Membrane Protein Extraction Kit

Cat: EX1110
Size: 50T/100T
Storage: 2-8℃, valid for 1 year.

Kit Components:

Kit Components	50T	100T	Storage
Reagent A: Protein Extract Solution A	20mL	40mL	2-8℃
Reagent B: Protein Extract B	20mL	40mL	2-8℃
Reagent C: Membrane Protein Solution C	10mL	20mL	2-8℃
Reagent D: Protease Inhibitor Mixture	200μL	400μL	-20℃

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!

Introduction:

Membrane protein extraction kit is a fast and efficient high-yield membrane protein extraction kit. This kit provides a complete set of reagents suitable for the extraction of total membrane protein from animal cells and animal tissues. The extraction process is simple and convenient and can be completed within 1h. The extracted membrane proteins are not only pure, maintain natural activity, and have little cross-contamination.

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 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.

This kit does not contain EDTA and is compatible with downstream applications such as metal chelation and chromatography.

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, extract protein from cells and tissues without grinding, repeated freeze-thaw, ultrasonic crushing and other pre-treatment.
2. The time of protein extraction is reduced to 1h.
3. Containing protein stabilizer, the extracted protein is stable.
4. The background interference is low when the protein concentration is detected by UV.
5. 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.

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. Do not mix with other brands of reagents, otherwise it will affect the use effect.
4. Contamination of the sample or reagent with bacteria or fungi or cross-contamination of reagents may result in false results.
5. All reagents used during 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.
6. If the solution of protease inhibitor precipitates during storage, it will not affect the use, and it will be used normally after dissolution.
7. 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.
8. Other protease inhibitor products can be added as needed for your own experiment.
9. 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, cell membrane protein extraction

1. Preparation of extraction solution:

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

   Add 2μL protease inhibitor mixture into every 500μL membrane protein solution C, mix well and put on ice for later use.
2. Take 5-10×10^6 cells, centrifuge at 4℃, 500×g for 5min, carefully absorb the medium, as far as possible to absorb the collection of cells.
3. Wash the cells twice with cold PBS, and drain the supernatant as much as possible after each wash.
4. Add 200μL-400μL of cold reagent A to the cell sample and mix thoroughly.
5. Oscillate at 2-8℃ for 20-30min until the cells are fully lysed and the cell precipitation is significantly reduced.
6. Centrifuge at 4℃, 12000×g, for 5min.
7. Inhale the supernatant into another clean centrifuge tube and bathe at 37℃ for 5-10min.
8. At 37℃, 1000×g centrifuge for 5min, at this time the solution is divided into two layers, the lower layer is the membrane protein about 30-50μL.
9. Carefully remove the upper liquid and collect the upper part for backup analysis.
10. Use 150-200μL cold reagent B to fully dissolve the lower layer of membrane protein, mix and ice bath for 2 minutes, 37℃ water bath for 5-10min, 37℃, 1000×g centrifugation for 5min, at this time the solution is divided into two layers, the lower layer is about 30-50μL of membrane protein. Carefully remove the upper layer of liquid and keep the lower layer.
11. Follow the steps above to extract the lower layer membrane protein again (this step is optional and generally not required).
12. Dissolve the lower layer of membrane protein with 50-200μL cold reagent C membrane protein solubilizing solution to obtain the membrane protein.

13. The protein extract was quantified and then divided into -80℃ refrigerator for reserve or directly used for downstream experiment.

Third,membrane protein extraction from tissue samples

1. Preparation of extraction solution:

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

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

2. Take an appropriate tissue sample of 50mg-100mg, wash it with cold PBS, then cut it as much as possible with surgical scissors, add 400μL of cold reagent A, and homogenize it with a tissue homogenizer/machine until there is no obvious visible solid.
3. The tissue homogenate was sucked into a pre-cooled clean centrifuge tube and oscillated at 4℃ for 20-30min.
4. Follow the step (5) of the cell protein extraction method to operate below.

Analysis of common problems:

1. Low protein concentration?

The abundance of membrane protein is relatively low, and if conditions permit, it is necessary to increase the amount of cell loading as much as possible to increase the membrane protein concentration. Some tissue samples may not be fully lysed when treated, 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 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. 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℃ for 30min 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.

4. 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.

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 membrane contact with the reagent.