Total Protein Extraction Kit (no Detergent)

Cat: EX1102
Size: 50T/100T
Storage: 2-8°C, valid for 1 year.

Kit Components:

Kit Components	50T	100T	Storage
Protein Extract Solution A	25mL	5mL	2-8℃
Protease Inhibitor Mixture B	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℃ 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 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 samples of plants, bacteria, fungi, yeast, etc.

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 proteins extracted from this kit can be used for Western Blotting, protein electrophoresis, ELISA, transcriptional activity analysis, Gel shift gel blocking assay, co-immunoprecipitation, enzyme activity determination and other protein studies.

All components of this kit do not contain detergent components, and the composition of the final protein sample has no influence on downstream applications such as NI column purification, molecular sieve, ion exchange, affinity purification, etc.

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. After dialysis or desalting treatment, the final protein sample will not contain detergent, high concentration of salt and other effects. Basically, it can meet any downstream proteomic related experimental research.

The protein extracted by this kit is 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 situation.

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

The protein samples extracted by this kit contain high concentration of salt components and cannot be directly used for 2D electrophoresis. The final samples are desalted and then used for 2D electrophoresis.

This kit is measured by 50mg cells/tissues per treated sample (about 50μL cell precipitation volume). If a large number of cells/tissues need to be treated, the extraction solution can be added to the cell precipitation at the rate of 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.

Applicable Sample:

Cells and tissues.

Self-prepared Reagents and Instruments:

Centrifuge, oscillator, vortex mixer, homogenizer/homogenizer, 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. Total protein extract contains a variety of active components, can fully release cytoplasmic protein, nuclear protein, but also can bind the released protein to prevent precipitation.
6. Protease inhibitor inhibits the degradation of protein, and the formula of protease inhibitor is optimized. The protease inhibitor mixture consists of five independent protease inhibitors Aprotinin, Leupeptin, Pepstatin A, Bestatin, and E-64, each of which can specifically inhibit the activity of one or more proteases. 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. Please centrifuge the reagent in the rotating cap centrifuge tube briefly before opening the cap, and throw the liquid on the inner wall of the cap to the bottom of the tube to avoid the liquid spilling when opening the cap. 2. All reagents in the process of the experiment must be pre-cooled; All appliances must be pre-cooled in the -20℃ refrigerator. The sample must be kept at a low temperature during the whole process.
2. If the solution of protease inhibitor precipitates during storage, it will not affect the use, and it will be used normally after dissolution.
3. If the kit can not be used up in a short time, the protease inhibitor mixture can not be added to the extraction solution at one time.
4. You can add other protease inhibitor products according to your own experimental needs.
5. In the downstream experiment, if the enzyme activity of a specific protease or phosphatase is detected, the extract can be performed without protease inhibitors or phosphatase inhibitors, and the extraction process should be kept at a low temperature to shorten the centrifugation time.
6. Beta-actin, GAPDH and Tubulin can be selected as parameters in Western experiments.
7. Protease inhibitor at 2-8℃ is a solid state, from the refrigerator after recovery to room temperature or 37℃ for a short time water bath, into a liquid
8. After the state, centrifuge to the bottom of the tube and then open the lid.

Second, extraction of total protein from cell samples

Suspension cell protein extraction

1. Preparation of the extraction solution:

Add 2μL protease inhibitor mixture into every 500μL cold protein extract, mix well and put on ice for later use.

[Note]:

(1) Prepare the protein extract solution according to the number of samples needed to be processed. The protease inhibitor mixture can not be added to the extract at one time. Phosphatase inhibitors can be added in one go.

(2) If the extract with the protease inhibitor has not been used completely within a week, the protease inhibitor should be added again before being used again.

(3) If the downstream experiment is to detect the enzyme activity of a specific protease or phosphatase, pay attention to adjust whether the inhibitor mixture is added according to the actual situation.

(4) Protein extract used in the following steps An extract containing protease inhibitor prepared for this step.

2. Take 5×10^6 cells, centrifuge at 4℃, 2500×g for 5min, carefully absorb the medium, blot as dry as possible, and collect the cells.

[Note]:

(1) The number of cells is adjusted according to the experimental situation, and the amount of lysate per time is not certain, please adjust according to the actual situation.

(2) Generally, about 10 times the cell volume can be added to the lysate. If you need to increase the

concentration of the obtained protein sample, you can.
(3) Adjust the cell: extract dosage ratio to 1:5.
3. Wash the cells twice with cold PBS, sucking up as much supernatant as possible after each wash. (Add PBS and mix well, centrifuge 2500×g for 5 min)
4. 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°C for 20-30min until the cells are fully lysed without obvious cell precipitation.

[Note]:
(1) Use the lower speed of the oscillator/shaker, the extraction liquid can be slightly shaken.
(2) No vibration conditions can also not oscillate, slightly extend the extraction processing time, in the middle of every few minutes with pipette blow evenly.

5. Centrifuge at 4°C, 12000×g for 15min.
6. The total protein is obtained by inhaling the supernatant into another pre-cooled clean centrifuge tube.
7. The protein extract was quantified and divided into -80°C refrigerator for reserve or directly used in downstream experiment.

[Note]:
(1) It is recommended to use BCA method for protein quantification.
(2) There is no problem in storing the protein sample at -80°C for one year. Be careful not to be hydrolyzed away by protease and not to be contaminated by bacteria.

8. The protein sample dialysis treatment or desalting column desalting treatment for downstream experiments.

[Note]:
(1) The protein sample contains salt, which needs to be desalted for two-dimensional electrophoresis.
(2) The protein samples treated by dialysis or desalting centrifuge column do not contain detergent and high concentration of salt.

Third, total protein extraction of tissue samples

1. Preparation of extraction solution:
Add 2μL protease inhibitor mixture into every 500μL cold protein extract, mix well and put on ice for later use.

[Note]:
(1) Prepare the protein extract solution according to the required sample size, and the protease inhibitor mixture can not be added to the extract at one time. Phosphatase inhibitors can be added in one go.
(2) If the extract with the protease inhibitor has not been used completely within a week, the protease inhibitor should be added again before being used again.
(3) If the downstream experiment is to detect the enzyme activity of a specific protease or phosphatase, pay attention to adjust whether the inhibitor mixture is added according to the actual situation.

2. The protein extract used in the following steps is an extract containing a protease inhibitor prepared for this step.
3. Take a 50-100mg tissue sample, wash it with PBS, then cut it as much as possible with surgical scissors, add 500μL total protein extract, and homogenize it with a tissue homogenizer/homogenizer until there are no visible solids.

[Note]:
(1) If the tissue sample is very small, it can be cut and directly added to the extraction liquid for 15 minutes, without homogenizer.
(2) Generally according to the tissue: the amount of extraction liquid 1:10 (w/v) can be added to the extraction liquid. If you need to improve the obtained protein. Sample concentration, the tissue: extract dosage ratio can be adjusted to 1:5.
(3) Can also be used liquid nitrogen grinding method, using the conventional liquid nitrogen grinding method can be.

4. The tissue homogenate is sucked into a pre-cooled clean centrifugal tube and oscillated at 4°C for 10-20min.

[Note]:
(1) Use the lower speed of the oscillator/shaker, the extraction liquid can be slightly shaken.
(2) No oscillating conditions can also not oscillate, slightly extend the processing time of the extraction liquid, every few minutes with the pipette blow mixing can be.

5. Centrifuge at 4°C, 10000-14000×g conditions for 15min.
6. Inhale the supernatant into another pre-cooled clean centrifuge tube to obtain the total protein.

7. The protein extract was quantified and divided into -80°C refrigerator for reserve or directly used in downstream experiment.

[Note]:

(1) It is recommended to use BCA method for protein quantification.
(2) There is no problem in storing protein samples at -80°C for one year. Be careful not to be hydrolyzed away by protease and not to be contaminated by bacteria.

8. The protein sample dialysis treatment or desalting column desalting treatment for downstream experiments.

[Note]:

(1) The protein sample contains salt, which needs to be desalted for two-dimensional electrophoresis.
(2) The protein sample treated by dialysis or desalting centrifuge column does not contain detergent and high concentration of salt.

Analysis of Common Problems:

1. Low protein concentration?

Processing part of the tissue sample may not fully lyse, resulting in low protein concentrations. Simply increase 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 is the method of quantifying the 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.

3. Is the extracted protein active?

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

4. Slow cell lysis rate?

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

5. Gelatinous precipitate occurs 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-kappa B, p53, etc., does not require ultrasound treatment.

Note:

1. Before the formal experiment, please select several samples for pre-experiment to optimize the experimental conditions and achieve the best experimental results.
2. The reagent in the screw cap trace reagent tube should be centrifuged briefly before opening the cap, and the liquid on the cap and inner wall should be centrifuged to the bottom of the tube to avoid reagent loss when opening the cap.
3. It is prohibited to 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 lead to wrong results.
5. It is best to use disposable suction heads, tubes, bottles or glassware, reusable glassware must be cleaned before use and thoroughly remove residual cleaners.
6. After the completion of the experiment, all samples and utensils in contact should be disposed of in accordance with the prescribed procedures.