Exosome Isolation Kit (from cell culture media, for NTA/TEM)

Catalog No.: EZB-exo201

Description

The *EZ*Bioscience® Exosome Isolation Kit (from cell culture media for NTA/TEM) provides a simple, reliable, and rapid method for isolating high-quality intact total exosome from cell culture media, urine, broncho alveolar lavage fluid (BALF), cerebro-spinal fluid (CSF) samples, without the need for ultracentrifugation.

Exosomes are small vesicles (30 ~ 120 nm) containing RNA and protein that are secreted by various types of cells in culture, and found in abundance in body fluids, such as blood, saliva, urine, and breast milk. Exosomes were reported to function as intercellular messengers, delivering their cargo of effector or signaling macromolecules between specific cells; however, their formation, the makeup of the cargo and biological pathways in which they are involved remain unclear.

The biological and medical study of exosome function and trafficking requires the isolation of intact exosomes, but the current methods used are tedious and difficult. The *EZ*Bioscience® Exosome Isolation Kit (from cell culture media) provides a simple and reliable method of concentrating intact exosomes from cell culture media samples. By tying up water molecules, the Exosome Precipitation Reagent forces less-soluble components (i.e. exosomes) out of solution, allowing them to be collected with a brief and relatively low-speed centrifugation. Then the resuspended exosomes are purified by a Purification Column (0.22 μm). After that, the exosomes could be used for RNA purification (for RNA sequencing, RNA Chip, RT-qPCR), protein extraction, particle size detection and electron microscopy, etc.

Components

Components	EZB-exo201-S	EZB-exo201-L
Exosome Precipitation Reagent (EPR)	50 ml	100 ml
Purification Column	10 preps	20 preps

The Exosome Precipitation Reagent contains enough reagent for processing up to 200 ml of cell culture media.

Storage

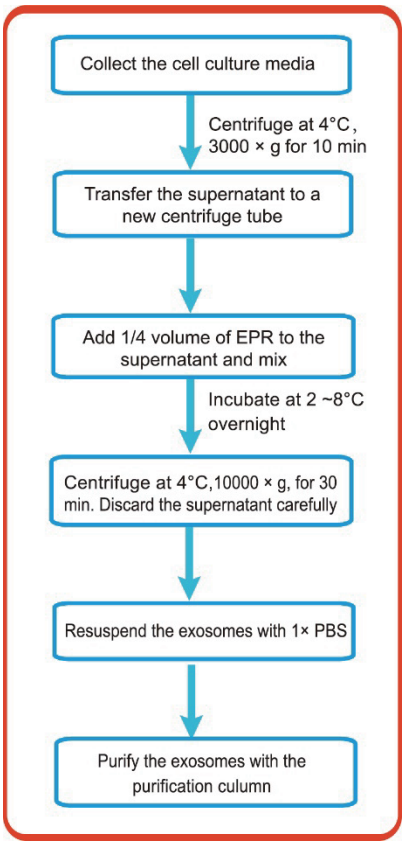
Store the Exosome Precipitation Reagent at 2°C to 8°C.

Protocol

General Guidelines

1. To ensure that isolated exosomes originate from your cells of interest, culture the cells with exosome depleted fetal bovine serum (FBS) or serum-free culture medium, because normal FBS contains extremely high levels of exosomes that will contaminate the cell derived exosomes. If you cannot obtain exosome depleted FBS, certain cell lines can be grown for up to 12 hours in media without FBS.
2. Exosome Precipitation Reagent (EPR) needs to be placed in a 30°C water bath for 30 minutes after removal from the 4°C refrigerator and mixed thoroughly before use.
3. The Exosome Precipitation Reagent (EPR) is not recommended for isolation of exosomes from other kinds of samples. If you are isolating intact exosomes from plasma, serum and the other body fluids, use the *EZ*Bioscience® Exosome Isolation Kit (from plasma/serum) (Cat. No.: EZB-exo1).
4. After exosomes are isolated, total RNA can be extracted using the *EZ*Bioscience® Exosome RNA Purification Kit (Cat. No.: EZB-exo-RN1).

Experimental Procedure Overview:



Prepare Sample

1. Harvest 5 ~ 20 ml cell culture media (or urine, broncho alveolar lavage fluid, cerebro-spinal fluid).
2. Centrifuge the cell media at 3,000 × g for 10 minutes to remove cells and debris.
3. Transfer the supernatant containing the cell-free culture media to a new tube without disturbing the pellet.

Isolate Exosomes

1. Transfer the required volume of cell-free culture media to a new tube and add 1/4 volume of the Exosome Precipitation Reagent (EPR).

Cell Culture Media	EPR
5 ml	1.25 ml
20 ml	5 ml

2. Mix the culture media/reagent mixture well either by vortex or inversion until the solution is homogenous. Incubate the sample at 2°C to 8°C overnight.
3. After incubation, centrifuge the sample at 10,000 × g for 30 minutes at 4°C.
4. Carefully aspirate the supernatant by pipetting and discard the supernatant. Exosomes are contained in the pellet at the bottom of the tube (not visible in most cases).

Resuspend Exosomes

1. Add 1× PBS or similar buffer to the pellet and vortex or pipette up and down to resuspend the exosomes.

Starting Cell Culture Media Volume	Resuspension Volume
1 ml	30 µl
10 ml	60 µl

2. Load the resuspended exosome on the Purifying Column, and centrifuge at 4,000 × g for 5 minutes. The purified exosomes are ready for downstream analysis or further purification through affinity methods.

Note: The purified exosome sample may be stored at 2°C to 8°C for up to 7 days or can be stored at -80°C for long term storage. To minimize the risk of RNA degradation, we recommend proceeding directly with further downstream sample processing.