Fungal Membrane Protein Extraction Kit

Item number: EX2090
Specification: 50T/100T
Validity: 2-8℃ storage, valid for one year.

Product content:

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

Note:

1. Protease inhibitors can also be stored at 2-8°C before use without open lid. Store at -20°C after opening the lid for use.
2. The protease inhibitor is solid at 2-8°C. Take it out of the refrigerator and return to room temperature or 37°C 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 various biological functions and play an important role in the occurrence and development of diseases. The preparation of membrane protein samples needs to be fully considered for downstream applications such as gel analysis and mass spectrometry, so the preparation of membrane protein samples becomes an insurmountable challenge.

Fungal membrane protein extraction kit is a fast and efficient high-yield membrane protein extraction kit. Fungal membrane protein extraction kit can extract membrane protein from a variety of large fungi (fungi), which 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 cell membrane components, including the plasma membrane, the nuclear membrane, and various organelle membranes. The kit contains a protease inhibitor mixture that prevents the protease from degrading the protein and ensures the extraction of 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. containing protein stabilizer, the extracted protein is stable.

3. UV detection of protein concentration, low background interference.
4. Protease inhibitor inhibited protein degradation, and the formula of protease inhibitor 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 A 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 the loadingbuffer should be avoided during membrane protein electrophoresis.
7. SDS of the loadingbuffer can be increased during membrane protein electrophoresis.
8. Centrifuge speed has relative centrifugal force (RCF, ×g) and speed per minute (RPM) two ways to express, some centrifuges have RPM and ×g display switching, but some centrifuges do not have automatic switching function. Need to use the following formula for conversion: $g=r\times1.118\times10^{-5}\times rpm^2$(r is the effective centrifugal radius, that is, the length from the centrifuge axis to the center of the centrifugal collection tube at the bottom, in centimeters) For example: the rotational speed is 3000rpm, the effective centrifugal radius is 10cm, then the relative centrifugal force (RCF, ×g) $=10\times1.118\times10^{-5}\times3000^2=1006.2$(×g).

2. Operation steps

1. Preparation of extraction liquid:

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

2. Take 100-200mg fungal sample and cut it up as much as possible with surgical scissors. Add 500μL of extract solution A and fully homogenize it with a homogenizer/homogenizer.
3. Oscillate continuously at 2-8℃ for 1-2 hours.
4. Centrifuge the extract at 10000×g at 2-8℃ for 5 minutes and take the supernatant.
5. Add 5μL of extract B to the supernatant and mix thoroughly.
6. Water bath at 37℃ for 10 minutes.
7. Centrifuge 1000×g at 37℃ for 5 minutes.
8. At this time the solution is divided into 2 layers, carefully remove the upper layer, leaving about

50μL liquid at the bottom of the lower tube.

9. Dissolve the solution with 1-2 times the volume of membrane protein dissolving solution C to obtain a membrane protein sample.
10. The protein extract was quantified and divided into -80℃ 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 sample as much fungus as possible. Some tissue samples may not be fully lysed when handled, 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 method is used to quantify the protein?

The 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 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.