WSE- 7423

EzBactYeastCrusher

Instruction Manual

June 22, 2026 4th edition

1. Precautions for safe use of this product

To use this product safely, please read this instruction manual carefully first. Please refrain from operating the product until you fully understand the contents of this instruction manual. This instruction manual describes only how to use this product for the specified purpose. Please refrain from using the product for purposes or in ways not described in this instruction manual. If you use the product for purposes or in ways not described in this instruction manual, you are solely responsible for all necessary safety measures and unforeseen circumstances. Also, please carefully read and understand the instruction manuals of any devices you will be using at the same time..

2. Purpose of use

This product is a kit for solubilizing E. coli and yeast cells and extracting proteins. The prepared protein extract can be used for biochemical and immunological analyses such as purification of expressed proteins, electrophoresis, immunoprecipitation, ELISA , and enzyme activity experiments.

3. Product Configuration

Name	Volume	Quantity	Strage
Yeast PreLysis buffer	100 mL	1	15-30℃
BactYeastLysis buffer	100 mL	1	15-30℃
Protease Inhibitor	1 mL	1	-20℃
DNase I	1 mL	1	-20℃

4. composition

Name	Main component
Yeast PreLysis buffer	Buffer solution
BactYeast Lysis buffer	Surfactants, buffer solutions
Protease Inhibitor	100 × concentration, aprotinin, pepstatin A , leupeptin, DMSO
DNase I	2kU/mL Deoxyribonuclease I

This product contains substances that exceed the exemption limits set by the Industrial Safety and Health Act . For details, please download the SDS for this product from the ATTO website (https://www.atto.co.jp/) .

5. Strage

- **Yeast PreLysis Buffer** (Yeast Pre-treatment Buffer: Yeast Pre-treatment Solution) and **BactYeast Lysis Buffer** (Bactoyster Extract Buffer) should be stored at room temperature (15-30 °C). It remains stable within its expiration date if unopened.
- **Protease** inhibitors and **DNase I** (**Deoxyribo nuclease**) should be stored frozen (-20 ° C). It remains stable within the expiration date if unopened .

6. Disposal method

- Please Dispose of each reagent in accordance with the disposal method of your affiliated institution.

7. Other items required besides this product

- DTT if necessary
- Microcentrifuge tubes
- Vortex Mixer
- Refrigerated centrifuge

8. Precautions for use

- This product is delivered by refrigerated goods transportation. Please store the product under the temperature suitable for each reagent after receiving.
- **Protease Inhibitor** contains DMSO, it may freeze at low temperatures. Please allow it to thaw completely at room temperature before use .
- If necessary, increase or decrease the amount of **Protease inhibitor** added, or add other inhibitors such as AEBSF or Bestatin.
- **Yeast PreLysis buffer** is only used for yeast pretreatment. If used for Escherichia, it may be solubilized since the solution is slightly alkaline.
- This kit is optimized for solubilizing budding yeast, Saccharomyces cerevisiae, and various Escherichia. To improve efficiency of extracting yeast protein, either add 10mM DTT (5~50mM) to Yeast PreLysis buffer or extend reaction time to 30-60minutes. If the additional of DTT or the extension of the reaction time might interfere with the downstream application, these should be omitted.
- For solubilizing fission yeast or Shizosaccharomyces pombe other yeast cells, the condition of the solubilization might be needed to be optimized. To improve the extraction efficiency from fission yeast, Shizosaccharomyces pombe, add 10mM DTT (5~50mM) to Yeast PreLysis buffer, extend the reaction time to 30-60minutes or raise the reaction temperature to 35-60℃. To improve efficiency of extracting protein or in case the method described influences experiments, acid washed glass bead (Φ0.5mm)can be used for disrupting cells.
- When using cryopreserved cells, always wash the cells with distilled water before freezing, remove excess water by centrifugation, and store the resulting cells (centrifugation sediment) at -80°C .
- This kit can be used with the addition of EDTA or DTT . Furthermore, since it does not inhibit the activity of enzymes such as lysozyme or zymolyase , it can be used with these additives.
- It hardly inhibits the activity of enzymes such as luciferase and β- galactosidase. Extraction at low temperatures (4 °C or on ice) maintains stable enzyme activity. Furthermore, the extract can be used directly for activity measurements.

9. How to use

I. Solubilization of E. coli

1. E. coli in 5-10 mL of culture medium until A600 = 0.5-1.0 (50-100 mg of bacterial mass).E. coli is collected by centrifugation at 2,000xg for 5 minutes.
2. Discard the supernatant after centrifugation, add 5 mL of distilled water to the cells and mix.Centrifuge again at 2,000xg for 5 minutes to collect cells. (washing step) .
3. Mix Protease Inhibitor and DNase I as the following table. Keep mixed solution at room temperature before use. For extracting protein at low temperature, store at 0-4°C.

(1 sample: (50-100 mg)	Required amount	Protease Inhibitor (blue lid)	DNase I (red lid)
BactYeast Lysis buffer	0.5 mL	5 µL	5 µL

Prepare 0.5mL reagent per 50-100mL culture cells, please adjust the reagent amount depending on the culture volume. The reagent mixed with Protease Inhibitor and DNase I cannot be stored.

For extracting protein from large volume of culture cells (50 mL or more of culture medium)2.5 to 3 time volume Lysis buffer of the cell pellet is required.

4. After centrifugation, discard the supernatant and completely suspend the bacterial cells (centrifugation precipitate) using a vortex mixer.
5. Add the mixed **BactYeast Lysis buffer** (0.5 mL/ sample) prepared in step 3 to the bacterial cells and mix by vortexing for at least 5 seconds.

   *Mix until all clusters of cells are dissolved.
6. Incubate at room temperature for 10 minutes.

   *If cells settle, invert and mix 2-3 times every 1-2 minutes .

   Incubate at 0-4℃ enzyme activity or extracting for unstable protein measuring.
7. Centrifuge 10,000xg at 4℃ for 5minutes .
8. Collect the supernatant after centrifugation (E. coli extracted protein) .

   *Soluble protein is collected to centrifuge supernatant. For extracting insoluble protein, the pellet should be dissolved in 8M urea-containing buffer or 6M guanidine hydrochloride-containing buffer and further treated to extract protein.

II. Solubilization of Yeast cells

1. Yeast cells are cultured in 5-10 mL of culture medium until A600 = 0.5-1.0 (50-100 mg of cells).
2. The yeast cells are collected by centrifugation at 2,000xg for 5 minutes.
3. Remove the supernatant, add 5mL distilled water to cells and mix. Centrifuge again 2,000xg for 5 minutes to harvest cells (washing step) .
4. Mix **Protease Inhibitor** and **DNase I** as the following table. Keep mixed solution at room temperature before use. For extracting protein at low temperature, store at 0-4℃ .

(1 sample : 50～100mg)	Required amount	Protease Inhibitor	DNase I	DTT (unattached)
Yeast PreLysis buffer (yeast cells only)	0.5mL	-	-	0~50mM
BactYeast Lysis buffer	0.5 mL	5µL	5µL	

*Prepare 0.5mL reagent per 50-100mL culture cells, please adjust the reagent amount depending on the culture volume. The reagent mixed with Protease Inhibitor and DNase I cannot be stored.

* DTT is not attached to this kit. The efficiency of extracting protein will be improved by adding DTT.

5. Discard the supernatant after centrifugation, and suspend the cells (centrifugation sediment) using a vortex to completely break up the cell clumps.
6. Add the mixed **Yeast PreLysis buffer** (described in, above Item 4) to cells and vortex them for 5 seconds.

   *If it does not interfere with the experimental setup, adding 5-50 mM DTT will increase protein extraction efficiency.

   *Mix until all clusters of cells are dissolved.

   * **Yest P r e L y sis buffer** is a weakly alkaline solution. Please note that it may occasionally affect the analysis of cell wall proteins.

   * Without using **Yeast PreLysis buffer**, it's possible to extract yeast protein even though the efficiency of extraction will be lowered. If you do not use **Yeast PreLysis buffer** , skip steps 6-8.
7. Incubate at room temperature for 5 minutes.

   Please incubate at 0-4℃ for measuring enzyme activity or unstable protein extracting.
8. Centrifuge the cell lysate at 10,000xg for 5 seconds (4 ℃) .
9. Discard the supernatant after centrifugation, and suspend the cells (centrifugation sediment) using a vortex to completely break up the cell clumps.

   *Please completely remove the supernatant using a paper towel or similar. If a large amount of supernatant remains , **Yeast P r e L y sis Buffer** is a weakly alkaline solution , so it will affect the pH.
10. Add the **BactYeast Lysis buffer** (0.5 mL/ sample) prepared in step 4 to the cells and mix by vortexing for at least 5 seconds.

    *Mix until all clusters of cells are dissolved.
11. Incubate at room temperature for 10 minutes.

    *Mix 2-3 times every1-2 minutes in case cells are settled on the bottom.

    Extending the incubation time to 30-60 minutes may improve extraction efficiency.

    Incubate at 0-4℃ for measuring of enzyme activity or unstable protein extracting.
12. Centrifuge the cell lysate at 10,000xg for 5 minutes (4 ℃) .
13. Collect the supernatant after centrifugation (yeast-extracted protein) .

    *Soluble proteins are recovered in the supernatant after centrifugation.
    *If the extraction efficiency is extremely poor, use acid-washed glass beads, etc(φ Please use the 0.5 mm size) .

10. others

*Even Experiment operation may have a significant variance in results due to a slight technical difference in same protocol. It is important to know the tips to obtain optimal result.
As our website provides various "tips on experiment" and you can download the document, please visit our website below and read articles; **https://www.atto.co.jp/**

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