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# Product Information

**Protein Extraction Reagent Type 2**

Catalog Number **C0606**
Store at Room Temperature

## TECHNICAL BULLETIN

### Product Description

Protein Extraction Reagent Type 2 is designed to solubilize many types of proteins during sample preparation. The solubilized proteins can be analyzed with two-dimensional (2D) gel electrophoresis, which separates proteins by their isoelectric point (pI) in the first dimension and molecular mass in the second dimension.[1] This reagent is one of the traditional combinations available for 2D electrophoresis and contains zwitterionic detergents. To improve the separation of proteins and reduce artifacts on an isoelectric focusing (IEF) gel, it is also suggested to use both tributylphosphine (Catalog Number T7567) and iodoacetamide (Catalog Number A3221) to reduce and alkylate disulfide bonds, respectively, prior to 2D analysis.

### Components

The product is supplied as a convenient, dry powder blend in 4 separate bottles. Upon reconstitution with water, each bottle of Protein Extraction Reagent Type 2 will contain 25 mL of the reagent. The final solution contains 5.0 M urea, 2.0 M thiourea, 40 mM Trizma® base, 2.0% CHAPS, and 2.0% SB3-10, pH 10.1.

### Reagents and Equipment Required But Not Provided

- Tributylphosphine Stock Solution (TBP), 10 × 0.5 mL flame-sealed ampules, Catalog Number T7567
- Alkylating Reagent, Iodoacetamide, 10 × 56 mg in brown glass vials, Catalog Number A3221
- High-purity water, Catalog Number W4502
- 30 °C water bath
- Micropipettes
- Sonicator (Branson digital sonicator, Model 450 or equivalent)
- Centrifuge and centrifuge tubes

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

### Optional Products

| Product Name (Catalog Number) | Suggested Application |
|---|---|
| *E. coli,* K12 strain cells (EC1) | 5 mg of *E. coli* cells per 1 mL of solublizing reagent |
| Protease Inhibitor Cocktail for use in tissue culture media (P1860) | Used to prevent proteolytic degradation |
| Protease Inhibitor Cocktail for use with bacterial cell extracts (P8465) | Used to prevent proteolytic degradation |
| Protease Inhibitor Cocktail for use with fungal and yeast extracts (P8215) | Used to prevent proteolytic degradation |
| Protease Inhibitor Cocktail for with mammalian cell and tissue extracts (P8340) | Used to prevent proteolytic degradation |
| Protease Inhibitor Cocktail for plant cell and tissue extracts (P9599) | Used to prevent proteolytic degradation |
| Benzonase (E1014) | Add to reduce viscosity |
| Benzonase, Ultrapure (E8263) | Add to reduce viscosity |
| Bradford Reagent (B6916) | Dilute solubilized sample 10-fold before assay |

### Preparation Instructions

Add 15 mL of high purity water to each bottle before use. The solution will become cold to the touch and needs to be warmed to 20–25 °C for all the solids to dissolve. A 30 °C water bath will aid in dissolving the material. The final volume of the reagent is 25 mL. The solution may be stored in 1 to 2 mL aliquots at –20 °C or below.

Alternatively, this material may be weighed out. Use 1 mL of high purity water per 907 mg of powdered product to generate 1.67 mL of fresh reagent.

**Storage/Stability**
The product as supplied is stable for at least 1 year.

Note: Do **not** allow the temperature of the prepared reagent to rise above 30 °C, since cyanates, which are detrimental to proteins, may form in the solution. During use, the temperature of the solution should also not fall below 15 °C, since urea and thiourea will precipitate.

**Procedure**
The following general procedure uses *E. coli* as the cell type to generate a total protein extract. Other cell types can be used with this procedure. However, the researcher needs to optimize the extraction procedure based on the specific source of protein. The method of cell disruption will depend upon the cell type. Protease inhibitors or protease inhibitor cocktails may be necessary to preserve the protein profile of certain samples. It may also be necessary to add nucleases such as Benzonase® (e.g. Catalog Numbers E8263 or E1014) to reduce the viscosity of some samples (nuclei), because of the presence of high molecular mass DNA. Addition of nonspecific nucleases will help reduce viscosity.[4] This extraction reagent will denature most enzymes, so it is best to add any enzymes prior to using this reagent.

The amount of starting material may be adjusted to fit the scale of the extraction. This procedure is given as a general guideline.

1. Suspend 10 mg of lyophilized *E. coli*, strain K12 cells (Catalog Number EC1) in 2 mL of the Protein Extraction Reagent Type 2. For other cell sources, it is suggested to use a minimum of 2 mL of the reagent per 50 to 100 mg of wet cell paste (any species) or 250 mg of tissue (liver, heart, brain, etc.).
2. Reduce the proteins by adding TBP (Catalog Number T7567) to a final concentration of 5 mM.
3. Sonicate this suspension with an ultrasonic probe for one to two minutes to disrupt the cells and break down the DNA. The temperature of the solution during sonication should be controlled using an ice bath. It should not be allowed to rise above 30 °C. If the urea and thiourea begin to precipitate, the sample should be warmed (ice bath removed) until the precipitate disappears.
4. Centrifuge the suspension at 14,000 × *g* at room temperature for 30 minutes to pellet cell debris.
5. Transfer the supernatant to a clean tube and discard the insoluble pellet.
6. Alkylate the proteins by adding iodoacetamide (e.g. Catalog Number A3221) to a final concentration of 15 mM in the supernatant. Incubate for 1.5 hours at room temperature.
7. After the incubation, centrifuge the reduced and alkylated sample at 20,000 × *g* for five minutes at room temperature (microcentrifuge) to pellet any insoluble material.
8. The supernatant is now ready for loading onto IPG strips. The samples may need to be diluted further with Protein Extraction Reagent Type 2 to obtain the desired results by 2D gel electrophoresis.
9. Optional: It is suggested that the Bradford assay be used to measure the protein concentration of extracts prepared with Protein Extraction Reagent Type 2. Better results for solutions prepared with this reagent are obtained with the Bradford procedure than with the Lowry and bicinchoninic acid (BCA) methods. However, it is necessary to dilute the solubilized protein sample 10-fold with high purity water before performing the Bradford assay. This reduces the concentration of detergent to a level which will not interfere with the Bradford Reagent (Catalog Number B6916).

Samples containing high concentrations of salts and buffers may not work with this product. These samples should be processed prior to use (dialysis or precipitation).

**References**
1. Molloy, M.P. *et al*., *Electrophoresis*, **19(5),** 837-844 (1998).
2. Herbert, B.R. *et al*., *Electrophoresis*, **19(5)**, 845-851 (1998).

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