ReadyPrep™ Protein Extraction Kit (Total Protein)

Instruction Manual

Catalog #163-2086

For technical service, call your local Bio-Rad office, or in the US, call 1-800-4BIORAD (1-800-424-6723)

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Section 1
Introduction

The ReadyPrep protein extraction kit (Total Protein) is designed as a simple, rapid and reproducible method to prepare total cellular protein extracts suitable for 2-D gel analysis. The strongly chaotropic extraction solution used in the kit contains the zwitterionic detergent ASB-14, making this solution one of the most powerful solubilizing reagents available for 2-D electrophoresis. The extraction process solubilizes many types of cell proteins, including membrane proteins, and the extraction protocol can be applied to a wide variety of biological samples, from animal cells and tissues to yeast, bacteria and plant tissues. At the end of the extraction process, the protein sample can be applied onto Immobilized pH gradient strips (IPGs) and subjected to isoelectric focusing and 2-D gel separation.

Section 2
Kit Specifications

Each ReadyPrep protein extraction kit (Total Protein) provides sufficient reagents to perform up to 20 extractions each starting with 50–100 mg of cells or tissue. The procedure can easily be scaled up to accommodate larger amounts of cells or tissue. The entire procedure can be completed in about 45 min.

Items Supplied With Kit

- **2-D Rehydration/Sample Buffer 1.** Two vials. Lyophilized. After reconstituting, each vial contains 10 ml of 7 M urea, 2 M thiourea, 1 % (w/v) ASB-14 detergent, 40 mM Tris base, and 0.001% Bromophenol Blue.
- **TBP Reducing Agent.** 1 ampoule containing 0.6 ml of 200 mM tributylphosphine (TBP) in 1-methyl-2-pyrrolidinone sealed under nitrogen gas.
- **Empty Vial.** 1 storage vial for TBP reducing agent.
- **Instruction Manual,** 1.

Items Required But Not Provided

- 2.0 ml microcentrifuge tubes (for example, VWR catalog #20170-237).
- Disposable round-bottom tubes for plant sample extraction (for example, VWR catalog #60818-725).

- ReadyPrep 2-D cleanup kit (Bio-Rad catalog #163-2130) for plant sample extraction. When working with plant leaf tissue, removal of interfering compounds, such as phenolics, is essential for high quality 2-D gels that are free of horizontal streaks.
- Microcentrifuge capable of spinning at 12–16,000 x g while maintaining a temperature between 18–20°C.
- Sonicator
- Protease inhibitor(s) (optional)
- Carrier ampholytes (for example, Bio-Lyte® 3/10 ampholyte, Bio-Rad catalog #163-2094)
- ReadyPrep proteomic grade water (Bio-Rad catalog #163-2091).
- *RC DC*™ Protein Assay (Bio-Rad catalog #500-0121 or #500-0122)

Section 3
Storage Conditions

Store the unopened kit at room temperature. After opening, unused reconstituted ReadyPrep 2-D Rehydration/Sample Buffer 1 should be aliquoted in 1 to 2 ml volumes and stored frozen at –80°C. After opening, transfer the ReadyPrep TBP reducing agent to the empty glass vial provided and store the vial at –20°C to prevent evaporation of the TBP.

Section 4
Reagent Preparation

ReadyPrep 2-D Rehydration/Sample Buffer 1: Add 5.6 ml of ReadyPrep proteomic grade water or similar quality water to one bottle. Swirl the vial gently until the contents are completely dissolved. The solution can be warmed slightly in the palm of the hand or in a water bath to speed the dissolution process. DO NOT heat the solution above 25 to 30°C to avoid the formation of cyanates. Cyanates can react with and modify the proteins in the sample.

ReadyPrep TBP Reducing Agent. Tributylphosphine (TBP) has an unpleasant odor and is very volatile. Work with TBP in a fume hood. Wear a laboratory coat and gloves when handling the ampoule of TBP reducing agent. Wipe up spills with wet towels. Open the ampoule by snapping the top off at the scored neck. Transfer the entire contents of the ampoule to the empty screw-cap storage vial provided. Screw the cap of the vial down tightly and store the vial at –20°C to prevent evaporation of the TBP. While using, keep the vial of TBP reducing agent on ice.

Section 5
Instructions for Use

Extraction of 50–100 mg of sample.

1. Immediately before performing an extraction, complete the preparation of 2-D Rehydration/Sample Buffer 1. Prepare only enough complete 2-D rehydration/sample buffer 1 for the number of extractions being performed. Each extraction will require 1.0 ml of the solution. The complete buffer is prepared by adding 10 µl of ReadyPrep TBP reducing agent and the appropriate ampholyte to 0.2 % (w/v) final concentrate to every 1 ml of reconstituted 2-D Rehydration/Sample Buffer 1. The ampholyte is chosen to match the pH range of the IPG strip to be used for 2-D analysis, though for most applications, Bio-Rad's Bio-Lyte 3/10 ampholyte (catalog #163-2094) can be used. Other additions, such as protease inhibitors can also be made at this time.

2. In a 2.0 ml microcentrifuge tube add 1 ml of complete 2-D Rehydration/Sample Buffer 1 (see Step 1) per 50–100 mg of animal tissue or 0.05 ml of wet cell pellet from sources such as cell culture, yeast, or bacteria. For plant tissue add 2–3 ml of 2-D Rehydration/Sample Buffer 1 per each gram of tissue in, for example, a disposable 14 ml round-bottom tube. The sample-to-buffer volume ratio indicated above is only a guide and may be adjusted depending upon the desired scale of the preparation and type of sample used.

   Insufficient volume of 2-D Rehydration/Sample Buffer 1 may result in poor cell lysis and incomplete solubilization of all protein types.

   Plant tissue should be ground to a fine powder using a mortar and pestle in liquid nitrogen before addition of 2-D Rehydration/Sample Buffer 1.

3. Place the sample on ice and sonicate the suspension with an ultrasonic probe to disrupt the cells and fragment the genomic DNA. Sonicate the sample using 30 sec bursts, typically 3–4 times, or until lysis is complete. Chill the suspension on ice briefly between each ultrasonic treatment. If necessary, transfer the extract to a microcentrifuge tube when this step is complete.

Note: If using sonication, care must be exercised to prevent heating of the sample. The temperature of the sample should not be allowed to rise above 30°C. Similarly, if the sample becomes too cold, precipitation of the urea and thiourea can occur. If this happens, gently warm the sample until the precipitate dissolves before proceeding further.

Note: Disruption of cells by sonication is dependent on the cell type. For example, *E. coli* requires longer sonication times than animal cells and tissues. Yeast cell disruption requires even more vigorous sonication The addition of glass beads or use of a Bead Beater (BioSpec Products) can greatly improve cell lysis of these sample types.

4. Centrifuge the tube at maximum speed in a microcentrifuge (~16,000 x g) for 20–30 min at 18–20°C to pellet cell debris.

5. Remove and transfer the supernatant to a clean tube and discard any insoluble pellet.

6. The sample is now ready to be loaded onto IPG strips. Unused extract should be stored in aliquots at –80°C.

   Note when working with plant leaf samples: for best 2-D separation results treat the sample using the ReadyPrep 2-D Cleanup Kit (Bio-Rad catalog #163-2130) prior to performing IEF.

7. Determine the protein concentration of the sample. The Bio-Rad *RC DC* Protein Assay (catalog #500-0121 or #500-0122) is recommended for this measurement. This assay allows for accurate protein quantitation in the presence of detergents, reducing agents, and other substances that typically interfere with other protein assays.

8. An appropriate dilution of the protein extract in 2-D rehydration/sample buffer 1 containing TBP reducing agent and ampholytes may be needed before IEF/2-D gel analysis. Refer to **Section 6** for guidelines on selecting the appropriate volume to use. Additional ReadyPrep 2-D rehydration/sample buffer 1 is available and sold separately for this purpose (see **Section 7**).

Section 6
Appendix

2-D Rehydration/Sample Buffer 1 Volume

Before IEF and 2D gel electrophoresis, the protein sample may need to be diluted to achieve the desired protein load for the chosen stain. To best determine the volume of diluent to use, consider the IPG strip length, the pH gradient of the IPG strip, and the staining or detection method. To assist with these calculations, the table that follows indicates appropriate volumes of 2-D rehydration/sample buffer needed to rehydrate IPG strips of specific lengths and the approximate amounts of protein required for detection using silver stain or Coomassie Blue G-250 stain.

IPG strip length	**7 cm**	**11 cm**	**17 cm**	**18 cm**	**24 cm**
Rehydration volume per strip	125 µl	185 µl	300 µl	315 µl	410 µl
Protein load–Silver stain	5–20 µg	20–50 µg	50–80 µg	50–80 µg	80–150 µg
Protein load–Coomassie G-250	50–100 µg	100–200 µg	200–400 µg	200–400 µg	400–800 µg

Section 7
Product Information

Catalog #	Description

Sample Preparation Kits

163-2086	ReadyPrep Protein Extraction Kit (Total Protein), 20 preps
163-2085	ReadyPrep Protein Extraction Kit (Soluble/Insoluble), 20 preps
163-2084	ReadyPrep Protein Extraction Kit (Membrane II), 10 preps
163-2130	ReadyPrep 2-D Cleanup Kit, 50 preps
163-2089	ReadyPrep Protein Extraction Kit (Cytoplasmic/Nuclear), 50 preps
163-2088	ReadyPrep Protein Extraction Kit (Membrane I), 50 preps
163-2087	ReadyPrep Protein Extraction Kit (Signal), 50 preps
163-2090	ReadyPrep Reduction-Alkylation Kit, 50 preps
163-2100	ReadyPrep Sequential Extraction Kit, 5–15 preps

Rehydration/Sample Buffers

163-2083	ReadyPrep 2-D Rehydration/Sample Buffer 1, 10 ml, containing 7 M urea, 2M thiourea, 1% ASB-14, 40 mM Tris base, and 0.001% Bromophenol Blue

Protein Quantitation Kits (also see bulletin 2610)

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

Buffer Components

163-2101	Tributylphosphine (TBP), 200 mM, 0.6 ml
163-2094	100X Bio-Lyte 3/10 Ampholyte, 1 ml
163-2091	ReadyPrep Proteomic Grade Water

Bio-Rad Laboratories, Inc.
2000 Alfred Nobel Dr.
Hercules, CA 94547 USA
(510) 741-1000

1-800-424-6723

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