Bacterial Protein Extraction Kit

Bacterial Protein Extraction Kit

Item No. B665764 (100 preps)
Storage condition: -20℃ storage.

Product content

individual parts making up a compound	B665764 100 preps
Bacterial Protein Extraction Reagent	100 ml
Protease Inhibitor Cocktail (100x)	1 ml
Lysozyme (50 mg/ml)	200 μl
DNase I (1,000 U/ml)	100 μl

Product Introduction

Bacterial Protein Extraction Reagent uses a mild non-ionic decontaminant and is suitable for the extraction of recombinant proteins expressed by E. coli and insect cells. The extraction process does not require ultrasonic crushing, 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 inhibitor on the basis of the extraction reagent, which can improve the protein extraction efficiency and alleviate the sticky phenomenon caused by DNA, and effectively avoid protein degradation. The extracted proteins maintain biological activity and can be used for downstream operations such as IP, Western blot and protein purification.

Caveat

1. This product is suitable for the extraction of proteins from fresh or frozen bacterial and insect cells.
2. This product uses Tris buffer system, please use the same buffer system for the purification operation after protein extraction.
3. Protein lysates obtained with this product can be used for protein quantification by BCA or Bradford method.
4. For special strains, if the extraction is not satisfactory, the sample may be frozen before protein extraction.
5. Protease inhibitors, salts, chelating agents, reducing agents, etc. may be added to the product according to specific conditions.

Operation process

Insect cell protein extraction

1. Collect the cells by low speed centrifugation. Add 10 μl Protease Inhibitor Cocktail to every 1 ml of Bacterial Protein Extraction Reagent to make 1× working solution.
2. Weigh the wet weight of the cells and add 1× working solution at 10 ml/g.
3. After resuspension, incubate on ice for 20 minutes (time on ice should be adjusted depending on cell type).
4. Centrifuge at 15,000 × g for 15 minutes to isolate soluble proteins.

● Bacterial soluble protein extraction

1. Centrifuge at 5,000 x g for 10 minutes and collect the organisms.
2. Optional: Add 1 μl DNase I (1,000 U/ml), 2 μl Lysozyme (50 mg/ml) and 10 μl Protease Inhibitor Cocktail per 1 ml of Bacterial Protein Extraction Reagent, and mix well by vortexing and shaking. 3. Add the extraction solution to the bacterial precipitate at the ratio of 20 ml of Bacterial Protein Extraction Reagent per gram of bacterial precipitate, and vortex thoroughly or blow up and down with a pipette until the bacteria are completely resuspended.
4. After resuspension, incubate at room temperature for 10-15 minutes (the time of placement should be adjusted depending on the cell type).
5. Centrifuge at 15,000 x 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 is in inclusion bodies, use an inclusion body proteolytic solution for solubilization or optimize expression conditions to increase soluble protein expression.

Common problems

concern	Possible causes
target protein is insoluble	The target protein is expressed as an inclusion body
The target protein was not extracted after the addition of Lysozyme.	too low a temperature
The target protein was not extracted after the addition of Lysozyme.	Decreased or inactivated Lysozyme activity
High extract viscosity	Decreased or inactivated DNase I activity
Most of the protein is still present in the precipitate after protein extraction	Too much protein
Protein extraction reagent with sediment precipitation	too low a temperature