Exo*Isolator* Exosome Isolation Kit
Exo*Isolator* Isolation Filter

Technical Manual

General Information

Exosomes, a form of secreted extracellular vesicles, contain various proteins and nucleic acids; therefore, exosomes have various effects on recipient cells[1]. Recently, there have been many reports on exosomes related, for example, to metastasis and malignant progression of cancer, therapeutic research, and diagnostic research. Many methods have been developed for the purification of exosomes for research use. Of these, the main method is ultracentrifugation (UC). However, UC is complicated and requires a long time to purify exosomes.

Dojindo's Exo*Isolator* Exosome Isolation Kit can collect exosomes from cell supernatants with a recovery rate equivalent to that of UC. Since Exo*Isolator* Exosome Isolation Kit requires only the filtration procedure unlike UC, exosomes are obtained quickly without any complicated operations.

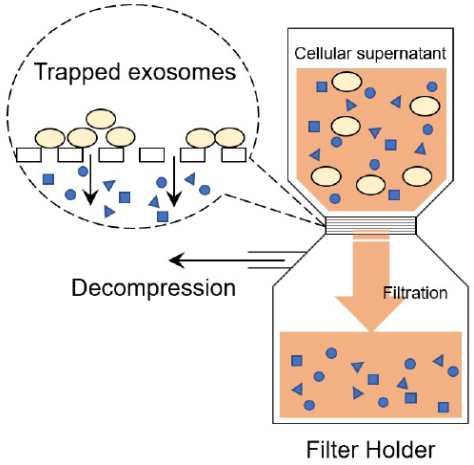


Figure 1. Principle of exosomes purification
(Patent pending)

Kit Contents

EX10 Exo*Isolater* Exosome Isolation Kit		EX11 Exo*Isolater* Exosome Isolation Filter	
- Filter Holder	x 1	- Isolation Filter	x 10
- Isolation Filter	x 3		
- Tweezers	x 1		

Storage Condition

Store at room temperature

Required Equipment and Materials

- Aspirator

Note: Prepare an aspirator as commonly used for cell experiments. Recommended aspiration pressure for use is −25 kPa or more lower aspiration pressure.

- 0.22-µm filters

Note: The 0.22-µm filter is used for pre-filtration of the cell supernatant to remove cellular fragments and cell debris.

- Micropipettes
- Microtubes
- Phosphate-buffered saline (PBS)

Precaution

- Before use, the Filter Holder should be washed and autoclaved.
- The Filter Holder is autoclavable and can be used repeatedly. The Isolation Filter is consumable. If there are not enough Isolation Filters, please purchase the Exo*Isolator* Isolation Filter (EX11), which contains 10 Isolation Filters.
- Be careful to handle the Isolation Filter because it is very thin and fragile. Please use a pair of tweezers in the kit to handle the Isolation Filter.
- The Isolation Filter should be correctly oriented. The shiny surface faced with a blue sheet should be oriented at the upside. The rough surface faced with a yellow sheet should be oriented at the downside (Figure 2).
- The recommended sample volume is 25 ml. Approximately 5-15 minutes is required for filtration of 25 ml sample. When the sample volume is more than 25 ml, the time required for filtration will be longer.

-Filtration of exosome-rich supernatant may result filter clogging. Divide the supernatant and serve them for filtration individually.

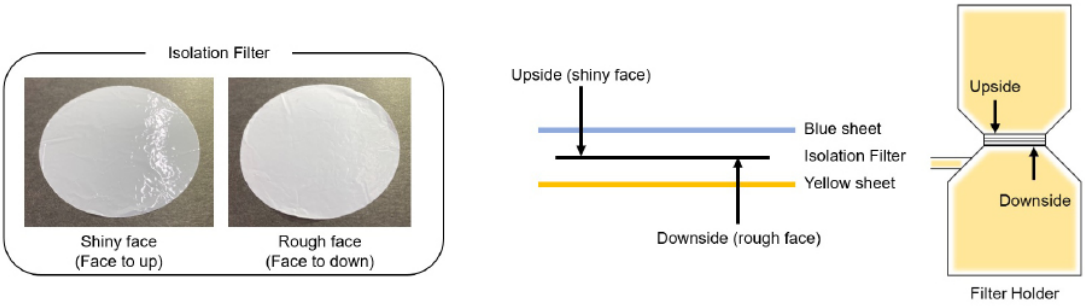


Figure 2. Upside(shiny face) and downside (rough face) of Isolation Filter

General Protocol

1. Prepare cell supernatant for the experiment.

Note: The recommended sample volume is 25 ml.

Note: The amount of exosomes collected from cell supernatant varies depending on the cell type and the culture protocol.

2. Pass the cell supernatant through a 0.22-µm filter; this produces the pre-filtered sample.
3. Assemble Filter Holder without the funnel (Figure 3).

Note: Three silicon O-rings are included in the Exo*Isolator*. Settle silicon O-ring correctly in the groove of the Filter Holder to improve the airtightness.

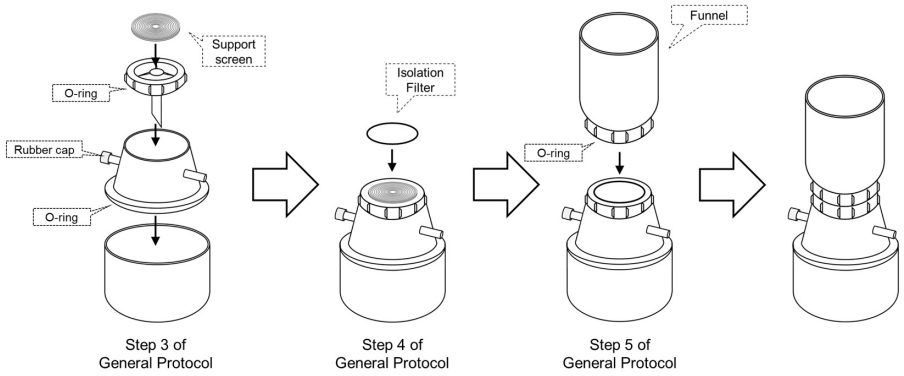


Figure 3. How to assemble Filter Holder and Isolation Filter

4. Put the Isolation Filter on the support screen using a pair of tweezers (Figure 4a).

Note: The Isolation Filter is very thin and susceptible to static electricity.

Note: Make sure there are no wrinkles on the surface of the filter (Figure 4b and 4c).

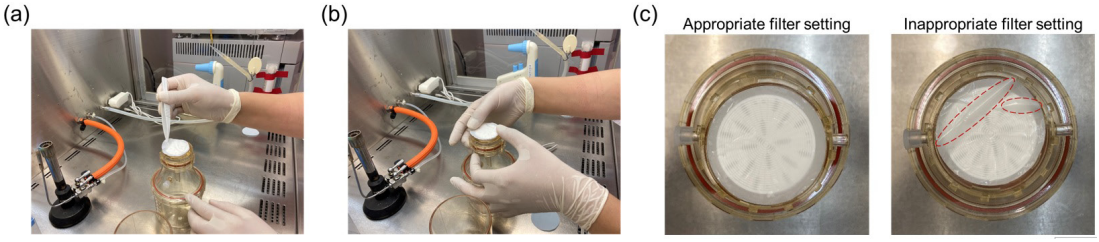


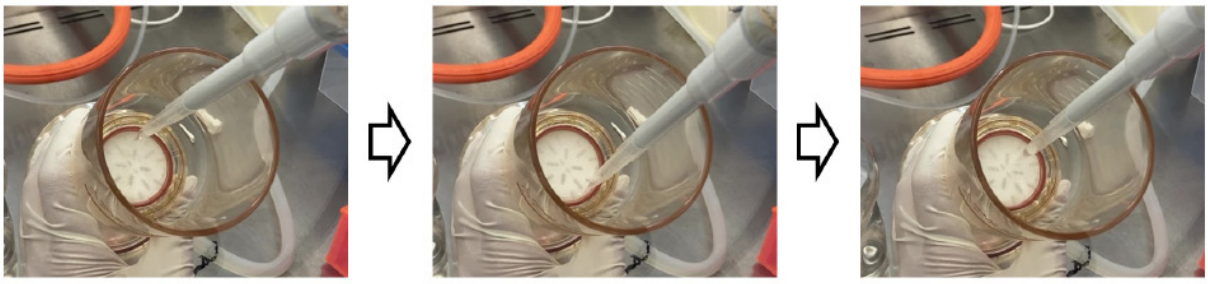
Figure 4. How to settle the Isolation Filter on the support screen

5. Settle the funnel on the Isolation Filter.
6. Pour 4 ml PBS onto the Isolation Filter and aspirate for wetting the Isolation Filter.
7. Pour the pre-filtered sample onto the Isolation Filter and aspirate.
8. Pour 2 ml PBS onto the Isolation Filter and aspirate for washing. Repeat this step twice.
9. To collect exosomes, flush repeatedly with 250 µl PBS on the surface of the Isolation Filter using a micropipette and collect the liquid by pipetting (Figure 5).

Note: A video of the operation is available on our website.

10. Transfer the PBS containing exosomes to a microtube.
11. Repeat step 9–10 once, giving a total volume of 500 µl exosome suspension.

Note: To increase the concentration of the exosomes, decrease the volume of PBS used in step 7-8. Be aware that reducing PBS volume may result in lower recovery of exosomes.



1. Flush the Isolation Filter with 250 µl PBS to collect exosomes.
2. Take the exosome-suspended 250 µl PBS by using a pipet.
3. Apply 250 µl of exosomes-suspended PBS again to flush the Isolation Filter. Repeat flushing and collecting process for 20 times.

Note: Make sure all surface of the Isolation Filter being flushed to collect the exosomes in high recovery rate.

Figure 5. Step 9 of General Protocol.

Experimental example

Isolation of exosomes from HEK293 cell supernatant using Exo*Isolator*

1. HEK293S cells suspended in 30 ml serum-free medium at 5×10^5 cells/ml were placed in a 125-ml baffled Erlenmeyer flask. The cells were shaken at 155 rpm at 37°C for 2 days in a 5% CO_2 incubator.
2. After centrifuging at 1500 rpm for 5 min, the cell supernatant was transferred to a 50-ml conical tube.
3. The collected cells were resuspended in 30 ml of fresh serum-free medium and cultured for another 2 days in the same conditions.
4. After centrifuging at 1500 rpm for 5 min, the cell supernatant was transferred to a 50-ml conical tube.
5. The cell supernatants collected in steps 2–4 were mixed and passed through a 0.22-µm filter to give the pre-filtered cell supernatant.
6. The Isolation Filter was put on the support screen and the funnel was settled on the Isolation Filter.
7. PBS (4 ml) was poured onto the Isolation Filter and aspirated.
8. After aspiration, 25 ml of pre-filtered cell supernatant was poured onto the Isolation Filter.

9. After aspiration, 2 ml of PBS was poured onto the Isolation Filter and aspirated. This operation was repeated twice.
10. After aspiration, the Isolation Filter was flushed repeatedly with 250 µl PBS using a micropipette and the liquid was collected by pipetting.
11. The PBS containing the exosomes was transferred to a microtube.
12. Steps 10–11 were repeated.
13. The PBS containing exosomes were collected as 500 µl of total volume and were examined by nanoparticle tracking analysis (NTA) and western blotting.

Comparison of exosome purity collected by UC and Exo*Isolator*

[Purification of exosomes by UC]
1. The cell supernatant from HEK293 cells was passed through a 0.22-µm filter to give the pre-filtered cell supernatant.
2. The pre-filtered cell supernatant (25 ml) was transferred to a centrifuge tube and centrifuged at 100000 × *g* for 2 hours (himac CP80NX, Hitachi).
3. After decantation, the pellet was then suspended in 6 ml PBS and centrifuged at 100000 × *g* for 2 hours.
4. After decantation, the pellet was then suspended in 100 µl PBS.
5. The PBS containing the exosomes was transferred to a microtube.
6. The centrifuge tube was washed with 100 µl PBS. PBS used for washing was transferred to the microtube. This step was repeated once.
7. The PBS containing the exosomes was centrifuged at 10000 × *g* for 5 min. The supernatant was transferred to a new microtube.

[Nanoparticle Tracking Analysis (NTA)]
Particle number of purified exosomes were measured using Nanosight NS300 (Quantum Design; camera level 13; detect threshold 5).

[Western blotting]
1. Purified exosomes were mixed with sample buffer without dithiothreitol and boiled at 95°C for 5 min.
2. Samples were separated by SDS-PAGE (SuperSep Ace, 10-20%, 13well; Wako) and transferred to PVDF membranes (Trans-Blot Turbo Transfer System Transfer Pack; Bio-Rad).
3. Following antibodies were used for detection of CD9, CD63, and CD81.

	Primary antibody	Secondary antibody
CD9	Monoclonal rabbit anti-CD9 antibody (diluted 1:1000; cat. no. ab236630; abcam)	Polyclonal goat anti-rabbit antibody conjugated with HRP (diluted 1:2000; cat. no. ab97051; abcam)
CD63	Monoclonal mouse anti-CD63 antibody (diluted 1:1000; cat. no. MEX002-3; MBL)	Polyclonal goat anti-mouse antibody conjugated with HRP (diluted 1:2000; cat. no. ab205719; abcam)
CD81	Monoclonal mouse anti-CD81 antibody (diluted 1:500; cat. no. MEX003-3; MBL)	Polyclonal goat anti-mouse antibody conjugated with HRP (diluted 1:2000; cat. no. ab205719; abcam)

4. The membranes were treated with SuperSignal West Pico PLUS Chemiluminescent Substrate (Thermo Fisher Scientific).
5. The protein bands were detected using a Syngene Pxi (Syngene).

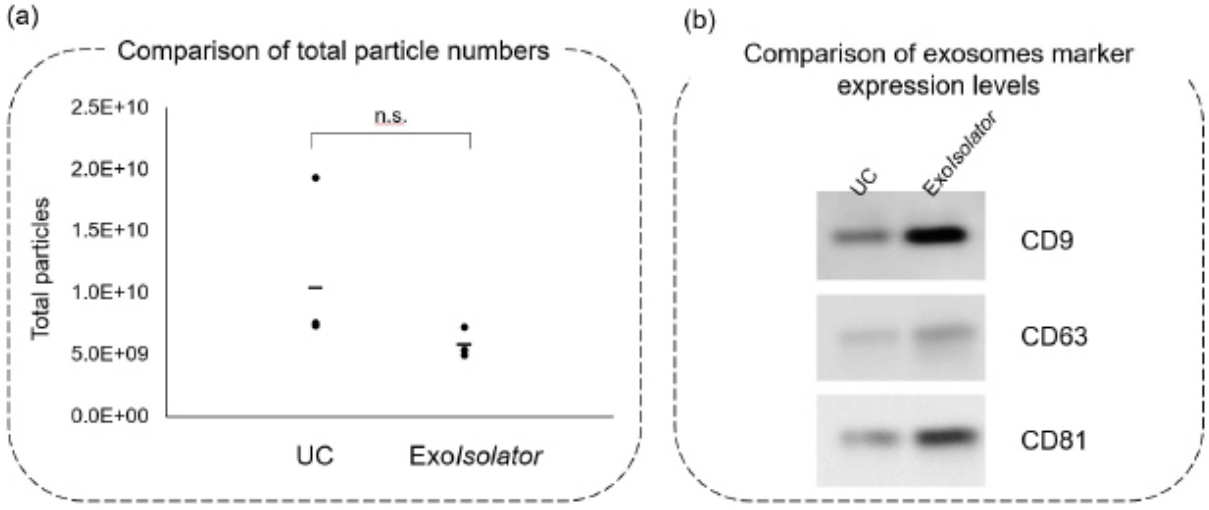


Figure 6. Exosomes collected by UC or using the ExoIsolator Exosome Isolation Kit were evaluated using (a) NTA and (b) western blotting.

References

1) Kalluri, R.; LeBleu, V. S. The biology, function, and biomedical applications of exosomes. *Science.* **2020**, *367(6478)*.