Exosome Isolation Kit (from plasma for NTA/TEM)

Catalog No.: EZB-exo101

Description

The *EZ*Bioscience® Exosome Isolation Kit (from plasma for NTA/TEM) uses purification column method to provide a simple, reliable and rapid method for isolating high-quality total exosome from human and animal plasma, 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 plasma for NTA/TEM) provides a simple and reliable method of concentrating intact exosomes from human and animal plasma 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 exosomes are further purified by centrifuge purification columns. The purified exosomes can be used for Nanoparticle Tracking Analysis (NTA), Transmission Electron Microscope (TEM), and co-culturing with cells.

Components

Component	EZB-exo101-S	EZB-exo101-L
Exosome Precipitation Reagent (EPR)	10 ml	20 ml
Purification Column	10 preps	20 preps

The Exosome Precipitation Reagent contains sufficient reagent for processing up to 30/ 60 ml of plasma.

Storage

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

Protocol

General Guidelines

a) The Exosome Precipitation Reagent (EPR) is not recommended for isolation of exosomes from any other body fluids or cell culture media. Specialized Exosome Isolation reagents are available for serum, urine, cell culture media, and other body fluids, each optimized for its specific type of biological sample.

b) For sample from blood, if downstream RNA expression profiling is planned, do not use hemolyzed samples. Even traces of red blood cells in serum or plasma will affect the RNA profile. Instead, we recommend using serum or citrate/EDTA plasma and discourage using heparin plasma (RNA isolated from heparin plasma can reduce PCR performance).

c) 100 ~ 1000 µl of plasma typically provides enough exosomes for most standard types of analysis. For exosome isolation from larger starting volumes, (>1 ml), we recommend extending or optimizing centrifugation time to account for the larger volume and ensure efficient recovery of exosomes.

Prepare Sample

1. Remove the plasma sample from storage and place on ice. If the sample is frozen, thaw the sample in a 25°C to 37°C water bath until it is completely thawed, and place on ice until needed.
2. Centrifuge the plasma sample at 3000 × g for 10 minutes at 20°C to remove cells and debris.
3. Transfer the supernatant containing the partially clarified plasma to a new tube without disturbing the pellet.
4. Centrifuge the new tube at 10000 × g for 20 minutes at 20°C to remove debris.
5. Transfer the supernatant containing the clarified plasma to a new tube without disturbing the pellet, and place it on ice until ready to perform the isolation.

Isolate Exosomes

1. Transfer the required volume of clarified plasma to a new tube and add 0.5 volume of 1× PBS. Mix the sample thoroughly by vortexing.
2. Add 20% total volume (i.e. Total volume = plasma + PBS) Exosome Precipitation Reagent (from plasma) to the sample.

Plasma + PBS	Reagent
200 µl + 100 µl	60 µl
1 ml + 0.5 ml	300 µl

3. Mix the plasma & reagent mixture well either by vortexing or inversion until the solution is homogenous. Incubate the sample at 2°C to 8°C for 2 hours.

   Note: This precipitation step can be extended to overnight, if needed.

4. After incubation, centrifuge the sample at 10000 × g for 30 minutes at 20°C.

   Note: For mouse plasma, centrifuge for 30 minutes at 4°C.

5. Carefully aspirate the supernatant by pipetting and discard.

   Note: Exosomes are contained in a pellet at the bottom of the tube.

6. Centrifuge the tube for 30 seconds at 10000 × g to collect any residual reagent.
7. Discard any residual supernatant by careful aspiration with a pipette.

Resuspend Exosomes

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

Starting Plasma Volume	Resuspension Volume
200 µl	20 µl
1 ml	100 µl

Purify Exosomes

1. The exosome suspension was added to a purification column, centrifuged at 4000 g for 5 minutes at 4°C, and the effluent was the purified exosomes, which can be used for Nanoparticle Tracking Analysis (NTA), Transmission Electron Microscope (TEM), and co-culturing with cells.

Note: The purified exosome sample may be stored at 2°C to 8°C for up to 2 days or can be stored below -20°C for long term storage. To minimize the risk of RNase contamination, we recommend proceeding directly with further downstream sample processing.