Yeast membrane protein extraction kit

Item No. : EX2100

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	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:

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

Yeast membrane protein extraction kit is an efficient and high-yield membrane protein extraction kit. Yeast membrane protein extraction kit can extract membrane protein from various yeast samples, and can be used for crude preparation of purified protein and membrane protein preparation. The extraction process is simple and convenient.

This kit contains a unique formulation that effectively dissolves the total membrane components of yeast, including the plasma membrane, nuclear membrane, and various organelle membranes. The kit contains a protease inhibitor mixture that prevents protease from degrading the proteins and provides a guarantee for extracting high purity proteins.

The protein extracted from this kit can be used for WesternBlotting, protein electrophoresis, immunoprecipitation, ELISA, transcriptional activity analysis, Gelshift gel retardation test, enzyme activity determination and other downstream protein research experiments.

The protein extracted by this kit is 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.

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、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 should not be added to the extraction solution all at once.

6、Other protease inhibitors can be added according to their own experimental needs.

Second, the 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.

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 the 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 500μLof cold (4℃) yeast protein extract B to the precipitate and mix well.

7. Gently shake at 2-8℃ for 15-45 minutes.

8. Centrifuge the extract 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, carefully remove the upper part, leaving about 30-50μL liquid in the lower part of the bottom of the tube.
12. Dissolve the lower layer solution with 50-100μL membrane protein solution C to obtain a yeast membrane protein sample.
13. 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. The Bradford method is not suitable because the reagent 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 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 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.