1. Instructions for ensuring the safe use of this Product

Please read this Instruction Manual thoroughly to ensure the safe use of this Product. Do not use the Product unless you fully understand the contents of this Instruction Manual. This Instruction Manual describes the method and purpose of use intended for the Product. Please refrain from using the Product for the purpose and method not described in this Instruction Manual. If the Product should be used for the purpose and method not described in this Instruction Manual, the user will be responsible for any necessary safety measures and any unpredictable consequences.

Also, please read thoroughly the instruction manuals for the devices to be used with the Product.

2. Purpose of use

The Ez-Bact-Yeast-Crasher is a kit for solubilizing Yeast & Bacterial cells and extracting protein. It's used for purification of expressed protein, electrophoresis, biochemical or immunological analysis such as immunoprecipitation, ELISA, enzymatic activity experiments.

3. Product configuration

Name	Content	Piece(s)	Strage
Yeast PreLysis buffer	100 mL	1	20-30℃
BactYeastLysis buffer	100 mL	1	20-30℃
Protease Inhibitor	1 mL	1	-20℃
DNase I	1 mL	1	-20℃

4. Composition

Name	Principal elements
Yeast PreLysis buffer	Detergent, buffer solution
BactYeast_Lysis buffer	Detergent, buffer solution
Protease Inhibitor	100×concentration, aprotinin, pepstatinA, leupepttin、DMSO
DNase I	2kU/mL deoxyribonuclease I

This product does not contain any poisonous and deleterious substances under the Poisonous and Deleterious Substances Control Act or any substances Control Act or any substances that are to be notified and exceed the exemption amount under the Industrial Safety and Health Act. Matters that are to be notified and exceed the exemption amount for the specified chemicals under the PRTR Act are partially included.

5. Storage

・Yeast PreLysis buffer and BactYeast Lysis buffer can be kept at room temperature(20-30℃).Unless it is opened, it will be stable until the expiration date.

・Protease Inhibitor and DNase I should be kept in frozen storage.(-20℃). Unless it is opened, it will be stable until the expiration date.

6. Disposal

Please comply with the disposal method of your organization when disposing reagents.

7. Required items other than the Product

・Distilled water

・DTT (if necessary)

・Micro-centrifuge tube

・Vortex mixer

・Cooled centrifuge

8. Precaution on use

・The product is delivered by refrigerated goods transportation. Please store the product under the temperature suitable for each reagent after receiving.

・As the protease Inhibitor includes DMSO, it may be crystallized at low temperature. Please dissolve it completely at room temperature before use.

・The amount of protease inhibitor should be increased or decreased according to your sample preparation, and other protease inhibitors like AEBSF, Bestatin can be added if needed.

・Yeast PreLysis buffer should be used only for yeast pretreatment. If Escherichia coli is treated with this reagent, the cells may be solubilized because of the slightly alkaline pH.

1 sample： 50～100mg	Amount required for 1 sample	Protease Inhibitor (blue lid)	DNase I (red lid)
BactYeast Lysis buffer	0.5 mL	5μL	5μL

・This kit is optimized for solubilizing budding yeast, Saccharomyces cerevisiae, and various bacterial cells. To improve the efficiency of extracting yeast protein, either add 10mM DTT (5~50mM) to Yeast PreLysis buffer or extend the reaction time to 30-60minutes. If the addition of DTT or the extension of the reaction time might interfere with the downstream application, these should be omitted.

・For solubilizing fission yeast, Shizosaccharomyces pombe or 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 the efficiency of extracting protein, or if the addition of DTT or raising extracting temperature might give any effects on sample, acid washed glass bead (Φ0.5mm)can be used for disrupting cells.

・When extracting protein from frozen cells, before freezing cells, wash with distilled water and remove extra moisture by centrifugation and store them in-80℃.

・EDTA and DTT can be added to this kit for use. This kit doesn't include inhibitors of enzymatic activity such as Lysozyme, Zymolyase and so on.

・Inhibitory effects of the enzymatic activity like luciferase or β-galactosidase activity are minimized. Enzymatic activity is more stable when the extraction is proceeded at low temperature; 4℃ or on ice. The protein extracts can be used for enzymatic activity measurement.

9. How to use

I. Solubilization of Escherichia coli

1. Cultivate Escherichia coli until A600=0.5~1.0 in 5-10mL culture medium. (50-100mg biomass)
2. To harvest Escherichia coli, centrifuge 2,000xg for 5 minutes.
3. Discard centrifuge supernatant, add 5mL distilled water to bacteria and mix. Centrifuge 2,000xg for 5 minutes to harvest cells.
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℃.

*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 (50mL＜)2.5 to 3 time volume Lysis buffer of the cell pellet is required.

5. Remove supernatant, loosen clusters of cells by vortex.
6. Add the mixed BactYeast Lysis buffer (described in above Item 4) to the cells and vortex them more than 5 seconds.
   *Mix until all clusters of cells are dissolved.
7. Incubate 10 minutes at room temperature.
   *Mix 2-3 times invertedly every1-2 minutes in case cells are settled on the bottom.
   *Incubate at 0-4℃for measuring enzyme activity or extracting unstable protein.
8. Centrifuge 10,000xg at 4℃ for 5minutes.
9. Collect the supernatant. (Bacterial cell 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. Cultivate yeast cells until A600=0.5~1.0 in 5-10mL culture medium. (50-100mg biomass)
2. To harvest yeast cells, centrifuge 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.
4. Mix Protease Inhibitor and DNase I as the following table. Keep the mixed solution at room temperature before use. For extracting protein at low temperature, store at 0-4℃.

*Prepare 0.5mL reagent per 50-100mL culture cells. 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. Remove supernatant, and loosen clusters of cells by vortex.
6. Add the mixed Yeast PreLysis buffer (described in, above Item 4) to cells and vortex them for 5 minutes.
   *The efficiency of extracting protein will be improved by adding 5-50mM DTT.
   *Mix until all clusters of cells are dissolved.
   *Yeast PreLysis buffer is slightly alkaline solution. It might possibly give some influences on periplasmic protein analysis.
   *Without using Yeast PreLysis buffer, it's possible to extract yeast protein even though the efficiency of extraction will be lowered. Please omit the steps 6 to 8.
7. Incubate 5 minutes at room temperature.
   *Please incubate at 0-4℃ for measuring enzyme activity or unstable protein extracting.
8. Centrifuge 10,000xg at 4℃ for 5minutes.
9. Remove supernatant, loosen clusters of cells by vortex.
   *Remove any liquid completely by using paper towels. The pH of the reaction mixture will be influenced by the residue of Yeast PreLysis buffer.
10. Add the mixed BactYeast Lysis buffer (described above in Item 4) to cells and vortex them more than 5 seconds.
    *Mix until all clusters of cells are dissolved.
11. Incubate 10 minutes at room temperature.
    *Mix 2-3 times every1-2 minutes in case cells are settled on the bottom.
    *The extraction efficiency may improve if the incubation time is extended to 30-60minutes.
    *Incubate at 0-4℃ for measuring enzyme activity or extracting unstable protein.
12. Centrifuge 10,000xg at 4℃ for 5minutes.
13. Collect the supernatant. (Yeast cells extracted protein)
    *soluble protein is collected to the supernatant.
    *Use acid washed glass bead (Φ0.5mm) in case the efficiency of extracting is very low.

(1 sample：50～100mg)	Amount required for 1 sample	**Protease Inhibitor** (blue lid)	**DNase I** (red lid)	***DTT*** (unattached)
Yeast PreLysis buffer（yeast cells only）	0.5 mL	-	-	0~50mM
BactYeast Lysis buffer	0.5 mL	5μL	5μL	

10. Others

*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;

http://www.atto.co.jp/