Exosome Isolation Kit (from plasma/serum, for NTA/TEM)

Catalog No.: EZB-exo101

Description

The *EZ*Bioscience® Exosome Isolation Kit (from plasma/serum for NTA/TEM) provides a simple, reliable and rapid method for isolating high-quality intact exosomes from human and animal plasma/serum/semen/follicular fluid, without the need for ultracentrifugation. 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 affinity method. The purified exosomes can be used for RNA analysis, Nanoparticle Tracking Analysis (NTA), Transmission Electron Microscope (TEM) detection, etc.

Components

Components	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 33/66 ml of plasma/serum.

Storage

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

Protocol

General Guidelines

1. 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 (i.e. Exosome Isolation Kit (from cell culture media)) are available for urine, cell culture media, and other body fluids, each optimized for its specific type of biological sample.
2. If extracting exosomes in plasma/serum samples, prevent hemolysis when collecting blood samples to avoid interference with the results. It is recommended to use citrate/EDTA plasma and discourage using heparin plasma.
3. 100 ~ 1000 µl of plasma/serum 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.
4. 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.
5. After exosomes are isolated, total RNA can be extracted using the *EZ*Bioscience® Exosome RNA Purification Kit (Cat. No.: EZB-exo-RN1).

Prepare Sample

1. Place the plasma/serum sample on ice until needed. If the sample is frozen, thaw the sample in a 25°C ~ 37°C water bath until it is completely thawed.
2. Centrifuge the plasma/serum sample at 3,000 × g for 10 minutes at room temperature (~20°C) to remove cells and debris.
3. Transfer the supernatant to a new tube without disturbing the pellet.
4. Centrifuge the new tube at 10,000 × g for 20 minutes at room temperature to remove debris.
5. Transfer the supernatant to a new tube without disturbing the pellet, and place it on ice until ready to perform the isolation.

Isolate Exosomes

6. Add 1/2 plasma/serum sample volume of 1× PBS to the above supernatant, mix the sample thoroughly by vortex.
7. Add 1/5 total volume (total volume = plasma/serum + PBS) Exosome Precipitation Reagent (EPR) to the above mixture.

Plasma/Serum + PBS	EPR
100 µl + 50 µl	30 µl
1 ml + 500 µl	300 µl

8. Mix well by vortex or inversion until the solution is homogenous. Incubate the sample at 2°C ~ 8°C for 30 minutes.
   Note: This precipitation step can be extended to overnight, if needed.
9. After incubation, centrifuge the sample at 10,000 × g for 30 minutes at room temperature.
   Note: For mouse plasma, centrifuge for 30 minutes at 4°C.

10. Carefully aspirate the supernatant by pipetting and discard.
    Note: Exosomes are contained in a pellet at the bottom of the tube.
11. Centrifuge the tube for 30 seconds at 10,000 × g to collect any residual reagent.
12. Discard any residual supernatant by careful aspiration with a pipette.

Resuspend Exosomes

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

Starting Plasma/Serum Volume	Resuspension Volume
100 µl	25 ~ 50 µl
1 ml	100 µl

Purify Exosomes

14. The exosome was added to a purification column and centrifuged at 4,000 × g for 5 minutes and 4°C, the effluent was the purified exosomes, which can be used for Nanoparticle Tracking Analysis (NTA), Transmission Electron Microscope (TEM).
    Note: The purified exosome sample may be stored at 2°C ~ 8°C for 2 days ~ 1 week or can be stored at -80°C for longer storage.

Serum and Plasma Preparation

Plasma Separation

1. Collect whole blood in Anticoagulant tubes containing EDTA (or other anticoagulant, e.g., citrate), then turn the anticoagulation tubes upside down 10 ~ 15 times to mix thoroughly.
   Note: Do not use heparin-containing blood collection tubes, as this anticoagulant can interfere with downstream assays, such as RT-PCR.
2. Store tubes at 4°C for 3 ~ 4 hours. Then centrifuge blood samples in primary blood collection tubes for 10 minutes at 1500 × g and 4°C using a swinging bucket rotor.
3. Carefully transfer the upper (yellow) plasma phase to a new tube (with conical bottom) without disturbing the intermediate buffy coat layer (containing white blood cells and platelets).
   Note: Carryover of white blood cells and platelets from the buffy coat layer is the most likely source of cellular miRNA/RNA contamination in plasma.
4. The collected plasma can be used directly for subsequent experiments. For longer storage, keep plasma frozen in aliquots at -80°C.

Serum Separation

1. Collect whole blood in a primary blood collection tube without anticoagulants such as EDTA or citrate. For complete clotting, leave tubes at 4°C for 3 ~ 4 hours (Note: The blood should be transferred to 4°C within 10 minutes), or at room temperature (~20°C) for 40 minutes to 1 hour.
2. Centrifuge tubes for 10 minutes at 1500 × g and 4°C using a swinging bucket rotor.
3. Carefully transfer the upper (yellow) serum phase to a new tube (with conical bottom) without disturbing the pellet containing cellular material.
   Note: Prevent transfer of cellular material from the lower phase.
4. Centrifuge serum samples in conical tubes for 10 minutes at 1500 × g and 4°C. This will remove additional cellular nucleic acids attached to cell debris.
5. Carefully transfer cleared supernatant to a new tube without disturbing the pellet, which forms a smear along the outer side of the centrifugation tube.
6. The collected serum can be used directly for subsequent experiments. For longer storage, keep serum frozen in aliquots at -80°C.