Total Protein Extraction Kit (for Proteome Test, Mass Spectrometry)

Cat: EX1101
Size: 50T/100T
Storage: 2-8℃, valid for 1 year.

Kit Components:

Kit Components	50T	100T	Storage
Component A: Protein Extract A1	22.5mL	45mL	2-8℃
Component B: Protein Extract A2	2.5mL	5mL	-20℃
Component C: Protease Inhibitor Mixture B	100μL	200μL	-20℃

Note:

1. Protein extract solution A2 and protease inhibitor can also be stored at 2-8℃ before use without open lid. After opening the lid, store at -20℃.
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:

Protein extraction kit (suitable for proteomics experiments) is suitable for extracting total protein from various primary or successive animal cells and various solid tissues, such as brain, spinal cord, nerve junction or fiber, fat, liver, digestive tract, kidney, heart, muscle, blood vessel, connective tissue and other animal tissues. The extraction process is simple and convenient, and can be completed within 1h.

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 protein extraction components of this kit do not contain detergent components that cannot be removed by dialysis, and do not contain SDS, TritonX-100, chaps and other components that may affect the mass spectrometry experiment. After dialysis or desalting treatment, the final protein sample will not contain detergent, high concentration of salt and other effects. It can basically meet the requirements of any downstream proteomic related experimental research.

The protease inhibitor mixture of this product does not contain AEBSF, which can avoid the Mass Spectrometry peak shift caused by AEBSF. Therefore, the protein samples extracted from this product can be used for mass spectrometry (MS) detection and analysis, proteomics and other related research.

The protein extracted by this kit is an active protein with natural protein conformation.

Self-prepared Reagents and Instruments:

Centrifuge, oscillator, 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 30min 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. Total protein extract contains a variety of active components, can fully release cytoplasmic protein, nuclear protein, but also can bind the released protein to prevent precipitation.
6. Protease inhibitor inhibits the degradation of protein, and the formula of protease inhibitor is optimized. The protease inhibitor mixture consists of five independent protease inhibitors Aprotinin, Leupeptin, Pepstatin A, Bestatin, and E-64, each of which can specifically inhibit the activity of one or more proteases. 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 micro-reagent 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. 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.
6. All samples and exposed glassware should be disposed of in accordance with the prescribed procedure after the experiment is completed.
7. All reagents must be pre-cooled during the experiment; All utensils must be pre-cooled in a -20℃ refrigerator. The sample must be kept at a low temperature during the whole process.
8. If the solution of protease inhibitor is precipitated during storage, it will not affect the use, and it should be used normally after dissolution
9. 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.
10. Beta-actin, GAPDH, and Tubulin can be used as parameters in Western Blot experiments.

Second, cell sample protein extraction

1. Extraction solution preparation:

Mix reagent A1 and reagent A2 to form protein extract solution A, thoroughly mix and set aside.

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

2. Collect the cells and centrifuge at 4℃, 2000×g, for 5-10min. Carefully absorb the medium and blot it as dry as possible.
3. Wash the cells twice with cold PBS, sucking up as much supernatant as possible after each wash.
4. Every 5×10^6-1×10^7 cells (about 50mg cells/50μL cell accumulations), add 500μL cold total protein extraction solution, blow and mix well, and shake at 4℃ for 20-30min until the cells are fully lysed without obvious cell precipitation.
5. Centrifuge at 4℃, 12000×g, for 15min.
6. Inhale the supernatant into another pre-cooled clean centrifuge tube to obtain the total cell protein.

7. The protein extract was quantified and divided into -80℃ refrigerator for reserve or downstream experiment.
8. The protein samples were treated by dialysis or desalting column and then used for downstream experiment.

Third, extraction of total protein from tissue samples

1. Preparation of extraction solution:

Reagent A1 and reagent A2 were mixed to form protein extract solution A, and then fully mixed for use.

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

2. Take 50-100mg tissue sample, wash it with PBS, then cut it as much as possible with surgical scissors, add 500μL total protein extract solution A, and homogenize it with a tissue homogenizer/homogenizer until there is no visible solid.
3. The tissue homogenate was sucked into a pre-cooled clean centrifuge tube and oscillated at 4℃ for 20-30min.
4. Centrifuge at 4℃, 12000×g, for 15min.
5. Inhale the supernatant into another pre-cooled clean centrifuge tube to obtain the total tissue protein.
6. The protein extract was quantified and divided into -80℃ refrigerator for reserve or directly used in downstream experiment.
7. The protein samples were treated by dialysis or desalting column and then used for downstream experiment.

Analysis of Common Problems:

1. Low protein concentration?

Some samples may not be fully lysed when processed, resulting in low protein concentration. 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 is the method of quantifying the protein?

BCA method is recommended.

3. 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 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, reusable glassware must be washed and thoroughly removed of residual cleaning agents before use.
3. After the completion of the experiment, all samples and contact utensils should be disposed of in accordance with the prescribed procedures.
4. Avoid skin or mucous membrane contact with the reagent.
5. Rinse the reagent with water immediately if it accidentally comes into contact with skin or eyes.