Membrane Protein Extraction Kit

Cat: EX1111
Size: 50T/100T
Storage: 2-8℃ (Store at 2-8℃ before using protease inhibitor without lid, store at -20℃ after using lid), valid for 1 year.

Kit Components:

Kit Components	50T	100T	Storage
Reagent A: Protein extract Solution A	27mL	55mL	2-8℃
Reagent B: Membrane Protein Solution B	11mL	22mL	2-8℃
Reagent C: Protease Inhibitor Mixture C	100μL	100μL×2	-20℃
Specification	1	1	

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:

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 not only have high purity, maintain natural activity, but also rarely 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.

Product Features:

1. Easy to use, extract protein from cells and tissues without grinding, repeated freezing and thawing, ultrasonic crushing and other pre-treatment.
2. The time of protein extraction can be shortened 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 mixture can inhibit almost all important protease activities, including serine protease, cysteine protease, aspartate protease, etc.

Protocols:

A. Cell membrane protein extraction

1. Extract solution preparation:
   Add 1μL of reagent C for every 500μL of reagent A, mix well and set aside on ice.
   Add 1μL of reagent C every 500μL of reagent B, mix well and set aside on ice.
2. Take more than 5-10×10^6 cells, centrifuge at 4℃, 500×g for 3-5min, carefully absorb the medium, blot as much as possible, and collect cells.
3. Wash the cells twice with cold PBS, sucking up the supernatant as much as possible after each wash.
4. Add 500μL of cold reagent A (with reagent C) to the cell sample and mix well.
5. It was oscillated at 2-8℃ for 30min until the cells were fully lysed and cell precipitation was significantly reduced.
6. Centrifuge at 4℃, 12000×g, for 5min.
7. The supernatant was sucked into another clean centrifuge tube and bathed in water at 37℃ for 10min.
8. At 37℃, centrifuge at 1000×g force for 5min. At this time, the solution is divided into two layers.
9. Remove the upper layer of liquid carefully and collect the upper layer to keep for further analysis.
10. The membrane protein is obtained by fully dissolving the lower membrane protein portion with reagent B (containing reagent C) of 100-200μL ice.
11. The protein extract was quantified and divided into -80℃ refrigerator for reserve or directly used in downstream experiment.

B. Tissue membrane protein extraction

1. Extract solution preparation:
   Add 1μL of reagent C for every 500μL of reagent A, mix well and set aside on ice.
   Add 1μL of reagent C every 500μL of reagent B, mix well and set aside on ice.
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 500μL of cold reagent A (including reagent C), 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 30min.
4. Follow Step 5 of A. cell membrane protein extraction to proceed.

Note:

1. This kit is intended for scientific research only and is not intended for diagnosis or treatment.
2. All samples and utensils touched upon completion should be disposed of in accordance with the prescribed procedures.