Serum Protein Extraction Kit (for Proteome Test and Mass Spectrometry)

Cat: EX1181
Size: 50T/100T
Storage: 2-8℃, valid for 1 year.

Kit Components:

Kit Components	50T	100T	Storage
Component A1: Protein Extract A1	22.5mL	45mL	2-8℃
Component A2: Protein Extract Solution A2	2.5mL	5mL	-20℃
Component B: Protein Diluent B	15mL	30mL	2-8℃
Component C: Protease Inhibitor Mixture C	100μL	200μL	-20℃

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:

Serum protein extraction kit is suitable for extracting total protein from various animal serum.

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, so 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.

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, 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.

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 cannot be added to the extract 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.

Second, serum protein extraction

1. Extraction solution preparation:
   Reagent A1 and reagent A2 are mixed to form serum protein extract solution A, and then fully mixed for use.
   Every 500μL of serum protein extract A, add 2μL of protease inhibitor mixture, mix well and put on ice for later use.
2. Take serum samples, add 200μL protein extract into every 100μL serum sample, mix thoroughly, and oscillate at 4℃ for 5-10min.
3. Centrifuge at 4℃, 14000×g, for 5min.
4. The serum protein can be obtained by inhaling the supernatant into another pre-cooled clean centrifuge tube.
5. The protein extract was quantified and divided into -80℃ refrigerator for reserve or directly used in downstream experiment.
6. Dilute the serum protein sample to the required concentration with protein diluent or other buffer according to the relevant experimental requirements.
7. The protein samples were treated by dialysis or column desalting and then used for downstream experiments.

Analysis of Common Problems:

1. Low protein concentration?
   Some 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. How to quantify 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.

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 skin or eyes, it should be rinsed with water immediately.