



## Cell Supernatant Exosome Isolation Kit Protocol

**Product Name: Cell Supernatant Exosome Isolation Kit**

**Catalog Number: CSB-EI0102(2T), CSB-EI0110(10T)**

### DESCRIPTION

Exosomes are a type of extracellular vesicles secreted by cells that contain a variety of RNA, proteins, and phospholipids with a diameter of about 30-150 nm. It is abundant in cell culture supernatants and body fluids (milk, serum, semen, plasma, saliva, urine, amniotic fluid, cerebrospinal fluid, etc.). Exosomes play an important role in the transfer of material and information between cells.

This product provides a simple and reliable method to extract intact exosomes from the cell culture supernatant. Exosomal vesicles isolated by this product are suitable for various downstream applications, such as electron microscopy analysis, NTA analysis, NanoFCM analysis, Western Blot, fluorescence quantitative (qPCR), and high-throughput sequencing, etc.

### ADVANTAGES

- High production
- High purity
- High efficiency
- Easy operation
- In tact vesicle structure
- No requirement for equipment (ultracentrifugation is unnecessary)

### MATERIALS PROVIDED

Reagent Composition	2T	10T
Reagent A	300ul*1	1.5ml*1
Reagent B	1 tube	5 tubes
Reagent C	100ul*1	500ul*1
Reagent D	2ml*1	7.5ml*1
Reagent E	25ml*1	45ml*2
Reagent F	2ml*1	5ml*1



## **STORAGE CONDITIONS**

Store at 2-10°C.

## **SHELF LIFE**

One year from the date of receipt.

## **OPERATION METHOD**

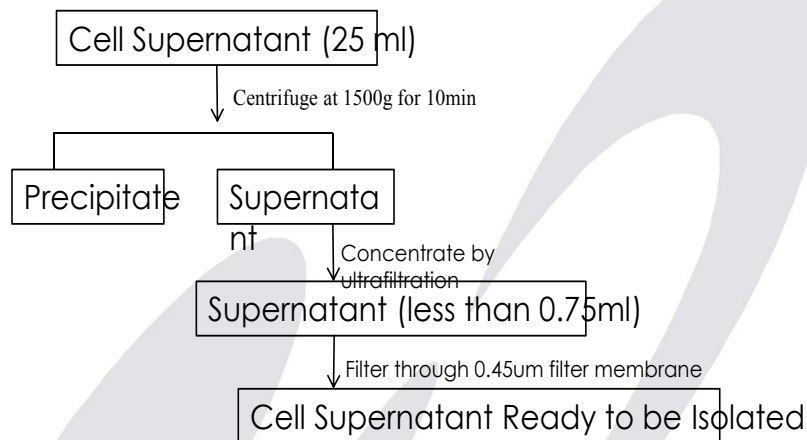
### ● **Sample Pretreatment**

1. Collect 25ml of cell supernatant from two 10cm culture dishes (no less than two dishes) (cell density is not less than  $1 \times 10^6$  cells/ml).
2. Centrifuge the cell supernatant at 1500g for 10min, and retain the supernatant.
3. The supernatant is concentrated to 0.5ml (the volume should not exceed 0.75ml).

[Note] It is recommended to use 10-100K ultrafiltration tube for concentration.

4. The concentrated sample is filtered at 0.45um and ready to be isolated.

### Cell Supernatant Processing Flowchart



### ● **Exosome Isolation**

1. Take 150ul of Reagent A (shake and mix before use) in a 1.5ml EP tube, add 1ml of Reagent E, mix by inversion, centrifuge at 850rpm for 1min, aspirate the liquid and retain the precipitate.
2. Take one vial of Reagent B and add 1000ul of sterile water to dissolve. After it is completely dissolved, transfer 500ul to the precipitate in step 1 and place on a level mixer and incubate at room temperature for 2h.

[Note] The remaining 500ul of Reagent B should be stored frozen at -20°C until the next isolation.

3. Centrifuge at 850rpm for 1min, aspirate the liquid and retain the precipitate.

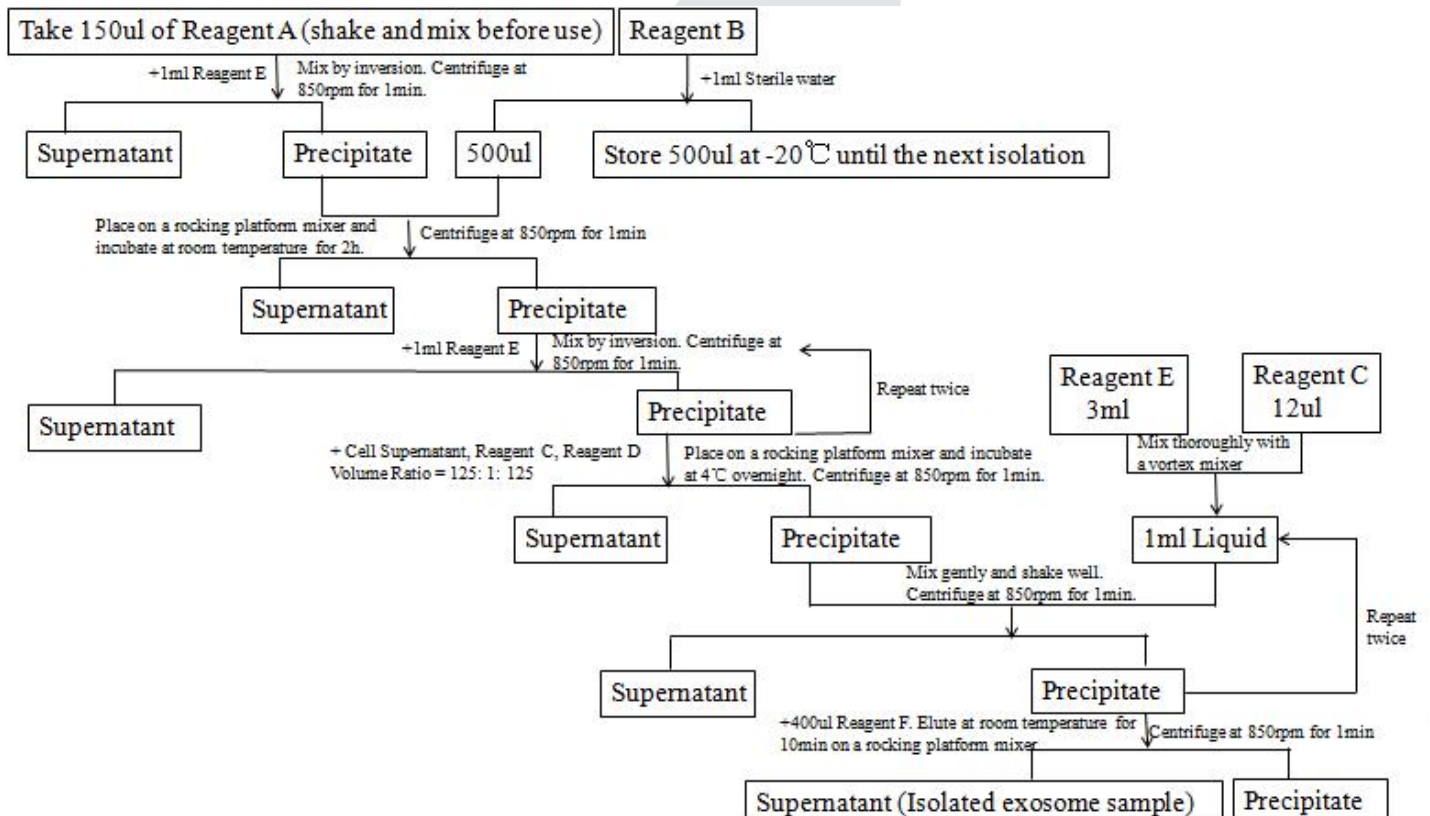


4. Add 1000ul of Reagent E, centrifuge at 850rpm for 1min, aspirate the liquid and retain the precipitate. Repeat this step twice.
5. Add the sample to be isolated to the precipitate in step 4. Add Reagent C and Reagent D in order, the volume ratio of the extracted sample: Reagent C: Reagent D is 125: 1: 125, and place them on a level mixer and incubate at 4°C overnight.
6. Centrifuge at 850rpm for 1min, aspirate the liquid and retain the precipitate.
7. Take 3ml of Reagent E, add 12ul of Reagent C, and mix thoroughly with a vortex mixer.  
[Note] Use this reagent as soon as it is prepared.
8. Take 1ml of the mixed buffer in step 7 and add to the precipitate in step 6. After gently mixing and shaking, centrifuge the liquid at 850 rpm to remove the liquid and retain the precipitate. Repeat this step twice.
9. Add 400ul of Reagent F to the precipitate in step 8 and elute at room temperature for 10min on a level mixer, centrifuge at 850rpm for 1min, and retain the liquid, which is the isolated exosome sample.

**[Note]** The amount of elution buffer can be appropriately increased or decreased according to the amount of exosomes. The recommended reference range is 100ul-500ul.

10. Short-term storage at 4 °C, long-term storage at -80 °C. Avoid repeated freeze-thaw cycles.

#### Exosome Isolation Flowchart





## **NOTE**

1. All steps are performed at room temperature.
2. It is recommended to use fresh cell supernatant for exosome isolation.
3. Make sure that the consumables used are low protein adsorption materials.
4. To ensure that the exosomes obtained are from your cells, it is best to use exosome-free serum for culture.
5. This product is only for exosomes isolation derived from cell culture supernatant.
6. This kit is only for scientific research, not for clinical diagnosis.