Platelet Membrane Protein Extraction Kit

Cat: EX1220
Size: 50T/100T
Storage: 2-8°C, valid for 1 year.

Kit Components:

Kit Components	50T	100T	Storage
Reagent A: Extract Solution A	25mL	50mL	2-8°C
Reagent B: Extract Solution B	25mL	50mL	2-8°C
Reagent C: Membrane Protein Solution C	15mL	30mL	2-8°C
Reagent D: Protease Inhibitor Mixture	250μL	500μL	-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!

Introduction:

The platelet membrane protein extraction kit is suitable for extracting membrane proteins from various animal platelets. The extraction process is simple and convenient and can be completed within 1h.

This kit contains a unique formula that effectively dissolves the membrane components. 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.

EDTA is not present in this kit 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, shorten the time of protein extraction to 30min.
2. Containing protein stabilizer, the extracted protein is stable.
3. The background interference is low when the protein concentration is detected by UV.

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 precipitates during storage, it will not affect the use, and it will 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, platelet membrane protein extraction

1. Extract solution preparation:
   Add 2μL protease inhibitor mixture to every 500μL of cold protein extract A, mix well and set aside on ice.
   Add 2μL protease inhibitor mixture to every 500μL protein solution C, mix well and set aside on ice.
2. Take 1-5mLACD anticoagulant and centrifuge at room temperature 200g for 10min.
3. Collect the upper plasma and discard the lower blood cells to precipitate.
4. Centrifuge the upper plasma at room temperature 3000g for 10min, discard the supernatant, and collect the precipitation.
5. Add 500μL extract B to the precipitation and wash it once, centrifuge 3000g for 10min, discard the supernatant, and collect the precipitation.
6. Add 300-500μL of cold protein extract solution A to the precipitation, mix well, and oscillate at 4°C for 20-30min.
7. Centrifuge at 4°C, 12000g for 10min and remove the supernatant.
8. Take a water bath at 37°C for 10min.
9. Centrifuge 1000g at 37°C for 3min.
10. At this point the liquid is divided into 2 layers, carefully remove the upper layer of solution and leave the lower layer at the bottom of the tube, about 30-50μL of liquid.
11. Dissolve the liquid with 1-2 times the volume of membrane protein dissolving solution C to obtain a sample of platelet membrane protein.

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

Analysis of Common Problems:

1. Low protein concentration?

Platelet membrane protein abundance is low, in the case of conditions, it is necessary to increase the amount of platelet samples as much as possible.

Some tissue samples may not be fully lysed when processed, resulting in low protein concentrations. Simply extend the processing time of the reagent 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?

It is recommended to use BCA method. 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 protein structure, does not destroy the original interaction between proteins, proteins maintain their natural conformation and activity.

4. Is there no band in membrane protein electrophoresis?

The concentration of membrane protein samples is usually low, and protein quantification must be carried out 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 buffer is added, the protein 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 used in the final electrophoresis.

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.