Yeast membrane protein/cytoplasmic protein extraction kit

Item No. : EX2110

Specification: 50T/100T

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

Product content:

Name	50T	100T	Storage conditions
Yeast membrane protein Extract A	25mL	50mL	Store at 2-8℃
Yeast membrane protein extract B	25mL	50mL	Store at 2-8℃
Membrane protein solution C	10mL	20mL	Store at 2-8℃
Protease inhibitor mixture	200μL	400μL	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:

Transmembrane proteins undertake a variety of biological functions, and the preparation of membrane protein samples needs to fully consider the support of downstream gel analysis and mass spectrometry applications, so the preparation of membrane protein samples has become an insurmountable challenge.

Yeast membrane protein/cytoplasmic protein extraction kit is a high-efficiency high-yield membrane protein extraction reagent box, yeast membrane protein/cytoplasmic protein extraction kit can extract membrane protein/cytoplasmic protein from various yeast samples, and can be used for crude preparation of purified proteins and membrane protein preparation. The extraction process is simple and convenient.

The kit contains a protease inhibitor mixture, which prevents protease from degrading the protein and provides a guarantee for extracting high purity protein.

The proteins 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, and enzyme activity determination.

The proteins extracted by this kit are active proteins with natural protein conformation.

EDTA is not present in this kit and is compatible with downstream applications such as metal chelation and chromatography.

Product Features:

1. Easy to use.
2. Contains 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.

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. All reagents must be pre-cooled during the experiment; All utensils must be pre-cooled in a -20℃ refrigerator. The sample must be kept at a low temperature during the whole process.
3. If the solution of protease inhibitor is precipitated during storage, it will not affect the use, and it should be used normally after dissolution.
4. You can add other protease inhibitor products according to your own experimental needs.
5. Extract solution B must be kept at 2-8℃ before use, otherwise it will not be easy to delaminate when downstream membrane protein is extracted.
6. Boiling of loadingbuffer should be avoided during membrane protein electrophoresis.
7. SDS of the loadingbuffer can be increased during membrane protein electrophoresis.

Two, operation steps

1. Extraction liquid preparation:

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

Add 2μL protease inhibitor mixture to every 500μL of membrane protein solution C, mix well and put on ice for later use.

2. Take an appropriate amount of yeast culture, centrifuge it at 4℃, 2000×g, for 5-10 minutes, carefully absorb the medium, blot it as dry as possible, and collect yeast precipitates.

3. Wash the yeast twice with cold PBS, sucking up the supernatant as much as possible after each wash.

4. Add 500μL yeast protein extract A to every 100-200μL volume of yeast sediment, mix well, and gently shake at room temperature or 37℃ for 30 minutes to 1 hour.

5. Centrifuge at 4℃, 2000×g, for 5-10 minutes, remove the supernatant and collect the precipitate.

6. Add 500μL of cold (4℃) yeast protein extract B to the precipitate and mix well.

7. Gently shake at 2-8°C for 15-45 minutes.
8. Centrifuge the extraction solution at 12000×g at 4°C for 5 minutes and remove the supernatant.
9. Put the supernatant in a water bath at 37°C for 10 minutes.
10. Centrifuge at 1000×g at 37°C for 3 minutes.
11. At this time, the solution is divided into 2 layers, the upper part is the yeast cytoplasmic protein, the lower part is the membrane protein.
12. Carefully remove the upper cytoplasmic protein portion, leaving about 30-50μL liquid in the lower part of the bottom of the tube.
13. Dissolve the lower layer solution with 50-100μL membrane protein solution C to obtain a yeast membrane protein sample.
14. 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, and it is necessary to increase the amount of cell samples. Some cell samples may not be fully lysed when treated, resulting in low protein concentrations. Just extend the processing time of reagents A and B 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 method is used to quantify the protein?

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

3. Is the extracted protein active?

This kit does not contain ionic detergent components, does not destroy the structure of the protein, does not destroy 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 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.