Adipose Tissue Protein Extraction Kit

Cat: EX1120
Size: 50T/100T
Storage: 2-8℃, valid for 1 year.

Kit Components:

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Reagent A: Protein Extract Solution A	25mL	50mL	2-8℃
Reagent B: Protease Inhibitor Mixture B	100μL	200μL	-20℃
Reagent C: Protein Centrifuge Tube C	50	100	2-8℃

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:

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 1h.

The kit contains a protease inhibitor mixture that prevents protease from degrading the protein and provides a guarantee for extracting high purity proteins. The kit contains a unique formula capable of dissolving cell membranes including the plasma membrane and nuclear membrane.

The protein 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, enzyme activity determination, 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.

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, 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. 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 composition of the mixture is optimized so that it can 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 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. 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. All reagents used during 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.
6. If the solution of protease inhibitor is precipitated during storage, it will not affect the use, and it should be used normally after dissolution.
7. 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.
8. Other protease inhibitor products can be added as needed for your own experiment.
9. 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.

Second, adipose tissue protein extraction

1. Extraction liquid preparation:
Add 2μL protease inhibitor mixture into every 500μL protein extract, mix well and put on ice for use.
2. Protein extraction
(1) Take 100-200mg tissue sample and cut it into pieces, add 500μL protein extract solution A, and homogenize it with a tissue homogenizer/homogenizer until there is no visible solid.
(2) The tissue homogenate was sucked into a pre-cooled clean centrifuge tube and oscillated at 4°C for 15-30min.
(3) The supernatant containing fat was sucked into the protein centrifuge tube, fitted with a liquid collection tube, and centrifuged at 4℃, 10000×g, for 5min.
(4) The liquid in the liquid collection tube is sucked into another clean centrifugal tube and centrifuged at 4℃, 14,000 ×g, for 10min.
(5) Inhale the supernatant into another pre-cooled clean centrifuge tube to obtain the protein sample.
(6) The protein extract was quantified and then packaged in a -80℃ refrigerator for reserve or directly used in downstream experiments.

Analysis of Common Problems:

1. Low protein concentration?

Some tissue samples may not be fully lysed when treated, 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. Some fat samples have very low protein content, so it is necessary to increase the amount of fat sample as much as possible, or to concentrate the protein.

2. What method is used to quantify protein?

It is recommended to use Lowry method (corresponding article number PC0030). 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 also 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.

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.