

Total Exosome Isolation (from other body fluids)

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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 other body fluids) reagent provides a simple and reliable method of concentrating intact exosomes from cerebrospinal fluid (CSF), ascitic fluid, amniotic fluid, milk, and saliva. By tying up water molecules, the Total Exosome Isolation (from other body fluids) 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 other body fluids) bottle contains sufficient reagent for processing up to 6 mL CSF, 12 mL ascitic fluid, 30 mL amniotic fluid, 6 mL milk, or 12 mL saliva.

Component	Amount	Storage
Total Exosome Isolation (from other body fluids)	6 mL	2°C to 8°C

General Guidelines

- The Total Exosome Isolation (from other body fluids) reagent is not recommended for isolation of exosomes from plasma, serum, urine, or cell culture media. Specialized Total Exosome Isolation reagents are available for these sample types, each optimized for its specific type of biological sample.
- 0.2–1 mL of each body fluid typically provides enough exosomes for all standard types of analysis.
- For exosome isolation from larger starting volumes (>1 mL), we recommend extending or optimizing the 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 body fluid 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.

Note: A significant amount of volume is lost from breast milk samples during centrifugation. Take the lost volume into account when deciding on initial sample volumes.

- Centrifuge the body fluid sample at 2000 × g to remove cells and debris as indicated in the following table:

Body fluid	Temperature and time
CSF	4°C, 30 min
Ascitic fluid	Room temp, 30 min
Amniotic fluid	4°C, 30 min
Milk	Room temp, 10 min
Saliva	Room temp, 10 min

- Transfer the supernatant containing the clarified or partially clarified fluid to a new tube without disturbing the pellet.
 - For ascitic fluid and saliva, proceed to “Isolate Exosomes”.
 - For CSF, amniotic fluid, and milk, proceed to step 4.
- Centrifuge CSF, amniotic fluid, and milk samples at 10,000 × g as indicated in the following table:

Body fluid	Temperature and time	
	2 nd Spin	3 rd Spin
CSF	4°C, 30 min	—
Amniotic fluid	4°C, 30 min	—
Milk*	Room temp, 30 min	Room temp, 10 min

* When transferring supernatant from milk samples, move the middle layer to a new tube. Avoid disturbing the upper layer and lower pellet, which contain contaminating material that can reduce/compromise the quality of the exosome preparation.

- Transfer the supernatant containing the clarified body fluid to a new tube without disturbing the pellet and proceed to “Isolate Exosomes”.

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Isolate Exosomes

- Transfer the required volume of clarified body fluid to a new tube and add the volume of Total Exosome Isolation (from other body fluids) reagent, as in the following table:

Body fluid	Reagent
CSF	1 vol
Ascitic fluid	0.5 vol
Amniotic fluid	0.2 vol
Milk*	0.5 vol
Saliva	0.5 vol

*Add 1 volume of 1X PBS to clarified milk samples and mix prior to adding Total Exosome Isolation (from other body fluids) reagent. The reagent volume is based on the total volume of milk plus PBS. For example, if you are isolating exosomes from 200 μ L of milk, add 200 μ L of 1X PBS and 200 μ L of reagent (0.5 volumes of 400 μ L).

- Mix the body fluid/reagent mixture by inverting the tube, vortexing, or pipetting up and down until the solution is homogenous.
- Incubate the sample according to the type of body fluid as indicated in the following table:

Body fluid	Temperature and time
CSF	2°C to 8°C, 1h
Ascitic fluid	Room temp, 30 min
Amniotic fluid	Room temp, 30 min
Milk	Room temp, 30 min
Saliva	2°C to 8°C, 1h

- After incubation, centrifuge the sample at 10,000 \times g according to the type of body fluid as indicated in the following table:

Body fluid	Temperature and time
CSF	2°C to 8°C, 1h
Ascitic fluid	Room temp, 10 min
Amniotic fluid	2°C to 8°C, 1h
Milk	Room temp, 10 min
Saliva	2°C to 8°C, 1h

- Aspirate the supernatant by pipetting and discard. Exosomes are contained in a pellet (which may not be visible) at the bottom of the tube.
- (Optional) Centrifuge the tube at room temperature for 5 minutes at 10,000 \times g to collect any residual reagent.
- Discard any residual supernatant by careful aspiration with a pipet and proceed to "Resuspend Exosomes."

Resuspend Exosomes

- Add 1X PBS or similar buffer to the pellet. Examples of typical volumes are shown in the following table:

Body fluid	Starting Volume (after clarification)	Resuspension Volume
CSF	700 μ L	50–75 μ L
Ascitic fluid	200 μ L	25–50 μ L
Amniotic fluid	200 μ L	25–50 μ L
Milk	200 μ L	25–50 μ L
Saliva	500 μ L	25–50 μ L

- Vortex or pipet up and down to resuspend the exosomes.
 - For ascitic fluid, amniotic fluid, and saliva samples, proceed to step 5.
 - For CSF and milk samples, proceed to step 3.
- Centrifuge resuspended exosomes at 10,000 \times g for 5 minutes at room temperature.
- Transfer the supernatant to a new tube. Avoid disturbing the pellet which contains non-organic particulate matter.
- Isolated 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 urine)	4484452
Total Exosome Isolation (from plasma)	4484450
Exosome- Human CD63 Isolation/Detection (from cell culture media)	10606D
Exosome Spin Columns (MW3000)	4484449
Exosome- Streptavidin Isolation/Detection	10608D
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
Conical Tubes (50 ml) (Racked)	AM12501
Conical Tubes (15 ml) (Racked)	AM12500

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