

EXORPTION™ Extracellular vesicles purification kit

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<Background>

Extracellular vesicles (EVs) are nano-sized particles enclosed by a lipid bilayer membrane secreted from various cells. EVs play important biological roles, including intercellular communication and immune responses. They contain diverse molecular markers such as proteins, nucleic acids, and glycans, both inside and on the surface of the vesicle membrane. These biomarkers strongly reflect the characteristics of their originating cells, making them promising tools for disease diagnosis and therapy.

Ultracentrifuge is the most commonly used method for isolating EVs from biological samples. However, this method requires specialized equipment, is time-consuming, and demands careful operation. Therefore, a simple and efficient EVs isolation method with a shorter processing time is highly desirable.

EXORPTION™ enables the rapid and high-purity isolation of EVs from biological solutions such as body fluids and conditioned medium. The purified EV can be utilized for various analyses and experiments, including protein and nucleic acid characterization and their biological activities.

<Storage>

15-25 °C

<Kit contents>

1. Purification column		10 tubes
2. LureCAP (attached to the bottom of the Purification column)		10 pieces
3. Wash Buffer (×10)	1.7mL	1 tube
4. Elution Buffer	1.7mL	1 tube
5. Dilution tube		1 tube
6. Product Manual		1 sheet

<Additional required materials>

Micropipette
Pipette tips※
Microcentrifuge tube (2 mL)※
Tabletop centrifuge
Microcentrifuge
Vortex mixer

※ Low protein absorption grade products are suitable.

<Precaution>

1. If you analyze the nucleic acid after the isolation of EV, it is recommended to use sterile or DNase and RNase-free microcentrifuge tubes and pipette tips.
2. Biohazardous waste including waste liquids and materials such as pipette tips, microcentrifuge tubes, and spin columns, must be disposed of according to institutional guidelines.

<Wash Buffer (×1) preparation>

Dilute Wash Buffer (×10) to ×1 with ultrapure water. 1.5 mL of Wash Buffer (×1) is required for each column. Diluted Wash Buffer should be prepared when needed.

e.g. (Prepare 1.6 mL of Wash Buffer (×1) for each column)

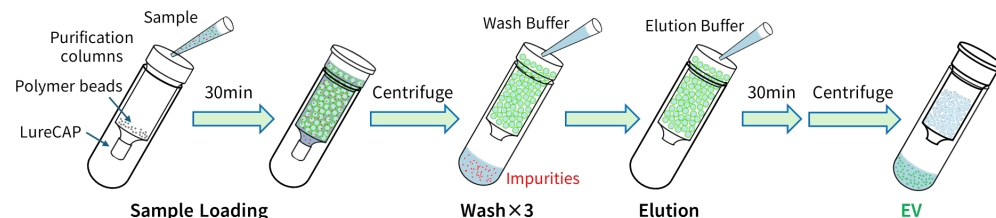
Number of columns	Wash Buffer (×10)	Ultrapure water
1 tube	0.16 mL	1.44 mL
2 tubes	0.32 mL	2.88 mL
5 tubes	0.80 mL	7.20 mL

<Pretreatment of samples>

Before purifying the samples, remove debris by centrifugation at 2000×g for 10 min at 4 °C. Please pretreat appropriately according to the type of samples. The samples containing the insoluble components may cause clogging of the bottom filter of the spin column.

<Purification protocols>

● Standard method



EVs can be purified from a 1 mL sample. If the sample volume is less than 1 mL, it is recommended to dilute it with PBS or saline to a final volume of 1 mL.

1. Tap a Purification column to drop the polymer beads down to the bottom. Remove the bottom cap, then attach the LureCAP to the bottom of the Purification column.
2. Place the Purification column into a 2 mL microtube. Remove the top cap, then add 1 mL of sample solution to the Purification column. After adding the sample, cover the Purification column with the removed top cap. Allow 30 minutes for EVs adsorption.
3. Remove the LureCAP, then place back the Purification column in the microtube.
4. Discard the remaining solution by centrifuge at 1,000~5,000×g for 30~60 seconds at room temperature.
For the Pulse-flow method, proceed to step P1 ~ P3.
5. Add 0.5 mL of Wash Buffer (×1), then centrifuge at 1,000~5,000×g for 30~60 seconds at room temperature.
Repeat this step a total of 3 times to wash the polymer beads.
Note 1: Since the swollen polymer beads can easily spill out, it is recommended to remove the top cap carefully.
6. Transfer the Purification columns to new 2 mL microtubes. Add 0.1 mL of Elution Buffer to each Purification columns.
7. Close the top cap of the Purification columns, then mix the beads for 10 seconds using a vortex mixer. After mixing, allow 30 minutes for EVs elution into the solution.

For research use only, Not for medical and diagnostic use.

8. Recover the elution solution by centrifuge at 1,000~5,000×g for 30~60 seconds at room temperature.

Note 2: The volume of the recovered EVs elution solution increases due to water release from the polymer beads

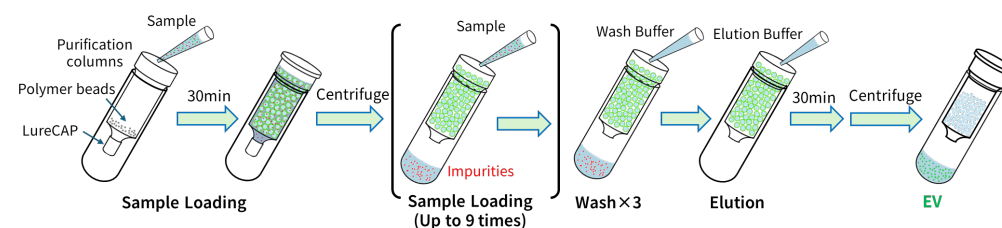
Note 3: The recovered EVs elution solution has a high salt concentration. If necessary, dilute or desalt the elution before use.

The following protocols are examples for evaluating recovered EVs using EXORPTION™.

ELISA: It is recommended to dilute the EV solution at least 3-fold with a salt-free diluent or 0.2 % BSA solution. The 0.2 % BSA solution is particularly preferable if evaluating EV surface markers.

RNA extraction: No dilution is necessary when using a commercially available RNA isolation column.

● Pulse-flow method



The pulse-flow method is suitable for samples with a low EV concentration. Repeatedly adding the sample solution up to a total volume of 4.6 mL increases the EV concentration.

P1. After step 4, add 0.4 mL of sample, then allow 10 minutes for EVs adsorption.

P2. Discard the flow through by centrifuge at 1,000~5,000×g for 30~60 seconds at room temperature.

P3. Repeat steps P1 to P2 up to 9 times.

After step P3, proceed to step 5.

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