



TECHNICAL MANUAL

Exosome Isolation Affinity Purification

- **SKU CODE:** KACO00001
- **SIZE:** 2T/10T
- **RUO:** Research-Use-Only

Exosome Isolation Affinity Purification

Please read entire manual carefully before starting experiment.

Table of Contents

1. General Information	3
2. Storage & Expiry	3
3. Product Description	3
4. Kit Contents	4
5. Important Notes	4
6. Protocol	4

1. General Information

Sample type:

Cell Culture Supernatant, Serum, Plasma, Breast Milk, Urine, Saliva, Yeast, Plant

2. Storage & Expiry

This product should be stored at 2-10°C up to 12 months. The expiration date is indicated on the outer label of the kit box.

3. Product Description

Exosomes are small extracellular vesicles, typically 30–150 nm in diameter, released by a wide range of cell types. They carry diverse molecular cargo—including RNA species, proteins, and phospholipids—and are abundant in cell-culture supernatants as well as biological fluids such as milk, serum, plasma, semen, saliva, urine, amniotic fluid, and cerebrospinal fluid. By mediating the transfer of molecular information between cells, exosomes play key roles in intercellular communication and regulation.

This kit provides a straightforward and reliable method for isolating intact exosomes from cell-culture supernatants and various biofluids, including serum, plasma, breast milk, urine, and saliva. The resulting vesicles are suitable for a broad range of downstream applications, such as electron microscopy, nanoparticle tracking analysis (NTA), NanoFCM particle characterization, Western blotting, quantitative PCR, and high-throughput sequencing.

This dual function kit includes validated Bradford Reagent to quantify total protein concentration for accurate sample normalization.

4. Kit Contents

No	Component Name	2 Tests	10 Tests
1	Reagent A	300 µl*1	1.5 mL*1
2	Reagent B	1 tube	5 tube
	Reagent C	100 µl*1	500 µl*1
3	Reagent D	2 ml*1	7.5 ml*1
4	Reagent E	25 ml*1	45 ml*1
5	Reagent F	2 ml*1	5 ml*1
6	Bradford Reagent	1 vial (2 mL)	1 vial (2 mL)

5. Important Notes

1. This product is intended for research use only and is not for diagnostic or therapeutic applications.
2. The reagent may cause irritation. Avoid direct contact with skin, eyes, and mucous membranes, and prevent inhalation of vapors or aerosols.
3. Appropriate personal protective equipment, including lab coats, disposable gloves, and eye protection, should be worn at all times. All procedures should follow standard laboratory safety guidelines for chemical reagents.

6. Protocol

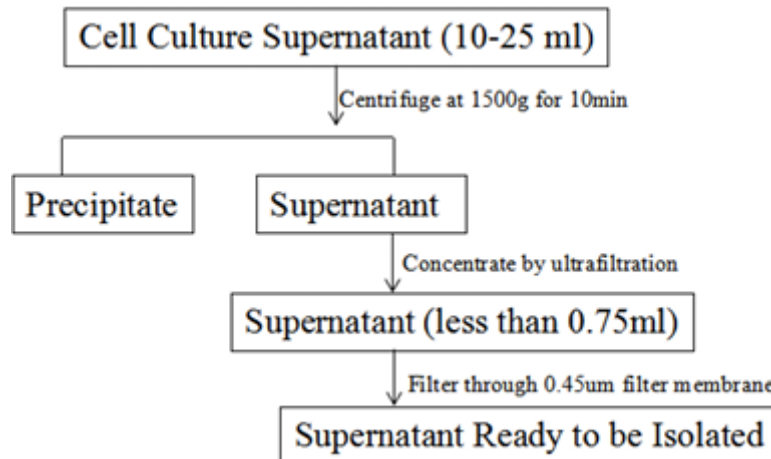
Sample Pretreatment

a. 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 1×10^6 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).

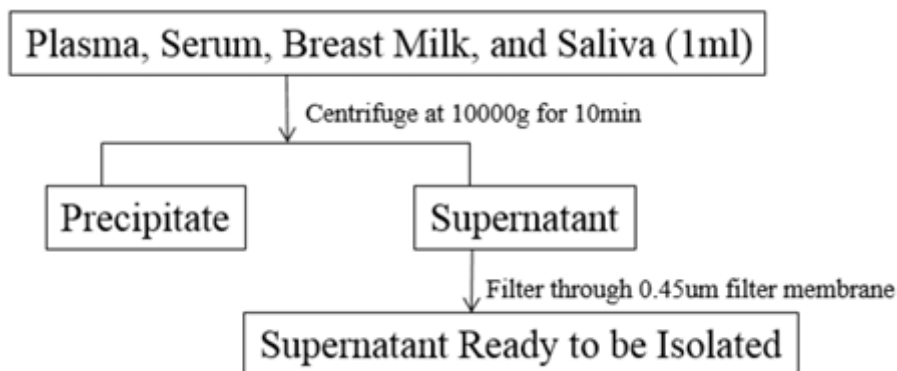
Note: It is recommended to use 10-100K ultrafiltration tube for concentration.

- 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.



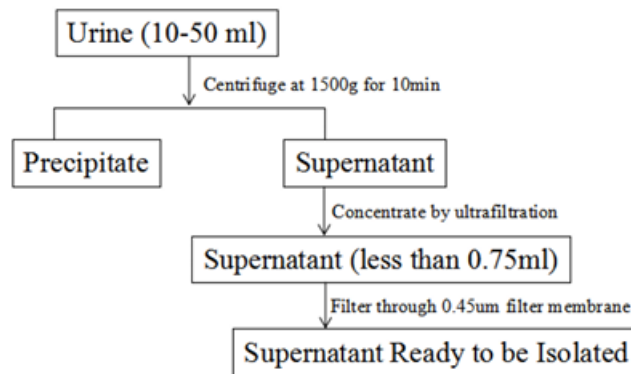
b. Serum, Plasma, Breast Milk, Saliva

- Take 1ml of plasma (serum, breast milk, saliva).
- Centrifuge the plasma (serum, breast milk, saliva) at 10000g for 10min, and retain the supernatant.
- The supernatant is filtered at 0.45um and ready to be isolated (volume no more than 0.75ml).



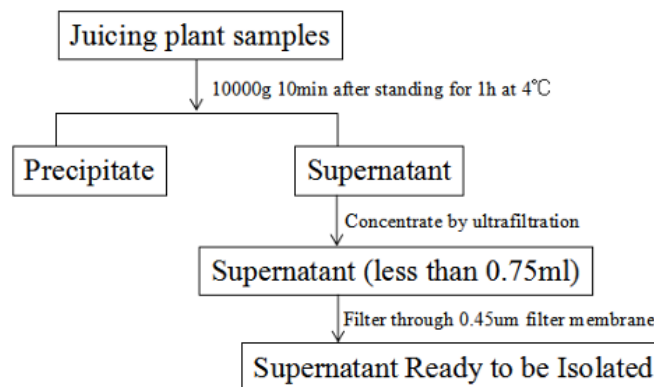
c. Urine

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.



d. Plant

- e. 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°C for 1 hour.
- f. Centrifuge the samples after standing (10000g for 10min) and save the supernatant. 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.*
- g. After concentration, the sample is filtered at 0.45um and ready to be extracted.



Exosome Precipitation

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°C 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.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.



Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.