Bacterial membrane protein extraction kit

Item No. : EX1940

Specification: 50T/100T

Validity: 2-8℃ storage, valid for one year.

Product content:

Name	50T	100T	Storage conditions
Bacterial membrane protein extract Solution A	25mL	50mL	Store at 2-8℃
Bacterial membrane protein extraction B	250μL	500μL	Store at 2-8℃
Membrane protein solution C	10mL	20mL	Store at 2-8℃
Protease inhibitor mixture	100μL	200μL	Store at -20℃

Note:

1. Protease inhibitors can also be stored at 2-8℃ before use without open lid. Store at -20℃ after opening the lid for use.
2. The protease inhibitor is solid at 2-8℃. Take it out of the refrigerator and return to room temperature or 37℃ water bath for a short time. When it becomes liquid, centrifuge it to the bottom of the tube and then open the lid.
3. Please use the reagent as soon as possible after unpacking!

Product Introduction:

Bacterial membrane protein extraction kit is an efficient and high-yield membrane protein extraction kit. Bacterial membrane protein extraction kit can extract membrane protein from various bacteria, which can be used for crude preparation of purified protein and membrane protein preparation. The extraction process is simple and convenient. The kit contains a unique formula that effectively dissolves cell membrane components. The kit contains a protease inhibitor mixture that prevents protease from degrading the proteins, ensuring the extraction of high quality proteins.

The protein extracted from this kit can be used for downstream protein research experiments such as Western Blotting, protein electrophoresis, immunoprecipitation, ELISA, transcriptional activity analysis, Gel shift gel blocking assay, enzyme activity determination, etc. The proteins extracted by this kit are active proteins with natural protein conformation.

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

Bring your own reagents and instruments:

Centrifuge, oscillator, vortex mixer, pipette, refrigerator, ice box, PBS buffer, protein quantification kit, centrifuge tube, suction tip, disposable gloves

Product Features:

1、Easy to use, protein extraction from bacteria does not need to go through ultrasonic crushing and other pretreatment.

2、Containing protein stabilizer, the extracted protein is stable.

3、The background interference is low when the protein concentration is detected by UV.

4、Protease inhibitors inhibited protein degradation, and the formulation of protease inhibitors

was optimized. The protease inhibitor mixture consists of 6 separate protease inhibitors; Each inhibitor specifically inhibits one or several protease activities. The composition of the mixture is optimized so that it can inhibit almost all important protease activities, including serine protease, cysteine protease, aspartate protease, alanyl-aminopeptidase, etc.

5、This product can be used on both gram-positive and Gram-negative bacteria.

6、This product does not contain EDTA and can be used for downstream applications such as metal chelation chromatography.

How to use:

First, use precautions:

1. The reagent in the rotating cap centrifuge tube should be centrifuged briefly before opening the cap, and the liquid on the inner wall of the cap should be thrown to the bottom of the tube to avoid the liquid spilling when opening the cap.
2. Protease inhibitor at 2-8℃ is a solid state, after taking out from the refrigerator, return to room temperature or 37℃ for a short time water bath, become a liquid state, centrifuge to the bottom of the tube and then open the lid.
3. All reagents used in the experiment must be pre-cooled; All utensils must be pre-cooled in a -20℃ refrigerator. The sample must be kept at a low temperature during the whole process.
4. If the solution of protease inhibitor is precipitated during storage, it will not affect the use, and it should be used normally after dissolution.
5. If the kit cannot be used up in a short time, the protease inhibitor mixture cannot be added to the extract all at once.
6. Other protease inhibitor products can be added as needed for your own experiment.
7. In the downstream experiment, if the enzyme activity of specific protease or phosphatase is detected, the extract can be without protease or phosphatase inhibitors. Pay attention to the low temperature operation during the extraction process to shorten the centrifugation time.

Second, the operation steps

1. Extraction liquid preparation:

Every 500μL cold extract A, add 5μL extract B and 2μL protease inhibitor mixture, mix well and put on ice for use.

2. Centrifuge the collector and wash the collector with PBS twice.
3. Add 500μL of cold extraction solution A (about 1:3-1:5 volume ratio of bacteria and extraction liquid) per 100-150mg wet heavy bacteria sample, blow and mix well, shake at 2-8℃ for 1-2 hours, until the bacteria are completely cracked, the liquid is clarified, and the bacteria precipitation is reduced.
4. The bacterial solution was centrifuged at a low temperature of 12,000×g at 2-8℃ for 5 minutes and the supernatant was taken.
5. Bathe in water at 37℃ for 10 minutes.
6. Centrifuge 1000×g at 37℃ for 3 minutes.

7. At this time, the liquid is divided into 2 layers. Carefully remove the upper solution and leave the lower part of the tube at the bottom, about 50μL of liquid.
8. Dissolve the solution with 50-200μL cold membrane protein solution to obtain a bacterial membrane protein sample.
9. The protein extract was quantified and divided into -80°C refrigerator for reserve or directly used for downstream experiment.

Analysis of common problems:

1. Low protein concentration?

The abundance of membrane protein is low, so it is necessary to increase the amount of cell samples as much as possible. Some tissue samples may not be fully lysed when processed, resulting in low protein concentrations. Just extend the processing time of reagent A appropriately. It is best to handle under the condition of continuous oscillation, and it can be mixed with a suction head at intervals of several minutes without an oscillator.

2. What is the method of quantifying the protein?

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

3. Is the extracted protein active?

This kit does not contain ionic detergent components, does not destroy the protein structure, does not disrupt the original interaction between the proteins, and the proteins maintain their natural conformation and activity.

What to note:

1. This kit is intended for scientific research only and is not intended for diagnosis or treatment.
2. It is best to use disposable suction heads, tubes, bottles, or glassware, and reusable glassware must be washed and thoroughly removed of residual cleaners before use.
3. All samples and exposed glassware should be disposed of in accordance with the prescribed procedure after the experiment is completed.
4. Avoid skin or mucous membranes coming into contact with the reagent.
5. If the reagent accidentally comes into contact with skin or eyes, it should be rinsed with water immediately.