Sample Prep/2-D Electrophoresis

ReadyPrep™ Sequential Extraction Kit

Part of the ProteomeWorks™ System

- **Efficient**
  Novel solubilizing and reducing agents
- **Innovative**
  Provides a third dimension of separation by protein solubility
- **Reproducible**
  Standardized reagents for reproducible separations
- **Convenient**
  Premixed reagents

Improve Analysis and Uncover Low-Abundance, Low-Solubility Proteins on 2-D Gels

Technique Overview

Sample preparation technique is one of the more difficult aspects of protein characterization and is a significant factor affecting 2-D gel quality.

The ReadyPrep sequential extraction kit is the first commercially available ready-to-use extraction kit based upon the differential solubility of proteins. The use of this ReadyPrep kit produces 2-D arrays in which the entire proteome is better represented, due to more complete solubilization of proteins. Moreover, the standardization of both protocol and reagents results in 2-D arrays that are reproducible run to run.

Expect These Results

- More total protein brought into solution through innovative solubilizing and reducing reagents, including thiourea, the detergent SB 3-10, and tributylphosphine (TBP)
- More protein spots overall are visualized per sample
- Reproducible sample prep for more consistent 2-D gels
- Each gel will contain a less complex 2-D pattern, resulting in easier analysis

These results are achieved because the protein sample is extracted successively by three reagents. The insoluble pellet from each step is solubilized by the next reagent (see Figure 1). Each fraction is then separated by 2-D gel electrophoresis.

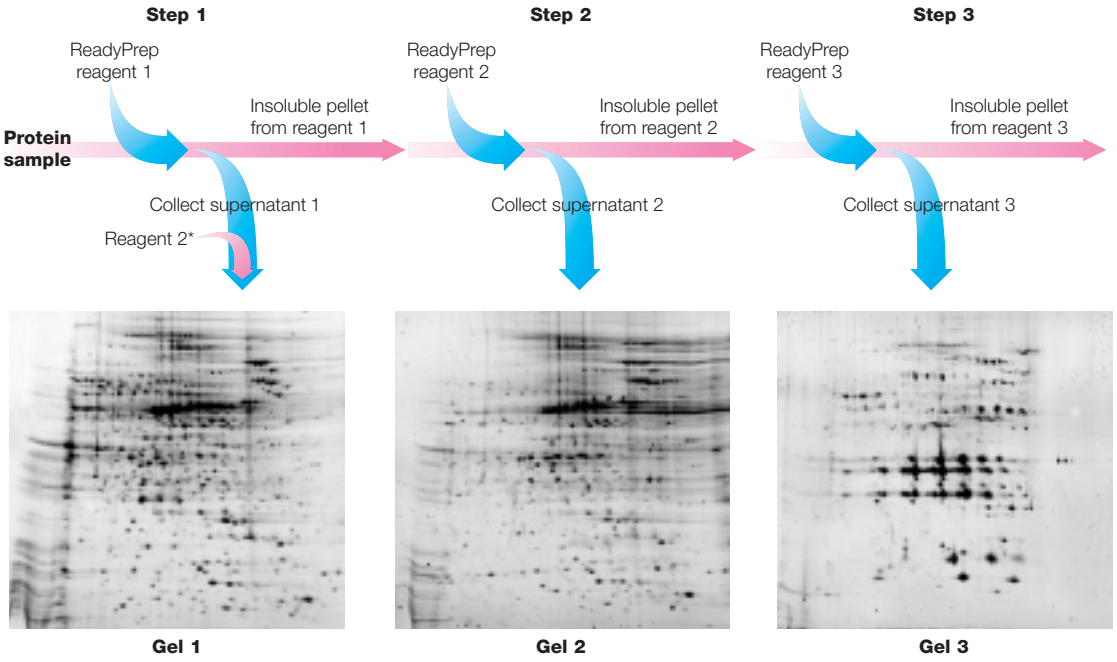

Fig. 1. ReadyPrep reagents distribute proteins into three different fractions based on differential solubility, and provide increased resolution of proteins in the resultant gels (Molloy et al. 1998).

*Supernatant 1 is diluted with reagent 2 for gel analysis.

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Efficient Protocol

The ReadyPrep sequential extraction kit utilizes three aggressive solubilizing reagents* and a novel reducing agent (tributylphosphine, or TBP). These allow the extraction of larger numbers of proteins and a 2-D separation that includes rare and low-abundance proteins (see Figure 2).

If desired, reagent 3 (the strongest solubilizing reagent) can be used to solubilize the entire sample at once. With this method, more proteins are resolved in the gel than with traditional reagents; however, the resulting 2-D pattern will also be more complex.

* Reagent 1 contains 40 mM Tris base; reagent 2 contains 8 M urea, 4% CHAPS, 40 mM Tris, and 0.2% Bio-Lyte® 3/10 ampholyte; reagent 3 contains 5 M urea, 2 M thiourea, 2% CHAPS, 2% SB 3-10, 40 mM Tris, and 0.2% Bio-Lyte 3/10 ampholyte.

Reference

Molloy MP et al., Extraction of membrane proteins by differential solubilization for separation using two-dimensional gel electrophoresis, Electrophoresis, 19, 837–844 (1998)

2-D gel electrophoresis has become an increasingly important tool in the emerging field of proteomics. In support of this new field, Bio-Rad has developed the integrated ProteomeWorks system, which provides the tools necessary for all aspects of 2-D/proteome research — from sample preparation and IEF to image analysis and protein identification. For more information, visit our ProteomeWorks web site at **ProteomeWorksSystem.com**

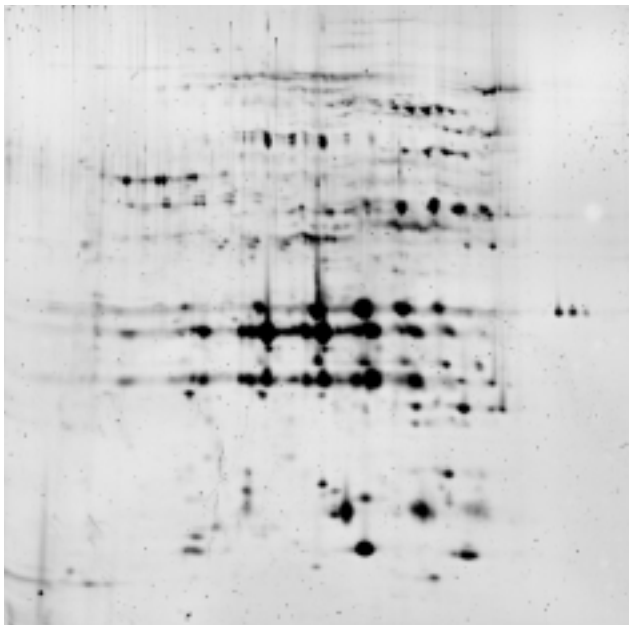

Fig. 2. Low-abundance, low-solubility proteins from *E. coli* become visible after the third extraction.

Ordering Information

Catalog #	Description
163-2100	ReadyPrep Sequential Extraction Kit, 5–15 preps
163-2101	Tributylphosphine (TBP), 200 mM, 0.6 ml
163-2102	ReadyPrep Sequential Extraction Kit Reagent 1, 1 vial
163-2103	ReadyPrep Sequential Extraction Kit Reagent 2, 1 vial
163-2104	ReadyPrep Sequential Extraction Kit Reagent 3, 1 vial

ReadyPrep reagents are premixed and are stable at room temperature.

The ProteomeWorks system is the global alliance between Bio-Rad Laboratories, Inc. and Waters Corporation (Micromass MS Technologies), dedicated to furthering proteomics research.

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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 21 507 6191* ***Canada*** *(905) 712-2771* ***Czech Republic*** *+ 420 2 41 43 05 32* ***China*** *(86-21) 63052255* ***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* ***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*** *52 5 534 2552 to 54* ***The Netherlands*** *0318-540666* ***New Zealand*** *64 9 415 2280* ***Norway*** *23 38 41 30* ***Poland*** *+ 48 22 8126 672* ***Portugal*** *351-21-472-7700* ***Russia*** *7 095 721 1404* ***Singapore*** *65-2729877* ***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*

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