Bacterial membrane protein/cytoplasmic protein extraction kit

Item No. : EX1990

Specification: 50T/100T

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

Product content:

Name	50T	100T	Storage conditions
Bacterial cytoplasmic protein extract A	25mL	50mL	Store at 2-8℃
Bacterial membrane protein extract 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/Cytoplasmic protein extraction kit can extract membrane protein and cytoplasmic protein from a variety of bacteria. 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 proteins extracted from this kit can be used for Western Blotting, protein electrophoresis, immunoprecipitation, ELISA, and transcriptional activity

Analysis, Gel shift gel retardation experiment, enzyme activity determination and other downstream protein research experiments. The protein extracted by this kit is the active protein with natural protein conformation.

EDTA is not present in this kit 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 precipitates during storage, 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 extraction solution 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:

Add 5μL extract B and 2μL protease inhibitor mixture into every 500μL cold extract A, mix well and put on ice for later use.

2. Centrifuge the bacteria and wash the bacteria with PBS twice.

3. Add 500μL of cold extract liquid A per 100-150mg of wet heavy bacteria sample (about 1:3-1:5 volume ratio of bacteria and extract liquid, completely submerged bacteria), blow and mix well, shake at 2-8℃ for 1-2 hours, until bacteria are completely cracked, liquid is clarified, and bacteria precipitation is reduced.

4. Centrifuge the bacterial solution at a low temperature of 12,000×g at 2-8℃ for 5 minutes, and take the supernatant.

5. Water bath 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, and the upper layer is carefully removed to get the cytoplasmic protein.
8. Leave the bottom layer of the tube, about 50μL of liquid.
9. Dissolve the lower layer solution with 50-200μL cold membrane protein solution to obtain the bacterial membrane protein sample.
10. 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?

Membrane protein abundance is low, and 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.

4. No bands in membrane protein electrophoresis?

Membrane protein samples are usually low in concentration and must be quantified before electrophoresis to ensure that the amount of protein on the electrophoresis is sufficient.

After the membrane protein is extracted and fully dissolved with the solution, it can be treated by ultrasound and then quantified.

After Loading the protein with Loading buffer, it can be kept at 50°C for 30 minutes without boiling.

The final concentration of SDS in protein Loading buffer can be increased to 3%-10%.

If the content of membrane protein in some samples is too low, acetone can be used to precipitate the membrane protein, and then dissolve the membrane protein in the loading buffer, usually clear protein bands can be produced.

Low current and low current electrophoresis is the best method for electrophoresis.

Precautions:

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 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.