PureExo® Exosome Isolation kit (for cell culture media)

Cat. #: P100S (2 reactions); P100 (10 reactions); P100L (40 reactions)

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

Product Size: Each reaction can process 2 ~ 4 ml cell culture medium. The yield of each reaction can yield pure exosome suspended in 50 ~ 200 µl PBS (from which 150 ~ 400 µg exosomal protein or 50 ~ 200 ng exosomal RNA can be extracted using our *Exosomal RNA and Protein Extraction Kit, Cat.#: P200*).

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

This kit can isolate / purify pure exosome at high yield from cell culture media.

- ✓ Easy to use: No ultra-centrifugation (< 2 hours)
- ✓ 10 fold higher yield (vs. other kits or ultracentrifuge method)
- ✓ Save cost (vs. antibody-bead method)
- ✓ Isolate pure exosome (exosome purity > 95%)
- ✓ Intact exosome (good morphology)

Use as little as 2 ml cell media to isolate high yield of exosomes for any downstream applications: EM study, exosome label, exosome subpopulation, qRT-PCR profiling of exosomal miRNAs, and gel analysis of exosomal proteins.

Product Components (store at room temperature)

Component	Amount		
	Cat. #: **P100S**	Cat. #: **P100**	Cat. #: **P100L**
Solution A (blue)	0.5 ml	2.5 ml	10 ml
Solution B	0.5 ml	2.5 ml	10 ml
Solution C	2.0 ml	10 ml	10 ml x 4
PureExo® Column	2	10	40

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

Protocol (example of processing **2 ml** cell culture medium)

Sample Preparation:

- Fetal bovine serum (FBS), even the so-called "exosome-free FBS" contains high level exosomes which will contaminate the cell derived exosomes. Use serum-free media to starve the cells for 48 hours before media harvest.
- If the cultured cells are highly proliferative cell, such as tumor cells, dilute the cell culture medium by 1:2 first.
- If the culture medium is from bioreactor system, dilute the medium to no more than 2 x 10^5 cells / 2 ml.

1. Collect **2 ml** cell culture medium.

2. Centrifuge the cell culture media at **3,000× g** for **15 minutes at 4°C** to remove cells and debris.

❖Important: skip this step may cause filter clog in step 15.

3. Transfer 2 ml clear supernatant (cell-free culture media) to a new **glass tube 1** and keep it on ice.

4. In **glass tube 2**, add Solution A/B/C in the following order to prepare 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: 0.125 ml; 2nd add Solution B: 0.125 ml; 3rd add Solution C: 0.5 ml

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

When process different volume of cell medium, please refer to "Reaction Volume Table" below for solution A/B/C recipe.

Reaction Volume Table (per reaction)

Cell culture media (clear supernatant)	Mixture A/B/C =	Solution A +	Solution B +	Solution C
2 ml (minimum)	0.750 ml =	0.125 ml +	0.125 ml +	0.50 ml
3 ml	1.125 ml =	0.187 ml +	0.187 ml +	0.75 ml
4 ml (maximum)	1.500 ml =	0.250 ml +	0.250 ml +	1.00 ml

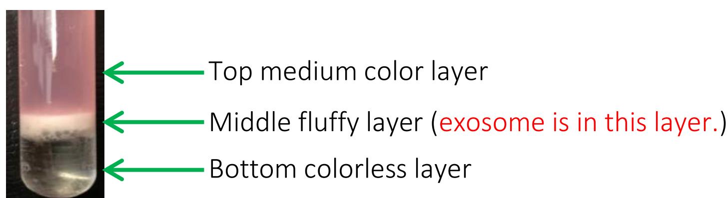
❖ The maximum medium volume of each reaction is **4 ml** from at most **5 x 10^5 cells**. One PureExo® Column can be used for only one reaction. **Do not exceed** the suggested sample volume or the cell number. Otherwise it may cause indistinct layer separation and column clogging.

5. **Vortex** the tube 2 (0.75 ml mixture A/B/C) for **10 seconds** to obtain a homogenous solution.

6. Add the 0.75 ml mixture A/B/C from **tube 2** to **tube 1** (2 ml cell-free culture media).

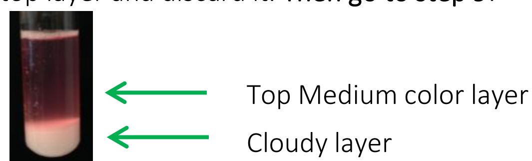
7. Tightly cap tube 1, **gently invert the tube for at least 10 times to mix well**, then incubate at **4°C** for **30 minutes**.

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



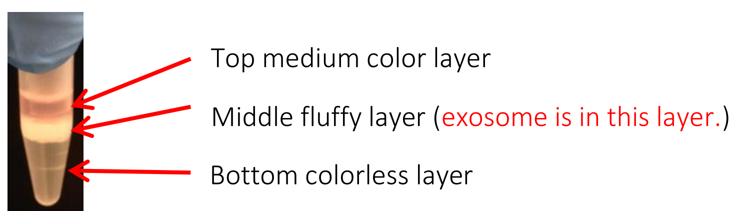
Without disturbing the Middle fluffy layer, carefully pipet out the Top medium color layer and discard it. **Then go to step 9.**

8b. When only two layers appear: Top medium color layer and white Cloudy layer, (as shown in the figure below).
Carefully remove the top layer and discard it. **Then go to step 9**.

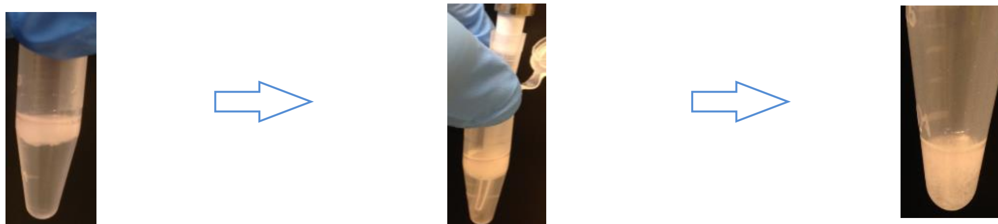


Notice: If layer separation are indistinct, add another 0.75 ml mixture A/B/C (0.75 ml is for this example experiment), **gently invert the tube for at least 10 times to mix well**, and incubate at 4°C for another 30 minutes. Then start again from step 8a.

9. Transfer the left over in the **glass tube 1** to a new **1.5 ml microcentrifuge tube** (not provided) and spin at **5,000x g** for **3 minutes**. A new three-layer separation will occur (Top medium color layer, Middle fluffy layer and Bottom colorless layer as shown in the figure below). Proceed to next step immediately.



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



Pipet out the Top medium color layer Remove the Bottom colorless layer **only keep the Middle fluffy layer**

11. Transfer the whole fluffy layer to a **new 0.5 ml microcentrifuge tube**. Spin at **5,000x g** for **3 minutes**, then repeat **step 10** for one time. Now only the **"fluff pellet"** is left in the tube, and its volume is about 25 µl in our case.

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

13. Add **1× PBS** equal to **4 times volumes** of the collected fluff pellet to the 0.5 ml tube. In our case, 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 0.5 ml tube on a horizontal shaker for **3 minutes** at high speed, then **pipet up and down vigorously** for 10 times. Repeat this "shake-pipet up and down" procedure 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 exosome. In such case, extend the pipetting and shaking time in step 13 and 14.

15. Spin the 0.5 ml tube at **5,000x g** for **5 minutes**. Transfer the **supernatant** carefully into one **PureExo® Column** (provided). Do not disturb the fluff pellet.
    Note: Keep the fluff pellet at 4°C. Do not discard it until the experiment is finished. See Trouble shooting 1.2 for detail.

16. Spin the Column at **1,000× g** for **5 minutes** to collect all the "flow-through", which is the **isolated pure exosome** (exosome suspended in PBS). Use the isolated exosome directly for downstream applications (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 store 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.

    Now we have easy version of P100, 3 steps, lower price and the same 95% purity. P100EZ link

Trouble Shouting:

1. **The final exosome yield is low.**

   1.1. Check if there is left over liquid in the column. If yes, it indicates that the column is clogged by contaminated protein. Several factors may cause the clogging: 1) cell debris is not removed completely in step 2; 2) serum was added in the medium; 3) some fluff pellet was incidentally pipetted up in step 15; 4) Over 4 mL cell medium sample is loaded in step 1. When clogging happens, you need to prepare the sample and start over again.

   1.2. For some type of sample, the fluff (in step 13) is very difficult to be resuspended, and the exosome may be trapped in the fluff. This can be examined by check exosome marker level in step 14 pellet and the final exosome flow-through using ELISA. If the signal from step 14 pellet is high, the exosome release step is incomplete.

   Add the final flow-through back to the fluff pellet stored in 4°C (in step 14), pipet up and down **vigorously 60 times**, and shake the tube on a horizontal shaker for **20 minutes**. **Repeat pipetting** up and down vigorously a few times in the middle. Go through another column to collect the exosomes.

   1.3. For some cell type, the production of exosome is low. Generally, the cells produce more exosomes when they are in fast proliferating phase. Tune the cell culture condition (seeding density, splitting intervals etc.) to achieve optimal cell growth condition to collect more exosome. Also increase the initial input medium volume to collect more exosome.

2. **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 top and bottom layer. Pass the sample through a new column to collect the flow-though.

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

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

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

   4.1. 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.

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

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

   5.1. Please check internal control amplification.

   5.2. Please check the primer sensitivity.