Exosome Extraction and Purification Kit (*Plasma or Serum*) Plus

Product Instruction Manual (Version 4.1)

Product Name: Exosome Extraction and Purification Kit (*Plasma or Serum*) Plus

Item No. Specification: UR52150 (2 T), UR52151 (20 T)

Transportation and Storage: Room temperature transportation, Solution A stored below -18℃, other components are stored at room temperature.

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 plasma, serum. 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, 1.5 mL centrifugal tube, 1×PBS buffer solution (sterile).

Product Composition:

Component Name	UR52150	UR52151
Solution A* (Stored at -18℃)	2 mL	20 mL
Solution B* (Stored at room temperature)	1 mL	6 mL
Exosome Purification Filter*	2 Tubes	20 Tubes

* Nuclease-free, Sterile

Operation Procedure:

I. Sample Pretreatment

1. The centrifuge shall be pre-cooled for 10 min at 4℃ before use;
2. 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;
3. Dispense samples at 500 µL per tube, adjusting the volume to 500 µL with 1×PBS;
4. Centrifugation to remove cell debris: Transfer the sample to a 1.5 mL centrifugal tube and centrifuge at 3,000 × g (~6,200 rpm*) for 10 min at 4°C to remove cellular debris from 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 small centrifuge with an effective centrifugal radius of approximately 7 cm (≤2 mL centrifugal tube), the same applies below.

5. 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°C to remove impurity debris from the sample;
6. Supernatant transfer: Transfer debris-free supernatant to new centrifugal tube.

II. Heteroprotein Removal

1. Adding Solution A: Add 400 µL **pre-cooled** Solution A into 500 µL blood sample and tightly cover the centrifugal tube immediately, and fully mix it using the vortex oscillator for 30 s;
2. Centrifuging to remove the protein: Centrifuge the mixed sample at 12,000 × g (~12,400 rpm) for 20 min at 4°C to remove heteroproteins from the samples;
3. Supernatant transfer: Transfer the heteroprotein-depleted supernatant into a new 1.5mL centrifugal tube;

III. Exosome Extraction

1. Adding Solution B: Add 120 µL Solution B into the heteroprotein-depleted supernatant;
2. Solution mixing: tightly cover the centrifugal tube and vortex mix） for 1 min, and then place it at 4℃ for more

than 30 min (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 15 min at 4°C. 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, transfer the resuspended solution to a new 1.5 mL centrifugal tube (Note: It is recommended to resuspend every 500 μL of plasma serum 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）.

IV. 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℃ in a cryogenic refrigerator for subsequent experiments.

Note:

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