# Fungal protein extraction kit (for proteome test, mass spectrometry)

**Item No.** : EX2541
**Specification:** 50T/100T
**Validity:** 2-8℃ 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℃ |
| Component B: protease inhibitor mixture B | 100μL | 200μL | Store at -20℃ |

***Note:***

1. Protein extract solution A2 and protease inhibitor can also be stored at 2-8℃ before use without open lid. After opening the lid, store at -20℃.
2. Protein extract solution A2 avoid repeated freeze-thaw.
3. Protease inhibitor is solid state at low temperature of 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 to the bottom of the tube and then open the lid.
4. Please use the reagent as soon as possible after unpacking!

**Product Introduction:**

Fungal protein extraction kit (suitable for proteomics experiments) is suitable for extracting total protein from various filamentous fungi. The optimized lysate formula can fully release a variety of membranous and non-membranous mycelium proteins as well as a variety of mycelium metamorphosis proteins. 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. 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. 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. Therefore, the protein samples extracted from this product can be used for mass spectrometry (MS) detection and analysis, proteomics and other related research.

**Prepare your 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.
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 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. 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.

**2. Fungal protein extraction:**

1. Extraction solution preparation:

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

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

2. Culture filamentous fungi, centrifuge at 4℃, 5000×g for 5-10 minutes, carefully absorb the medium, blot as much as possible, and collect the bacteria.

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

4 Place the cells in a mortar and grind them to powder with liquid nitrogen.

5. Add 300μL-500μL protein extract to every 100mg of bacteria, mix well, and shake at 2-8℃ for 20-30 minutes.

6. Centrifuge at 4℃, 12000×g, for 10 minutes.

7. Inhale the supernatant into another pre-cooled clean centrifuge tube to obtain the total fungal protein.

8. The protein extract was quantified and divided into -80℃ refrigerator for reserve or downstream experiment.

9. 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 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. 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.