# Exosome Extraction and Purification Kit (*Milk*)

**Product Instruction Manual (Version 4.1)**

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

**Item No. Specification:** UR52146 (20 T), UR52149 (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 milk. 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 | UR52146 | UR52149 |
|---|---|---|
| Solution A* | 50 mL | 5 mL |
| Solution B* | 60 mL | 6 mL |
| Solution C* | 60 mL | 6 mL |
| Solution D* | 120 mL | 12 mL |
| 50 mL Centrifugal Filter Column | 20pcs | 2pcs |

* 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 recommended amount of emulsion for a single extraction is not less than 25 mL;
3. Centrifugation to remove the lipids: Transfer the sample to a centrifugal tube and centrifuge at 10,000 × g (~9,500 rpm**) for 20 min at 4°C to remove lipids and some proteins from the sample (Note: After centrifugation, the sample separates into three layers, with a lipid layer in the upper layer, a protein sediment in the lower layer, and whey in the middle layer. After centrifugation, the upper layer should appear “compact, stable and not easily dispersed”. If the upper layer is “fluffy and easily dispersed” and there is a lot of sediments in the lower layer, repeat this step and collect the middle layer of liquid 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. Whey transfer: Transfer the lipid-removed whey (middle layer of liquid) to a new 50 mL centrifugal tube(Note: The upper layer of lipid can be poked with a pipette tip and poured slowly, or transferred using a pipette; a small amount of lipid and sediment in the transferred whey is normal and will not affect subsequent experiments).

**II. Heteroprotein Removal**

1. Whey clarification: Add Solution A into the whey. Invert the centrifugal tube to mix it thoroughly until it becomes “translucent”. Then add Solution B and invert the tube to mix thoroughly. Place it at 2°C to 8°C for 10 min; (Note: After standing is completed, shake gently the centrifugal tube. “Tofu pudding-like” solid will be presented and the fluid will be “transparent”. If the state of “tofu pudding” is not presented or the sample is still “milk white”, add an

appropriate amount of Solution B until the fluid turns "transparent");

| Whey Volume | Solution A Dosage | Solution B Dosage |
|---|---|---|
| 20 mL | 2 mL | 2.5 mL |

Note: The exact amount to be added depends on the volume of whey and is calculated proportionally according to the table above.

2. Centrifugation to remove the protein: Centrifuge the clarified whey at 10,000 × g (~9,500 rpm**) for 10 min at 4°C and collect the supernatant;

3. Supernatant filtration: Transfer the collected supernatant to a 50 mL centrifugal filter and centrifuge at 5,200 rpm (~3,000 × g) for 2 min at 4°C. (Note: Repeat this step if the filtration is not complete. 50mL Centrifugal filter is a disposable material and repeated use is not recommended);

4. Transfer the filtered supernatant to the new centrifugal tube, add Solution C and invert it to thoroughly mix it; (Note: The dosage of Solution C shall be consistent with the dosage of Solution B)

**III. Exosome Extraction**

1. Supernatant pretreatment: Add Solution D to the supernatant after adding Solution C. The specific dosage to be added is as follows (For other dosages, please calculate other dosages proportionally):

| Sample Name | Sample Dosage | Added Solution D Dosage |
|---|---|---|
| Whey | 20 mL | 5 mL |

2. Solution mixing: After adding Solution D, tightly cover the centrifugal tube and vortex mix for 1 min, then place it at 4°C for more than 1 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°C 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 sediment with an appropriate amount of 1× PBS by gently pipetting.） After complete dissolution, transfer the resuspended solution into a new 1.5 mL centrifugal tube(Note: It is recommended that each 25 mL of milk be resuspended with about 200 μL of 1× PBS);

6. Exosome particles harvesting: Centrifuge 1.5 mL centrifugal tube containing resuspended solution at 12,000 × g (~12,400 rpm*) for 2 min at 4°C, 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. The exosome solution may appear faint milky white, which is a normal phenomenon).

*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°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!