PROMETHEUS™
Protein Biology Products

Instruction Manual

Yeast Protein Extraction Buffer/Kit

Catalog No.: 18-411, 18-412, 18-413 & 18-414

Introduction:

Our Yeast Protein Extraction Buffer is designed for gentle and efficient extraction of soluble proteins from yeast cells, which doesn't require lysis with mechanical disruption. Yeast protein extraction and purification have traditionally been difficult and time consuming, since yeast cells are difficult to lyse due to its complex and rigid cell wall. Techniques for protein extraction from yeast cells 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 the low yields of proteins commonly obtained with this technique are the result of denaturation and proteins nonspecifically binding to the glass beads. In contrast, our Yeast Protein Extraction Buffer uses a simple protocol that can be completed in much shorter time with no special equipment required.

The Yeast Protein Extraction Buffer is supplied as buffer only as well in a kit format with Lyticase enzyme.The Yeast Protein Extraction kit containing Lyticase enzyme provides a convenient method for a highly efficient spheroplast formation and protein extraction from different yeast strains (e.g. *Saccharomyces cerevisiae*, *Pichia Pastoris*, and *Schizosaccharomyces pombe*). The procedure involves spheroplast formation by enzymatic digestion of the yeast cell wall by Lyticase enzyme, followed by cell protein extraction, while avoiding protein degradation and interference with protein immunoreactivity and biological activity. Our yeast protein extraction buffer contains a proprietary nonionic detergent with an organic buffer system and other agents, which does not require sonication or any other mechanical steps to lyse the yeast cells. The unique composition offers adaptability for different applications and in addition, it offers several folds increase in the yield of soluble protein when compared with other yeast lysis extraction buffers.

Depending on the particular application, additional components, such as protease inhibitor cocktail, reducing agents, etc., may be added to the extraction buffer. The kit method involves the yeast cell pellets to be incubated with the supplied extraction buffer along with Lyticase enzyme, that help to remove the yeast cell walls and centrifuged to separate cell debris and insoluble proteins. The supernatant contains the soluble protein fraction, which can be further purified or directly analyzed by SDS-PAGE, Western blot and other techniques. The protein yields obtained with our Yeast Protein Extraction Buffer are much higher than those obtained from standard methods and extracted proteins are in their native state, which can be used for assaying biological activities and other downstream applications. The cell extract is compatible with reporter gene expression assays (e.g., β-galactosidase, alkaline-phosphatase), immunoassays (Western blots, immunoprecipitation), affinity-based purification (e.g. FLAG, glutathione S- transferase (GST) and histidine-tagged fusion proteins), DNA-protein interaction assay (e.g., gel-shift), Coomassie and silver staining.

Kit Components:

Item Name	Cat. No. 18-411	Cat. No. 18-412	Cat. No. 18-413 (100 Preps)	Cat. No. 18-414 (200 Preps)	Storage Condition*
Yeast Protein Extraction Buffer	250 ml	500 ml	125 ml	250 ml	4 °C
Yeast Suspension Buffer	N/A	N/A	15 ml	30 ml	4 °C
Lyticase Enzyme	N/A	N/A	1 ml	2 x 1 ml	-20 °C

**The individual buffer and complete kit with enzymes are shipped at ambient temperature. Upon receipt, store the individual kit components as marked. The kit components are stable for one year, if stored and used as recommended.*

Items Needed (Not Supplied with the Kit):

- Appropriate Yeast Cell culture
- Centrifuge
- Incubator
- DTT and/or β-mercaptoethanol
- Optional Items: Protease Inhibitor Cocktail (Yeast/Fungi or His-Tag) and EDTA if desired.

Important Notes on the Product

- The Yeast Extraction Buffer volume required depends on the amount of yeast cell pellet size and can be adjusted in the protocol step.
- The yeast extraction buffer is capable of extracting proteins equally from both recently harvested cells as well as frozen cells.
- Protease inhibitor cocktail, Yeast/Fungi (Cat. No. 18-437) or His-Tag (Cat. No. 18-434) can be added in the extraction buffer to prevent protein degradation.
- Reducing agents (e.g. 5mM DTT final concentration) and chelating agents (e.g. 2mM EDTA final concentration) can be added to the extraction buffer as some proteins are better extracted when DTT or EDTA is present in the Extraction buffer.

Procedures for Protein Extraction from Yeast:

1. Grow the yeast culture with OD_{600} between 1.5 to 3.0 and pellet the yeast cells by centrifugation at 3,000-5,000 x *g* for 5 minutes.
2. Resuspend the cell pellet (~50µl) with equal volume of Yeast Suspension Buffer with 5mM DTT or 1µl of β-mercaptoethanol per 100µl Yeast suspension. **Note:** The DTT should be freshly added to each buffer mixture.
3. Remove a small sample for OD_{800} measurement (for spehroplast formation).
4. Mix the Lyticase Enzyme tube by tapping with finger and add 10µl of Lyticase Enzyme **Note:** Each yeast strain requires a different concentration of Lyticase enzyme for efficient spheroplast formation (10µl Lyticase Enzyme for *S. cerevisiae/ Pichia pastoris* would be sufficient, but the yeast strain *Schizosaccharomyces pombe* would require more ~15µl of Lyticase Enzyme, which can be determined with the OD_{800} measurement)
5. Incubate the tube at 37 °C for 15-30 minutes (for *S. pombe* 30-45 minutes).
6. Monitor the spheroplast formation according to the Spheroplasts Lysis Monitoring procedure below* and finalize the incubation time.
   ***Measuring the Spheroplast Formation by Checking the OD of the sample at 800nm:** Before the addition of Lyticase Enzyme enzyme (Step 4 pre-lysed sample), place a 10µl sample from the reaction mixture into 990µl water and read the OD at 800 nm. After the addition of the Lyticase enzyme (during the incubation of the sample at 37^0C, step 5), at every 10 minutes, take a 10µl sample, add 990µl extraction buffer and read the OD at 800nm. When the OD is 15-20% of the pre-lysed sample reading, end the incubation. Longer digestion times than required may have deleterious effects on subsequent procedures.
7. Pellet the spheroplasts by centrifugation at 5,000 x *g* for 5 minutes and carefully remove the supernatant, leaving the spheroplast pellet in the tube. The spheroplasts (pellet) at this stage can be stored at -20^0C, if needed or can be used directly for protein extraction.
8. For protein extraction, resuspend the spheroplast pellet in 3 volumes of Yeast Protein Extraction Buffer. Pipette up and down, and then vortex to homogenize the spheroplasts in suspension.
9. Incubate the spheroplast suspension for 20-30 minutes at room temperature, while shaking.
10. Centrifuge at 14,000 x *g* for 20 minutes at 4^0C.
11. Transfer the supernatant (soluble proteins) to a clean, chilled test tube. Typically, more than 90% of the soluble proteins are extracted at this stage and may be used for further purification, analysis and downstream applications. For storage, snap-freeze aliquots of the supernatant in liquid nitrogen and store at -70^0C.

Related Products

1. **Protein Extraction Buffers/ Kits (Cat. No. 18-400, 18-402, 18-404, 18-406, 18-409)**
   For extracting proteins from Bacteria, Insects Cells, Mammalian cells, and Tissue samples.
2. **RIPA Lysis & Extraction Buffer (18-415, 18-416 and 18-417)**
   For extracting proteins from different species samples.
3. **Protein Loading Buffer [2X], Cat. No. 20-309**
   Non-reducing ready to use buffer for loading protein samples on to the gel.
4. **Protein Loading Buffer [2X], Cat. No. 20-310**
   Reducing ready to use buffer for loading protein samples onto the gel.

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