubility conditions that precipitate a protein of interest or selectively precipitate unwanted proteins or contaminants. The ReadyPrep™ line offers a variety of kits for sample preparation, and requires no hardware or instrumentation, making it an easy approach to try with minimal time investment.

Chromatography — Convenient One-Step Methods

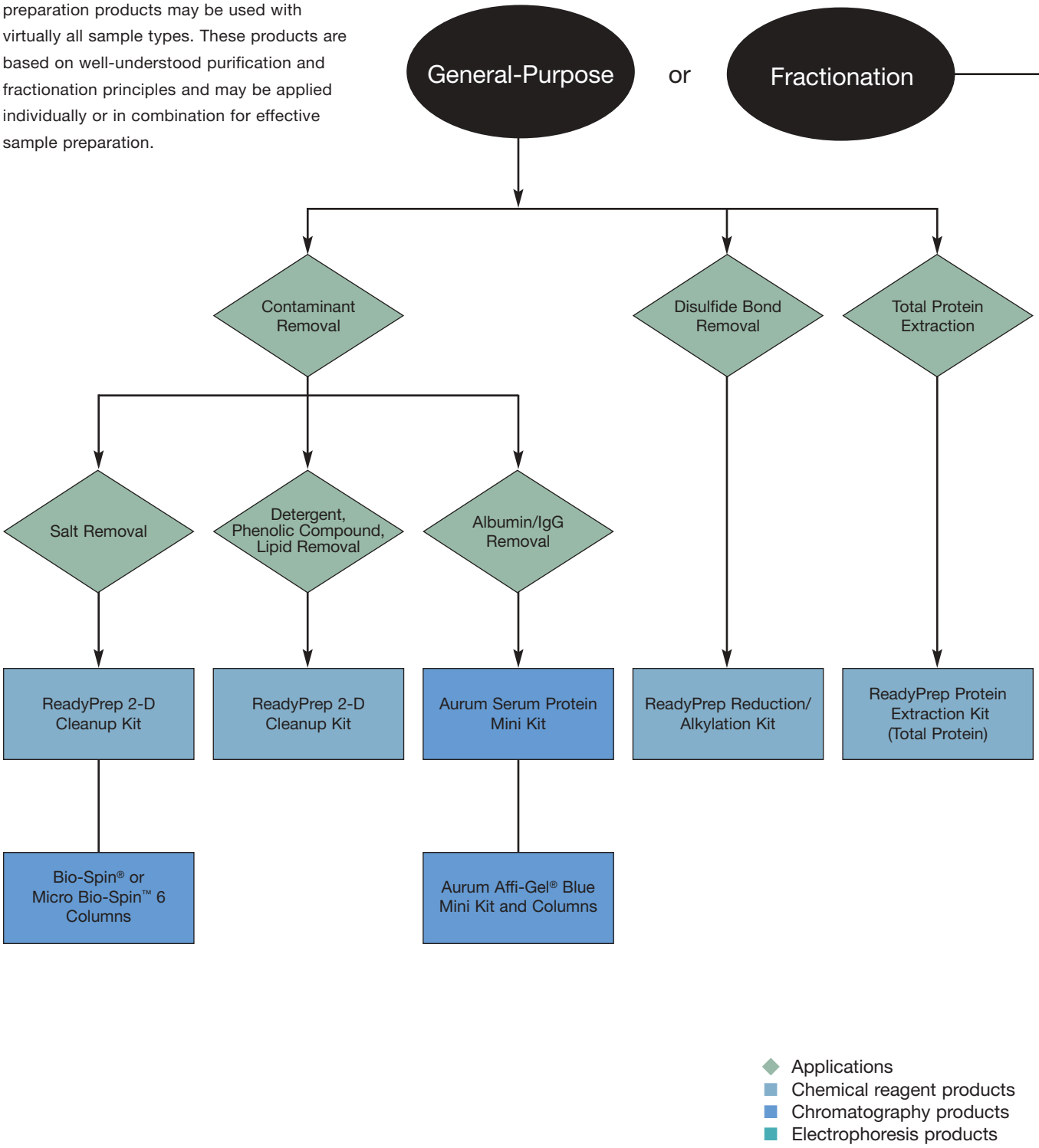
Chromatographic methods separate, enrich, and purify proteins from complex samples by exploiting the differential binding properties of proteins to solid supports. Ion exchange, affinity, and size exclusion methods may be used alone or in combination to remove contaminants and reduce complexity of a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

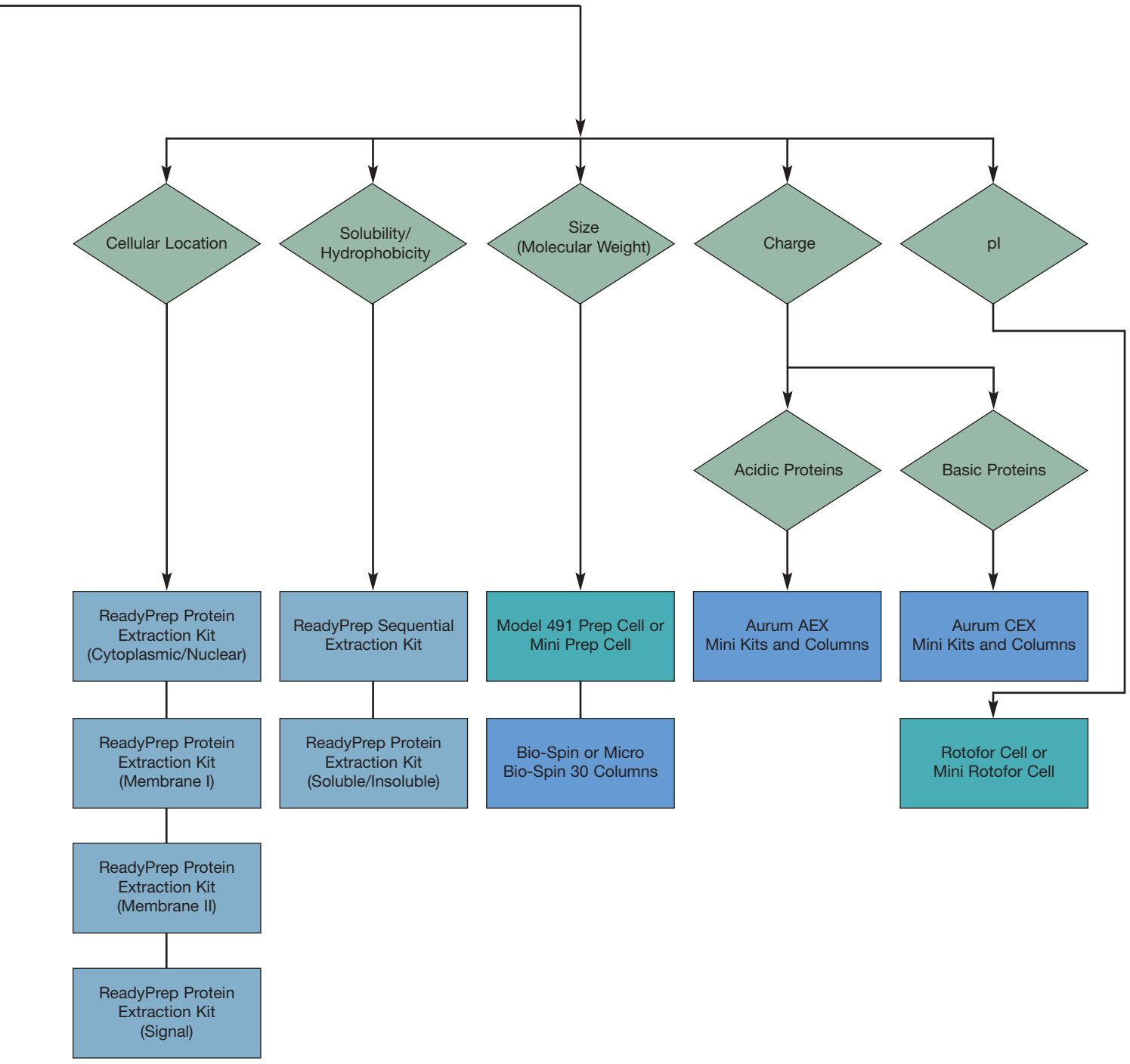
Electrophoresis — Effective Tools for Enrichment of Low-Abundance Proteins

Electrophoretic tools fractionate proteins according to their isoelectric point (pI) or molecular weight. The Rotofor® and mini Rotofor 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.

Product Selection Guide

Bio-Rad's comprehensive suite of sample preparation products. Bio-Rad's sample preparation products may be used with virtually all sample types. These products are based on well-understood purification and fractionation principles and may be applied individually or in combination for effective sample preparation.

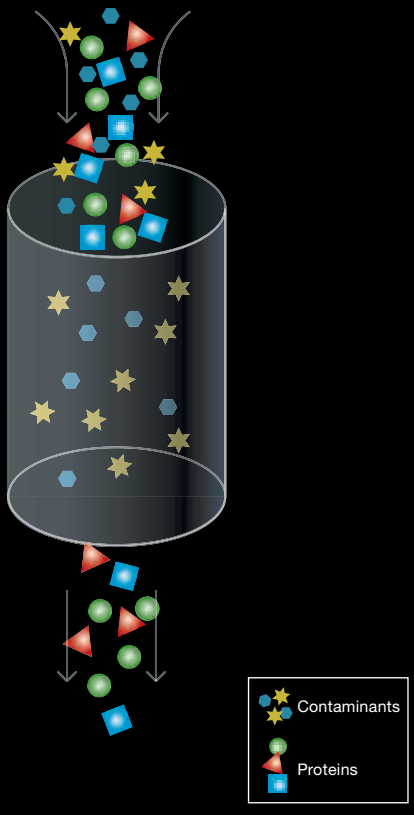



Cellular Location
Solubility/ Hydrophobicity
Size (Molecular Weight)
Charge
pI
Acidic Proteins
Basic Proteins
ReadyPrep Protein Extraction Kit (Cytoplasmic/Nuclear)
ReadyPrep Protein Extraction Kit (Membrane I)
ReadyPrep Protein Extraction Kit (Membrane II)
ReadyPrep Protein Extraction Kit (Signal)
ReadyPrep Sequential Extraction Kit
ReadyPrep Protein Extraction Kit (Soluble/Insoluble)
Model 491 Prep Cell or Mini Prep Cell
Bio-Spin or Micro Bio-Spin 30 Columns
Aurum AEX Mini Kits and Columns
Aurum CEX Mini Kits and Columns
Rotofor Cell or Mini Rotofor Cell

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

General-Purpose Cleanup

Success of any protein analysis depends on sample purity. Contaminants, such as salts, detergents, ionic compounds, and even high-abundance proteins, can ruin a 2-D experiment by interfering with protein separation or masking signals from proteins of interest. It is crucial to eliminate these contaminants prior to analysis. Bio-Rad provides a variety of effective general-purpose cleanup options to ensure high-quality results.



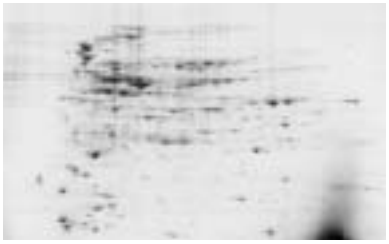
Fractionation

Fractionation can greatly improve visualization of low-abundance proteins by removing other proteins that might mask their detection. Fractionation reduces overall sample complexity and enriches samples for the proteins of interest. Bio-Rad has developed a comprehensive suite of approaches for fractionating complex protein mixtures.

General-Purpose

Before

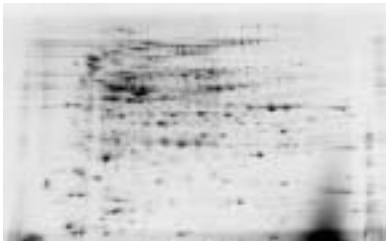
After



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 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

General-purpose cleanup may provide the only sample preparation steps you need to ensure good resolution and limit variability on your 2-D gels.

Salt Removal

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

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 µl of sample

ReadyPrep 2-D Cleanup Kit

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

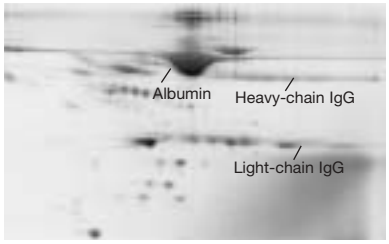
Detergent, Lipid, and Phenolic Compound Removal

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

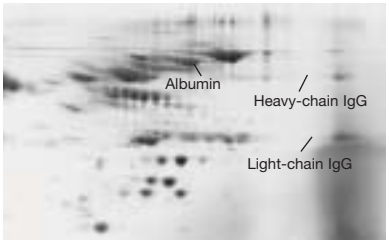
ReadyPrep 2-D Cleanup Kit

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

Before

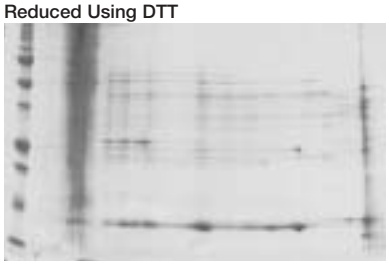


After



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. Samples (200 µg of protein) were focused on 11 cm ReadyStrip pH 3–10 IPG strips, then run on Criterion 8–16% Tris-HCl precast gels for the second dimension.

Reduced Using DTT



Reduced Using ReadyPrep Kit

Disulfide bond removal using the ReadyPrep reduction-alkylation kit. Protein samples were reduced either using 50 mM DTT in rehydration/sample buffer or by treatment with 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.

Albumin and IgG Removal

Proteomic analysis of serum presents its own unique challenges. Albumin and IgG contribute up to 90% of the total protein in a serum sample. These proteins obscure lower-abundance proteins and limit loading capacity on 2-D gels. To obtain meaningful results, these proteins must be removed prior to downstream separation or analysis.

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
- Increase sample load of other proteins 3- to 4-fold
- Use a quick and easy spin-column format
- Provide eluted proteins ready for IEF analysis

Disulfide Bond Removal

Disulfide bond removal 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

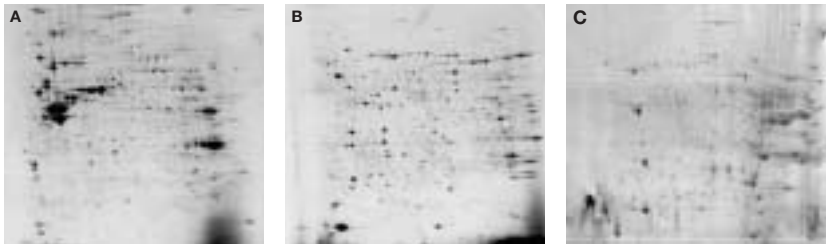
Total Protein Extraction

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

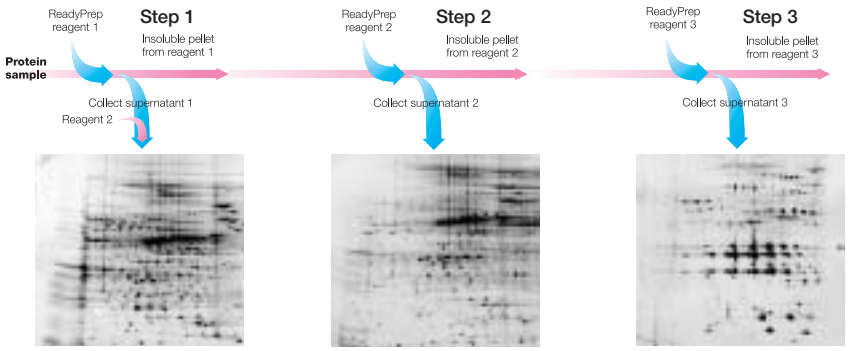
ReadyPrep Protein Extraction Kit (Total Protein)

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

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 protein (~450 µg) was 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 in addition to the general-purpose kits to uncover proteins of lower abundance or specific proteins of interest.

Fractionation by Cellular Location

Bio-Rad offers a variety of choices for fractionation by cellular location.

ReadyPrep Protein Extraction Kit (Cytoplasmic/Nuclear)

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

ReadyPrep Protein Extraction Kit (Membrane I)

- Uses a quick and effective protocol
- Does not require ultracentrifugation or preparation of density gradients

ReadyPrep Protein Extraction Kit (Membrane II)

- Isolates more complex membrane proteins than the membrane I kit

ReadyPrep Protein Extraction Kit (Signal)

- Specifically isolates membrane proteins involved in intracellular membrane trafficking and signaling

Fractionation by Differential Solubility

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 the strongest 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 the strongest solubilizing solution

Fractionation by Protein 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 protein
- Can be used as a complementary separation strategy to 2-D gels and for downstream protein purification
- Are effective for enrichment of low-abundance proteins

Bio-Spin and Micro Bio-Spin 30 Columns

- Separate proteins <40 kD by size exclusion chromatography
- Accommodate up to 100 µl of sample
- Use a quick, easy spin-column format

Fractionation by Protein 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

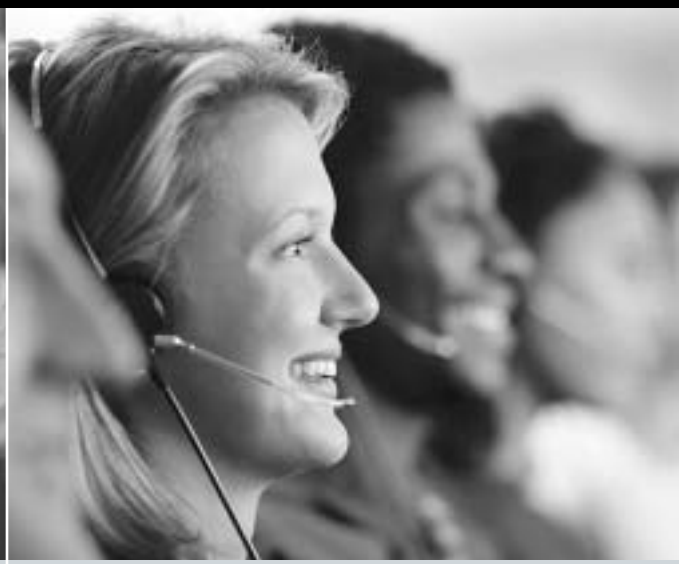
Fractionation by Protein pI

Protein fractionation by pI is an effective enrichment strategy that improves downstream sample loading and separation on narrow- and micro-range IPG strips by eliminating proteins outside the pH region of interest.

Rotofor and Mini Rotofor Cells

- Fractionate proteins by preparative liquid-phase isoelectric focusing
- Separate and concentrate proteins into 20 discrete fractions
- Separate up to 1 g of protein
- Can be used as a complementary separation strategy to 2-D gels and for downstream protein purification
- Are particularly effective for enrichment of low-abundance proteins

Support



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.

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**

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