Filamentous Fungal Membrane Protein Extraction Kit

Cat: EX1820
Size: 50T/100T
Storage: 2-8℃, valid for 1 year.

Kit Components:

Kit Components	50T	100T	Storage
Component A: Filamentous Fungal Membrane Protein Extract A	25mL	50mL	2-8℃
Component B: Filamentous Fungal Membrane Protein Extract B	250μL	500μL	2-8℃
Component C: Membrane Protein Solution C	10mL	20mL	2-8℃
Component D: Protease Inhibitor Mixture D	100μL	200μL	-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!

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.

Filamentous fungi membrane protein extraction kit is a fast, efficient and high-yield membrane protein extraction kit. The filamentous fungi membrane protein extraction kit can extract total membrane proteins from a variety of small filamentous fungi (molds) in addition to large fungi (fungoides). The optimized lysate formula can fully release membrane proteins from all kinds of membranous mycelia and non-membranous mycelia proteins, and extract membrane proteins from various mycelia metamorphosis proteins, which can be used for crude preparation of purified proteins and membrane protein preparation. The extraction process is simple and convenient.

This kit contains a unique formula that effectively dissolves cell membrane components, including the plasma membrane, 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 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, which can be used for different downstream applications.

EDTA is not contained in this kit and is compatible with metal chelates and chromatography, among others.

Self-prepared Reagents and Instruments:

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

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 of which specifically inhibits one or several protease activities. The optimized composition of this mixture allows it to inhibit almost all important protease activities, including serine protease, cysteine protease, aspartate protease, alanyl-aminopeptidase, etc.

Protocols:

First, use precautions

1. Before the formal experiment, please select several samples to do pre-experiment, in order to optimize the experimental conditions and achieve the best experimental results
2. Centrifuge the reagent in the screw cap microreagent tube briefly before opening the cap, and centrifuge the liquid on the cap and inside wall to the bottom of the tube to avoid reagent loss when opening the cap.
3. All reagents in the process of 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 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.
8. It is prohibited to mix with other brands of reagents, otherwise the effect will be affected.
9. Contamination of the sample or reagent with bacteria or fungi or cross-contamination of reagents may result in false results.

Second, filamentous fungal membrane protein extraction

1. Extraction solution preparation:
   Add 1μL protease inhibitor mixture into each 500μL cold extract A and C, mix well and put on ice for use.
2. Collect 50-200mg fungal samples, wash with PBS twice, centrifuge at 4℃, 5000×g for 5min, carefully absorb PBS, blot as dry as possible, and collect bacteria.
3. Add 500μL extract solution A and then homogenize fully with a homogenizer/homogenizer.
4. Keep oscillating at 2-8℃ for 1-2h.
5. Centrifuge the extraction solution at 10000×g at 2-8℃ for 5min and remove the supernatant.
6. Add 5μL of extract B to the supernatant and mix thoroughly.

7. Water bath at 37°C for 10min.
8. Centrifuge 1000×g at 37°C for 5min.
9. At this time the solution is divided into 2 layers, carefully remove the upper layer, leaving about 50μL of liquid at the bottom of the lower tube.
10. Dissolve the solution with 1-2 times the volume of membrane protein dissolving solution C to obtain a membrane protein sample.
11. The protein extract was quantified and divided into -80°C refrigerator for reserve or directly used for downstream experiment.

Analysis of Common Problems:

1. What method is used to quantify 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.

2. 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.

3. No bands in membrane protein electrophoresis?

Membrane protein samples are usually low in concentration, and protein quantification must be performed 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.

Membrane protein abundances are usually low, so try staining with silver if possible.

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.