Bacterial ribosomal protein extraction kit

Article number: EX1920

Specification: 50T/100T

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

Product content:

Name	50T	100T	Storage conditions
Component A: Ribosome extract Solution A	50mL	100mL	Store at 2-8℃
Component B: Ribosome extract B	500μL	1mL	Store at 2-8℃
Component C: Ribosome extract C	25mL	50mL	Store at 2-8℃
Component D: Ribosomal protein extract solution D	10mL	20mL	Store at 2-8℃
Component E: protease inhibitor mixture	100μL	250μL	Store at -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!

Product Introduction:

Ribosome is a kind of ribonucleoprotein particle in the cell, mainly composed of RNA and protein, its only function is to synthesize amino acids into protein polypeptide chains according to the instructions of mRNA, so ribosome is a molecular machine for protein synthesis in the cell. The ribosome has no membrane structure and is mainly composed of protein (40%) and RNA (60%). Ribosomes are divided into two types according to sedimentation coefficient, one type (70S) exists in prokaryotes such as bacteria, and the other type (80S) exists in the cytoplasm of eukaryotic cells. Some of them float in the cell, and some clump together.

Bacterial ribosome extraction kits can be used to extract ribosomal proteins from various prokaryotic bacteria samples.

The protein extracted from this kit can be used for downstream protein research experiments such as Western Blotting, protein electrophoresis, immunoprecipitation, ELISA, enzyme activity determination, etc.

The protein extracted by this kit is an active protein with natural protein conformation, which has a wide range of downstream applications. The ability of the extract to lyse cells is mild, and the lyse time should be optimized according to the actual sample situation.

This kit does not contain EDTA 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、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 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 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℃ 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.

2. Bacterial ribosomal protein extraction:

1. Extraction solution preparation:

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

2. Collect bacterial cells in logarithmic growth phase and drain the medium as much as possible after centrifugation.

3 Wash twice with cold PBS, sucking up as much supernatant as possible after each wash.

4. Add 1mL of cold reagent A and 10μL of cold reagent B to 500mg of wet heavy bacteria, mix well and put on ice for 30 minutes.

5. Use high pressure cell crusher or ultrasonic cell crusher to break the cells.

6. Centrifuge at 4°C, 1000×g, for 5min. Discard the precipitation and collect the supernatant.

7. Centrifuge the supernatant at 20000×g at 4°C for 30min. Discard the precipitation and collect the supernatant.

8. Centrifuge the supernatant at 4°C, 170,000×g, for 60 minutes. Discard the supernatant and collect the precipitation.

9. Add 400μL of cold reagent C to the precipitate and mix well.

10. Centrifuge at 4°C, 170000×g for 60min.

11. Discard the supernatant, add 50-100μL ribosomal protein extract D to the precipitation, and mix thoroughly.

12. The ribosomal protein sample was obtained, which was frozen in the refrigerator at -80°C for use or directly used in downstream experiments.

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. Plant ribosomal protein abundance is relatively low, in the case of conditions, it is necessary to increase the sample loading amount as much as possible to improve the protein concentration.

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 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.