Thick-wall microbial protein extraction kit

Article number: EX1840

Specification: 50T/ 100T

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

Product content:

Name	50T	100T	Storage conditions
Component A: Thick-walled microbial protein extract A	25mL	50mL	Store at 2-8℃
Component B: Protein stabilizer B	250μL	500μL	Store at -20℃
Component C: Protease inhibitor mixture C	100μL	200μL	Store at -20℃

Note:

1. Protease inhibitors can also be stored at 2-8℃ before use without open lid. After opening the lid, store at -20℃.
2. The protease inhibitor is in solid state at 2-8℃. Take it out of the refrigerator and return to room temperature or 37℃ water bath for a short time. After it becomes a liquid state, centrifuge to the bottom of the tube and then open the lid.
3. Please use the reagent as soon as possible after unpacking!

Product Introduction:

Thick-walled microbial total protein extraction kit can extract total protein from various thick-walled microbial bodies. It includes all kinds of yeast, filamentous fungi, gram-positive bacteria, spores, microalgae, eggs and other samples with thick fine cell walls that are difficult to crack.

The extraction process is simple and convenient, with no need for mechanical grinding or organic solvents. The kit contains a protease inhibitor mixture that prevents the protease from degrading the protein, providing a guarantee for extracting high-quality protein.

The protein extracted from the kit can be used for SDS-PAGE electrophoresis detection, Western blotting, gel block experiment and other downstream experiments. About 5-10mg of protein can be extracted per milliliter of bacterial solution. The protein was quantified by BCA or Lowry method.

EDTA is not present in this kit and is compatible with downstream applications such as metal chelation and chromatography.

Bring your 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.

2, containing protein stabilizer, the extracted protein is stable.

3, UV detection of protein concentration, low background interference.

4. Protease inhibitor inhibited protein degradation, and the formula of protease inhibitor was optimized. The protease inhibitor mixture consists of 6 independent protease inhibitors, 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.

How to use:

First, use precautions:

1. Before the formal experiment, please select a few samples for pre-experiment, in order 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 the loss of the reagent when opening the cap.
3. All reagents in the process of the experiment must be pre-cooled; All appliances must be pre-cooled in -20℃ refrigerator. The sample must be kept at a low temperature during the whole process.
4. If the solution of protease inhibitor precipitates during storage, it will not affect the use, and it should be used normally after dissolution.
5. 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.
6. You can add other protease inhibitor products according to your own experimental needs.
7. If the downstream experiment is to test the enzyme activity of specific protease or phosphatase, the extract can be without protease inhibitor or phosphatase inhibitor, and pay attention to the low temperature operation during the extraction process to shorten the centrifugal time.
8. It is prohibited to mix with other brands of reagents, otherwise it will affect the effect of use.
9. Contamination of the sample or reagent with bacteria or fungi or cross-contamination of reagents may lead to wrong results.

2. Thick-walled microbial protein extraction:

1. Extraction solution preparation:

According to the sample size needed to be extracted, 2μL protease inhibitor mixture and 5μL protein stabilizer were added to extract solution A every 500μL, thoroughly mixed and put on ice for use.

2. Centrifuge the bacterial solution at 4℃, 10000×g, for 5min, discard the supernatant, absorb the remaining liquid as much as possible, and collect the bacterial bodies.

3. Wash the bacteria with PBS twice. If you are freezing the bacteria, do the following steps directly.

4. Add 500μL extract solution for every 100mg-150mg wet heavy bacteria sample, blow and mix well, shake at 2-8℃ for 30-45 minutes.

5. At 150w-300w, 10s ultrasonic /10s interval, the ice bath was ultrasonic until the bacterial solution became clear.

6. Centrifuge the extract solution at 4℃ and 12000×g for 5 minutes, and transfer the

supernatant into a cold clean centrifuge tube.

7. The total protein sample is obtained.

8. The total protein sample was quantified and then divided into -80℃ refrigerator or directly 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 reagents A and B 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. Some gram-positive bacteria may be more difficult to crack and are best treated with ultrasound.

2. What method is used to quantify the protein?

The 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 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 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. Reusable glassware must be cleaned and thoroughly removed before use.
3. After the completion of the experiment, all samples and contact utensils should be disposed of in accordance with the prescribed procedures.
4. Avoid skin or mucous membrane contact with the reagent.
5 Rinse the reagent with water immediately if it accidentally comes into contact with skin or eyes.