

Total Exosome Isolation (from plasma)

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Store at 2°C to 8°C

Product Description

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 including blood, saliva, urine, and breast milk. Exosomes are thought 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 incompletely understood.

The biological study of exosome function and trafficking requires the isolation of intact exosomes, but the current methods used are tedious and difficult. The Total Exosome Isolation (from plasma) reagent provides a simple and reliable method of concentrating intact exosomes from human and mouse plasma samples. By tying up water molecules, the Total Exosome Isolation (from plasma) reagent forces less-soluble components (i.e. exosomes) out of solution, allowing them to be collected with a brief, low-speed centrifugation.

Product Contents

The Total Exosome Isolation (from plasma) bottle contains sufficient reagent for processing up to 20 mL of plasma.

Component	Amount	Storage
Exosome Precipitation Reagent (from plasma)	6 mL	2°C to 8°C
Proteinase K	1.25 mL	-20°C

General Guidelines

- The Total Exosome Isolation (from plasma) reagent is not recommended for isolation of exosomes from any other body fluids or cell culture media. Specialized Total Exosome Isolation reagents are available for serum, urine, cell culture media, and other body fluids (cerebrospinal fluid, saliva, amniotic fluid, ascitic fluid, and milk), each optimized for its specific type of biological sample.
- 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.
- After exosomes are isolated, total RNA and protein can be extracted using the Total Exosome RNA and Protein Isolation Kit.

Prepare Sample

- 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 liquid, and place on ice until needed.
- Centrifuge the plasma sample at 2000 × g for 20 minutes at room temperature to remove cells and debris.
- Transfer the supernatant containing the partially clarified plasma to a new tube without disturbing the pellet.
- Centrifuge the new tube at 10,000 × g for 20 minutes at room temperature to remove debris.
- 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.
- Proceed to “Isolate Exosomes (with proteinase treatment),” or “Isolate Exosomes (without proteinase treatment).”

Isolate Exosomes (with proteinase treatment)

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).”

- Transfer the required volume of clarified plasma to a new tube and add 0.5 volumes of 1X PBS.
- Mix the sample thoroughly by vortexing.
- Add 0.05 volumes of Proteinase K to the sample. For example, for 100 µL starting volume of plasma, add 5 µL of Proteinase K.
- Vortex the sample and then incubate the tube at 37°C for 10 minutes.
- Add 0.2 volume (i.e. Total volume = plasma + PBS) of the Exosome Precipitation Reagent (from plasma) to the sample.

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

- Mix the Proteinase K -treated plasma/reagent mixture well either by vortexing or inversion until the solution is homogenous.
Note: The solution should have a cloudy appearance.
- Incubate the sample at 2°C to 8°C for 30 minutes.
- After incubation, centrifuge the sample at 10,000 × g for 5 minutes at room temperature.
Note: For mouse plasma, centrifuge for 30 minutes at 4°C.
- Aspirate the supernatant by pipetting and discard. Exosomes are contained in a pellet at the bottom of the tube.
- (Optional) Centrifuge the tube for 30 seconds at 10,000 × g to collect any residual reagent.
- Discard any residual supernatant by careful aspiration with a pipet and proceed to “Resuspend Exosomes.”

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Isolate Exosomes (without proteinase treatment)

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

Plasma + PBS	Reagent
100 μ L + 50 μ L	30 μ L
1 mL + 0.5 mL	300 μ L

4. Mix the plasma/reagent mixture well either by vortexing or inversion until the solution is homogenous.
Note: The solution should have a cloudy appearance.
5. Incubate the sample at room temperature for 10 minutes.
6. After incubation, centrifuge the sample at 10,000 \times g for 5 minutes at room temperature.
7. Aspirate the supernatant by pipetting and discard.
Note: Exosomes are contained in a pellet at the bottom of the tube.
8. (*Optional*) Centrifuge the tube for 30 seconds at 10,000 \times g to collect any residual reagent.
9. Discard any residual supernatant by careful aspiration with a pipet and proceed to "Resuspend Exosomes."

Resuspend Exosomes

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

Starting Plasma Volume	Resuspension Volume
100 μ L	25–50 μ L
1 mL	100–500 μ L

2. Once the pellet is resuspended, the exosomes are ready for downstream analysis or further purification through affinity methods.
Keep isolated exosomes at 2°C to 8°C for up to 1 week, or at –20°C or colder for long-term storage.

Related Products

Product	Cat. no.
Total Exosome RNA and Protein Isolation Kit	4478545
Total Exosome Isolation (from serum)	4478360
Total Exosome Isolation (from cell culture media)	4478359
Total Exosome Isolation (from other body fluids)	4484453
Total Exosome Isolation (from urine)	4484452
Exosome- Human CD63 Isolation/Detection (from cell culture media)	10606D
Exosome- Streptavidin Isolation/Detection	10608D
Exosome Spin Columns (MW3000)	4484449
Exosome Immunoprecipitation (Protein A)	10610D
Exosome Immunoprecipitation (Protein G)	10612D
Nuclease-Free Water (not DEPC-Treated) (1 x 100 mL)	AM9938
PBS - Phosphate-Buffered Saline 10X, pH 7.4	AM9624
Non-Stick RNase-free Microfuge Tubes (2.0 ml)	AM12475
Non-Stick RNase-free Microfuge Tubes (1.5 ml)	AM12450

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