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ExoFast™ Exosome Isolation Reagent from plasma

Catalog Number: D032

Content and Storage

Component	Amount	Shipping Condition	Storage Condition
ExoFast™ Exosome Isolation Reagent from plasma	10 mL	Room temperature	2~8°C for one year

Product Description

Exosomes are small vesicles (30–120 nm) containing protein and RNA that are secreted by various types of cells in culture, and found in abundance in body fluids including blood, saliva, urine, and breast milk. Exosomes are thought to function as intercellular messengers, signaling macromolecules between specific cells, however, their formation, and biological pathways in which they are involved remain incompletely understood.
The biological study of exosome function and trafficking requires the isolation of intact exosomes, but the current methods used are tedious, non-specific, and difficult. The ExoFast™ Exosome Isolation Reagent from plasma provides a simple and reliable method of concentrating intact exosomes from human and mouse plasma samples. By tying up water molecules, the ExoFast™ Exosome Isolation Reagent forces less-soluble components (i.e. exosomes) out of solution, allowing them to be collected after brief, low-speed centrifugation.

Prepare Sample

1. Remove the plasma sample from storage and place it on ice. If the sample is frozen, thaw the sample in a 25°C water bath until it is completely liquid, and place on ice until needed.
2. Centrifuge the plasma sample at 3000 × g for 15 minutes at room temperature 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 10,000 × g for 20 minutes at room temperature 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 with proteinase treatment

Note: Proteinase K treatment is recommended to remove the bulk of protein from plasma, but it may result in partial degradation of proteins exposed on the surface of the exosomes. If this is of concern to your study, proceed to “Isolate Exosomes without proteinase treatment.”

1. Transfer the required volume of clarified plasma to a new tube and add 0.5 volumes of 1× PBS.
2. Mix the sample thoroughly by vortexing.
3. Add 0.05 volumes of Proteinase K (20 mg/mL) to the sample. For example, for 100 µL starting volume of plasma, add 5 µL of Proteinase K (20 mg/mL).
4. Vortex the sample and then incubate the tube at 37°C for 10 minutes.
5. Add 0.2 volume (i.e. Total volume = plasma + PBS) of the ExoFast™ Exosome Isolation Reagent to the sample.

Plasma + PBS	Reagent
100 µL + 50 µL	30 µL
1 mL + 0.5 mL	300 µL

6. Mix the plasma/reagent mixture well by inverting or vortexing until there is a homogenous solution, and incubate at 2~8°C for 30 min.
7. After incubation, centrifuge the samples at 10,000 × g for 5 min at room temperature.
   Note: For mouse plasma, centrifuge at 10,000 × g for 30 min at 4°C.
8. Aspirate and discard the supernatant. Exosomes are contained in a beige or white pellet at the bottom of the tube.
9. Resuspend the pellet in a convenient volume of 1X PBS or similar buffer.
10. Once the pellet is resuspended, the exosomes are ready for downstream analysis or further purification through affinity methods.
    Keep isolated exosomes at 2~8°C for up to 1 week, or at -20°C for long-term storage.

Isolate Exosomes without proteinase treatment

Note: Proteinase K treatment is recommended to remove the bulk of protein from plasma, but it may result in partial degradation of proteins exposed on the surface of the exosomes. If this is of concern to your study, proceed to “Isolate Exosomes without proteinase treatment.”

1. Transfer the required volume of clarified plasma to a new tube and add 0.5 volumes of 1× PBS.
2. Mix the sample thoroughly by vortexing.
3. Add 0.2 volume (i.e. Total volume = plasma + PBS) of the ExoFast™ Exosome Isolation Reagent to the sample.

Plasma + PBS	Reagent
100 µL + 50 µL	30 µL
1 mL + 0.5 mL	300 µL

4. Mix the plasma/reagent mixture well by inverting or vortexing until there is a homogenous solution, and incubate at room temperature for 10 min.
5. After incubation, centrifuge the samples at 10,000 × g for 5 min at room temperature.
   Note: For mouse plasma, centrifuge at 10,000 × g for 30 min at 4°C.
6. Aspirate and discard the supernatant. Exosomes are contained in a beige or white pellet at the bottom of the tube.
7. Resuspend the pellet in a convenient volume of 1X PBS or similar buffer.
8. Once the pellet is resuspended, the exosomes are ready for downstream analysis or further purification through affinity methods.
   Keep isolated exosomes at 2~8°C for up to 1 week, or at -20°C for long-term storage.