



ExoFast™ Exosome Isolation Reagent from other body fluids

Catalog Number: D034

Content and Storage

Component	Amount	Shipping Condition	Storage Condition
ExoFast™ Exosome Isolation Reagent from other body fluids	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 other body fluids 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 ExoFast™ Exosome Isolation Reagent forces less-soluble components (i.e. exosomes) out of solution, allowing them to be collected after brief, low-speed centrifugation.

Protocol

1. Remove the body fluid sample from storage and place it 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.
2. 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	RT, 30 min
Amniotic fluid	4°C, 30 min
Milk	RT, 10 min
Saliva	RT, 10 min

3. 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 step 6.
For CSF, amniotic fluid, and milk, proceed to step 4.
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*	RT, 30 min	RT, 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.
- Add the volume of the ExoFast™ Exosome Isolation Reagent to the clarified body fluid, 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 1× PBS to clarified milk samples and mix prior to adding ExoFast™ Exosome Isolation Reagent. The reagent volume is based on the total volume of milk plus PBS. For example, if you are isolating exosomes from 200 µL of milk, add 200 µL of 1× PBS and 200 µL of reagent (0.5 volumes of 400 µL).

- Mix the body fluid/reagent mixture well by inverting or vortexing until there is a homogenous solution.
- Incubate the sample according to the type of body fluid as indicated in the following table:

Body fluid	Temperature and time
CSF	2–8°C, 1 h
Ascitic fluid	RT, 30 min
Amniotic fluid	RT, 30 min
Milk	RT, 30 min
Saliva	2–8°C, 1 h

9. After incubation, centrifuge the sample at 10,000 × g according to the type of body fluid as indicated in the following table:

Body fluid	Temperature and time
CSF	2–8°C, 1 h
Ascitic fluid	RT, 10 min
Amniotic fluid	2–8°C, 1 h
Milk	RT, 10 min
Saliva	2–8°C, 1 h

10. Aspirate and discard the supernatant. Exosomes are contained in a beige or white pellet at the bottom of the tube (which may not be visible).
11. (Optional) Centrifuge the sample at 10,000 × g for 5 minutes to collect any residual reagent.
12. Discard any residual supernatant by careful aspiration with a pipette.
13. Add a convenient volume of 1× PBS or similar buffer to the pellet.
14. Vortex or pipet up and down to resuspend the exosomes.
For ascitic fluid, amniotic fluid, and saliva samples, proceed to step 17.
For CSF and milk samples, proceed to step 15.
15. Centrifuge resuspended exosomes at 10,000 × g for 5 minutes at room temperature.
16. Transfer the supernatant to a new tube. Avoid disturbing the pellet which contains non-organic particulate matter.
17. Isolated 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.