

PureExo® Exosome Isolation kit (for stem cell culture media)

Cat. #: [P107S](#) (2 reactions); [P107](#) (10 reactions)

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

Product Size: Each reaction can process 20 mL stem cell culture medium. The yield of each reaction can yield pure exosome suspended in 50 ~ 200 µL PBS (from which 100 ~ 1000 µg exosomal protein or 50 ~ 300 ng exosomal RNA can be extracted using our *Exosomal RNA and Protein Extraction Kit, Cat.#: P200*).

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

This kit can isolate / purify pure exosome at high yield from stem cell culture media.

- ✓ Easy to use: No ultra-centrifugation (< 2 hours)
- ✓ 10 fold higher yield (vs. other kits or ultracentrifuge method)
- ✓ Save cost (vs. antibody-bead method)
- ✓ Isolate pure exosome (exosome purity > 95%)
- ✓ Intact exosome (good morphology)

This kit isolate high yield pure 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)

Component	Amount	
	Cat. #: P107S	Cat. #: P107
Solution A (blue)	1.5 mL	7.5 mL
Solution B	1.5 mL	7.5 mL
Solution C	6 mL	10 mL x 3 bottles
PureExo® Column	2	10

* Cap Solution A, B and C bottles immediately after each use.

Protocol

Sample Preparation:

- Fetal bovine serum (FBS) contains high level exosomes which may contaminate the cell derived exosomes. Use serum-free conditioned media to **starve stem cells for 48 hours** before media harvest.

1. Collect **20 mL** cell culture medium.
2. Centrifuge the cell media at **3,000x g** for **15 minutes at 4°C** to remove cells and debris.

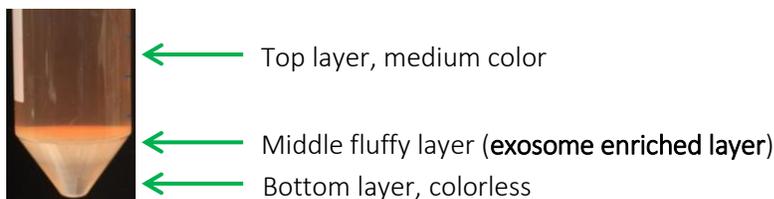
❖ **Important:** skip this step may cause filter clog in step 14.

3. Transfer 20 mL clear supernatant (cell-free culture media) to a new **50 mL centrifuge tube (tube 1)** and keep it on ice.
- ❖ The maximum medium volume of each reaction is **20 mL** from at most **1 x 10⁷ cells**. **Do not exceed** the suggested sample volume or the cell number. Otherwise it may cause indistinct layer separation and column clogging. One PureExo[®] Column can be used for only one reaction.
4. In another **50 mL centrifuge tube (tube 2)**, add the solutions **in the following order** to prepare A/B/C mixture (always prepare A/B/C mixture **right before use**):

1st Solution A: 0.75 mL; **2nd** Solution B: 0.75 mL; **3rd** Solution C: 3 mL

** Cap Solution A, B and C bottles immediately after each use.*

5. **Vortex** the tube 2 (4.5 mL A/B/C mixture) for **5 ~ 10 seconds** to obtain a homogenous solution.
6. Add the 4.5 mL A/B/C mixture from **tube 2** to **tube 1** (20 mL cell-free culture media).
7. Tightly cap tube 1, **gently invert the tube for at least 10 times to mix well**, and then incubate at **4°C** for **30 minutes**.
- 8a. The mixture now appears as 3 layers (as shown in the figure below):



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

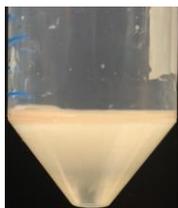
- 8b. Occasionally, only two layers (medium color top layer and white cloudy / fluffy layer) are visible. Remove and discard the top layer, and then **go to step 9**.

Notice: If no layer separation was clearly seen at all, add another 1 volume (4.5 mL in this example) of solution A/B/C mixture, **gently invert the tube for at least 10 times to mix well**, and incubate for another 30 minutes in 4°C. Proceed as step 8a described.

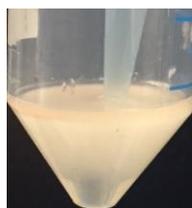
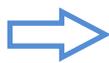
9. Transfer the left over in the tube to a new **50 mL centrifuge tube** (not provided) and spin at **5,000x g** for **3 minutes**. A new three-layer separation will occur. **Proceed to next step immediately.**



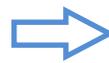
10. Pipet out and discard the top layer. Insert the pipette tip down to the tube bottom to remove the colorless bottom layer **completely**. Only keep the fluff middle layer in the tube.



(remove top layer)



(remove bottom layer)



(only keep the fluffy middle layer)

11. Transfer the “fluff” to a **new 1.5 mL microcentrifuge tube**, and repeat spinning at **5,000x g** for **3 minutes** and repeat step 10 once.
12. Leave the tube cap open to **air dry** for 5 ~ 10 minutes at room temp. (**Do not over dry**).
13. Add **1x PBS** as much as **1-2 volumes** of the collected fluff to the tube (~200 μ L in this example). Resuspend the “fluff” by pipetting up and down **vigorously for 40 times**. Shake the 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” procedure for another 2 times.

Note: This step is important. If the fluff is not well re-suspended, the exosome may be trapped in the fluff and the final yield will be low. For some type of samples, the pellet is sticky and difficult to be dissociated, from which the exosome is difficult to be released. Extend the pipetting and shaking time as needed. To examine if the exosome is trapped in the fluff precipitation, check exosomal marker level in step 14 pellet and the final exosome flow-through using ELISA. If the signal from step 14 pellet is high, the exosome release step is incomplete.

14. Spin the tube at **5,000x g** for **5 minutes**. Transfer the **supernatant** carefully into **PureExo® Column** (provided). Do not disturb the pellet.

Note: Keep the fluff pellet at 4°C. Do not throw it until the experiment is finished.

15. Spin the Column at **1,000x g** for **5 minutes** to collect all the flow-through.
16. The “flow-through” is the **isolated pure exosome** (suspended in PBS). 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.

-- The end --

Customer also buy:

DNA Extraction / PCR	Cat. #	Feature
1-Drop PCR Mix (squeeze 1 drop do PCR, no pipetting)	W2599-5	squeeze bottle makes PCR easier
Plasmid Miniprep	W0500-50	40 % below market price
Endotoxin-Free Plasmid Maxiprep	W2104-10	40 % below market price
Plasmid 96 Miniprep (4 x 96 rxn)	W0506-496	50 % below market price
2x Gold Master Mix (with dyes, hot start, HiFi)	W0655-5	25 % below market price

Virus Packaging	Cat. #	Feature
Lenti / Retrovirus 10x Titer-Up	P906 / P909	package 10x more virus
Transfection Reagent	P901	Higher efficiency than lipid-based kits

Exosome	Cat. #	101Bio.com exosome purity	other vendors exosome purity
Exosome Isolation Kit - cell media / serum	P100 / P101	95%	25% ~ 30%
Exosomal RNA / Protein Extraction kit	P200		

Services	Turnaround	
Cloning service	1 week / step	
Cell line gene editing - special expertise	3 ~ 6 months	State-of-the-art
Lentivirus packaging high titer / ultra high	2 weeks	10-year experience
3rd Generation Aptamer designing service	3 ~ 6 months	Unique