Insect cytoplasmic protein extraction kit

Item No. : EX1680

Specification: 50T; 100T

Store: 2-8 ° **C,** valid for 1 year.

Product Contents:

Name	50T	100T	Storage conditions
Reagent A: Protein extract Solution A	25mL	50mL	Store at 2-8°C
Reagent B: protease inhibitor mixture	100μL	200μL	Store at -20°C

Note: Please use the reagent as soon as possible after unpacking.

After the reagent bottle is opened, each component shall be stored according to the required conditions.

Product introduction:

Insect cytoplasmic protein extraction kit provides a complete set of reagents, suitable for extracting cytoplasmic protein from various insect cells and various insect solid tissues. The extraction process is simple and convenient, and can be completed within 1 hour. The prepared cytoplasmic protein not only has high purity and natural activity, but also has little cross-contamination.

The kit contains a protease inhibitor mixture, which prevents the protease from degrading the protein and provides a guarantee for the extraction of high purity protein.

The proteins extracted from this kit can be used for Western Blotting, transcriptional activity analysis, Gel shift gel block assay, immunocoprecipitation, enzyme activity determination and other protein studies.

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

The protein extracted in this kit is an active protein with a natural protein conformation.

The protein samples extracted by this kit contain high concentration of salt components and cannot be directly used for 2D electrophoresis. If the downstream experiments need to be directly used for isoelectric focusing or two-dimensional electrophoresis, please use the kit of other product numbers. It is also possible to desalt the final sample and then use it for 2D electrophoresis, desalting with a desalting tube.

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, reducing the protein extraction time to 30 minutes to 1 hour.
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, Pepstatin A, 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.

Own reagents and instruments:

Centrifuge, oscillator, homogenizer/homogenizer, vortex mixer, pipette, refrigerator, ice box, PBS buffer, protein quantification kit, centrifuge tube, suction tip, disposable gloves.

Directions to use:

I、**Precautions for use**:

1. Pre-experimentation is important. It is necessary to do pre-experiment, take a small number of samples before the formal experiment to optimize the experimental conditions, and carefully carry out like the formal sample to see whether the experimental results are feasible and whether the experimental conditions are suitable for your sample. Due to the diversity of biological samples, the experimental conditions of different samples are usually very different, and the experimental conditions required under different models of the same cell may also be different. In order to avoid wasting formal samples and reagents, we must pre-experiment to optimize the experimental conditions in advance.
2. The reagent in the rotating cap centrifuge tube should be centrifuged briefly before opening the cap, and the liquid on the inner wall of the cap should be thrown to the bottom of the tube to avoid the liquid spilling when opening the cap.
3. Protease inhibitor at 2-8℃ is a solid state, after taking out from the refrigerator, return to room temperature or 37℃ for a short time water bath, become a liquid state, centrifuge to the bottom of the tube and then open the lid.
4. All reagents used in 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.
5. If the solution of protease inhibitor is precipitated during storage, it will not affect the use, and it should be used normally after dissolution.
6. 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.
7. Other protease inhibitor products can be added as needed for your own experiment.
8. 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.

II、Cell protein extraction

1. Extract solution preparation:

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

[Note] :

① Prepare the protein extract according to the number of samples to be processed, the protease inhibitor mixture cannot be added to the extract all at once.

② If the extract with a protease inhibitor is not used up within a week, the protease inhibitor should be added again before being used again.

③ The protein extract used in the following steps is a protease inhibitor-containing extract prepared for this step.

2. Take 5-10×10^6 cells, centrifuge at 4℃, 500×g for 2-3 minutes, 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, the amount of lysate per time is not certain, please adjust according to the actual situation.

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

4. Add 400μL cold protein extract into every 5-10×10^6 cells, swirl for 15 seconds at high speed or blow and mix well, and oscillate at 2-8℃ for 20-30 minutes.

【 Note 】 :

① Use the lower speed of the oscillator/shaker and just keep the extract shaking slightly.

② No oscillating conditions can not be oscillated, slightly extend the processing time, every few minutes in between with a pipette blow to mix.

5. Then centrifuge at 4℃, 12000×g, for 10 minutes.

6. Inhale the supernatant into another pre-cooled clean centrifuge tube to obtain cytoplasmic protein.

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

[Note] :

① BCA method is recommended for protein quantification.

② The protein sample is stored at -80℃ for one year without problem. Be careful not to be hydrolyzed off by protease and not to be contaminated by bacteria.

III、Histone extraction

1. Extraction solution preparation:

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

[Note] :

① Prepare the protein extract according to the number of samples to be processed, the

protease inhibitor mixture cannot be added to the extract all at once.

② If the extract with a protease inhibitor is not used up within a week, the protease inhibitor should be added again before being used again.

③ The protein extract used in the following steps is a protease inhibitor-containing extract prepared for this step.

2. Take an appropriate amount of insect tissue sample, cut it up as much as possible with surgical scissors, cool extract A, homogenize it with a tissue homogenizer/homogenizer until there are no visible solids, rest on ice for 3 minutes, and then inhale the homogenate into another pre-cooled clean centrifuge tube.

【 Note 】 :

① Small insects such as fruit flies can be cut up and then homogenized directly.

② Larger insects require removal of wings, legs, carapaces, etc.

③ Add 200μL of cold extract solution A for every 20-50μL precipitation volume.

3. Vortex oscillation at high speed for 15 seconds or blow and mix well, and oscillate at 2-8℃ for 20-40 minutes.

【 Note 】 :

① Use the lower speed of the oscillator/shaker and just keep the extract shaking slightly.

② No oscillating conditions can not be oscillated, slightly extend the processing time, every few minutes in between with a pipette blow to mix.

③ Can also be measured by tissue weight, every 20mg to add 200μL extract A.

4 Then centrifuge at 4°C, 12000×g, for 10 minutes.

5. Inhale the supernatant into another pre-cooled clean centrifuge tube to obtain cytoplasmic protein.

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

[Note] :

① BCA method is recommended for protein quantification.

② The protein sample is stored at -80°C for one year without problem. Be careful not to be hydrolyzed off by protease and not to be contaminated by bacteria.

Analysis of common problems:

1. Low protein concentration?

Note that treatment with protein extract solution A does not lyse completely, resulting in low protein concentration. As long as the treatment time of reagent A is extended appropriately. It is best to deal with it under the condition of continuous oscillation, without an oscillator, it can also be mixed with a suction head at intervals of a few minutes, until there is no obvious precipitation.

2. What method is used to quantify protein?

BCA method is recommended. The Bradford method is not suitable because reagent B 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 destroy the original interaction between proteins,

The proteins maintain their natural conformation and activity.

What to watch for:

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.