PureExo® Exosome Isolation kit (for serum or plasma)

Cat. #: P101

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

Product Size: 10 reactions (100~600 μL serum / reaction). The yield of each reaction is 100~200 μL exosome, from which 300~400 μg exosomal protein or 200~300 ng exosomal RNA can be extracted.

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

P101 kit can isolate / purify pure exosome at high yield from serum or plasma.

- ✓ **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 100 μL serum 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

This kit contains reagents sufficient for processing 6 mL of serum.

Component	Amount	Storage
Solution A (orange)	1 mL	Room temperature
Solution B	1 mL	Room temperature
Solution C *	4 mL	Room temperature
PureExo® Column	10 x (1.5 mL)	Room temperature

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

Reaction volumes table (Important)

Serum	Mixture A/B/C	=	Solution A	+	Solution B	+	Solution C
100 µl	**100 µl**	=	17 µl		17 µl		66 µl
200 µl	**200 µl**	=	33 µl		33 µl		134 µl
300 µl	**300 µl**	=	50 µl		50 µl		200 µl
400 µl	**400 µl**	=	67 µl		67 µl		266 µl
500 µl	**500 µl**	=	83 µl		83 µl		334 µl
600 µl	**600 µl**	=	100 µl		100 µl		400 µl

❖ Suggested starting volume: 100 µl serum. One PureExo® Columns is only for one reaction. **Do not process more than 600 µl serum** per reaction. Otherwise it will cause indistinct layer separation and column clogging.

Protocol (example for processing **100 µl** serum)

1. Collect the serum sample and keep it on ice. Or thaw the frozen sample completely at room temperature, and keep it on ice.
2. Centrifuge the serum sample at **2,000× g** for **10 minutes** at **4ºC** to remove debris.
   - ❖ Important: skip this step may cause filter clog in step 14.
3. Transfer **100 µl** clear supernatant to **glass tube 1** without disturbing the pellet, and keep it on ice.
4. In **glass tube 2**, add the solutions in the following order to prepare mixture A/B/C:

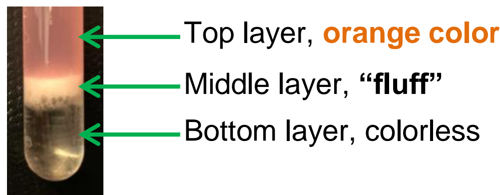
1st	Solution A (**orange**)	17 µl
2nd	Solution B	17 µl
3rd	Solution C *	66 µl

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

*** If more than 100 µl serum is processed, please refer to "Reaction Volume Table" for the recipe of solution A/B/C.*

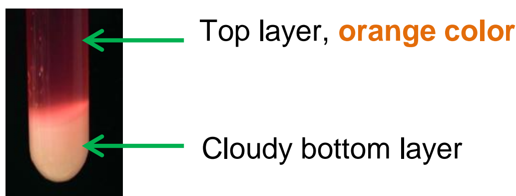
5. **Vortex** the tube 2 (mixture A/B/C) for **5-10 seconds** to obtain a homogenous solution.
6. Add **tube 2** (100 µl mixture A/B/C) to **tube 1** (100 µl serum).
7. Tightly cap tube 1, vigorously vortex for **30 seconds**, then incubate at **4°C** for **1 hours**.

8a. The mixture now appear as 3 layers:



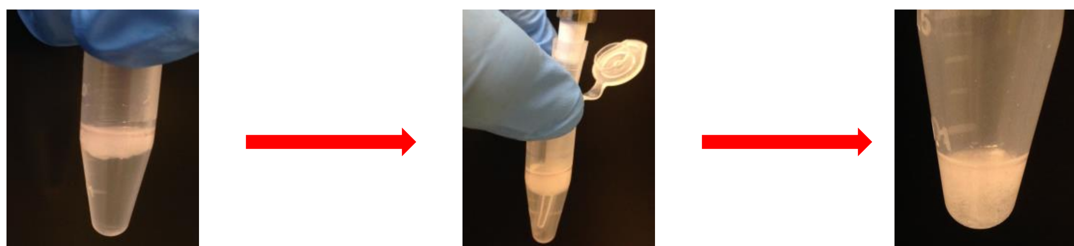
Aspirate the top layer using a pipette without disturbing the middle fluff layer and discard it. Go to step 9.

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 **1,000× g** for **3 minutes**. A new three-layer separation will appear: top orange color layer, middle fluff layer and bottom colorless layer. (See figure on next page for detail.) 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 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 –