SunLong Biotech Co.,LTD
Tel: 0086-571-56623320 Fax:0086-571-56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

Bacterial Transmembrane Protein Extraction Kit

Catalog Number: EXP0108

Size: 50T/100T

Storage: Store at 2-8℃, valid for one year.

Kit Contents

Component	50T	100T	Storage
Bacterial Transmembrane Protein Extract Solution A	25ml	50ml	2-8℃
Bacterial Transmembrane Protein Extract Solution B	500ul	1ml	2-8℃
Membrane Protein Solution C	10ml	20ml	2-8℃
Protease Inhibitor Mixture D	100ul	200ul	-20℃

Note:

1. Protease inhibitor can also be stored at 2-8 ° C before use. Store at -20 ° C after use.
2. Protease inhibitor is in the solid form at 2-8 ° C. Remove from the refrigerator and return to room temperature or 37 ° C for a short time water bath, turn into the liquid form and centrifuge to the bottom of the tube before opening the cap.
3. Please use up the reagents as soon as possible after unpacking!

Product Description

Bacterial Transmembrane Protein Extraction Kit is a high-efficiency and high-yield membrane protein extraction kit, which can extract transmembrane proteins from all kinds of bacteria, and can be used for the crude preparation of purified proteins and the preparation of membrane proteins, the extraction process is simple and convenient. This kit contains a unique formulation that effectively solubilises cell membrane components. The protease inhibitor mixture prevents protein degradation by proteases, providing for the extraction of high quality proteins.

The proteins extracted in this kit are active proteins with natural protein conformation， which can be used in downstream protein research experiments such as Western Blotting, protein electrophoresis, immunoprecipitation, ELISA, transcriptional activity analysis, Gel shift gel block assay, enzyme activity assay and so on.

This kit does not contain EDTA and is compatible with downstream applications such as metal chelation chromatography.

Reagents and Equipment Required but Not Provided

Centrifuge, shaker, vortex mixer, pipette, refrigerator, ice box, PBS buffer, protein quantification kit, centrifuge tubes, pipette tips, disposable gloves

Product Features

1. Easy to use, protein extraction from bacteria does not need to go through ultrasonic crushing and other pre-treatment.
2. Reduce the time of protein extraction to 1 hour.
3. Containing protein stabiliser, the extracted protein is stable.
4. Low background interference in UV detection of protein concentration.
5. Protease inhibitors inhibit protein degradation with an optimised formulation. The Protease Inhibitor Mixture contains 6 separate protease inhibitors; each inhibitor specifically inhibits one or more protease activities. The optimised composition of the mixture allows it to inhibit almost all important protease activities, including serine proteases, cysteine proteases, aspartate proteases, alanyl-aminopeptidases and others.
6. This product can be used for Gram-positive and Gram-negative bacteria.

Protocol

I. Notes

1. Please centrifuge the reagents in spin-cap centrifuge tubes for a short time before opening the cap to shake the liquid to the bottom of the tube, so as to avoid spilling the liquid when opening the cap.

2. Protease inhibitor is in the solid form at 2-8 ° C. Remove from the refrigerator and return to room temperature or 37 ° C for a short time water bath, turn into the liquid form and centrifuge to the bottom of the tube before opening the cap.

3. All the reagents during the experiment should be pre-cooled; all the instruments should be pre-cooled in the refrigerator at -20℃. The samples should be kept at low temperature throughout the process.

4. If precipitation occurs during storage of protease inhibitor, it will not affect the use, and it will be used normally after dissolution.

5. If the kit cannot be used up in a short time, the protease inhibitor mixture should not be added to the extract all at once.

6. Other protease inhibitors can be added according to your experimental needs.

7. For downstream experiments where the enzymatic activity of a specific protease or phosphatase is being measured, the extract can be used without the addition of protease inhibitors or phosphatase inhibitors. Keep the extraction process at low temperature to shorten the centrifugation time.

II. Procedure

1. Extract preparation:

Add 5ul of Extract Solution B and 2ul of Protease Inhibitor Mixture D to every 500ul of cold Extract Solution A. Mix well and set aside on ice.

2. Collect the organisms by centrifugation and wash them twice with PBS.
3. Add 500ul of cold Extract Solution A to every 100-150 mg of wet weight sample of bacterium(the

volume ratio of bacterium and extract is about 1:3- 1:5, completely submerge the bacterium), blow and mix well. Shake at 2-8℃ for 1-2 hours until the bacterium is completely lysed, and the bacterial precipitation is reduced.

4. Centrifuge the bacterial solution at 12000×g for 5 minutes at 2-8 ° C and take the supernatant.
5. Water bath at 37 ° C for 10 minutes.
6. Centrifuge at 37 ° C, 1000 x g for 3 minutes.
7. At this point the liquid is divided into 2 layers, carefully remove the upper layer, leaving the lower layer at the bottom of the tube, about 50ul.
8. Dissolve the solution with 1-2 times the volume of membrane protein dissolution solution to obtain a sample of bacterial membrane protein.
9. Quantify the above protein extracts and store them at -80 。C or use them directly in downstream experiments.

Q&A

1. Low protein concentration?

Lower abundance of membrane proteins requires a larger volume of cells to be sampled. Some tissue samples may not be lysed completely when processed, resulting in low protein concentrations. Simply extend the processing time of Extract Solution A appropriately. It is best to process under continuous shaking conditions, or if the shaker is not available, mix by pipetting at intervals of a few minutes.

2. What methods are used to quantify protein?

The BCA method is recommended. The Bradford method is not suitable because Extract Solution A contains components that interfere with the Bradford method, resulting in inaccurate quantification. The Bradford method can be used for quantification if dialysis has been performed or if the buffer system has been changed with a desalting column.

3. Are the extracted proteins active?

This kit does not contain ionic detergent components, does not destroy the structure of proteins, there is no damage to the original interactions between proteins, so proteins maintain their natural conformation and activity.

Notes

1. This kit is for scientific research use only, not for diagnostic or medical treatment.
2. It is best to use disposable tips, tubes, bottles or glassware. Reusable glassware must be washed and thoroughly cleaned of residual cleaning agents before use.
3. All samples and contacted instruments should be disposed of according to prescribed procedures after completion of the experiment.
4. Avoid contact of skin or mucous membranes with reagents.
5. If the reagents accidentally come in contact with the skin or eyes, flush with water immediately.