

Y-PER™ Plus, Dialyzable Yeast Protein Extraction Reagent

78998 78999

1164.3

Number	Description
78998	Y-PER Plus, Dialyzable Yeast Protein Extraction Reagent, 25mL
78999	Y-PER Plus, Dialyzable Yeast Protein Extraction Reagent, 500mL

Storage: Upon receipt store at room temperature. Product shipped at ambient temperature.

Introduction

The Thermo Scientific Y-PER Plus Reagent uses a mild detergent formulation for protein extraction that is superior to the classical methods of protein isolation from yeast. Y-PER Plus Reagent may also be used to extract soluble proteins from *E. coli* and vegetative *B. subtilis*, as well as a variety of other gram negative and positive bacteria. The protocol requires 20 minutes and can be performed at room temperature. In studies with *Saccharomyces cerevisiae*, yields of soluble protein typically exceed those achieved by the standard glass bead disruption method.

Important Product Information

- **Fresh Cells and Frozen Cells:** The Y-PER Plus Reagent is capable of extracting proteins equally well from both freshly harvested and frozen cells.
- **Cell Density and Strain Variation:** Differences in growth rate among organisms, growth temperature and media composition affects the number of cells harvested from a given culture volume. Therefore several suggestions for the amount of Y-PER Plus Reagent to use for a given cell pellet (wet cell paste) weight are included.
- ***Pichia pastoris*:** To achieve lysis of cells grown in rich media such as YEPD, cells must be harvested during log-phase growth. To enhance lysis of cells in stationary phase, add DTT or TCEP•HCl (to a final concentration of 0.1M and 20-50mM, respectively) directly to the Y-PER Plus Reagent before lysis to release much more of the soluble proteins. For best results use 2.5-5.0mL of Y-PER Plus Reagent for 1g of cell paste; scale up or down accordingly. An alternative to adding reducing agents is adding a protease inhibitor and incubating at 45°C.
- ***Saccharomyces cerevisiae*:** For best results, harvest cells in log-phase. To increase yield for cells in stationary-phase, add DTT or TCEP•HCl (to a final concentration of 0.1M and 20-50mM, respectively) directly to the Y-PER Plus Reagent before extraction. No differences in efficiency results for either rich- or synthetic-defined media. For best results use 2.5-5.0mL of Y-PER Plus Reagent for 1g of cell paste; scale up or down accordingly.
- ***Schizosaccharomyces pombe*:** For best results, use the original Thermo Scientific Y-PER Reagent (Product No. 78990) for extracting proteins from *S. pombe*.
- ***Bacillus subtilis*:** The Y-PER Plus Reagent will not extract protein from *B. subtilis* spores. When using a strain that is able to sporulate, harvest cells during log-phase. For strains unable to sporulate, cells may be grown to saturation before harvesting. Use 2.5-5.0mL of Y-PER Plus Reagent per 1g of cell paste; scale up or down accordingly.
- ***Escherichia coli*:** Use 2.5-5.0mL of Y-PER Plus Reagent per 1g of cell paste; scale up or down accordingly.
- **Enzyme Activity:** Because all proteins differ in structure, solubility and stability, a particular protein might not retain optimum activity in the presence of Y-PER Plus Reagent. For example, the original Y-PER Reagent works well for assaying β -galactosidase activity, but Y-PER Plus Reagent slows down its kinetics. Y-PER Plus Reagent is compatible with the standard, affinity-based purification protocols for glutathione S-transferase (GST) and His-tagged proteins.

- **Optional/Supplemental Materials:** The protein concentration of extracts can be determined using the Thermo Scientific BCA Protein Assay Kit (Product No. 23225) or the Coomassie Plus (Bradford) Protein Assay Reagent (Product No. 23236). Protease inhibitors (Product No. 78410 or 78415) are compatible and may be added directly to the reagent before extraction.

Procedure for Extracting Protein from Yeast Cells

1. Pellet cells by centrifuging at $\sim 3000 \times g$ (e.g., 5000 RPM for Beckman JA-20 rotor) for 5 minutes at 4°C. The cells can be processed immediately after centrifugation, or the cell pellet can be frozen at -20°C or -80°C until ready to use.
2. Resuspend the cells in an appropriate amount of Y-PER Plus Reagent (see the Important Product Information Section). Vortex gently or pipette up-and-down until the mixture is homogenous. Adding protease inhibitors to the sample during lysis prevents protein degradation.
3. Agitate the mixture gently at room temperature for 20 minutes. To increase yield of *S. pombe* or *P. pastoris* cultures grown past log-phase, increase the temperature to 45°C and add a protease inhibitor cocktail.
4. Collect the cell debris by centrifuging at $13,000 \times g$ for 5 minutes. Typically, greater than 50% of the soluble protein is extracted and may be used for further purification analysis. A second extraction will increase the total protein yield.

Related Thermo Scientific Products

89835	DNase I
78425	Halt Protease Inhibitor Single-Use Cocktail EDTA-Free (100X), 100 μ L \times 24 microtubes
78430	Halt Protease Inhibitor Single-Use Cocktail, 100 μ L \times 24 microtubes
78990	Y-PER Yeast Protein Extraction Reagent
78994	Y-PER 6xHis Column Purification Kit
78997	Y-PER GST Column Purification Kit
75768	Yeast β -Galactosidase Assay Kit
78870	Yeast DNA Extraction Kit

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