Bacterial Protein Extraction Kit

B665764-100T

Introduction:

Bacterial protein extraction reagents use mild non-ionic detergents and are suitable for extracting recombinant proteins expressed in Escherichia coli and insect cells. During the extraction process, there is no need for ultrasonic fragmentation, effectively avoiding contamination of exogenous proteins. This product can be applied to extract soluble proteins from bacterial lysates. The bacterial protein extraction kit adds a mixture of lysozyme, DNase I, and protease inhibitors to the extraction reagent, which can improve the efficiency of protein extraction and reduce the viscosity caused by DNA, effectively avoiding protein degradation. The extracted protein maintains biological activity and can be subjected to downstream operations such as IP, Western blot, and protein purification.

Contents:

Bacterial Protein Extraction Kit			
Cat No.	Component	Size	Storage
B665764A	Bacterial Protein Extraction Reagent	100mL	RT
B665764B	Protease Inhibitor Cocktail (100x)	1mL	-20℃
B665764C	Lysozyme (50 mg/mL)	200μL	-20℃
B665764D	DNase Ⅰ (1000 U/mL)	100μL	-20℃

Notes:

1. This product is suitable for extracting proteins from fresh or frozen bacterial and insect cells.
2. This product uses Tris buffer system. Please use the same buffer system for protein purification after extraction.
3. The protein lysis solution obtained from this product can be used for protein quantification using BCA or Bradford method.
4. For special strains, if the extraction effect is not ideal, the sample can be frozen before protein extraction.
5. Depending on the specific situation, protease inhibitors, salts, chelating agents, reducing agents, etc. can be added to this product.

Operation steps:

- Insect cell protein extraction

1. Collect cells by low-speed centrifugation. Add 10μL The Protease Inhibitor Cocktail to every 1 mL of Bacterial Protein Extraction Agent is 1 x working fluid.
2. Weigh the wet weight of the cells and add 1 x working solution at a rate of 10 mL/g.
3. After resuspension, incubate on ice for 20 minutes (the ice storage time should be adjusted

according to different cell types).

4. Centrifuge at 4.15000 × g for 15 minutes to isolate soluble proteins.

- Extraction of soluble bacterial proteins

1. Centrifuge for 10 minutes at a rate of 5000 × g and collect the bacterial cells.

2. Optional steps: Add 1 mL of Bacterial Protein Extraction Reagent every 1 μL DNase I (1000 U/mL), 2 μL Lysozyme (50 mg/mL) and 10 μL Protease Inhibitor Cocktail, vortex oscillation and mixing.

3. Add 20 mL of Bacterial Protein Extraction Reagent to each gram of bacterial precipitate, and add the extraction solution to the bacterial precipitate. Vortex thoroughly or use a pipette to blow up and down until the bacterial precipitate is completely resuspended.

4. After resuspension, incubate at room temperature for 10-15 minutes (the storage time should be adjusted according to different cell types).

5. Centrifuge at 15000 × g for 5 minutes.

6. Transfer the supernatant to a new centrifuge tube (the supernatant is soluble protein) for protein quantification and downstream experiments.

Note: If the target protein exists in the form of inclusion bodies, inclusion body protein solution can be used for dissolution or expression conditions can be optimized to increase the expression of soluble proteins.

Frequently asked questions:

Problem	Possible reasons	Resolvent
The target protein is insoluble	The target protein is expressed as an inclusion body	Optimize expression conditions or add Lysozyme and DNase I to protein extraction reagents using inclusion body protein solution
After adding Lysozyme, the target protein has not been extracted yet	Temperature too low	Restore the reagent to room temperature
After adding Lysozyme, the target protein has not been extracted yet	Lysozyme Decreased or inactivated activity	Add more Lysozymes or replace with new enzymes
Extract has high viscosity	DNase I Decreased or inactivated activity	Add more DNase I or replace with a new DNase I to increase the final concentration of magnesium ions to 2 mM
After protein extraction, most of the proteins still exist in the precipitate	Excessive protein content	Add Lysozyme and DNase I
The protein extraction reagent has sediment precipitation	Temperature too low	Restore the protein extraction reagent to room temperature