PureExo® Exosome Isolation kit (for cell culture media)

Cat. #: P100

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

Product Size: 10 reactions (2 mL~4 mL medium / reaction). The yield of each reaction is 50~200 µL exosome, from which 150~400 µg exosomal protein or 50 ~ 200 ng exosomal RNA can be extracted.

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

P100 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 and ultracentrifuge)
- ✓ **Save** cost (vs. antibodies-beads method)
- ✓ Isolate **Pure** exosome (>95%)
- ✓ **Intact** exosome (good morphology)
- ✓ Use as little as **2 mL** cell media to achieve 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 Contents

Component	Amount	Storage
Solution A (**blue**)	2.5 mL	Room temperature
Solution B	2.5 mL	Room temperature
Solution C *	10 mL	Room temperature
PureExo® Column	10 x (1.5 mL)	Room temperature

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

Reaction Volume Table (important)

Cell culture media	Mixture A/B/C =	Solution A	+	Solution B	+	Solution C
2 mL (min / Rxn)	0.75 mL	= 0.125 mL	+	0.125 mL	+	0.5 mL
3 mL	1.125 mL	= 0.187 mL	+	0.187 mL	+	0.75 mL
4 mL (max / Rxn)	1.5 mL	= 0.25 mL	+	0.25 mL	+	1 mL

- ❖ The maximum medium volume of each reaction is **4 mL** from at most **5 x 10^5 cells**. One PureExo® Column is only for reaction. **Do not exceed** the suggested sample volume or the cell number. Otherwise it will cause indistinct layer separation and column clogging.
- ❖ If the cultured cells are highly proliferative cell, such as **tumor cells** or **stem cells**, reduce loading amount of cell culture medium by half to process.

Protocol (example of processing **2 mL** medium)

Sample Preparation: Normal fetal bovine serum (FBS) contains high level exosomes which may contaminate the cell derived exosomes. Use serum-free media to starve the cells for 48 hours before media harvest.

1. Collect **2 mL** cell culture medium. If the culture medium is from Bioreactor system, dilute the medium to less than 2 x 10^5 cells / 2 mL.

2. Centrifuge the cell media at **2,000× g** for **10 minutes at 4°C** to remove cells or debris.

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

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

4. In **glass tube 2**, add the solutions in the following order to prepare mixture A/B/C:

1st	Solution A (**blue**)	0.125 mL
2nd	Solution B	0.125 mL
3rd	Solution C *	0.5 mL

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

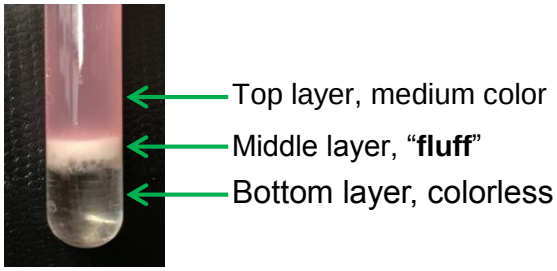
   ** *If more than 2 mL medium is processed, please refer to "Reaction Volume Table" for the recipe of solution A/B/C.*

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

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

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

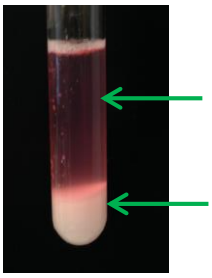
8a. The mixture now appear as 3 layers (as shown in figure):



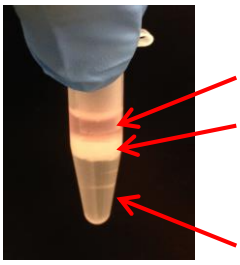
Carefully aspirate the top layer using a pipette without disturbing the middle fluff layer and discard it, then go to step 9.

8b. Sometimes, only two layers (medium color layer and white cloudy / fluffy layer) are visible (as shown in the following figure).

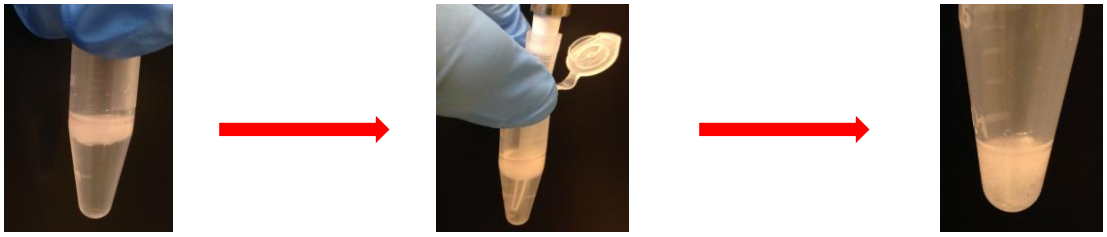
Remove and discard the top layer, then go to step 9.



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 medium 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" 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 resuspend 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.

-- The end --