# DiagExo® Urinary Exosome Isolation kit

Cat. #: P120S (2 rxn); P120 (10 rxn); P120L (40 rxn)

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

**Application:** to isolate exosome from urine.

**Product Size:** 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 (purity > 95%)
- **Intact** exosome (good morphology)
- Use as little as 1 mL urine 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)

| Components | Amount | | |
|---|---|---|---|
| | Cat.#: P120S | Cat.#: P120 | Cat.#: P120L |
| 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 |
| DiagExo® Column | 2 | 10 | 40 |

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

- Reaction Volume Table (important)

| Volume of urine | Mixture A/B/C = | Solution A + | Solution B + | Solution C |
|---|---|---|---|---|
| 1 mL (minimum) | 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 (maximum) | 1.5 mL = | 0.25 mL + | 0.25 mL + | 1.0 mL |

❖ Maximum urine volume per reaction is 3 mL. Processing more than 3 mL urine may cause indistinct layer separation and column clogging.

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

1. **Sample prepare:** Centrifuge **2 mL** urine sample 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.
2. Without disturbing pellets, transfer 2 mL clear supernatant to a new **glass tube 1** and keep it on ice.
3. In a new **glass tube 2**, add Solution A/B/C 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 all Solution A, B, C bottles well immediately after each use to prevent evaporation.*

*When process different volume of urine, please refer to "Reaction Volume Table" on page 1 for the solution A/B/C recipe.*

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

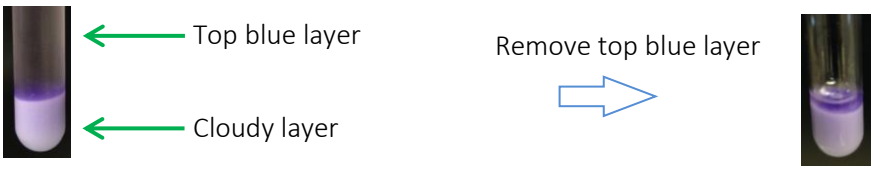
6. Transfer all mixture A/B/C (1 mL) from **glass tube 2** to **glass tube 1** (to mix with the 2 mL urine).

7. Cap **glass tube 1** well, **gently invert the tube for at least 10 times to mix well**, then incubate at **4°C** for **1 hour.**

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

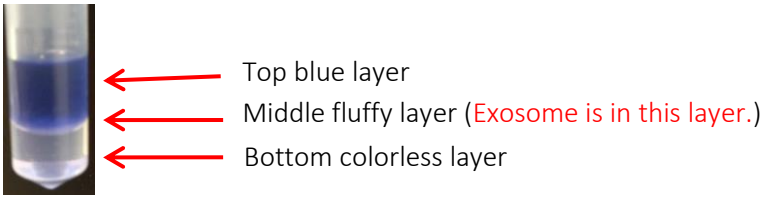

Without disturbing the middle fluffy layer, carefully pipet out the top blue layer and discard it. **Then go to step 9.**

8b. Sometimes, only two layers are visible, Top blue layer and white Cloudy layer at bottom (as shown in the following figure). Carefully remove the Top blue layer and discard it. **Then go to step 9.**

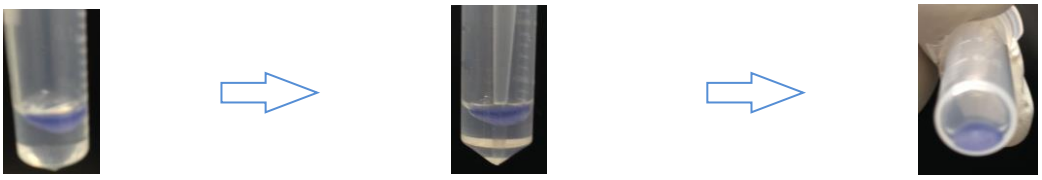



**Notice:** If layer separation are indistinct, add another 1 mL mixture A/B/C (1 mL is for this example experiment), **gently invert the tube for at least 10 times to mix well**,, and incubate at 4°C for another 30 minutes. Then start again from step 8a.

9. Transfer the left over in the glass tube 1 to **a new 2.0 mL microcentrifuge tube** (not provided in the kit) and spin at **5,000× g** for **3 minutes**. A new three-layer separation will occur (Top blue layer, Middle fluffy layer and Bottom colorless layer as shown in the following figure). Proceed to next step immediately, or the layer separation may become blur.




10. Pipet out the Top blue 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 blue 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 this example.

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 0.5 mL tube. In this example, 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" 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").

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

17. The "flow-through" is the **isolated pure exosome** (exosome suspended in PBS). The whole protocol is **completed** here. 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.

## 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) debris is not removed completely in step 1; 2) some fluff pellet was incidentally pipetted up in step 15; 3) Over 3 mL urine sample was loaded in step 1. When clogging happens, you need to prepare the sample and start over again.

   1.2. For some types of sample, the fluff (in step 13) is very difficult to be re-suspended, 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 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 sample, the exosome is low. Increase the initial input urine volume to collect more exosome.

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

   There are 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 column to collect flow though.

3. **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 P200 kit 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.

## Customer also buy

| DNA Extraction / PCR | Cat. # | Virus Packaging | Cat. # |
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| 1-Drop PCR Mix<br>( squeeze 1 drop do PCR, no pipetting ) | W2599-5 | Lenti / Retrovirus 10x Titer-Up<br>( package 10x more virus ) | P906 / P909 |
| Plasmid Miniprep | W0500-50 | Transfection Reagent (better than Lipid-based kit) | P901 |
| Endotoxin-Free Plasmid Maxiprep | W2104-10 | Lentivirus packaging service high titer/ultra high | Inquiry form |