Membrane Protein Extraction Kit

Cat.No: PE003-1 50T
PE003-2 100T

PE003

I. Introduction

The Membrane Protein Extraction Reagent Kit is for the enrichment of integral membrane proteins from cultured mammalian cells or from mammalian tissue using a mild detergent -based protocol. The cells are first lysed with a detergent, after which a second detergent is added to solubilize the membrane proteins. The cocktail is incubated at 37°C to separate the hydrophobic proteins from the hydrophilic proteins through phase partitioning.

Extraction efficiencies will vary depending on the number of times the integral membrane proteins of interest spans the lipid bilayer. Membrane proteins with up to five transmembrane domains are typically extracted with an efficiency of up to 90%. Cross -contamination of cytosolic proteins into the membrane fraction is typically < 10%.

II. Kit contents:

Reagent A	10ml	20ml
Reagent B	20ml	40ml
Reagent C	10ml	20ml
Protease Inhibitor Cocktail	250μl	500μl

1. Protein Extraction Solution: includes some effective components, which could release the membrane protein completely; the solution also includes phosphatase inhibitor cocktail.

2. Protease Inhibitor Cocktail: includes 7 independent protease inhibitor, AEBSF、Aprotinin、Leupeptin、Pepstatin A、Bestatin、E-64、EDTA.

Note:

1. The Cocktail should be avoid repeated freeze / thaw cycles.
2. If the kit could not be used up in one time, please do not add all the cocktail into the extraction solution.

III. Storage

1. Storage the Protease Inhibitor Cocktail at -20℃.
2. Storage the extraction solution at 2-8℃.

IV. Period of validity

One Year

V. Protocol:

A. Extraction of cell protein

1. Take 5-10×10^6 cells①, centrifugate them at 4℃,500 Xg for 2-3 mins, draw the medium carefully and dry, then collect the cells.
2. Wash the cells twice using cold PBS. Carefully remove and discard the supernatant each time.
3. Add 200ul cold reagent A and 2ul cocktail to the cell sample, Pipette up and down

to obtain a homogeneous cell suspension，then put it on ice bath for 10-15 mins.

4. Then shake it again in the high-speed vortex for 5s , Centrifuge tubes at 10,00 Xg for 5 mins at 4°C.
5. Transfer supernatant to new tubes and incubate 5-10 mins in 37°C water bath. then centrifuge tube at 200Xg for 5mins at 37℃.You will see 2 layers in the solution ,the top layer is the hydrophilic phase, the bottom layer is the hydrophobic protein phase with the amount of 20ul.
6. Carefully remove the hydrophilic phase (top layer) from the hydrophobic protein phase (bottom layer) and save in a new tube.
7. Collect the hydrophilic phase for analysis.③.
8. Dilute the hydrophobic phase with 150-200μl cold reagent B, blending it then put it on ice bath for 2 mins，37℃water bath for 5-10mins，centrifugate at 37℃②,2000 Xg for 5mins , There are 2 layers in the solution ,the top is hydrophilic phase, the bottom is hydrophobic phase with the amount of 20ul.
9. Repeat step 7 to extract hydrophobic phase (you could choose to do this step)
10. Dilute the hydrophobic phase with 80-200μl cold reagent C, then get the membrane protein.
11. Quantitation the extraction④ and package it ,then store at -80℃ refrigerator or use it for the following experiments directly⑤.

Note:

① Adjust the quantity of cell according to the experiment, the usage of lysis buffer is not the same every time, so please adjust it . Due to the low content of membrane protein, more cells are needed to ensure the yield.

② Centrifugate at 37℃,or at least 30℃.If there is no centrifugal machine with temperature control, please use the machine to centrifugate at RT, shorten the time to 2-3 mins.

③ When doing the the following experiments, dilute the hydrophilic phase and hydrophobic phase with reagent C. Analysis the hydrophilic phase at the same time.

④ Using BCA method to quantitation the protein.

⑤The protein sample is also available after store at －80℃ for one year. Keep it away from protease and bacteria.

B. Extraction of tissue protein.

1. Take proper quantity of tissue sample and cut it into layers, add cold PBS, homogenate it with Tissue Grinders till there is no solid could be seen(or grinding it with Liquid nitrogen),then put it on ice bath for 5 mins,
   Carefully transfer the homogenate to another pre-cooling clean centrifugal tube.
2. Centrifugate the tube at 4℃②,500 Xg for 2-3 mins, discard the supernatant.
3. Following the 3rd step of cell protein extraction to do the next procedures.
4. Storage the quantified extraction at -80℃ refrigerator for use.