

Plant protein extraction kit (for proteome test, mass spectrometry)

Item No.: EX2502 Specification: 50T/100T

Validity: 2-8°C storage, valid for one year.

Product content:

Name	50T	100T	Storage conditions
Component A1: Protein extract A1	22.5mL	45mL	Store at 2-8℃
Component A2: Protein extract solution A2	2.5mL	5mL	Store at -20°C
Component B: protease inhibitor mixture B	100μL	200μL	Store at -20°C

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!

Product Introduction:

Plant protein extraction kit (suitable for proteomics experiments) is suitable for extracting total protein from various fresh plant tissue samples. The extraction process is simple and convenient and can be completed within 1 hour.

This kit contains a unique formula that effectively dissolves cell membrane components, including the plasma membrane, 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 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, which 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 Mass Spectrometry peak shift caused by AEBSF. Therefore, the protein samples extracted from this product can be used for mass spectrometry (MS) detection and analysis, proteomics and other related research.

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

The proteins extracted from this kit can be used for downstream protein research experiments such as protein spectrometry, Western Blot, protein electrophoresis, 2-D, IEF, Pull-down, co-IP, IP, ELISA, transcriptional activity analysis, Gel shift gel blocking assay, enzyme activity determination, etc.

Own 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, extract protein from cells and tissues without grinding, repeated freeze-thaw, ultrasonic crushing and other pre-treatment.
- 2. Containing 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 types

There are separate protease inhibitors, each of which can specifically inhibit one or more 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.

How to use:

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°C 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. Extract A must be kept at 2-8°C before use, otherwise downstream membrane protein extraction will not easily delaminate.
- 7. Boiling should be avoided in loading buffer during membrane protein electrophoresis.
- 8. The SDS content of loading buffer can be increased during membrane protein electrophoresis.
- 9. Do not mix with other brands of reagents, otherwise the effect will be affected.
- 10. Contamination of the sample or reagent with bacteria or fungi or cross-contamination of reagents may lead to false results.

2. Plant tissue protein extraction:

1. Extraction solution preparation:

Mix reagent A1 and reagent A2 to form protein extract solution A, thoroughly mix and set aside.

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

- 2. The 100-200mg fresh plant tissue samples, which were washed and dried and the leaf stems and thick veins were removed, were cut as much as possible with surgical scissors, and were fully homogenized with A homogenizer/homogenizer after 500µL extract solution A was added.
- 3. The homogenate was sucked into a pre-cooled clean centrifuge tube and oscillated at 4° C for 30-60 minutes.
- 4 Centrifuge at 4°C, 12000×g, for 15 minutes.
- 5. The total plant 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°C refrigerator for reserve or downstream experiment.
- 7. The protein samples were treated by dialysis or desalting column and then 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 the protein?

The BCA method or Bradford method is recommended.

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.

What to 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.



