Adipose Tissue Protein Extraction Kit (Protein Degreasing Filter Column)

Cat: EX1130
Size: 50T/100T
Storage: 2-8℃, valid for 1 year.

Kit Components:

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Reagent A: Total Protein Extract Solution A	25mL	50mL	2-8℃
Reagent B: Protease Inhibitor Mixture B	100μL	200μL	-20℃
Reagent C: Protein Degreasing Centrifuge Column C	10	20	RT

Note:

1. The protein defatting centrifuge column can be reused (generally repeated no more than 5 times, after use, 1×PBS is required to clean the centrifuge several times, please replace the column after blockage occurs)
2. Protease inhibitors can also be stored at 2-8℃ before use without opening the lid. Store at -20℃ after opening the lid for use.
3. 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.
4. 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 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 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 extraction process is simple and convenient, and the protein extraction time 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. 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 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. Other protease inhibitor products can be added as needed for your own experiment.
7. In the downstream experiment, if the enzyme activity of a specific protease or phosphatase is tested, the extract can be performed without protease or phosphatase inhibitors, and the extraction process should be kept at a low temperature 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, 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. Histone extraction

(1) Take 100-200mg tissue sample cut, add 500μL protein extract solution A, and fully homogenize with a tissue homogenizer/homogenizer.
(2) The tissue homogenate was sucked into a pre-cooled clean centrifuge tube and oscillated at 4°C for 20-45min.
(3) Centrifuge the oscillating homogenate for 15min at 4℃, 12000-16000×g, discard the precipitation and collect the supernatant.
(4) Centrifuge the supernatant at 4℃, 12000-16000×g, for 5min, discard the precipitation and collect the supernatant.
(5) The supernatant was sucked into the protein defatting centrifuge tube, fitted with a liquid collection tube, and centrifuged at 4℃, 10000-16000×g, for 10-15min.
(6) The liquid in the liquid collection tube is sucked into another clean centrifuge tube to obtain the protein sample.
(7) The protein extract was quantified and divided into -80℃ refrigerator for reserve or directly used for downstream experiment.

Analysis of common problems:

1. Low protein concentration?

Some tissue samples may not be fully lysed 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 protein?

It is recommended to use BCA method to measure protein concentration. Bradford method is not suitable, because reagent A contains components that interfere with 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.

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.