Ribosome extraction kit

Item number: EX1380

Store: 2-8°C, valid for 1 year.

Product Information:

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. Ribosome has a membraneless structure and is mainly composed of protein (40%) and RNA (60%). Ribosomes are divided into two classes 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.

Ribosome extraction kits can be used to extract ribosomes from a variety of animal cells and solid soft and hard tissue samples.

The ribosome extraction of this kit requires high requirements for laboratory centrifuge equipment, and requires centrifugal force to reach more than 100000×g. The operation of the experiment requires a high degree of detail.

Product composition:

Product components	50T	100T	Storage conditions
Reagent A: Ribosome extract Solution A	50mL	100mL	Store at 2-8°C
Reagent B: ribosome extract B	25mL	50mL	Store at 2-8°C
Reagent C: Ribosome preservation solution C	20mL	40mL	Store at 2-8°C

Note:

Please use the reagent as soon as possible after unpacking, long-term use can be stored at -20°C.

Reagents and equipment should be prepared by yourself

Centrifuge, oscillator, homogenizer/homogenizer, vortex mixer, pipette, refrigerator, ice box, 1×PBS buffer, centrifuge tube, suction head, disposable gloves, cell screen

How to use (for informational purposes only) :

1. Precautions for use:

1. The reagent in the rotating cap centrifuge tube should be centrifuged briefly before opening the cap, and the liquid on the inner wall of the cap should be thrown to the bottom of the tube to avoid the liquid spilling when opening the cap.
2. All reagents must be pre-cooled during the experiment; All utensils must be pre-cooled in a -20°C refrigerator. The sample must be kept at a low temperature during the whole process.
3. All fluids and utensils used in the test should be treated with DEPC. Treatment with 0.1% DEPC for 12 hours and then high pressure.
4. Downstream for protein experiments can be treated without DEPC.
5. Cells should preferably be freshly collected or stored at -80°C immediately after collection.
6. Standard Dounce homogenizers are preferable. If standard Dounce homogenizers are not available, ordinary glass homogenizers may also be used. However, ribosome recovery may be reduced.

2. Cell ribosome extraction:

1. Take 1-2×10^7 cells, centrifuge at 4°C, 500-1000×g for 5min, carefully absorb the medium, blot as much as

possible, and collect cells.

2. Wash twice with cold PBS, and blot the supernatant as dry as possible after each wash.
3. Add 500μL-1mL of cold reagent A and put on ice for 10min.
4. Homogenize with a Dounce homogenizer for 20-30 strokes.
5. Centrifuge the homogenate at 4°C, 1000×g, for 5min. Discard the precipitation and collect the supernatant.
6. Centrifuge the supernatant at 20000×g at 4°C for 10min, discard the precipitation, and collect the supernatant.
7. Centrifuge the supernatant at 4°C, 100000× g-120000 ×g for 60min. Discard the supernatant and collect the precipitation.
8. Add 400μL of cold reagent B to the precipitate and mix well.
9. Centrifuge at 4°C, 100000×g -120000×g for 60min.
10. Discard the supernatant and re-suspend the precipitation with ribosome preservation solution.
11. The ribosome sample was obtained, which was frozen in the refrigerator at -80°C for use or directly used for downstream experiment.

3. Tissue ribosome extraction:

1. Take 50-100mg fresh animal tissue sample and wash it with PBS.
2. Cut up as much as possible with scissors and wash twice with cold PBS.
3. Add 500μL-1mL of cold reagent A and put on ice for 10min.
4. Fully homogenize with a Dounce homogenizer for 20-30 times until there are no obvious solid clumps. Then centrifuge at 4°C, 1000×g, for 5min.
5. Inhale the supernatant into another pre-cooled clean centrifuge tube.
6. Centrifuge at 20000×g at 4°C for 10min, discard the precipitation, and collect the supernatant.
7. Centrifuge the supernatant at 4°C, 100000× g-120000 ×g, for 60min. Discard the supernatant and collect the precipitation.
8. Add 400μL of cold reagent B to the precipitate and mix well.
9. Centrifuge at 4°C, 100000×g-120000×g for 60min.
10. Discard the supernatant and re-suspend the precipitation with ribosome preservation solution.
11. The ribosome sample was obtained, which was frozen in the refrigerator at -80°C for use or directly used for downstream experiment.

Notes:

(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) 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 samples or reagents with bacteria or fungi or cross-contamination of reagents may lead to 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) All samples and exposed glassware should be disposed of in accordance with the prescribed procedure after the experiment is completed.