Bacterial Ribosome Extraction Kit

Cat: EX2950

Size: 50T/100T

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

Kit Components:

Kit Components	50T	100T	Storage
Component A: Ribosome extract solution A	50mL	100mL	2-8℃
Component B: Ribosome extract solution B	0.5mL	1mL	2-8℃
Component C: Ribosome extract solution C	25mL	50mL	2-8℃
Component D: Ribosome preservation solution D	10mL	20mL	2-8℃

Notes:

1. The validity period is the validity period that the kit is stored according to the required conditions before it is unwrapped.

2. Please use the reagent as soon as possible after unpacking!

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 ribosomes from various prokaryotic bacteria samples.

Self-prepared reagents and instruments:

Centrifuge, ultrasonic crusher, pipette, PBS buffer, centrifugal tube, suction head, disposable gloves

Protocols:

First, notes for use:

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. For downstream applications of nucleic acids, all fluids and instruments used in the test should be treated with DEPC. Treatment with 0.1% DEPC for 12h and then high pressure.
5. Downstream for protein experiments can be treated without DEPC.
6. The key to bacterial ribosome extraction is the quality of the sample to be extracted. In general, it is best to use E. coli samples where RNase activity is missing.
7. For bacteria, it is best to use freshly collected samples, or samples stored at -80 ° C immediately after collection.

Second, bacterial ribosome extraction:

1. Collect bacterial cells in logarithmic growth phase and drain the medium as much as possible after centrifugation.
2. 2 Wash twice with cold PBS, sucking up as much supernatant as possible after each wash.
3. 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 30min.
4. Use a high pressure cell crusher or ultrasonic cell crusher to break the cells.
5. Centrifuge at 4°C, 1000×g, for 5min. Discard the precipitation and collect the supernatant.
6. Centrifuge the supernatant at 20000×g at 4℃ for 30min, discard the precipitation, and collect the supernatant.
7. Centrifuge the supernatant at 4℃, 170,000×g, for 60min. Discard the supernatant and collect the precipitation.
8. Add 400μL of cold reagent C to the precipitate and mix well.
9. Centrifuge at 4°C, 170000×g for 60min.
10. Discard the supernatant and precipitate with ribosome preservation solution D.
11. The ribosome sample is obtained and stored in refrigerator or directly used for downstream experiment.

Notes:

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.