Total Protein Extraction Kit (Suitable for Protein Interaction (Pull-down, Co-IP, etc.))

Cat: EX1100
Size: 50T/100T
Storage: 2-8℃, valid for 1 year.

Kit Components:

Kit Components	50T	100T	Storage
Reagent A: Total Protein Extract Solution A	25mL	50mL	2-8℃
Reagent B: Protease Inhibitor Mixture B	100μL	200μL	-20℃
Reagent C: Phosphatase Inhibitor Mixture C	100μL	200μL	2-8℃

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℃. 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:

Total protein extraction kit provides a complete set of reagents, suitable for extracting total protein from various primary or subculture animal cells and various animal 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 tissue samples. When combined with other reagents, it can also be used to extract total protein from plant, bacteria, fungi, yeast and other samples.

This kit contains a unique formulation that effectively dissolves cell membrane components, including the plasma membrane, the 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 extracted from this kit can be used for Pull-down, IP and other protein interaction studies, as well as WB, protein electrophoresis, ELISA, transcriptional activity analysis, Gel shift gel blocking experiment, enzyme activity determination and other downstream protein research experiments.

The protein extracted by this kit is an active protein with natural protein conformation, which has a wide range of downstream applications. The ability of the extract to lyse cells is mild, and the lyse time should be optimized according to the actual sample.

This kit does not contain EDTA and is compatible with downstream applications such as metal chelation and chromatography.

The protein samples extracted from this kit contain a high concentration of salt components and cannot be used directly for 2D electrophoresis. The final sample can also be demineralized with a column and then used for 2D electrophoresis.

This kit is measured by 50mg cells/tissue per treated sample (about 50μL cell precipitation volume). If a large number of cells/tissue need to be processed, the extraction solution can be added to the cell precipitation by 1:10 of the cell volume. Each 50T kit will be able to extract approximately 2.5g of total protein from each cell/tissue sample. Depending on the cell type, the total protein yield of 100mg cell precipitate (10^7 cells) is approximately 6mg or so.

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. Total protein extract contains a variety of active components, can fully release cytoplasmic protein, nuclear protein and membrane protein, but also can bind the released protein to prevent precipitation.
6. Protease inhibitors inhibit the degradation of protein, and the formulation of protease inhibitors is optimized. The protease inhibitor mixture consists of 6 independent protease inhibitors AEBSF, Aprotinin, Leupeptin, PepstatinA, Bestatin, E-64, each of which can specifically inhibit 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 for pre-experiment 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 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.
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.
10. Beta-actin, GAPDH, and Tubulin can be used as parameters in Western Blot experiments.

Second, total protein extraction from suspended cells

1. Preparation of the extraction solution:

Add 2μL protease inhibitor mixture and 2μL phosphatase inhibitor mixture into every 500μL cold total protein extraction solution, mix well and put on ice for use.

2. Cell protein Extraction

(1) Take 5×10^6 cells, centrifuge at 4℃, 2500×g for 5min, carefully absorb the medium, and drain the collected cells as much as possible.

(2) Wash the cells twice with cold PBS, and drain the supernatant as much as possible after each wash.

(3) Every 5×10^6-1×10^7 cells (about 50mg cells/50μL cell precipitation volume), add 500μL cold total protein extraction solution, blow and mix well, and shake at 4℃ for 20-30min until the cells are fully lysed and there is no obvious cell precipitation.

(4) Centrifuge at 4℃, 12000×g, for 15min.

(5) The total protein can be obtained by inhaling the supernatant into another pre-cooled clean centrifuge tube.

(6) The protein extract was quantified and divided into -80℃ refrigerator for reserve or directly used in downstream experiment.

Third, total protein extraction from adherent cells

1. Preparation of extraction solution:

Add 2μL protease inhibitor mixture and 2μL phosphatase inhibitor mixture into every 500μL cold total protein extraction solution, mix well and put on ice for use.

2. Cell protein Extraction

(1) Carefully absorb the culture solution of the adherent cells.

(2) Wash the cells twice with cold PBS, sucking up as much supernatant as possible after each wash.

(3) Add an appropriate amount of cold total protein extract, shake for 15-30min, until the cells are fully lysed, scrape with a cell scraper, and inhale the lysate into another clean centrifuge tube.

(4) Centrifuge at 4℃, 12000×g, for 15min.

(5) The total protein can be obtained by inhaling the supernatant into another pre-cooled clean centrifuge tube.

(6) The protein extract was quantified and divided into -80℃ refrigerator for reserve or directly used in downstream experiment.

Fourth, total protein extraction from tissue samples

1. Preparation of extraction solution:

Add 2μL protease inhibitor mixture and 2μL phosphatase inhibitor mixture into every 500μL cold total protein extraction solution, mix well and put on ice for use.

2. Histone extraction

(1) Take 50-100mg tissue sample, wash it with PBS, then cut it as much as possible with surgical scissors, add 500μL total protein extraction solution, and homogenize it with tissue homogenizer/homogenizer until there is no obvious visible solid.

(2) The tissue homogenate was sucked into a pre-cooled clean centrifuge tube and oscillated at 4°C for 10-20min.

(3) Centrifuge at 4℃, 12000×g, for 15min.

(4) The total 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.

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.

2. Slow cell lysis rate?

In order to fully ensure the activity of the extracted protein, the extract adopts a unique formula of protective protein, which has moderate cracking ability and wide range of downstream applications. The cracking time can be extended appropriately.

3. What method is used to quantify protein?

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.

4. Is there a gelatinous precipitate during extraction?

Protein extract treatment products sometimes appear a small amount of transparent glue, is a normal phenomenon. The transparent glue is a complex containing genomic DNA, etc. Without detecting specific proteins that bind particularly closely to genomic DNA, the supernatant can be directly centrifuged for subsequent experiments. If it is necessary to detect the protein closely bound to the genome, it can be treated by ultrasound, 300w/10s interval of 10s, ultrasound for 3min, and then centrifuge the supernatant for follow-up experiment. The detection of some common transcription factors, such as NF-kappaB, p53, etc., does not require ultrasound treatment.

5. 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.