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	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 **3,000× 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 10^7 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 glass 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):

Top layer, medium color

Middle fluffy layer (**exosome enriched layer**)

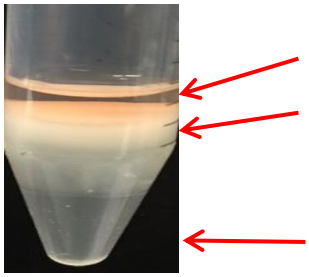
Bottom layer, colorless

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.

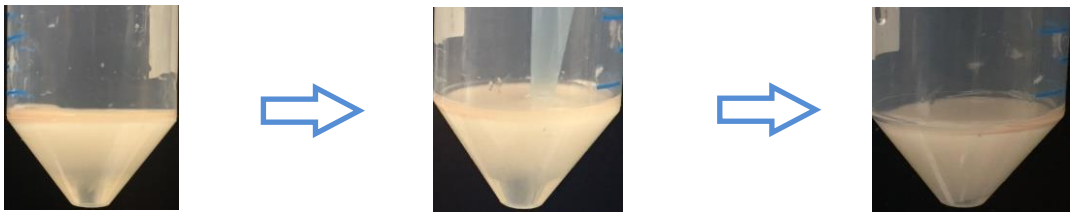


Medium color top layer

Fluffy middle layer (exosome enriched layer)

Colorless bottom layer

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 **1× PBS** as much as 1-**2 volumes** of the collected fluff to the tube (~200 µL in this example). Resuspend the "fluff" by pipetting up and down **vigorously for 40 times**. Shake the tube on a horizontal shaker for **3minutes** 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 **1,000× 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 may also like

Better than Matrigel	*Real 3D* Cell Culture Gel, 3-stiffness for different cells # P720
Mouse Tail DNA Extraction *2x Genotyping PCR (# T403)*	20-Min. fast. Ready for PCR # T605 (free sample available) → *This PCR mix is a good match* for Mouse Tail DNA kit
1-drop PCR Master Mix	Squeeze a drop for PCR # W2599-5 (free sample available)
Mycoplasma Detection qPCR Kit (# T42030)	Probe qPCR detecting all Mycoplasma Species in One reaction tube. High sensitivity
Lentiviral Expression Vectors	4 promoters: SFFV, CAG, CMV, EF1. 4 selections
10x Virus Titer-up Boost	Increases Lenti/Retro virus titer up to 10-fold # P906/ P909
Water Bath Clean Tablet	1 tablet, whole bath clean / week. Cat. # T20
Simple and Reliable	SV40T / hTERT *Cell Immortalization Kits*
Pure Plasmid Miniprep	High adsorption efficiency of plasmid DNA Cat. # W0500-50
Endotoxin-Free Plasmid Miniprep Kit	No need to precipitate, concentrate or desalt. Cat. # W2106-50