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Product information

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Yeast Protein Lysis Buffer

Catalog #: BSP026
Size: 20 preps
Storage: Mixed components storage*

*: Product will be shipped with ice pack. Check storage conditions.

Product Description:

Yeast Protein Lysis Buffer uses a mild detergent formulation for protein extraction that is superior to the classical methods of protein isolation from yeast. In studies with Pichia pastoris, yields of soluble protein typically exceed those achieved by the standard glass bead disruption method. The unicellular nature of yeast, combined with its ability to perform post-translational modifications that closely mimic processes in higher eukaryotes, have made them important research tools and recombinant expression systems.

The yeast cell is difficult to lysis, this is because yeast cells possess very complex proteinaceous cell wall that provides rigidity to the weak plasma membrane. Techniques for yeast cell lysis 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. However, Yeast Protein Lysis Buffer uses a simple room temperature protocol that can be completed in 20 minutes and require no special equipment. The lysis buffer is supplied with PMSF, protease inhibitors and DTT to improve protein stability, to maintain protein integrity and extraction efficiency. Depending on the required downstream applications, additional agents such as phosphatase inhibitor and chelating agents may be added into the kit. 20 ml is sufficient for 20 x 50 mg wet weight of the sample.

Features:

- Simple procedure, no extreme mechanical disruption, lengthy enzymatic treatments, or glass beads are required.
- Target proteins may maintain almost of biological functions, entire formulation (including the detergent) is dialyzable.
- Effective for cell lysis and protein solubilization extracts more than twice as much protein as glass bead methods.
- Effective for many different organisms, including Gram-negative bacteria, such as E.Coli.

Composition:

Lysis Buffer	20 ml
Protease Inhibitor	25 µl
DTT(1M)	1 ml
PMSF	250 µl

Storage:

Product shipped at ambient temperature. Upon receipt, store Lysis Buffer at 2-8°C. Store DTT(1M), Protease Inhibitor, PMSF at -20°C.

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Procedures for Extracting Protein from Yeast Cells:

1. Pellet cells by centrifuging at ~3000 × g (e.g., 5,000 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 Yeast Protein Lysis Buffer (for example, add 500 µl yeast Lysis buffer, 10 µl DTT(1M), 0.5 µl Protease Inhibitor and 5 µl PMSF into per 50mg wet yeast cell pellet). Vortex gently or pipette up-and-down until the mixture is homogenous.

   NOTE: Maintaining DTT at final concentration >0.02M is essential for efficient lysis.

3. Drastically (platform mixer) agitate the mixture about 280 rpm at room temperature for 20 minutes.

   NOTE: Typically, greater than 60% of the soluble protein is extracted at this point and may be used for further purification or analysis. A second extraction may increase the total protein yield.

4. Collect the cell debris by centrifuging at 13,000 × g for 5 minutes.
5. Reserve the supernatant (lysate) for analysis, further purification and/or protein concentration determination.

   NOTE: The lysis buffer contains detergent and, therefore, is not compatible with protein assays that are incompatible with detergents. After two extractions, more than 90% soluble yeast protein can be released into supernatant.

Additional Notes:

1. Before use, transfer small amount of yeast protein lysis buffer into a new tube. Add DTT solution into the lyisis buffer so that final concentration of DTT is > 0.02M.

   NOTE: DTT at final concentration >0.02M is essential for efficient lysis.

2. Fresh Cells and Frozen Cells: The yeast lysis buffer is capable of extracting proteins equally well from both freshly harvested and frozen cells.
3. 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 yeast protein lysis buffer to use for a given cell pellet (wet cell paste) weight are included in the following items.
4. Pichia pastoris and Saccharomyces cerevisiae: 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 TCEPHCl (to a final concentration of 0.1 M and 20-50 mM, respectively) directly to the lysis buffer before lysis to release much more of the soluble proteins and increase the extraction temperature to 42°C. For best results use 10.0 ml of the yeast lysis buffer for 1 gram of cell paste; scale up or down accordingly. For Saccharomyces cerevisiae No differences in efficiency results for either rich- or synthetic-defined media.
5. Escherichia coli: Use 5.0-10 ml of Extraction buffer per 1 gram of cell paste; scale up or down accordingly.
6. Enzyme Activity: Because all proteins differ in structure, solubility and stability, a particular protein might not retain optimum activity in the presence of the yeast Lysis buffer. The buffer is compatible with the standard, affinity-based purification protocols for glutathione S-transferase (GST).
7. Compatible with modified Lowry Protein Assay Kit (SK3041) or Non-Interfering Protein Concentration Determination Kit (SK3071).

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