

# **Fungal protein Extraction Kit (no detergent)**

Item No.: EX2540 Specification: 50T/100T

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

#### **Product content:**

Name	50T	100T	Storage conditions
Component A: Fungal protein extract	25mL	50mL	Store at 2-8°C
Component B: Protein stabilizer	200μL	400μL	Store at 2-8°C
Component C: protease inhibitor mixture	100μL	200μL	Store at -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°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:**

Fungal protein extraction kit (Small fungus) is suitable for extracting total protein from a variety of fungal samples other than large fungi (fungi). It includes various filamentous fungi (molds) and small single-celled fungi samples.

Kit components are optimized for thick cell wall samples of fungi, and the extraction process is simple and convenient and can be completed within 1 hour. The kit contains a protease inhibitor mixture that prevents protease from degrading the protein and ensures the extraction of high purity proteins.

If you need to extract the proteins of large fungi (fungi, etc.), please use another kit. For yeast protein extraction, please use another kit

The protein extracted from this kit can be used for downstream protein research experiments such as Western Blotting, protein electrophoresis, immunoprecipitation, ELISA, transcriptional activity analysis, Gel shift gel blocking experiment, enzyme activity determination, etc.

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

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

## Own reagents and instruments:

Centrifuge, oscillator, 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 time of protein extraction is reduced 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. The protein extract contains a variety of effective components, which can fully release cytoplasmic protein and nuclear protein, and 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 6 independent protease inhibitors AEBSF,



Aprotinin, Leupeptin, Pepstatin A, Bestatin, E-64; Each inhibitor can specifically inhibit the activity of one or several proteases. 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. Do not 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 result in false results.
- 5. 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.
- 6. After the completion of the experiment, all samples and utensils in contact should be disposed of in accordance with the prescribed procedures.
- 7. All reagents used in the experiment should 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.
- 8. If the solution of protease inhibitor is precipitated during storage, it will not affect the use, and it should be used normally after dissolution
- 9. 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.

## 2. Protein extraction

1. Extraction solution preparation:

Every  $500\mu L$  of cold fungal protein extract, add  $2\mu L$  protease inhibitor mixture and  $4\mu L$  protein stabilizer, mix well and put on ice for use.

- 2. Take a 50-100mg fungal sample and place it in a mortar and grind it with liquid nitrogen.
- 3 Add 500µL fungal protein extract after grinding.
- 4. Mix well and oscillate at 4°C for 20-60 minutes.
- 5. Centrifuge at 4°C, 12000×g, for 10 minutes.
- 6. Inhale the supernatant into another pre-cooled clean centrifuge tube to obtain the total fungal protein.
- 7. The protein extract was quantified and divided into -80°C refrigerator for reserve or directly used in downstream experiment.

# **Analysis of common problems:**

1. Low protein concentration?

Some samples may not be fully lysed when processed, resulting in low protein concentration. The treatment time of reagent A may be extended 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 protein?
  - BCA method is recommended. Bradford method is not suitable, because reagent A contains components that interfere with Bradford method, resulting in inaccurate determination. If dialysis has been performed or the buffer system has been replaced with a desalting column, the Bradford legal dosage can be used.
- 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.

## What to note:

- 4. This kit is intended for scientific research only and is not intended for diagnosis or treatment.
- 5. 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.
- 6. All samples and exposed glassware should be disposed of in accordance with the prescribed procedure after the experiment is completed.
- 7. Avoid skin or mucous membranes coming into contact with the reagent.
- 8. If the reagent accidentally comes into contact with skin or eyes, it should be rinsed with water immediately.