

Adipose Tissue Protein Extraction Kit (Proteome Test, Mass Spectrometry) - Filter Solid Impurities

Item No. : EX2600

Specification: 50T/100T

Validity: 2-8°C storage, valid for one year.

Product contents:

Name	50T	100T	Storage conditions
Component A1: Protein extract A1	22.5mL	45mL	Store at 2-8°C
Component A2: Protein extract solution A2	2.5mL	5mL	Store at 2-8
Component B: Protease inhibitor mixture B	100μL	200μL	Store at -20
Component C: Protein centrifuge tube C	50 sets	100 sets	Store at room temperature

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°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!

Product Introduction:

Adipose tissue protein extraction kit is suitable for extracting soluble total protein from adipose tissue and various solid tissues and cells with high fat content, such as adipose cells, fatty liver cells, brain, fat, fatty liver, connective tissue, thymus tissue and other animal tissues. The extraction process is simple and convenient, and can be completed within 1 hour.

The kit contains a mixture of protease inhibitors, which prevents the protease from degrading the protein, providing a guarantee for the extraction of high purity protein. The kit contains a unique formula capable of dissolving cell membranes including the plasma membrane and nuclear membrane.

The protein extraction components of this kit do not contain detergent components that cannot be removed by dialysis, and do not contain SDS, Triton X-100, chaps and other components that may affect the mass spectrometry experiment. The final protein sample will not contain detergent, high concentration salt and other components after dialysis or desalting treatment. 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 from this kit can also be used for downstream protein research experiments such as Western Blotting, protein electrophoresis, immunoprecipitation, ELISA, transcriptional activity analysis, Gel shift gel blocking assay, enzyme activity assay, etc.

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.

Bring your own reagents and instruments:

Centrifuge, oscillator, homogenizer, vortex mixer, pipette, refrigerator, ice box, PBS buffer, protein quantification kit, centrifuge tube, suction tip, disposable gloves

Product Features:

- 1、 Easy to use.

- 2、Contains protein stabilizer, the extracted protein is stable.
- 3、The background interference is low when the protein concentration is detected by UV.
- 4、Protease inhibitors inhibited protein degradation, and the formulation of protease inhibitors was optimized. The protease inhibitor mixture consists of 6 separate protease inhibitors, each of which specifically inhibits one or several protease activities. 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.

How to use:**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°C refrigerator. The sample must be kept at a low temperature during the whole process.
4. If the solution of protease inhibitor is precipitated during storage, it will not affect the use, and it should 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. Do not mix with other brands of reagents, otherwise it will affect the effectiveness of use.
7. Contamination of the sample or reagent with bacteria or fungi or cross-contamination of reagents may lead to false results.

2. Fat tissue protein extraction:**1. Extraction solution preparation:**

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

Every 500μL protein extract A, add 2μL protease inhibitor mixture B, mix well and put on ice for later use.

2. Take 100-200mg tissue sample and cut it into pieces, add 500μL protein extract solution A, 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 15-45 minutes.
4. Inhale the supernatant containing fat into the protein centrifuge tube, put on a liquid collection tube, and centrifuge at 4°C, 10000×g, for 5 minutes.
5. The liquid in the liquid collection tube is sucked into another clean centrifugal tube and centrifuged at 4°C, 12000×g, for 5 minutes.
6. Inhale the supernatant into another pre-cooled clean centrifuge tube to obtain the protein sample.
7. The protein extract was quantified and divided into -80°C refrigerator for reserve or directly used in downstream experiment.
8. 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 tissue samples may not be fully cleaved when processed, 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 the protein?

The 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. Is the extracted protein active?

This kit does not contain ionic detergent components, does not destroy the structure of the protein, does not destroy the original interaction between the proteins, and the proteins maintain their natural conformation and activity.

What to 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.
5. If the reagent accidentally comes into contact with the skin or eyes, rinse immediately with water.