

SAMPLE PREPARATION

ReadyPrep™ Sample Preparation Kits

Expression Proteomics // Tools for Protein Separation and Analysis

- **Minimize streaking on 2-D gels**
- **Elimination of salts, detergents, and disulfide bond complexes**
- **Optimized protocols for fractionation or general-purpose applications**
- **Multiple options for flexibility in 2-D sample preparation**

A Family of Tools for 2-D Electrophoresis

Bio-Rad Expression Proteomics

Bio-Rad's approach to expression proteomics focuses on three technologies: sample preparation, two-dimensional (2-D) electrophoresis, and imaging and analysis. Each technology area is ideally suited to a 2-D gel electrophoresis approach to analysis of sample proteins. By providing choices in methodology, protocols, and products, Bio-Rad's expression proteomics tools help you to achieve optimal results in any 2-D experiment.

Solutions to Your Sample Preparation Needs

One of the most critical steps in 2-D electrophoresis is the sample preparation step, which occurs even before the first-dimension separation. No sample preparation protocol is universally suitable; each experiment offers unique challenges due to the variety and complexity of sample sources. Many samples contain impurities that cause streaking and spurious spots. Some samples require fractionation to enrich for low-abundance proteins. Bio-Rad offers a comprehensive suite of products available for general-purpose sample cleanup and sample fractionation. As part of Bio-Rad's sample preparation solutions, the ReadyPrep kits provide tested tools and proven protocols, enabling reproducible 2-D results.

General-Purpose Sample Preparation

General-purpose sample preparation applies to most protein samples. It involves the general isolation of total protein or the removal of common contaminating agents, such as salts, detergents, peptides, lipids, and phenolic compounds, which can cause problematic streaking and smearing on 2-D gels. The ReadyPrep line of products offers a selection of kits for this purpose.

Sample Fractionation

For effective 2-D analysis, it may be necessary to reduce the complexity of the protein sample. Reduced sample complexity is especially important when analysis of low-abundance proteins is the goal. Ideally, any method employed should be simple, reproducible, and applicable to a wide variety of cell types. It should result in a protein sample of low conductivity free of substances that interfere with 2-D analysis. Bio-Rad offers several approaches for convenient and efficient extraction of cellular proteins into discrete, more manageable fractions enriched in certain classes of proteins, such as cytosolic, nuclear, and membrane proteins.



BIO-RAD

Total Protein Isolation

Total protein isolation is the first step in many protein studies.

The ReadyPrep protein extraction kit (total protein) provides a simple, rapid, and reproducible method to prepare total cellular protein suitable for 2-D analysis.

Remove Contaminants

Smearing on 2-D gels can be reduced by removing salts, detergents, peptides, lipids, and phenolic compounds (Figure 1). The ReadyPrep 2-D cleanup kit facilitates preparation of low-conductivity samples suitable for IEF and 2-D. Additionally, the kit uses a TCA-like precipitation protocol that can help concentrate dilute samples, allowing higher protein loads and therefore improved spot detection. This cleanup protocol is quantitative and can be completed in under an hour.

Reduce Smearing of Basic Proteins on Gels

Reduction of disulfide bonds is required for meaningful alkaline 2-D results. The reduction-alkylation kit irreversibly treats the protein sample prior to IEF so that disulfide bonds cannot re-form. This treatment helps to prevent spurious spot formation and enables transfer of higher amounts of protein from an IPG strip to an SDS-PAGE gel (Candiano et al. 2002). Figure 2 shows an example of improved separation using this kit.

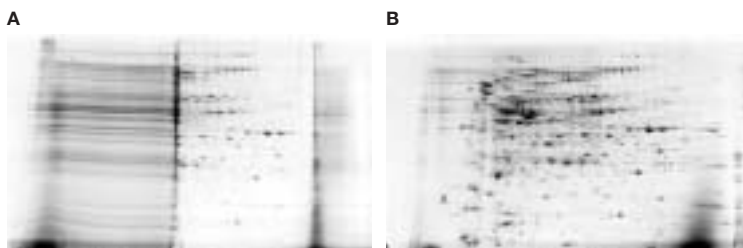


Fig. 1. Effective detergent removal by the ReadyPrep 2-D cleanup kit leads to reduced smearing. *E. coli* extracts were spiked with 1% SDS. A, untreated sample; B, sample treated with the ReadyPrep kit.

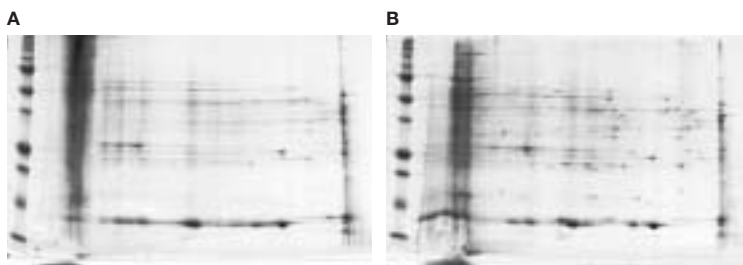


Fig. 2. Improved visualization of basic proteins from mouse liver extract after treatment with the ReadyPrep reduction-alkylation kit. Both samples were applied by cup loading onto 11 cm ReadyStrip™ pH 7–10 IPG strips. A, reduced with 50 mM DTT in rehydration/sample buffer; B, treated with the ReadyPrep kit.

Reduce Sample Complexity by Fractionation

Reduction of sample complexity through sample fractionation is key to identifying low-abundance proteins. Fractionation based on known cellular location is one strategy for reducing sample complexity. Figures 3 and 4 illustrate the enrichment of certain proteins based on known cellular location. However, if the cellular location of the protein of interest is not known, or the goal is to view the whole proteome, fractionation based on differential solubility is the best approach.

Three Kits for Membrane-Protein Enrichment

Sample-dependent optimization is a fundamental strategy for any protein analysis. Bio-Rad offers three ReadyPrep protein extraction kits that selectively isolate membrane proteins. The ReadyPrep protein extraction kits (membrane I and II) provide options for proteins of varying complexity. The membrane I kit uses a quick and effective protocol for isolating membrane proteins. More complex membrane proteins (e.g., multiple transmembrane domains) are better isolated with the membrane II protocol. The ReadyPrep protein extraction kit (signal) is specific for isolating proteins involved in intracellular membrane trafficking and signaling pathways. These include proteins such as GPI-anchored proteins, caveolin (and associated proteins), acylated tyrosine kinases, and G proteins.

Two Kits for Whole-Proteome Fractionation

The ReadyPrep sequential extraction kit and the ReadyPrep protein extraction kit (soluble/insoluble) both reduce sample complexity using differential solubilization. The sequential extraction kit isolates three different fractions of increasing solubility. These fractions are isolated sequentially, allowing the visualization of proteins that might not otherwise be seen. Stronger detergents are used for each subsequent fraction to increase solubilizing power. The solution with the strongest solubilizing power is reagent 3, containing the detergent SB 3-10. In contrast, the ReadyPrep protein extraction kit (soluble/insoluble) has a single fractionation step and uses the detergent ASB-14, a stronger detergent than SB 3-10. The two kits can be used independently, or the soluble/insoluble kit can be used to create a fourth fraction for even better resolution.

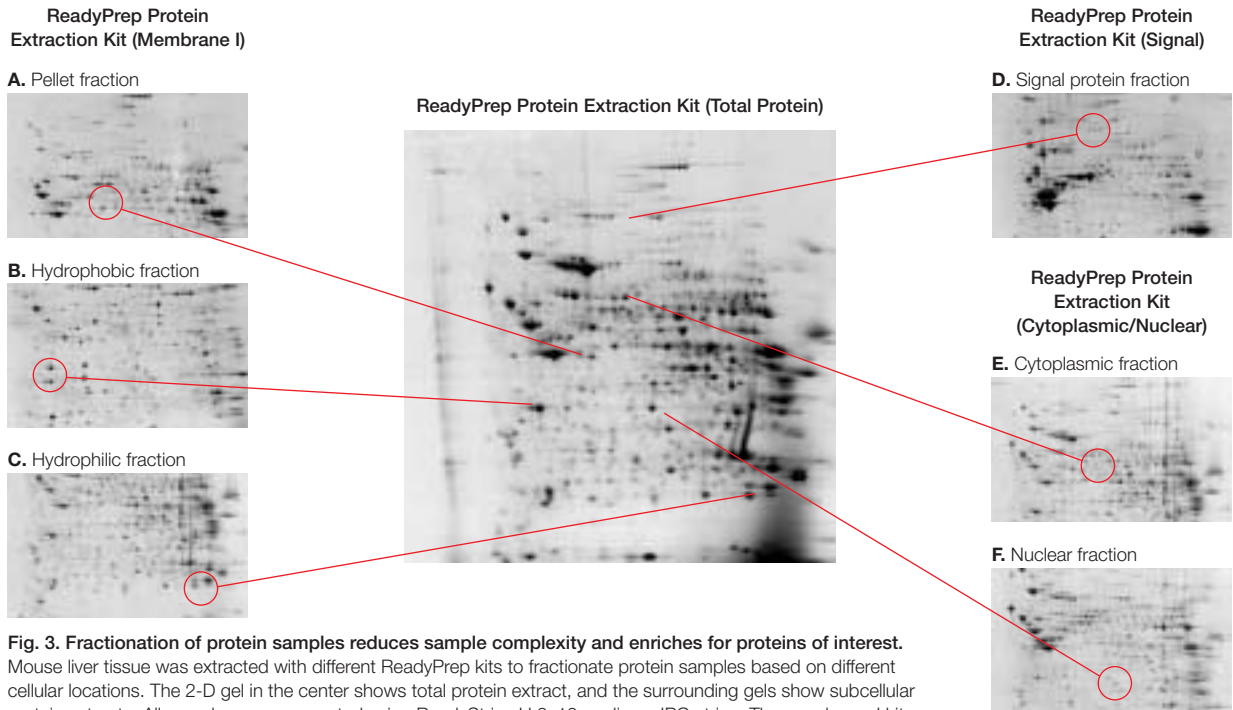


Fig. 3. Fractionation of protein samples reduces sample complexity and enriches for proteins of interest. Mouse liver tissue was extracted with different ReadyPrep kits to fractionate protein samples based on different cellular locations. The 2-D gel in the center shows total protein extract, and the surrounding gels show subcellular protein extracts. All samples were separated using ReadyStrip pH 3–10 nonlinear IPG strips. The membrane I kit generates three fractions (A, B, and C), the signal kit generates 1 fraction (D), and the cytoplasmic/nuclear kit generates two fractions (E and F). Enlarged regions of interest for those fractions are shown below in Figure 4.

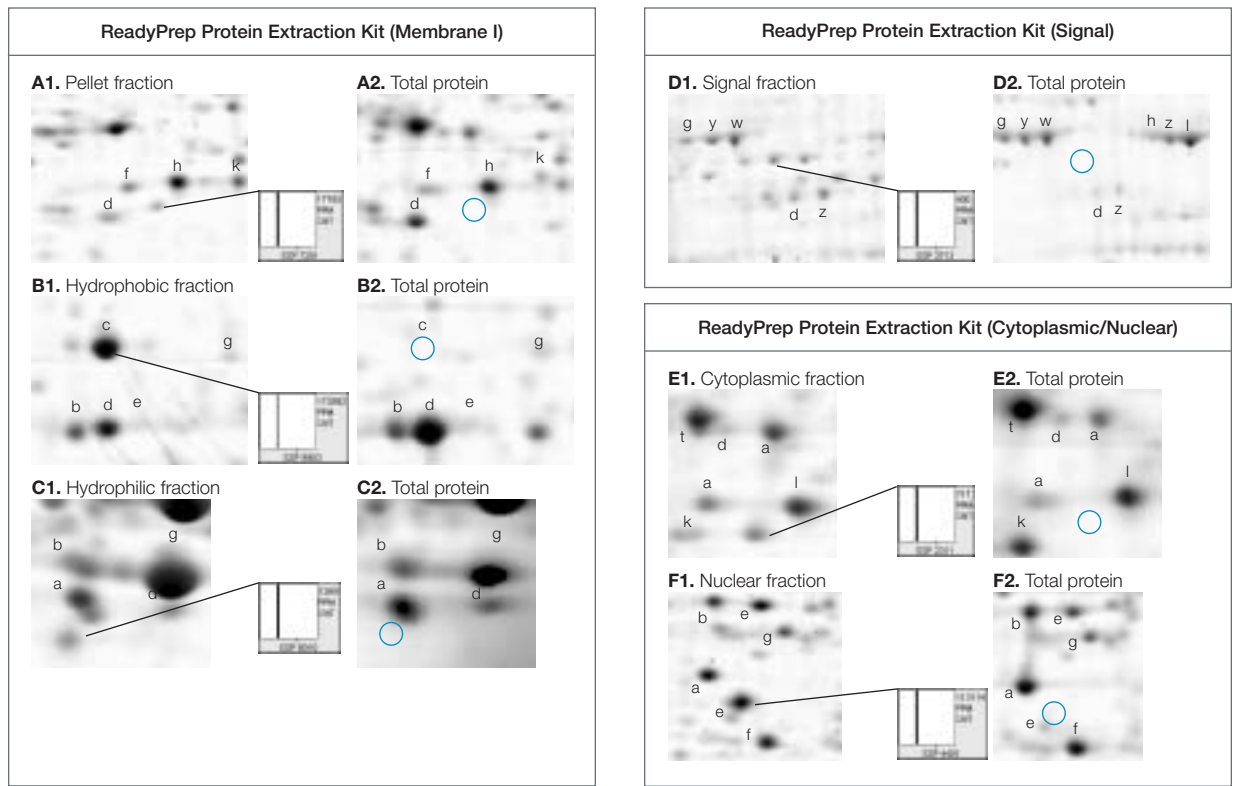


Fig. 4. Magnified gel regions show the presence of proteins in fractionated samples that were not detectable in the total protein sample (blue circles). Gel images were analyzed using PDQuest™ 2-D analysis software version 7.3. Enriched protein spots are indicated by quantity graphs showing presence of protein spots (full bar in the graph) versus no spot detected (no bar) in the comparable region of the total protein extract. Matched spots serving as landmarks are indicated by letters.

A Family of Sample Preparation Tools for 2-D Electrophoresis

Whether you need to improve spot resolution or enrich for specific proteins, ReadyPrep kits offer convenient, effective choices to meet those needs. For information on other 2-D sample preparation methods, such as abundant serum protein removal or large-volume sample preparation options, request bulletin 3096.

Worldwide Technical Support

Bio-Rad has a technical support network to address your 2-D sample preparation needs. In most parts of the world Bio-Rad provides field application specialists who troubleshoot and train on-site, as well as effective and knowledgeable technical telephone support. Bio-Rad wants to help make you successful in your research.

Ordering Information

Catalog # Description

General-Purpose Kits

- 163-2130 ReadyPrep 2-D Cleanup Kit, 50 preps, includes 15 ml precipitation agent 1, 15 ml precipitation agent 2, 2 ml wash reagent 1, 50 ml wash reagent 2, 0.25 ml wash 2 additive, instructions
- 163-2090 ReadyPrep Reduction Alkylation Kit, 100 preps, includes 2 ml alkylation buffer, 3 x 0.6 ml TBP reducing agent, 3 empty TBP storage vials, 5 x 56 mg iodoacetamide, instructions
- 163-2086 ReadyPrep Protein Extraction Kit (Total Protein), 20 preps, includes 2 x 10 ml ReadyPrep 2-D rehydration/sample buffer 1, 0.6 ml 200 mM TBP reducing agent, empty vial, instructions

Fractionation Kits

- 163-2089 ReadyPrep Protein Extraction Kit (Cytoplasmic/Nuclear), 50 preps, includes 50 ml cytoplasmic protein extraction buffer (CPEB), 25 g protein solubilization buffer (PSB), 30 ml PSB diluent, ReadyPrep 2-D cleanup kit, instructions
- 163-2088 ReadyPrep Protein Extraction Kit (Membrane I), 50 preps, includes 50 ml membrane protein extraction buffer 1 (M1), 50 ml membrane protein extraction buffer 2 (M2), 25 g protein solubilization buffer (PSB), 30 ml PSB diluent, ReadyPrep 2-D cleanup kit, instructions
- 163-2087 ReadyPrep Protein Extraction Kit (Signal), 50 preps, includes 50 ml signal protein extraction buffer 1 (S1), 50 ml signal protein extraction buffer 2 (S2), 25 g protein solubilization buffer (PSB), 30 ml PSB diluent, instructions
- 163-2085 ReadyPrep Protein Extraction Kit (Soluble/Insoluble), 20 preps, includes 2 x 10 ml ReadyPrep 2-D rehydration/sample buffer 1, 0.6 ml 200 mM TBP reducing agent, empty vial, 5 pouches lysis buffer, instructions

Catalog # Description

- 163-2084 ReadyPrep Protein Extraction Kit (Membrane II), 10 preps, includes 2 x 10 ml ReadyPrep 2-D rehydration/sample buffer 1, 0.6 ml 200 mM TBP reducing agent, empty vial, 5 pouches lysis buffer, 5 pouches membrane-protein concentrating reagent, instructions
- 163-2100 ReadyPrep Sequential Extraction Kit, 15 preps, includes 50 ml reagent 1, 3 x 10 ml reagent 2, 2 x 10 ml reagent 3, 0.6 ml 200 mM TBP reducing agent, empty vial, instructions

Kit Components and Related Products

- 163-2101 Tributylphosphine (TBP), 200 mM, 0.6 ml
- 163-2102 ReadyPrep Sequential Extraction Kit Reagent 1, 50 ml, 40 mM Tris base
- 163-2103 ReadyPrep Sequential Extraction Kit Reagent 2, 10 ml, 8 M urea, 4% CHAPS, 40 mM Tris, 0.2% Bio-Lyte® 3/10 ampholyte
- 163-2104 ReadyPrep Sequential Extraction Kit Reagent 3, 10 ml, 5 M urea, 2 M thiourea, 2% CHAPS, 2% SB 3-10, 40 mM Tris, 0.2% Bio-Lyte 3/10 ampholyte
- 163-2091 ReadyPrep Proteomics Grade Water, 500 ml
- 163-2083 ReadyPrep 2-D Rehydration/Sample Buffer 1, 10 ml, 7 M urea, 2 M thiourea, 1% ASB-14, 40 mM Tris
- 163-2106 ReadyPrep 2-D Starter Kit Rehydration/Sample Buffer, 10 ml, 8 M urea, 2% CHAPS, 50 mM DTT, 0.2% Bio-Lyte 3/10 ampholyte, 0.001% Bromophenol Blue
- 500-0121 RC DC™ Protein Assay Kit I, 500 standard assays, bovine γ -globulin standard
- 500-0122 RC DC Protein Assay Kit II, 500 standard assays, bovine serum albumin standard

Reference

Candiano G et al., Two-dimensional maps in soft immobilized pH gradient gels: a new approach to the proteome of the Third Millennium, *Electrophoresis* 23, 292-297 (2002)

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