PureExo® Exosome Isolation Kit (for serum or plasma)

Cat. #: P101 (10 reactions); P101L (40 reactions); P101S (2 reactions)

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

Product Size: Each reaction can process 100-500 µl serum or plasma. 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.)

This 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 or plasma 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 (store at room temperature)

Component	Amount		
	Cat. #: **P101**	Cat. #: **P101L**	Cat. #: **P101S**
Solution A	3 ml	12 ml	0.6 ml
Solution B	3 ml	12 ml	0.6 ml
Solution C	3 ml	12 ml	0.6 ml
Sample Buffer	20 ml	80 ml	4 ml
PureExo® Column	10	40	2

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

Protocol (for processing 100-500 µl serum/plasma)

❖ **Important**: **Do not process more than 500 µl serum or plasma** for each reaction. Otherwise it will cause indistinct layer separation and column clogging.

1. Collect 100-500 µL serum or plasma sample and keep it on ice. If starting with frozen sample, thaw the sample completely at room temperature, and then keep it on ice. (For serum / plasma collection and preparation instruction, please refer to the link: http://www.101bio.com/files/P101_serum_plasma_prep.pdf).
2. Centrifuge the serum/plasma sample at **3,000× g** for **15 minutes** at **4°C** to remove debris.

❖ **Important**: Skipping this step may cause filter clogging in Step 17.

Optional: If high lipid content serum/plasma sample is processed (eg. hyperlipidemia patient serum sample), transfer the supernatant to a new centrifuge tube without disturbing the pellet. Incubate the supernatant at 4°C for 2 hours, and then centrifuge at 1,000x g for 10 minutes at 4°C to remove fat precipitation.

3. Transfer **100-500 µl** clear supernatant to a **15 ml centrifuge tube** without disturbing the pellet. Add **Sample Buffer** to the supernatant to make a total volume of **2 ml diluted serum/plasma sample**, and keep it on ice. This dilution works well for all starting volumes between 100 to 500 µl. **Do not** process more than 500 µL sample per reaction.

4. In **a 1.5 ml microcentrifuge glass tube**, add Solution A, B and C in the following order to prepare 0.9 ml mixture A/B/C (always prepare this mixture A/B/C immediately before use. The mixture A/B/C cannot be stored):

   1st add Solution A: 0.3 ml 2nd add Solution B: 0.3 ml 3rd add Solution C: 0.3 ml

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

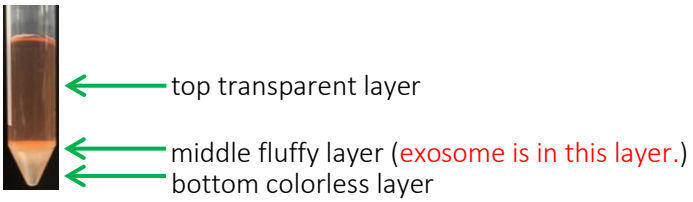
5. **Vortex** the mixture A/B/C for **10 seconds** to obtain a homogenous solution.

6. Add the 0.9 ml mixture A/B/C to the 2 ml diluted serum/plasma sample (from Step 3).

7. Cap the 15 ml tube, **gently invert the tube for at least 10 times to mix well**, and then incubate at **4°C** for **30 minutes**.

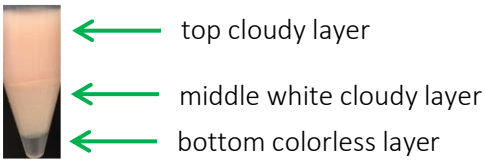
8. Spin the tube at **5,000× g for 3 minutes**.

9. **a.** The mixture now appears as 3 distinct layers:



Do not disturb the middle fluffy layer, and go to Step 10 (refer to Step 9b only if there are not 3 distinct layers).

b. For some samples, layer separation is not distinct. You can imprecisely see 3 layers (as shown in the figure below): top cloudy layer (aqueous layer), middle fluffy layer (thicker and less transparent than top layer) and bottom colorless layer.



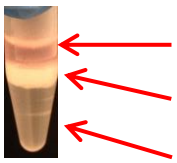
Carefully remove the top layer and discard it. Because the separation is not sharp, be careful, not to disturb or remove the middle fluffy layer because exosome is in this layer.

Prepare **another 0.9 ml mixture A/B/C** as described in Step 4 and 5, and add it to the tube containing the middle fluffy layer and bottom colorless layer. **Gently invert the tube for at least 10 times to mix well**. Incubate at 4°C for another 30 minutes. Spin the tube at **5,000× g for 3 minutes**. Now the mixture appears as 3 layers as shown in Step 9a. Then continue on to Step 10.

* Extra Solution A, B and C can be purchased separately from 101Bio.com (Cat.#: P101H).

10. Pipet out the top transparent layer and discard it without disturbing the middle fluffy layer. Transfer the middle fluffy layer (exosome is in this layer) to **a fresh 1.5 ml microcentrifuge tube**. Spin it at **5,000× g for 3 minutes**. A new 3-layer separation will appear: top transparent layer, middle fluffy layer and bottom colorless layer (see figure below).

❖ Important: Proceed to the next step immediately, without waiting.

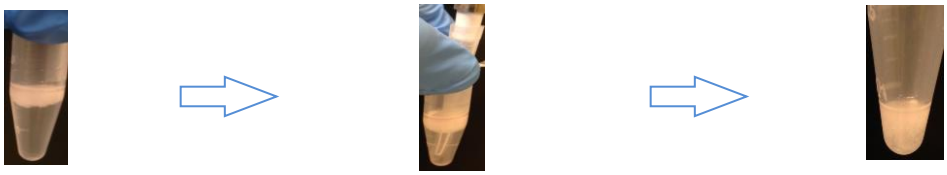


top transparent layer

middle fluffy layer (exosome is in this layer)

bottom colorless layer

11. Pipet out the top transparent layer and discard it. Insert pipette tip down to the tube bottom to completely remove the bottom colorless layer and discard it. Only keep the middle fluffy layer in the tube. (Exosome is in this layer.)



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

12. Spin again at **5,000x g for 3 minutes,** and 3 layers will appear again. Now, repeat **step 11** for one more time**.** Now only the **"fluff pellet"** is left in the tube. The **"fluff pellet"** volume is about 25 µl in this example experiment.

13. Leave the tube cap open to **air dry** for 5-10 minutes at room temp (do not over dry).

14. Add **1× PBS** equal to **4 times volumes** of the collected fluff pellet to the tube. In this example experiment, we added 100 µl PBS (4 x 25 µl fluff pellet). Resuspend the fluff pellet by pipetting up and down **vigorously for 40 times**.

15. Shake the tube on a horizontal shaker at high speed for **3 minutes**, and then **pipet up and down vigorously** for 10 times. Repeat this "shake-pipet up and down" procedure for another 2 times.

❖ **Important:** If the fluff pellet is not re-suspended well, the exosome may be trapped in the fluff pellet resulting in low exosome purity and yield. For some types of samples, eg. Hyperlipidemia patient sample, it is difficult to dissociate the fluff pellet to release exosome. In such cases, extend the pipetting and shaking time in this step.

16. Spin the tube at **5,000x g** for **5 minutes**. Without disturbing the "fluff pellet", transfer the **supernatant** carefully into one **PureExo® Column** (provided).

❖ **Important:** Keep the "fluff pellet" at 4°C. Do not discard it until the experiment is finished. See "Trouble shooting" 1.2 for detail.

17. Spin the PureExo® Column at **3,000× g** for **5 minutes** to collect the "flow-through", which is the **isolated pure exosome** (exosome suspended in PBS).

    The isolated pure exosome may be used directly for downstream assays (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 at –80°C for up to 3 months. Concentrated exosome will precipitate after sitting. Pipet up and down to re-suspend it well before each use.

Trouble shouting

1. The final exosome yield is low.

1.1. Check if there are left over liquid in the column. If yes, it indicates that the column is clogged by contaminated protein. Several reasons could cause the clogging, such as debris was not removed completely in Step 2; too much lipid protein in the sample (eg. Hyperlipidemia); some precipitation was pipet up in Step 16; too much sample was loaded, etc. If a clogging happens, prepare the sample again, input lower amount of sample, and pay more attention in steps 2 and 16.

1.2. For some types of samples, the fluff (Step 15) is sticky and it is difficult to release the exosome from the fluff. Some of the exosome may be trapped in the fluff. Add the final flow-through back to the fluff pellet stored at 4°C (from Step 16, exosomes are trapped in this fluff pellet), add **EDTA** to a final concentration of **3 mM.**

For example, if you use 0.5 M EDTA stock solution (500 mM EDTA), you need to add 0.6 µl EDTA stock solution to the 100 µl flow through. The calculation is as following:

EDTA stock solution volume = (final total volume x final concentration) / EDTA stock solution concentration

= (100 µl x 3 mM) / 500 mM

= 0.6 µl

Pipet up and down **vigorously for 60 times**, and shake the tube on a horizontal shaker for **20 minutes**. **Repeat pipetting** up and down vigorously a few times during the shaking. Go through another PureExo® column to collect the exosomes.

1.3. For some sample type, the content of exosome is low (the middle fluff layer is thin in Step 9a). Increase the initial input sample volume to collect more exosome.

2. The flow through has multiple layers.

There was bottom layer left in the fluff during steps 11-12. Spin the tube at **5,000× g** for **3 minutes**, and carefully pipet out and discard the 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 lyse than cells. Normal lysis buffer for cells, such as RIPA buffer, is not strong enough to completely lyse exosome to release exosomal protein. We suggest using 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 the 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.