Strategies for Proteomics Sample Preparation

Two-dimensional (2-D) gel electrophoresis is a popular and proven separation technique for proteome analysis. The 2-D procedure is straightforward: Proteins are first separated according to their isoelectric point (pI) by isoelectric focusing (IEF) and then by their molecular weight by SDS-PAGE. For most researchers, 2-D gel electrophoresis is easy to learn, because advances in immobilized pH gradient (IPG) technology have eliminated the need for tricky and tedious IEF in ampholyte gel gradients. Nevertheless, problems with smearing, streaking, and poor resolution and reproducibility tend to leave researchers dissatisfied with the results of 2-D experiments. These common complaints are often due to improper sample preparation.

One of the most undervalued aspects of the 2-D process, sample preparation prior to the first-dimension IEF separation contributes significantly to the overall reproducibility and accuracy of protein expression analysis. Some important considerations include:

- Care must be taken to prevent proteolysis during protein extraction, and proteins must be solubilized in a buffer that is compatible with IEF
- Contaminants such as salts and detergents must be removed to ensure successful separation
- Fractionation is essential to reduce protein sample complexity when analysis of subpopulations or low-abundance proteins is required

Without proper sample preparation, protein precipitation, gel streaking, and overall poor resolution are often the unfortunate result.

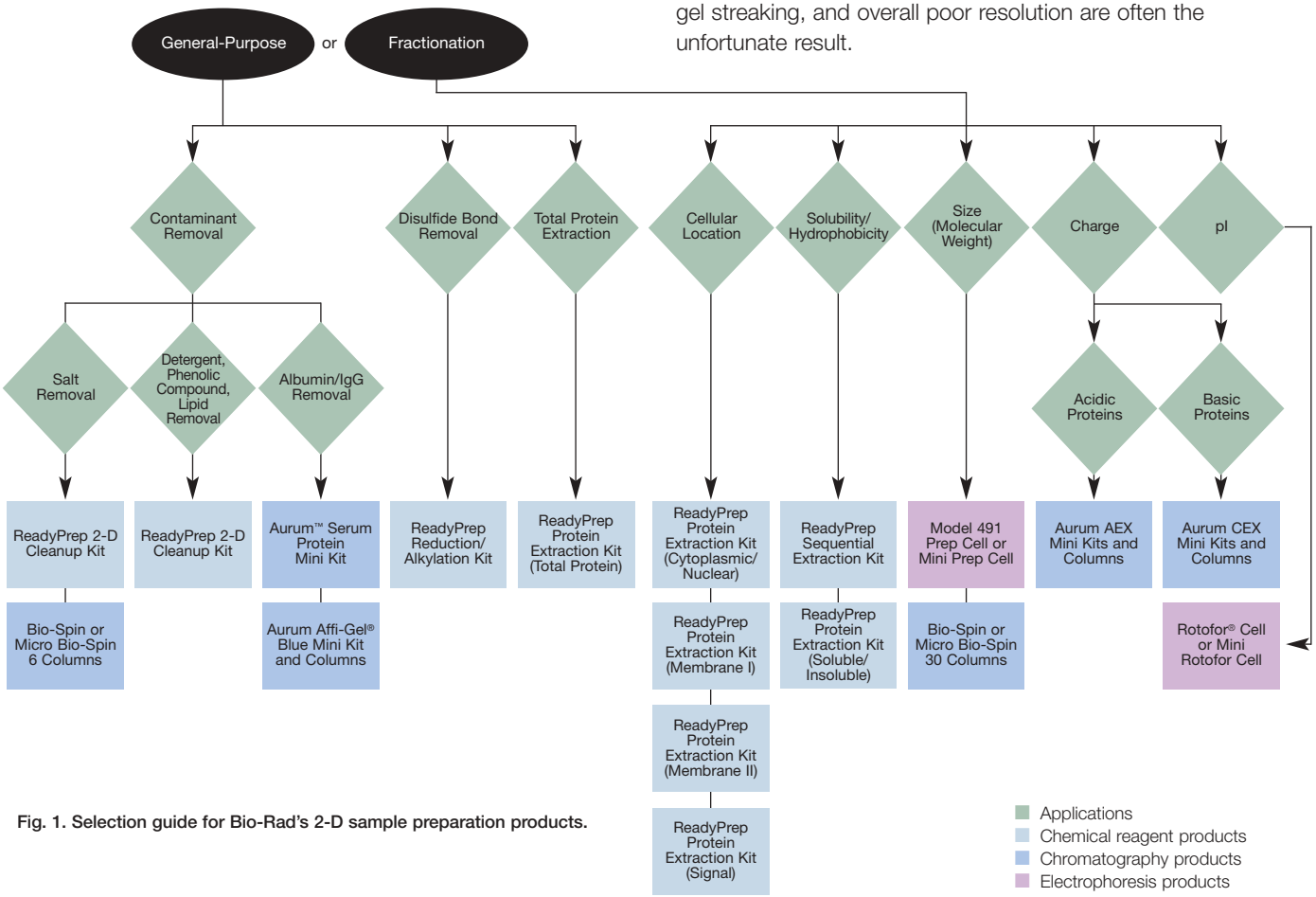

Fig. 1. Selection guide for Bio-Rad's 2-D sample preparation products.

BIO-RAD

Considering the incredible diversity of proteins and protein sample sources, today's proteomics researchers require a variety of protein separation and purification tools that can accommodate varying cleanup, solubilization, and fractionation needs. With this in mind, Bio-Rad has applied its expertise in solution chemistry, chromatography, and electrophoresis to develop the most comprehensive suite of products available for general-purpose sample cleanup and sample fractionation (Figure 1).

General-Purpose Cleanup: Enhancing Resolution of 2-D Gels

Bio-Rad offers a number of prepackaged kits for convenient and effective contaminant removal, disulfide bond reduction, and total protein extraction. These general-purpose kits may be used with all types of sample sources to enhance resolution and improve reproducibility of 2-D gel experiments. Kits are available for the applications described below.

Contaminant Removal

Salts, detergents, phenolic compounds, nucleic acids, and other contaminants are often present in cellular extracts. These compounds interfere with the establishment of the high voltage required for effective first-dimension separation on IPG strips. The result is streaking and poor separation (Figure 2).

The ReadyPrep™ 2-D cleanup kit uses a TCA-like precipitation process to quantitatively concentrate proteins while washing away ionic detergents, salts, lipids, etc., all of which interfere with IEF (Figure 2).

Micro Bio-Spin™ and Bio-Spin® 6 columns separate salts and other low molecular weight contaminants from proteins quickly and easily using gel filtration in a convenient spin format.

Serum Samples

Proteomic analysis of serum presents its own unique challenges. Albumins and immunoglobulin (IgG) contribute up to 90% of the total protein in a serum sample. Together, these proteins obscure other, less-abundant proteins and limit loading capacity on 2-D gels. In order to obtain meaningful results from serum samples, these proteins must be removed.

The Aurum Affi-Gel Blue and Aurum serum protein mini kits use affinity chromatography to easily and effectively remove albumin (Affi-Gel Blue) or albumin and IgG (serum protein kit).

Disulfide Bond Removal

During protein extraction, reducing agents help to solubilize proteins by breaking and preventing re-formation of disulfide bonds. During IEF, these reducing agents tend to migrate off the IPG strip, allowing disulfide bridges to re-form between proteins. The result is streaking and other random spot patterns, particularly in the alkaline regions of the IPG strip.

The ReadyPrep reduction-alkylation kit eliminates the potential for disulfide bond formation during IEF by first reducing the disulfide bonds with tributylphosphine (TBP) and then alkylating the sulfhydryl groups with iodoacetamide prior to IEF separation. This produces a 2-D pattern with more spots, fewer streaks, and greater reproducibility.

Total Protein Extraction

A standardized extraction protocol is useful for initial proteomic analyses and for consistent sample preparation.

The ReadyPrep protein extraction kit (total protein) uses the powerful zwitterionic detergent ASB-14 in a strongly chaotropic solubilization buffer to prepare total cellular protein extracts suitable for 2-D gel analysis. During protein extraction, contaminants are effectively removed from the sample.

Fractionation: The Quest for Low-Abundance Proteins

Fractionation can greatly improve visualization of low-abundance proteins. Through fractionation, overall sample complexity is reduced and samples are enriched for proteins of interest. Bio-Rad offers a comprehensive collection of approaches for fractionating complex protein mixtures.

Fractionation by Cellular Location

Proteins of interest may be in known subcellular locations, for example, in membranes, in the nucleus, or in the cytoplasm. Various combinations of extraction reagents, detergents, and temperatures may be used to fractionate and enrich samples for proteins of interest in these locations.

Three ReadyPrep kits have been designed for isolating integral membrane and membrane-associated proteins, and one kit for isolating nuclear and cytoplasmic proteins. Because the solution chemistry underlying each kit is different, each kit produces fractions with slightly different protein compositions (Figure 3).

Before

After

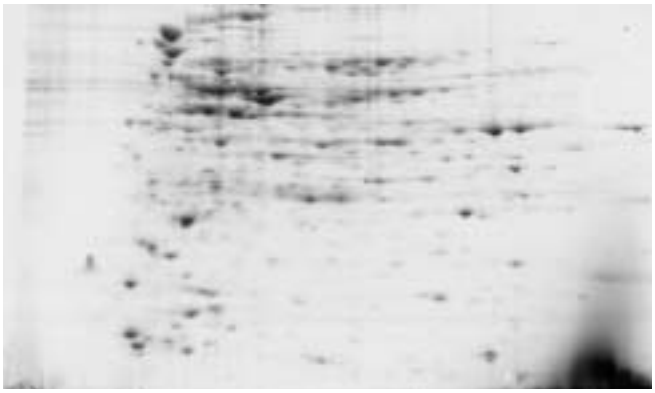

Fig. 2. Removal of high salt concentrations from samples using the ReadyPrep 2-D cleanup kit. *E. coli* extracts containing 1 M NaCl before (left) and after (right) removal of salts.

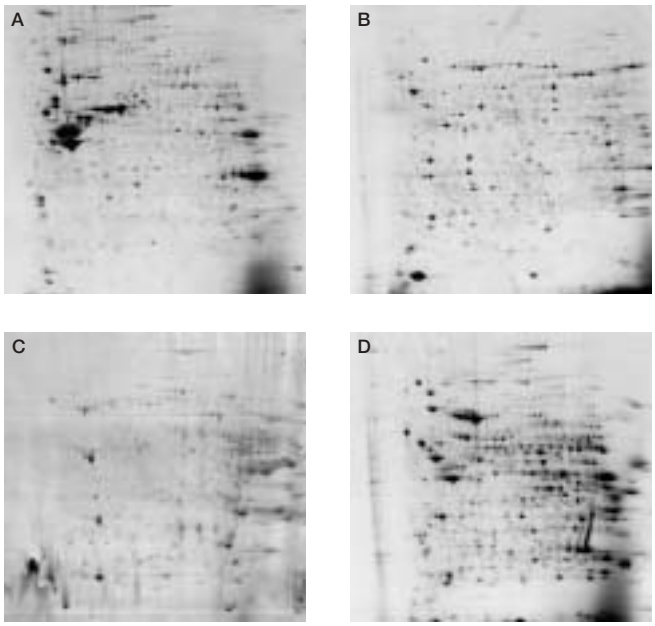

Fig. 3. Differences in 2-D patterns obtained using ReadyPrep signal (A), membrane I (B), membrane II (C), and total protein (D) kits. Mouse liver samples were extracted using the recommended protocols for each kit. Purified protein (~450 µg) was loaded onto 17 cm (A, B, C) or 24 cm (D) pH 3–10 nonlinear ReadyStrip™ IPG strips. Overall spot patterns differ for A, B, and C, even though all three kits isolate membrane proteins. Also observed is an enrichment of certain proteins in the membrane fractions that is not readily visible in the total protein extract (D).

The ReadyPrep protein extraction kits (membrane I and membrane II) use different solubilization techniques to isolate integral membrane and membrane-associated proteins without the preparation of density gradients. The membrane I kit is a quick and effective protocol for isolating most membrane proteins without the need for ultracentrifugation. More complex membrane proteins (those with larger numbers of transmembrane domains) are better isolated with the membrane II protocol, which does require ultracentrifugation. For an example of use of the ReadyPrep membrane I kit, request bulletin 3103.

The ReadyPrep protein extraction kit (signal) takes advantage of the limited solubility of plasma membrane microdomain structures (for example, lipid rafts and caveolae) in nonionic detergents at 4°C to yield a protein pellet that is enriched in membrane-associated signaling proteins, including GPI-anchored proteins, caveolin and associated proteins, acetylated tyrosine kinases, and G proteins.

The ReadyPrep protein extraction kit (cytoplasmic/nuclear) uses a specially formulated buffer and differential centrifugation to isolate intact nuclei and a strongly chaotropic extraction buffer to quickly prepare highly enriched fractions of cytoplasmic and nuclear proteins from eukaryotic samples.

Fractionation by Differential Protein Solubility

The ReadyPrep sequential extraction kit and the ReadyPrep protein extraction kit (soluble/insoluble) both fractionate proteins on the basis of their solubility in detergents. Because they use different sets of detergents, the two kits may be used independently or sequentially for even greater resolution.

Fractionation by Size (Molecular Weight)

Size-dependent separation is a powerful fractionation strategy in studies focused on a particular protein or protein family and all their posttranslational modifications because these proteins tend to be of similar molecular weight (for more information, request bulletin RP0026).

The Model 491 prep cell and mini prep cell perform size-dependent, high-resolution fractionation of proteins by continuous-elution gel electrophoresis (using native PAGE or SDS-PAGE). The large sample capacity (ranging from 50 µl to 15 ml and from 0.5 to 500 mg protein) of these cells makes them particularly effective tools for the enrichment of low-abundance proteins.

The Bio-Spin and Micro Bio-Spin 30 columns use prepacked Bio-Gel® P-30 resin to separate proteins >40 kD from small sample volumes (≤100 µl) by size exclusion chromatography.

Fractionation by Protein Charge

The Aurum AEX (anion exchange) and CEX (cation exchange) mini kits and columns employ ion exchange chromatography in an easy-to-use spin column format for fractionating and concentrating acidic and basic proteins from small sample volumes (<1 ml). Micro Bio-Spin 6 columns are included for salt removal from the fractionated samples.

Fractionation by pI

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

The Rotofor and mini Rotofor cells separate and concentrate proteins into 20 fractions according to pI by liquid-phase IEF. This unique separation method can also be coupled to analytical or preparative SDS-PAGE for a powerful, complementary first-dimension separation and enrichment strategy for high molecular weight, membrane, hydrophobic, or other proteins that are often underrepresented in IPG-based 2-D gels. The large sample capacity (ranging from 18 to 60 ml and from microgram to gram quantities of protein) of these cells makes them powerful tools for the enrichment of low-abundance proteins.

Summary

Regardless of the sample source, Bio-Rad's comprehensive suite of sample preparation products optimize 2-D gel-based analysis. These products are based on known purification and fractionation principles and may be applied individually or in combination for effective sample preparation. Initially, general-purpose cleanup may be the only step required to ensure good resolution and limit sample-to-sample variability on 2-D gels. As investigations progress, various fractionation tools may be used in addition to the general-purpose kits to uncover proteins of lower abundance or proteins of particular interest.

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *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 84-0* ***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*

Bulletin 3145 US/EG Rev A *04-0403 0604 Sig 1103*