

Y-PER™ Yeast Protein Extraction Reagent

78990 78991

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Number	Description
78990	Y-PER Yeast Protein Extraction Reagent, 500mL, sufficient reagent for 100-200g of wet cell pellet
78991	Y-PER Yeast Protein Extraction Reagent, 200mL, sufficient reagent for 40-80g of wet cell pellet

Storage: Store product at room temperature.

Introduction

The Thermo Scientific Y-PER Yeast Protein Extraction Reagent is a mild detergent formulation that is superior to the classical methods of protein isolation from yeast. In studies with *Saccharomyces cerevisiae*, yields of soluble protein typically exceed those achieved by glass bead disruption. Y-PER Reagent is effective for extracting soluble proteins from *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Bacillus subtilis* and *Escherichia coli* as well as a variety of gram-positive bacteria.

Yeast protein extraction and purification has traditionally been difficult and time consuming. The yeast cell is difficult to lyse because of its complex proteinaceous cell wall that provides rigidity to the weak plasma membrane. Techniques for protein extraction from yeast often involve harsh mechanical treatment while using strong reducing agents, chemicals and pH and temperature extremes. The popular glass bead lysis protocol requires special equipment and must be performed at 4°C. The low yields of protein commonly obtained with this technique are the result of denaturation and proteins nonspecifically binding to the glass beads. In contrast, Y-PER Reagent uses a simple room temperature protocol that can be completed in 20 minutes and requires no special equipment.

General Guidelines for Yeast Cell Disruption

- **Fresh Cells and Frozen Cells:** Y-PER Reagent is capable of extracting proteins equally well from recently harvested cells and frozen cells.
- **Cell Density and Strain Variation:** Differences in growth rate among organisms, growth temperature and media composition can have dramatic effects on the number of cells harvested from a given volume of culture. For this reason, several suggestions for the amount of Y-PER Reagent to use for a given cell pellet (wet cell paste) weight are provided.
- ***Saccharomyces cerevisiae*:** Y-PER Reagent is equally effective on cells grown to saturation or cells isolated from log-phase growth in either rich or synthetic defined media.
- ***Schizosaccharomyces pombe*:** Y-PER Reagent performs best on cells grown in synthetic defined media such as Edinburgh Minimal Media (EMM). To achieve adequate results from cells grown in rich media such as YES, cells must be harvested during log-phase growth. To increase protein yield from *S. pombe* cultures grown past log-phase, increase temperature to 45°C during lysis and use protease inhibitors.
- ***Bacillus subtilis*:** Y-PER Reagent will not lyse *B. subtilis* spores. When using sporulating strains, cells must be harvested during log-phase growth. For strains unable to sporulate, cells may be grown to saturation before harvesting.
- **Enzyme Activity:** Because all proteins differ in structure, solubility and stability, there is no guarantee that a particular protein will retain optimal activity in the presence of Y-PER Reagent. However, Y-PER Reagent does not interfere with the activity of β -galactosidase. Y-PER Reagent is also compatible with affinity-based purification protocols for glutathione S-transferase (GST) and histidine-tagged fusion proteins.

Note: Y-PER Reagent contains detergent and, therefore, is not compatible with protein assays that are incompatible with detergents.

Procedure for Protein Extraction

1. Pellet cells by centrifugation at approximately $3000 \times g$ (e.g., 5000 rpm for Beckman JA-20 rotor) for 5 minutes at 4°C .

Note: Cells may be processed immediately after centrifugation or the cell pellet may be frozen at -20°C or -80°C .

2. Resuspend cells in an appropriate amount of Y-PER Reagent as indicated in Table 1. Vortex gently or pipette up and down until the mixture is homogeneous.

Note: To prevent degradation of proteins, add protease inhibitors (e.g., Thermo Scientific Halt Protease Inhibitor Cocktail, EDTA-Free, Product No. 87785) to the sample.

Table 1. Volume of Thermo Scientific Y-PER Reagent to add per milligram of cell pellet.

Wet Cell Pellet Weight (mg)	Y-PER Reagent Volume (μL)
50	125-250
100	250-500
250	625-1250
500	1250 - 2500

3. Agitate the mixture at room temperature for 20 minutes.

4. Pellet the cell debris by centrifuging at $14,000 \times g$ for 10 minutes.

Note: Typically, greater than 90% of the soluble protein is extracted at this point and may be used for further purification or analysis. A second extraction may increase yield, but is usually not necessary.

5. Reserve the supernatant (i.e., lysate) for analysis, further purification and/or protein concentration determination.

Note: Y-PER Reagent contains detergent and, therefore, is not compatible with protein assays that are incompatible with detergents.

Related Thermo Scientific Products

89835	DNase I, 5000 units
87785	Halt™ Protease Inhibitor Cocktail, EDTA-Free (100X), 1mL
87786	Halt Protease Inhibitor Cocktail, contains sufficient reagents to treat 100mL of sample
78248	B-PER™ Bacterial Protein Extraction Reagent, 500mL
77720	Bond-Breaker™ CEP Solution, 5mL, enhances lysis of stationary-phase yeast cells
78870	Yeast DNA Extraction Kit, for extraction of genomic and plasmid DNA from <i>S. cerevisiae</i>
75768	Yeast β-Galactosidase Assay Kit

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