Exosome Extraction and Purification Kit (*Tissue*)

Product Instruction Manual (Version 4.1)

Product Name: Exosome Extraction and Purification Kit (*Tissue*)

Item No. Specification: UR52160 (2 T), UR52161 (20 T)

Transportation and Storage: Solution A2 is shipped in ice packs and stored below -18℃;

Transportation and storage: Other components are transported and stored at room temperature; 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.

I 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 a wide range of tissues (including brain, heart, liver, lung, muscle, spleen, lymph node, thymus, embryo, tumor and other tissues). In combination with the purification and filtration device, 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, 2 mL centrifugal tube, 1.5 mL centrifugal tube, 1×PBS buffer solution (sterile).

Product Composition:

Component Name	UR52160	UR52161
Solution A* (Stored at -18℃)	5 mL	48 mL
Solution B* (Stored at room temp)	1.2 mL	12 mL
Exosome Purification Filter*	2 Tubes	20 Tubes

* Nuclease-free, Sterile

Operation Procedure:

I. Sample Pretreatment

1. Melt and mix Solution A2 at 4℃ or on ice in advance;
2. Tissue shearing: Shear the tissue in a sterilized vessel (on ice) into pieces of about 1 mm^3 ~ 3 mm^3 (smaller fragments are preferable).
3. Tissue debris cleaning: Transfer the tissue debris to a centrifugal tube, add not less than 10 times the volume of 1 × PBS buffer solution, shake and mix well, then centrifuge at 300 × g (~2,000 rpm*) for 10 min, and discard the supernatant;

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

4. Tissue digestion: According to the ratio of 1 mL of Solution A2 for every 0.1 g of tissue block, mix the tissue block with Solution A2 in a 2 mL centrifugal tube, and incubate horizontally at 37 ℃, 80 rpm on a constant-temperature shaker for 20 min (or incubate in a 37 ℃ water bath for 30 min, mix every 5 min);
5. Centrifugation for supernatant: Centrifuge the reaction solution at 8,000 × g (~10,100 rpm) for 10 min, and transfer the supernatant to a new centrifugal tube;
6. Centrifugation to remove impurity debris: Transfer the centrifugal supernatant to a new centrifugal tube and centrifuge at 12,000 × g (~12,400 rpm) for 10 min at 4℃ to remove impurity debris from the sample;
7. Supernatant transfer: Transfer the supernatant with cell debris removal to a new centrifugal tube;

II. Exosome Extraction

1. Add Solution B2 reagent: Add 1/4 volume of Solution B2 reagent to the supernatant obtained in the previous step;
2. Solution mixing: Tightly cap the centrifugal tube, mix it evenly by vortex oscillator for 1 min, and then place it at 4℃ for more than 1 h. (Note: Extending the standing time can enhance exosome yield, but should not exceed 24 hours);
3. Precipitation of exosome: Remove the centrifugal tube containing the mixture and centrifuge at 12,000 × g (~12,400 rpm) for 30 min at 4℃. 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 12,000 × g (~12,400 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 sediment with an appropriate amount of 1× PBS by gently pipetting. After complete dissolution, and transfer the resuspended solution to a new 1.5 mL centrifugal tube (Note: It is recommended to resuspend every 0.1 g of tissue with about 200 μL of 1×PBS);
6. Exosome particles harvesting: Centrifuge the 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 enriched with the 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).

III. Exosome Purification

1. Purification of exosome: Transfer the harvested crude exosome particles into the upper chamber of the Exosome Purification Filter (EPF column) and centrifuge at 3,000 × g (~6,200 rpm) for 10 min at 4°C. After centrifugation, collect the liquid at the bottom of the EPF column tube, which is the purified exosome particles (Note: The EPF column cannot be reused);
2. Preservation of exosome: Aliquot the purified exosomes into appropriate volumes and store at -80°C in a cryogenic refrigerator for subsequent experiments.

Note:

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

Common Problems and Solutions:

I. Product Reagent Preservation Problems:

1. Solution A2 should be stored at -18°C before and after use, it is recommended to use it after dispensing, and repeated freezing and thawing should be avoided;
2. Solution B2 should always be stored at room temperature. If
the temperature is too low, white crystals may appear in the solution and are difficult to dissolve (requiring high temperature). A small amount of sediment does not affect the extraction effect.

II. Purification Column Usage Problems:

1. In general, each sample can be purified in a single centrifugation step at "3,000 × g (~6,200 rpm) for 10 min" according to the instructions. If there is still liquid left in the upper column, place the column in the centrifuge in a different direction (different from the previous direction) and repeat the centrifugation again;
2. If there is still more liquid left in the upper column after the above operation, please transfer the liquid in the upper column to the new purification column and continue to repeat the centrifugation operation until all the liquid in the upper column is transferred to the lower column.

III. Exosome Attainment Rate Problem:

In general, 0.1 g of tissue sample (take myocardial tissue as an example) can extract 100~200 μg of exosomes (5E+9~1E+10 Particles in total). However, due to variations in exosome content across different tissues, the extraction yield may vary）.