Endoplasmic reticulum extraction kit

Item number: EX1370

Store: 2-8°C, valid for 1 year.

Product Introduction:

The endoplasmic reticulum is found in various eukaryotic cells except the mature red blood cells of mammals. The endoplasmic reticulum is a system of interconnecting lamellar Spaces or small tubules formed by biofilms. The Spaces between the membranes are called cisterna, and are usually not directly connected to the extracellular Spaces and the cytoplasmic matrix. On the one hand, this membranous duct system constitutes the transport pathway of intracellular substances, on the other hand, it provides a broad reaction area for various enzyme reactions in the cell. The function of the endoplasmic reticulum is related to the synthesis of proteins, the synthesis of sugars and lipids, detoxification and assimilation, and it also has the function of transporting proteins.

The ER extraction kit can be used for the complete ER extraction of various animal cell and tissue samples, and can also be used for downstream ER protein extraction and other experiments.

Product composition:

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

Note: If the extract is not used for a long time, it can be stored at -20°C. If it is used several times within 15 days, it can be stored at 4°C.

Reagents and equipment should be prepared by yourself

Centrifuge, oscillator, homogenizer/homogenizer, scroll mixer, pipette, refrigerator, ice box, 1xPBS buffer, centrifuge tube, suction head, disposable gloves, cell screen.

Product use (for reference only) :

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 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. It is best to homogenize the sample using a Dounce homogenizer. If a Dounce homogenizer is not available, a regular 1ml glass homogenizer may also be used, but ER recovery may decrease.
4. Centrifugal force 50000×g centrifugation is required, if there is no condition, 30000× G-50000 ×g centrifugal force can be used, and it is best to reach about 45,000 ×g. The minimum centrifugal force needs to ensure 30000×g.
5. If all smooth ER vesicles need to be recovered, the centrifugal force of the last centrifugation step needs to be increased to 100,000 ×g.

Extractive ER of cells:

1. Take 1-2×10^7 cells, centrifuge at 4°C, 500×g for 5min, carefully absorb the medium, blot as much as possible, and collect cells.
2. Wash twice with cold PBS, sucking up as much supernatant as possible after each wash.
3. Add 500μL-1mL of cold reagent A and place on ice for 10min.
4. Homogenize with a Dounce homogenizer for 30-40 strokes.
5. Centrifuge the homogenate at 4°C, 1000×g, for 5min. Discard the precipitate and collect the supernatant.

6. Centrifuge the supernatant at 4℃, 11000×g for 10 minutes, discard the precipitation, and collect the supernatant.
7. The supernatant was centrifuged at 4℃, 50000×g, for 45min. 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, 50000×g, for 45min.
10. The supernatant was discarded and precipitated with endoplasmic reticulum preservation solution.
11. The endoplasmic reticulum samples were obtained and stored in refrigerator or directly used for downstream experiments.

Tissue endoplasmic reticulum extraction:

1. Take 50mg-100 mg fresh animal tissue samples and wash them clean 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 place on ice for 10min.
4. Homogenize thoroughly with a Dounce homogenizer for 30-40 ounces until there are no obvious solid masses. Then centrifuge at 4°C, 1000×g, for 5min.
5. Inhale the supernatant into another pre-cooled clean centrifuge tube.
6. Centrifuge at 4°C, 11000×g, for 10min, discard the precipitation, and collect the supernatant.
7. The supernatant was centrifuged at 50000×g at 4℃ for 45min. 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, 50000×g, for 45min.
10. The supernatant was discarded and precipitated with endoplasmic reticulum preservation solution.
11. The endoplasmic reticulum samples were obtained and stored in refrigerator or directly used for downstream experiments.

Points for attention:

(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 the sample or reagent with bacteria or fungi or cross-contamination of reagents may result in 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.