Cell Supernatant Exosome Extraction Kit

Cat: EX0011
Size: 20T
Storage: RT, Valid for 2 year. Mix well before use.

Kit Components:

Kit Components	Size
Exosome Concentration Solution*	100mL
Exosome Purification Filter*	20

Note: *RNase/DNase Free, Sterile.

Introduction:

Exosomes are small vesicles (30-150nm) secreted by cells containing RNA and protein, 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. After optimized treatment, this kit is suitable for exosome extraction from cell culture supernatant, and with purification filter device, high purity exosome particles can be obtained quickly and efficiently, 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 10000×g centrifugal force); Scroll oscillator; 2mL centrifugal rotor; 1.5mL centrifugal tube; 1×PBS buffer (sterile).

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 amount of cell culture supernatant used for a single extraction should be at least 20mL.

(3) Centrifuge to remove cell debris: The sample was transferred to a centrifuge tube and centrifuged at 4℃ at 3000×g (~5200rmp) for 10min to remove cell debris in the sample;
(Note: If there is much precipitation, centrifuge 3000×g/10min for several times until there is no obvious precipitation, and take the centrifuge supernatant each time).

(4) Supernatant transfer: The centrifuge supernatant with the removal of cell debris is transferred to a new 50mL centrifuge tube.

2. Extract exosomes

(1) Supernatant pretreatment: Add Exosome Concentration Solution (ECS reagent) to the centrifugal supernatant to remove impurities, and the specific dosage is as follows: (Other doses are converted according to the reagent dosage and other proportions in the table).

Sample name	Sample dosage	Add the ECS dose
Cell culture supernatant	20mL	5mL

(2) Solution mixing: After adding ECS reagent, cover the centrifuge tube tightly, mix it well by vortex oscillator for 1min, and then place it at 4℃ for at least 2h; (Increasing the standing time can improve the exosome yield, but the standing time should not exceed 24h).

(3) Precipitation of exosomes: The centrifuge tube containing the mixed liquid was removed and centrifuged at 10000×g (~9500rmp) at 4℃ for 60min. The supernatant was discarded, and the precipitation was 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 centrifuge tube after it is dissolved (Note: Other dosages should be converted according to the reagent dosage in the table).

Cell supernatant fluid volume	Add PBS dose
20mL	0.2mL

(5) Collection of exosome particles: A 1.5mL centrifuge tube containing the heavy suspension was centrifuged at 4℃ for 2min at 12000×g (~12400rmp) and the supernatant, which was rich in exosome particles, was retained. (Note: If there is much precipitation, the supernatant can be centrifuged at 12000×g/2min for several times until there is no obvious precipitation, and the centrifugal supernatant can be taken each time).

3. Purify exosomes

(1) Purification of exosomes: The harvested crude Exosome particle was transferred to the upper chamber of Exosome Purification Filter (EPF column) and centrifuged at 3000×g (~6200rmp) for 10min at 4℃. After centrifugation, the liquid at the bottom of the EPF column was collected. The liquid was the purified exosome particle (Note: EPF column should not be reused).

(2) Preservation of exosomes: The purified exosomes were stored in 50-100μL in a low-temperature refrigerator at -80℃ for further experimental use.

Note:

This product is intended for life science research only and is not intended for medical diagnosis or other purposes.