Gram Negative Bacterial Protein Extraction Kit

Cat: EX1730
Size: 50T/100T
Storage: 2-8℃, valid for 1 year.

Kit Components:

Kit Components	50T	100T	Storage
Component A: Gram Negative Bacterial Protein Extract A	25mL	50mL	2-8℃
Component B: Protein Stabilizer	250μL	500μL	2-8℃
Component C: Protease Inhibitor Mixture	100μL	200μL	-20℃

Note:

1. Protease inhibitors can also be stored at 2-8℃ before use without open lid. Store at -20℃ after opening the lid for use.
2. The protease inhibitor is solid at 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 it to the bottom of the tube and then open the lid.
3. Please use the reagent as soon as possible after unpacking!

Introduction:

Gram-negative bacteria total protein extraction kit can extract total protein from a variety of Gram-negative bacteria, can be used for purified protein crude preparation and total protein preparation. The extraction process is simple and convenient.

This kit contains a protease inhibitor mixture, which prevents protease from degrading the protein and provides a guarantee for extracting high quality protein

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.

The proteins extracted by this kit are active proteins with natural protein conformation, which can be used for different downstream applications.

EDTA is not contained in this kit and is compatible with metal chelates and chromatography, among others.

Self-prepared Reagents and Instruments:

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

Product Features:

1. Easy to use, without expensive equipment.
2. It can handle all kinds of bacteria, including fresh bacteria and frozen bacteria.
3. Shorten the time of protein extraction to 1 hour.
4. A mild neutral cracking component is used to keep the protein active.
5. Containing protein stabilizer, the extracted protein is stable.
6. The background interference is low when the protein concentration is detected by UV.
7. 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 optimized composition of this mixture allows it to inhibit almost all important protease activities, including serine protease, cysteine protease, aspartate protease, alanyl-aminopeptidase, etc.

Protocols:

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. The reagent in the screw cap microreagent 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 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℃ 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. Other protease inhibitor products can be added as needed for your own experiment.
7. In the downstream experiment, if the enzyme activity of specific protease or phosphatase is detected, the extract can be without protease or phosphatase inhibitors. Pay attention to the low temperature operation during the extraction process to shorten the centrifugation time.
8. It is prohibited to mix with other brands of reagents, otherwise the effect will be affected.
9. Contamination of the sample or reagent with bacteria or fungi or cross-contamination of reagents may result in false results.

Second, gram-negative bacterial protein extraction

1. Extraction solution preparation:

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

2. Centrifuge the bacterial solution for 5min at 4℃, 10000×g, discard the supernatant, blot 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 protein extract every 50-100mg wet weight bacteria sample, blow and mix well, and shake at 4℃ for 20-30min.
5. At 150-300w, 10s ultrasound /10s interval, the ice bath was ultrasonic until the bacterial solution became clarified.
6. Centrifuge the extraction solution at 4℃ and 12000×g for 5-10min, discard the precipitation, and collect the supernatant.
7. Transfer the supernatant into another clean centrifuge tube to obtain Gram-negative bacterial protein sample.

8. The total protein sample was quantified and divided into -80°C refrigerator for use or directly for downstream experiment.

Analysis of Common Problems:

1. Low protein concentration?

Some 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. 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. Gelatinous precipitate during extraction?

Protein extract treatment products sometimes appear a small amount of transparent glue, is a normal phenomenon. The transparent glue is a complex containing genomic DNA, etc. Without detecting specific proteins that bind particularly closely to genomic DNA, the supernatant can be directly centrifuged for subsequent experiments. If it is necessary to detect the protein closely bound to the genome, it can be treated by ultrasound, 300w/10s interval of 10s, ultrasound for 3min, and then centrifuge the supernatant for follow-up experiment. The detection of some common transcription factors, such as NF-kappaB, p53, etc., does not require ultrasound treatment.

4. 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.

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 the skin or eyes, rinse immediately with water.