Exosome Extraction Kit (emulsion)

Cat: EX0013
Size: 20T
Storage: RT, Valid for 2 year. Mix well before use.

Kit Components:

Kit Components	Size
Solution A	50mL
Solution B	50mL
Solution C	50mL
Solution D	100mL
50mL Centrifugal Filter Column	20

Introduction:

Exosomes are small vesicles (30-150nm) secreted by cells containing RNA and proteins, which exist in large quantities in body fluids such as blood, saliva, urine and milk. Exosomes are thought to function as intercellular messengers, transporting their effectors or signaling molecules between specific cells; However, their structure, effector composition, and the biological pathways involved are still unclear.

In the study of the biological function of exosomes, complete exosome particles need to be isolated, and the traditional ultracentrifugation method is complicated, demanding in hardware and difficult to operate. The exosome rapid extraction kit developed by Solarbio Biological, with optimized components, is suitable for exosome extraction in emulsion, and can quickly and efficiently obtain high-purity exosome particles, which can be used for electron microscope analysis, NTA particle size analysis, nucleic acid analysis, protein analysis, cytology experiments and animal experiments.

Self-prepared Material:

High speed centrifuge (can reach 10000g centrifugal force), vortex oscillator; 50mL centrifugal rotor, 50mL centrifugal tube, 2mL centrifugal rotor, 1.5mL centrifugal tube, PBS buffer.

Protocols:

1. Sample pretreatment

(1) Sampling: If the sample is frozen, remove it from the refrigerator and thaw it in a water bath at 25℃. Place the completely melted sample on ice; If it is a fresh sample, collect the sample and place it on ice.

(2) Initial sample dosage: The optimal emulsion volume for a single extraction is 50mL.

(3) Centrifuge lipid removal: The sample was transferred to a centrifuge tube and centrifuged at 10000g at 4℃ for 20min to remove lipids and some proteins in the sample; (Note: After centrifugation, the sample was divided into three layers, the upper layer was lipid layer, the lower layer was protein precipitation, and the middle layer was whey. After centrifugation, the upper layer is "dense, stable and not easy to fall off", if the upper layer is "soft and easy to fall off" and the lower layer is more precipitated, this step can be repeated, and the middle layer liquid can be taken each time by centrifugation).

(4) Whey transfer: The lipid-removed whey (intermediate layer liquid) is transferred to a new 50mL centrifuge tube (Note: the upper layer of lipids can be poked at the head of a gun and slowly dumped, or transferred with a pipette, a small amount of lipids and precipitation in the transferred whey is normal, and does not affect subsequent experiments).

2. Removal of impurity protein

(1) Whey clarification: Add Solution A to whey, mix the centrifuge tube upside down until it is "translucent", then add Solution B, and let it stand at 2℃ to 8℃ for 10min after mixing upside down; (Note: After standing, gently shake the centrifuge tube to show "beanflower-like" solid, and the liquid part is "transparent". If there is no "beanflower-like" or the sample is still "milky", you can add Solution B appropriately until the liquid is transparent "transparent").

Whey volume	Solution A	Solution B
40mL	4mL	3mL

Note: The specific dosage should be converted according to the table above.

(2) Centrifuge deproteinization: Centrifuge the clarified whey at 10000g at 4℃ for 10min and collect the supernatant.

(3) Filtration of supernatant: Transfer the collected supernatant to a 50mL centrifugal filter column and centrifuge it at 3000g at 4℃ for 2min. (Note: If not completely filtered, this step can be repeated. The 50mL centrifugal filter column is a disposable consumable and is not recommended for reuse).

(4) Transfer the filtered supernatant to a new centrifugal tube, add Solution C and mix it upside-down; (Note: the dosage of Solution C should be consistent with that of Solution B)

Sample volume after centrifuge filtration	Solution C dose
40mL	3mL

3. To extract exosomes

(1) Supernatant pretreatment: Add Solution D to the supernatant after adding Solution C, the specific dosage is as follows:

Sample dose	Add the Solution D dose
40mL	10mL

Note: For other doses, please convert according to the table above.

(2) Solution mixing: After adding the Solution D, cover the centrifugal tube tightly, mix it with a vortex oscillator for 1 min, and then place it at 2℃ to 8℃ for at least 2h; (Note: Increasing the standing time can improve the exosome yield, but the standing time should not exceed 24h).

(3) Precipitation exosomes: Take out the centrifuge tube containing the mixed liquid and centrifuge it at 10000g at 4℃ for 60min. Discard the supernatant, and the precipitation is rich in exosomes. (Note: Absorb the supernatant as much as possible).

(4) Exogenic weight suspension: Take 1×PBS to evenly blow the centrifugal precipitate (specific dosage added in the table below), and transfer the suspension to a new 1.5mL centrifugal tube after it is dissolved;

Sample volume	PBS dosage added
40mL	1mL

Note: For other doses, please convert according to the table above.

(5) Harvest exosome particles: Centrifuge a 1.5mL centrifuge tube containing the heavy suspension at 4℃ at 12000g for 5min and retain the supernatant, which is rich in exosome particles. (Note: If there is a lot of precipitation, this step can be repeated several times until there is no obvious precipitation, and the centrifugal supernatant is taken each time. The exosome solution may have a light milky white color, which is normal).

(6) Preservation of exosomes: The purified exosomes are stored in 50-100μL in a cryogenic refrigerator at -80℃ for further experimental use.

Note:

This product is only for life science research, not for medical diagnosis and other purposes!