DiagExo® Human Body Fluid Exosome Isolation kit

Cat. #: P121 (10 reactions); P121L (40 reactions); P121S (2 reactions)

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

Application: This is 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 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. The yield of exosome varies depending on the sample type.

Product Contents (store in room temperature)

Component	Amount		
	Cat. #: **P121**	Cat. #: **P121L**	Cat. #: **P121S**
Solution A	3 mL	12 mL	0.6 mL
Solution B	3 mL	12 mL	0.6 mL
Solution C	3 mL	12 mL	0.6 mL
Sample Buffer	20 mL	80 mL	4 mL
PureExo® Column	10	40	2

* *Tightly cap all bottles immediately after each use to prevent evaporation.*

Do not process more than 2mL body fluid for each reaction. Otherwise it will cause indistinct layer separation and column clogging.

Protocol (we suggest to processing 0.5 – 1 mL body fluid)

1. Prepare Sample: **Collect 0.5 – 1 mL body fluid sample** and keep it on ice. If start with frozen sample, thaw the sample completely at room temperature, and keep on ice.

2. Centrifuge the body fluid sample at **3,000× g** for **15 minutes** at 4°C to remove cells and debris.

   Important: skipping this step may cause filter clogging in step 15.

3. Transfer **0.5 – 1mL** clear supernatant to a **15 mL centrifuge tube** without disturbing the pellet. Add **Sample Buffer** to the supernatant to make a total volume of **2 mL diluted body fluid sample**, and keep it on ice. (This dilution works well for all starting sample volume between 0.5 – 1 mL.)

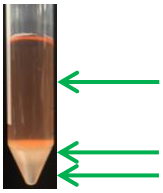
   Important: If start with 1 – 2 mL sample, add the **same volume of Sample Buffer** and keep it in ice. For example, if start with 1.2 mL saliva, add 1.2 mL Sample Buffer to dilute it. (Do not process more than 2 mL body fluid sample.)

4. In **another 1.5 mL microcentrifuge tube**, add Solution A/B/C in the following order to prepare 0.9 mL mixture A/B/C (always prepare mixture A/B/C right before use. The mixture A/B/C cannot be stored):

1st	add Solution A	0.3 mL
2nd	add Solution B	0.3 mL
3rd	add Solution C	0.3 mL

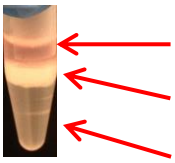
* *Cap all bottles well immediately after each use to prevent evaporation.*

5. **Vortex** the mixture A/B/C for **10 seconds** to obtain a homogenous solution.
6. Add the 0.9 mL mixture A/B/C to the diluted body fluid sample (from step 3).
7. Cap the 15 mL tube, **gently invert the tube for at least 10 times to mix well**, then incubate at **4°C** for **30 minutes**.
8. The mixture now appears as 3 layers:

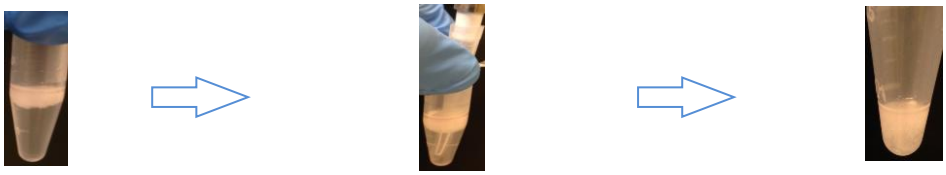


If the layer separation is not clear, centrifuge at 5,000 rpm for 3 minutes. Pipet out the Top transparent layer and discard it without disturbing the Middle fluffy layer.

9. Transfer the Middle fluffy layer (Exosome is in this layer) to **another 1.5 mL microcentrifuge tube**. Spin the tube at **5,000× g for 3 minutes**. A new three-layer separation will appear: Top transparent layer, Middle fluffy layer and Bottom colorless layer. (see figure below) Proceed to the next step immediately.



10. Pipet out the Top transparent layer and discard it. Insert pipette tip down to the tube bottom to completely remove the Bottom colorless layer. Only keep the Middle fluffy layer in the tube. Exosome is in this layer.



Pipet out the Top transparent layer | Remove the Bottom colorless layer | **only keep the Middle fluffy layer**

11. Spin again at **5,000x g for 3 minutes,** and 3 layers will appear again. Now, repeat **step 10** for one more time. Now only the **"fluff pellet"** left in the tube. The **"fluff pellet"** volume is about 25 µL in this example experiment.
12. Leave the tube cap open to **air dry** for 5-10 minutes at room temp (do not over dry).
13. Add **1× PBS** equal to **4 times volumes** of the collected fluff pellet to the tube. In this example experiment, we added 100 µL PBS (4 x 25 µL fluff pellet). Resuspend the fluff pellet by pipetting up and down **vigorously for 40 times**.

14. Shake the tube on a horizontal shaker at high speed for **3 minutes**, then **pipet up and down vigorously** for 10 times. Repeat this "shake-pipet up and down" for another 2 times.

    Note: This step is important. If the fluff pellet is not well re-suspended, the exosome may be trapped in the fluff pellet resulting in low exosome purity and yield. For some types of samples, it is difficult to dissociate the fluff pellet to release the exosome. In such case, extend the pipetting and shaking time in step 13 and 14.

15. Spin the tube at **5,000x g** for **5 minutes**. Without disturbing the "fluff pellet", transfer the **supernatant** carefully into one **PureExo® Column** (provided).

    Note: Keep the "fluff pellet" at 4°C. Do not discard it until the experiment is done. See "Trouble shooting" 2.2 for detail.

16. Spin the PureExo® Column at **1,000× g** for **5 minutes** to collect the "flow-through".

17. The "flow-through" is the **isolated pure exosome** (exosome suspended in PBS). The whole protocol is **completed** here. Use it directly for downstream assays (e.g. use 101Bio *Exosomal RNA and Protein Extraction Kit, Cat.#: P200,* to extract exosomal RNA/Protein), or store at 4°C for up to 1 week , or at –80°C for up to 3 months. Concentrated exosome will precipitate after sitting. Pipet up and down to resuspend it well before each use.

Trouble shouting

1. When there are only 2 layers (top pink/transparent layer and fluffy layer at the bottom) but not 3 layers as expected after step 7 (as shown in the picture at step 8).

Pipet out and discard the top pink/transparent layer, then go to step 9 and proceed.

For some samples, layer separation is not sharp. You can vaguely see 3 layers: Top cloudy layer (aqueous layer), middle fluffy layer (thicker and less transparent than top layer) and bottom colorless layer. Carefully remove the top layer and discard it. Because the separation is not sharp, be careful, not to disturb or remove the middle fluffy layer because exosome is in this layer.

Prepare another 0.9 mL mixture A/B/C as described in step 4 and 5, and add it to the tube containing the middle fluffy layer and bottom colorless layer. Gently invert the tube for at least 10 times to mix well. Incubate at 4°C for another 30 minutes. Spin the tube at 5,000× g for 3 minutes. Now the mixture appears as 3 layers as shown in step 8. Then Pipet out the Top transparent layer and discard it without disturbing the Middle fluffy layer and proceed to step 9.

* Extra Solution A, B and C can be purchased separately from 101Bio.com (Cat.#: P121H).

2. The final exosome yield is low.

2.1. Check if there are left over liquid in the column. If yes, it indicates the column is clogged by contaminated protein. Several reasons could cause the clogging, such as debris was not removed completely in step 2; too much lipid protein in the sample; some precipitation was pipet up in step 15; too much sample was loaded, etc. If this clogging happens, prepare the sample again, input lower amount of sample, and pay more attention in step 2 and 15.

2.2. For some types of samples, it is difficult to re-suspend the fluff (in step 13 and 14), and the exosome may be trapped in the fluff. Add the final flow-through back to the fluff pellet stored in 4°C (in step 15, a lot of exosomes are trapped in the fluff), pipet up and down **vigorously for 60 times**, and shake the tube on a horizontal shaker for **20**

minutes. **Repeat pipetting** up and down vigorously a few times during the shaking. Go through another column to collect the exosomes.

2.3. For some sample type, the content of exosome is low (the Middle fluffy layer is thin in step 8). Increase the initial input sample volume to collect more exosome.

3. **The flow through has multiple layers.**

There was bottom and/or top layer left in the fluff during step 9~11. Spin the tube at **5,000× g** for **3 minutes**, and carefully pipet out the bottom layer. Pass the sample through a new PureExo® column to collect the flow-though.

4. **Exosome yield is good, but exosomal protein level is low.**

Exosome membrane is more difficult to be lysed than cells. Normal lysis buffer for cells, such as RIPA, is not strong enough to completely lysis exosome to release exosomal protein. We suggest to use our Exosomal RNA and Protein Extraction Kit (Cat.#: P200) to extract exosomal protein.

5. **Exosome yield is good, but exosomal RNA level is low.**

5.1. RNA degradation. Please check the working environment for RNase free. Also can add spike-in RNA to isolated exosome and then do RNA isolation to control the RNA extraction procedure.

5.2. We suggest to use our P200 kit to extract exosomal RNA.

6. **Exosomal RNA yield is good, but cannot get RT-PCR amplification.**

6.1. Please check internal control /spike-in control amplification.

6.2. Please check the primer sensitivity.

Customer also buy:

Exosome	Cat. #	101Bio.com exosome purity	other vendors exosome purity
Exosome Isolation Kit - cell media / serum	P100 / P101	95%	25% ~ 30%
Exosomal RNA / Protein Extraction Kit	P200		
Exosomal DNA Extraction Kit	P230	Unique	
Exosome-TEM-easy Kit	P130	Unique	