Exosome Extraction Kit *(Urine)*

Product Instruction Manual (Version 4.1)

Product Name: Exosome Extraction Kit *(Urine)*

Item No. Specification: UR52126 (20 T), UR52129 (2 T)

Transportation and Storage: Room temperature transportation and storage, valid for 2 years.

Product Description:

Exosomes are nano-sized vesicles (30-150nm) secreted by cells, containing RNA and proteins, which are abundant in body fluids such as blood, saliva, urine and milk. Exosomes function as intercellular messengers, transmitting effectors or signal molecules between specific cells. However, their structures, compositions of effectors and biological pathways involved currently remain unclear.

In the biological functional study of exosomes, it is necessary to separate their complete particles. However, the conventional ultracentrifugation method involves complicated steps, high hardware requirements, and complex operational procedures. The Exosome Extraction and Purification Kit independently developed by Umibio is optimized for exosome extraction from urine. It enables rapid and efficient isolation of high-purity exosome particles, which can be used in electron microscopy analysis, Nanoparticle Tracking Analysis (NTA）, nucleic acid analysis, protein analysis, cytological experiments, animal experiments, etc.

Self-provided Material:

High-speed centrifuge, vortex oscillator, 50 mL centrifugal tube, 1.5 mL centrifugal tube, 1×PBS buffer solution (sterile).

Product Composition:

Component Name	UR52126	UR52129
Exosome Concentration Solution*	120 mL	12 mL
Exosome Solution Buffer*	10 mL	1 mL
50 mL Centrifugal Filter Column	20 pcs	2 pcs

* Nuclease-free, Sterile

Operation Procedure:

I. Sample Pretreatment

1. Sample preparation: For frozen sample: take out from the fridge and thaw in 25 °C water bath, then place the fully-thawed sample on ice; For fresh sample, collect and immediately place the sample on ice;
2. Initial dosage of the sample: The volume of urine in a single extraction is recommended to be not less than 20 mL;
3. Centrifugation to remove cell debris: Transfer the sample to the centrifugal tube. Centrifuge at 3,000×g (~5200 rpm **) for 10 min at 4 ℃ to remove the cell debris in the sample; (Note: For significant sediment, repeat centrifugation at 3,000 × g for 10 min until no obvious sediment remains, collecting the supernatant each time.

**Converted by a large centrifuge with an effective centrifugal radius of approximately 10 cm (≥15 mL centrifugal tube), the same applies below.

4. Centrifugation to remove impurity debris: Transfer the centrifugal supernatant to a new centrifugal tube and centrifuge at 10,000 × g (~9,500 rpm**) for 10 min at 4°C to remove impurity debris from the sample;
5. Sample filtration: Transfer the centrifugal supernatant with impurity debris removed to a 50 mL Centrifugal filter. Centrifuge at 3,000 × g (~5,200 rpm**) for 10 min at 4°C, and collect the filtrate from the lower chamber. (Note: If residual liquid remains in the upper chamber, repeat this step to obtain a larger sample volume);
6. Supernatant transfer: Transfer debris-free supernatant to new centrifugal tube.

II. Exosome Extraction

1. Supernatant pretreatment: Add Exosome Concentration Solution (ECS reagent) to the purified centrifuged supernatant, with a specific dosage of 4 mL ECS reagent per 20 mL of urine;
2. Solution mixing: After adding ECS reagent, tightly cover the centrifugal tube and vortex mix for 1 min, then place it at 4℃ for at least 8 h; (Note: Extending the standing time

can enhance exosome yield, but should not exceed 24 hours);

3. Precipitation of exosome: Take out the centrifugal tube with the mixed solution and centrifuge at 4℃ at 10,000 ×g （~9,500 rpm**） for 60 min. Discard the supernatant, and the sediment is rich in exosome particles(Note: Aspirate the supernatant as much as possible);

4. Re-centrifugation: The centrifugal tube containing the sediment is centrifuged again at 10,000 × g (~9,500 rpm**) for 2 min at 4°C, and the supernatant is discarded in order to remove any residual liquid from the wall of the tube (Note: Aspirate the supernatant as much as possible);

5. Resuspension of exosome: Resuspend the centrifugal precipitate with an appropriate volume of Exosome Solution Buffer (ESB reagent). After complete dissolution, transfer the resuspension solution to a new 1.5 mL centrifuge tube（） (Note: It is recommended to resuspend about 200 μL of ESB reagent for every 20 mL of urine);

6. Exosome particles harvesting: Centrifuge a 1.5 mL centrifugal tube containing the resuspended solution at 12,000 × g (~12,400 rpm**) for 2 min at 4°C, and collect the supernatant, which is rich in exosome particles (Note: If significant sediment persists, repeat centrifugation at 12,000 × g for 2 min until no obvious sediment remains, collecting the supernatant each time.)

*Converted by a small centrifuge with an effective centrifugal radius of approximately 7 cm (≤2 mL centrifugal tube).

7. Preservation of exosome: Aliquot the purified exosomes into appropriate volumes and store at -80℃ in a cryogenic refrigerator for subsequent experiments.

Note: This product is for life science research only, and medical diagnosis or other purposes are prohibited!