# Exosome Isolation Kit Protocol

**Product Name: Exosome Isolation Kit for Cell Culture Supernatant, Serum, Plasma, Breast Milk, Urine, Saliva, Yeast, Plant**

**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, serum, plasma, breast milk, urine, saliva. 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℃.

## SHELF LIFE

One year from the date of receipt.

## OPORATION METHOD

- **Sample Pretreatment**

**1. Cell Culture Supernatant**

1. Collect 10-25ml of cell culture supernatant from two 10cm culture dishes (no less than one dish) (cell density is not less than 1x10 ^ 6 cells/ ml).

2. Centrifuge the cell culture supernatant at 1500g for 10min, and retain the supernatant.

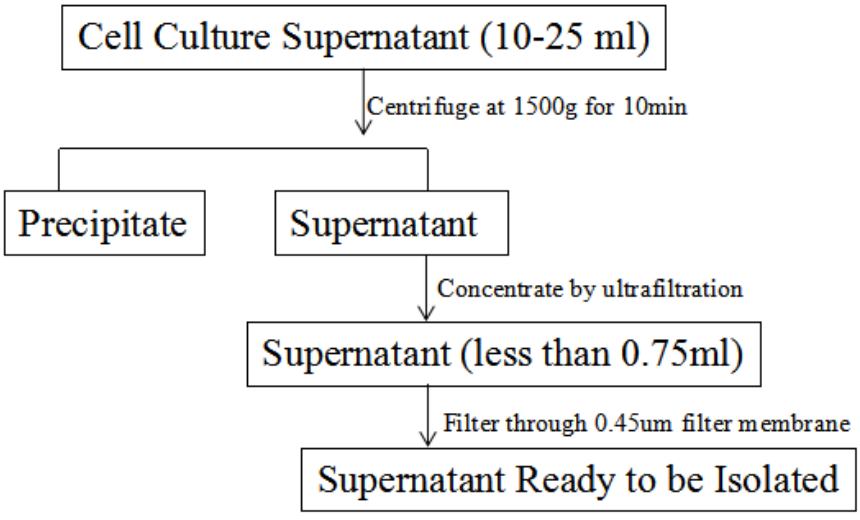
3. The supernatant is concentrated to 0.5ml (the volume should not exceed 0.75ml).

[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.

[Note] For the pretreatment of yeast samples, please refer to the above-mentioned cell supernatant pretreatment method.

Cell Culture Supernatant Processing Flowchart

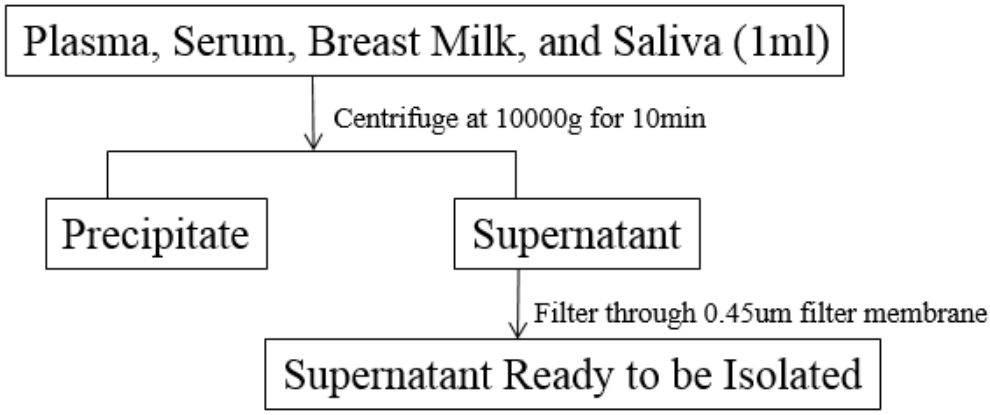



**2. Serum, Plasma, Breast Milk, Saliva**

1. Take 1ml of plasma (serum, breast milk, saliva).

2. Centrifuge the plasma (serum, breast milk, saliva) at 10000g for 10min, and retain the supernatant.

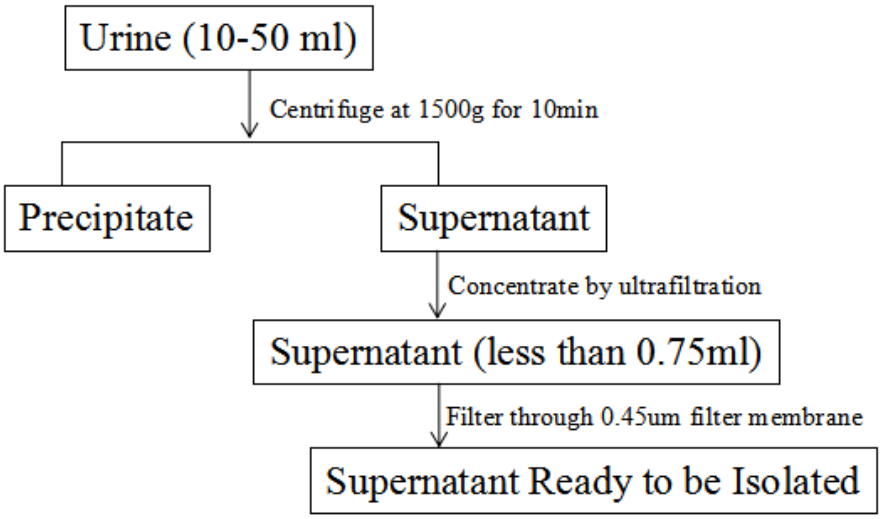
3. The supernatant is filtered at 0.45um and ready to be isolated (volume no more than 0.75ml).




**3. Urine**

1. Collect 10-50ml of the first morning mid-stream urine samples.
2. Centrifuge the urine at 1500g for 10min, and retain the supernatant
3. Concentrate the supernatant to 0.5ml (volume no more than 0.75ml)

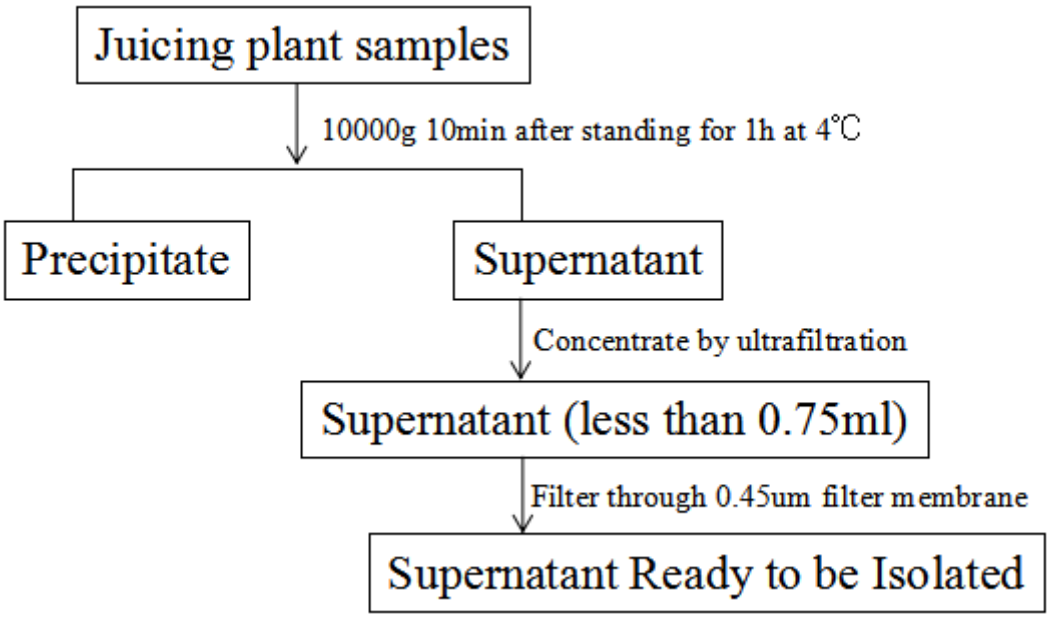
   [Note] It is recommended to use 10-100K ultrafiltration tube for concentration.
4. The concentrated sample is filtered at 0.45um and ready to be isolated.




**4. Plant**

1. Take an appropriate amount of plant samples, use a manual juicer to squeeze out about 35ml of the juice in the sample, and place it at 4℃ for 1 hour.
2. Centrifuge the samples after standing (10000g for 10min) and save the supernatant.
3. Concentrate the supernatant to 0.5ml (volume no more than 0.75ml).

   [Note] It is recommended to use a 50-100K ultrafiltration tube for concentration.
4. After concentration, the sample is filtered at 0.45um and ready to be extracted.




- **Exosome Isolation**

1. Take 150ul (It is recommended to cut off part of the pipette tip about 1cm when sucking) 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.

[Note] Reagent A contains microspheres and particulate matter, and visible precipitation is normal. Just mix and absorb before use, and there will be a little residue during the absorption process that will not affect the extraction effect.

2. Take one vial of Reagent B and add 2000ul of sterile water to dissolve. After it is completely dissolved, transfer 1000ul to the precipitate in step 1 and place on a level mixer and incubate at room temperature for 2h.

[Note] The remaining 1000ul Reagent B is stored at -20 in the freezer, and will be used for next extraction. If there is no horizontal mixer similar equipment, it can be mixed upside down and incubate at room temperature for 2 hours. The operation of the horizontal mixer in the following steps can be used for static incubation.

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℃ overnight (or incubate at room temperature for 2 hours on a level mixer).

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.

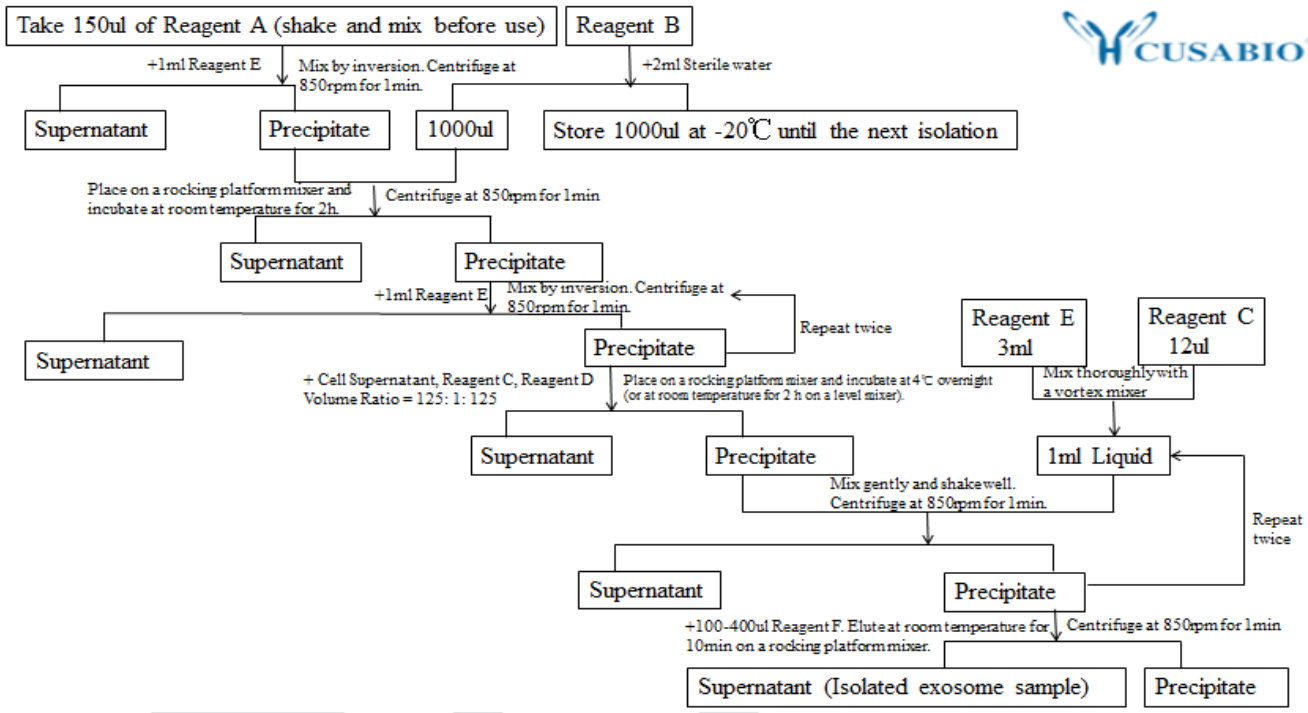
8. Take 1ml of the mixed buffer in step 7 and add to the precipitate in step 6. After gently mixing and shaking, centrifuge the liquid at 850 rpm to remove the liquid and retain the precipitate. Repeat this step twice.

9. Add 100-400ul 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 100-400ul.

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

Exosome Isolation Flowchart




## NOTE

1. All steps are performed at room temperature.
2. It is recommended to use fresh samples for exosome isolation.
3. Make sure that the consumables used are low protein adsorption materials.
4. This product is suitable for the isolation of exosomes from cell culture supernatant, plasma, serum, breast milk, urine, saliva, yeast, plant, etc.
5. This kit is only for scientific research, not for clinical diagnosis.