DiagExo® Urinary Exosome Isolation kit

Cat. #: P120

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

Application: to isolate exosome from urine.

Product Size: 20 reactions (3 mL Urine / 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.)

P120 kit can isolate / purify pure exosome at high yield from urine.

- **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 **1 mL** urine 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)	5 mL	room temperature
Solution B	5 mL	room temperature
Solution C *	20 mL	room temperature
DiagExo® Column	20 x (1.5 mL)	room temperature

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

- **Reaction Volume Table** (important)

Volume of urine	Mixture A/B/C	=	Solution A	+	Solution B	+	Solution C
1 mL (min / Rxn)	0.5 mL	=	0.083 mL	+	0.083 mL	+	0.334 mL
2 mL	1.0 mL	=	0.166 mL	+	0.166 mL	+	0.668 mL
3 mL (max / Rxn)	1.5 mL	=	0.25 mL	+	0.25 mL	+	1.0 mL

❖ The suggested maximum urine volume of each reaction is 3 mL. One PureExo® Columns per reaction. **Do not process more than 3 mL** urine to avoid indistinct layer separation and column clogging.

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

1. **Sample prepare:** Centrifuge the **2 mL** urine sample 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.

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

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

1st	Solution A (**blue**)	0.166 mL
2nd	Solution B	0.166 mL
3rd	Solution C *	0.668 mL

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

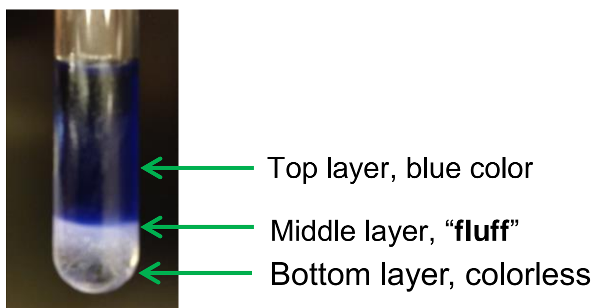
*** If other than 2 mL urine is processed, please refer to "Reaction Volume Table" for the recipe of solution A/B/C.*

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 1.0 mL) to **tube 1** (2 mL urine).

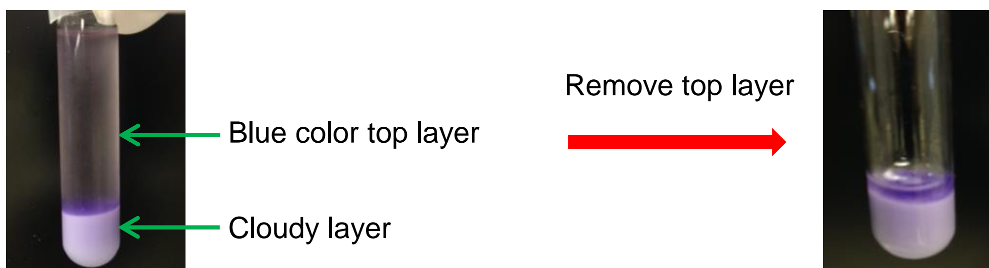
7. Tightly cap tube 1, vigorously vortex for **30 seconds**, then incubate at **4°C** for **1 hour.**

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

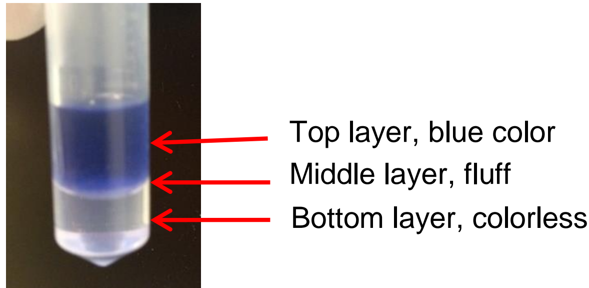


Carefully, aspirate the top layer using a pipette without disturbing the middle fluff layer and discard it. Then go to step 9.

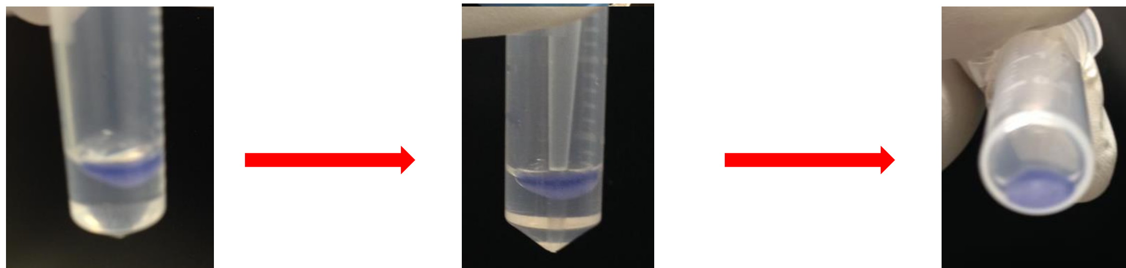
8b. Sometimes, only two layers (blue color top layer and white fluffy bottom layer) are visible (as shown in the following figure). Carefully remove / aspirate the top layer and discard it. Then go to step 9.



9. Transfer the left over in the tube to a new Eppendorf tube and spin at **1,000g ×** for **3 minutes**. A new three-layer separation will occur (top blue 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 "fluffy" or gel-like 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 min.** at room temp (do not over dry).

13. Add 1× PBS as much as 1-2 volumes of the collected fluff to re-suspend 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 –