DiagExo® Human Body Fluid Exosome Isolation kit

Cat. #: P121

Storage: keep all bottles upright, in cool and dark place. **Shelf Life:** 12 months

Application: For isolating exosome from, Cerebrospinal fluid (CSF), Amniotic fluid, Inflammatory fluid, Lymph fluid, Breast milk, Saliva, Gastrointestinal fluid (GI), and Broncho alveolar lavage fluid.

Product Size: 20 reactions (0.1~2 mL body fluid / reaction). The yield of exosome varies depending on the sample type.

Product Description (This product is for research use only.)

Use 0.5 - 2 mL human body fluid to achieve high yield of exosomes for any downstream applications: EM study, exosome labeling, exosome subpopulation, qRT-PCR profiling of exosomal miRNA, ELISA and gel analysis of exosomal proteins.

Product Contents

Component	Amount	Storage
Solution A (orange)	2 mL	room temperature
Solution B	2 mL	room temperature
Solution C *	8 mL	room temperature
DiagExo® Exosome Columns	20 x (1.5 mL)	room temperature

* *Cap the Solution C bottle immediately after each use.*

Reaction Volume Table (Important):

Solution A/B/C	Serum	CSF	Amniotic fluid	Inflamma-tory fluid	Lymph fluid	Breast milk	Saliva	GI fluid	Broncho alveolar lavage fluid
Solution A 20 µl Solution B 20 µl Solution C 80 µl	100 µl	100 µl	100 µl	100 µl	100 µl	200 µl	400 µl	400 µl	400 µl
Solution A 40 µl Solution B 40 µl Solution C 160 µl	200 µl	200 µl	200 µl	200 µl	200 µl	400 µl	800 µl	800 µl	800 µl
Solution A 60 µl Solution B 60 µl Solution C 240 µl	300 µl	300 µl	300 µl	300 µl	300 µl	600 µl	1.2 mL	1.2 mL	1.2 mL
Solution A 80 µl Solution B 80 µl Solution C 320 µl	400 µl	400 µl	400 µl	400 µl	400 µl	800 µl	1.6 mL	1.6 mL	1.6 mL
Solution A 100 µl Solution B 100 µl Solution C 400 µl	500 µl	500 µl	500 µl	500 µl	500 µl	1 mL	2 mL	2 mL	2 mL

- **Do not exceed** the suggested sample volume in the above table. Otherwise it will cause indistinct layer separation and column clogging.

Protocol (example for processing **100 µl of Cerebrospinal fluid** [CSF])

1 **Prepare Sample:** Collect suggested volume of specific body fluid (eg. 100 µl CSF) indicated in the above table. If start with frozen sample, thaw the sample completely at room temperature, and keep it on ice.

2 Centrifuge at **2,000× g** for **10 minutes** at room temperature to remove cells and debris.

- ❖ Important: skip this step may cause filter clog in step 14.

3 Without disturbing pellets, transfer clear supernatant to a new **glass tube 1** and keep it on ice.

4 Prepare mixture of A/B/C: In **glass tube 2**, add solutions in the **following order**:

1st Solution A (orange)	20 µl
2nd Solution B	20 µl
3rd Solution C *	80 µl

* *Cap the Solution C bottle immediately after each use.*

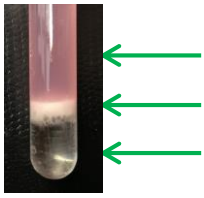
** *If other sample than "100 µl CSF" is processed, refer to the "Reaction Volume Table" for the proper volume of solution A, B, and C.*

5 **Vortex gently** tube 2 (mixture A/B/C) for **5 - 10 seconds** to obtain a homogenous solution;

6 Add **tube 2** (mixture A/B/C) to **tube 1** (Clear 100 µl CSF).

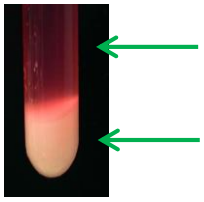
7 Tightly cap tube 1, vigorously vortex for **30 seconds**, then incubate at **4°C** for **1 hour**.

8a. The mixture now appear as 3 layers (as shown in the following figure):

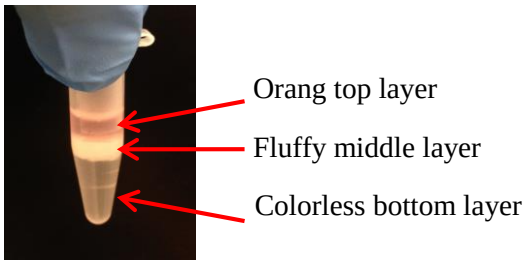


Carefully, aspirate the top layer using a pipette without disturbing the middle fluff layer and discard it. Go to step 9.

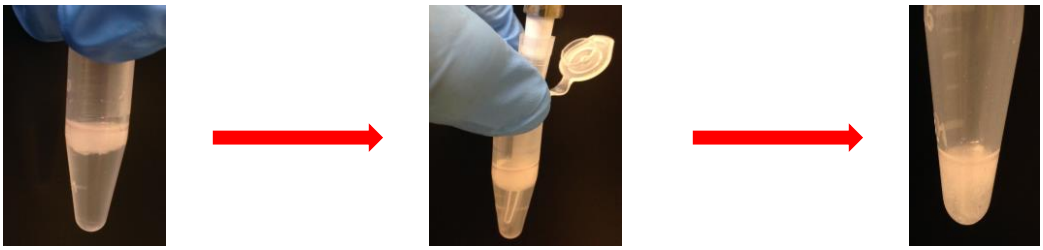
8b. Sometimes, only two layers (orange color top layer and cloudy bottom layer) are visible, remove and discard the top layer. Go to step 9.



9. Transfer the left over in the tube to a new Eppendorf tube and spin at **1,000x g** for **3 minutes**. A new three-layer separation will occur: top orange color layer, middle fluff layer and bottom colorless layer (as shown in the following figure). Proceed to next step within 10 seconds after centrifugation. (The layer separation becomes indistinct 60 seconds after centrifugation.)



10. Pipet out and discard the top layer. Insert pipette tip down to the tube bottom to remove the colorless bottom layer completely. Therefore only the "fluff" is left in the tube.



(Top layer removed) (To remove bottom layer) (Only "fluff" layer left in the tube)

11. **Repeat step 9 and step 10 once.**

12. Leave the Eppendorf tube cap open to **air dry for 5-10 minutes** at room temp. (Do not over dry).

13. Add **1× PBS** as much as 1-2 volumes of the collected fluff to the tube, and resuspsend the "fluff" by pipetting up and down **vigorously**.

14. Transfer the suspension carefully into **PureExo® Column** (provided) and spin the Column at **2,000× g** for **5** minutes to collect all the flow-through.

15. The "flow-through" is the isolated pure exosome (exosome suspended in PBS). Pipet up and down to resuspend the isolated pure exosome. Use it directly or store at 4°C for up to 1 week, or at ≤80°C for up to 3 months. Concentrated exosome will precipitate. Re-suspend well before each use.

Remark:

For human body fluid sample volume other than the suggested volume in the table, scale up or down the solution A, B and C **proportionally.**

The PH value in the body fluids will not affect our exosome isolation efficiency.

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