Exo-i

Exo-i manual
(For research use only)

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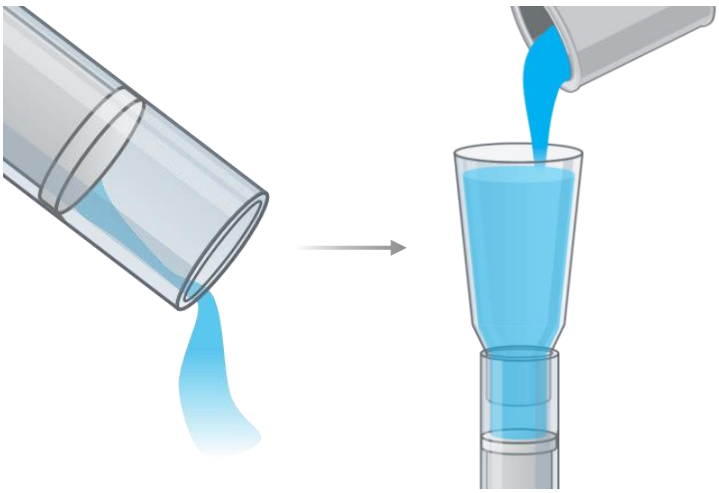
1. Sample Preparation

1) Centrifuge the sample and collect only the supernatant except the pellet .

   * If the sample is too dilute, it is recommended to concentrate it using a 100 kDa filter tube.
   If the sample is too concentrated, dilute it appropriately before use.

2. Exo-i Column Preparation



1) Hold the column at its center and set it upright. Open both the top and bottom caps.
2) Discard the preservation solution above the top disk and attach the funnel to the column.
3) Fix the column vertically on a clamp stand and place a waste container underneath.
4) Fill the column with DPBS up to the marked line and let it flow through completely.
5) After all DPBS has passed through and dripping has stopped, close the bottom cap to complete the preparation..

   * Caution: Do not allow the top disk to dry during this step.

3. Fraction Tube Setup

1) Arrange 1.5 mL microcentrifuge tubes in order on a microtube rack.
2) Place the microtube rack on an electronic balance and tare it.
3) Align the bottom outlet of the Exo-i column with the opening of the first tube.

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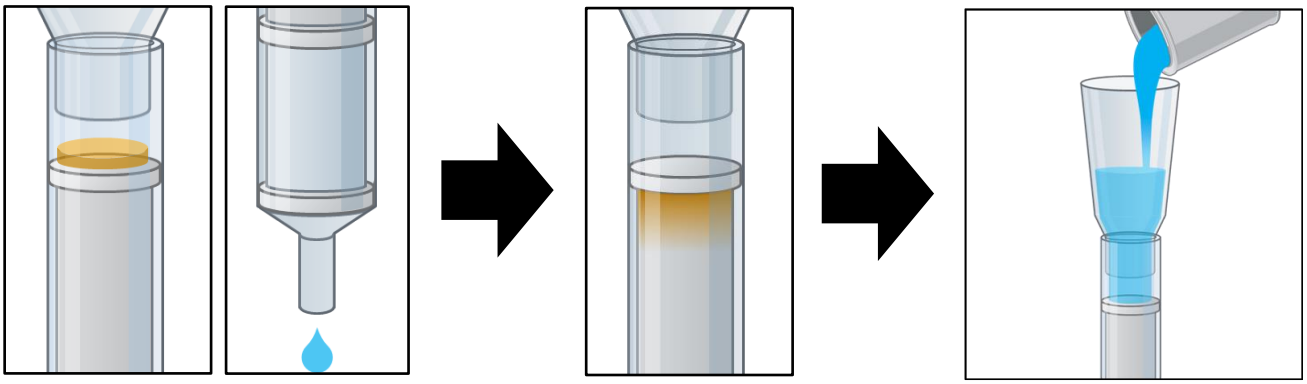
4. EV Isolation



1) Open the bottom cap of the column and load 500 μL of the prepared sample.
2) As the sample begins to flow, immediately collect the eluted solution into the first tube.
3) When flow ceases, align the bottom of the column with the second tube and fill the column with DPBS.
4) Collect 0.5 g of eluate into the second tube, then proceed to the next tube to collect the same amount.
5) Repeat the fraction collection process sequentially across all tubes.
6) After collection, conduct a characterization step to identify and select the appropriate EV fraction.

* To identify the fractions containing extracellular vesicles (EVs),
fractions 8 to 14 are typically analyzed using techniques such as Western blotting,
nanoparticle tracking analysis (NTA), or ELISA

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Trouble shooting

Case 1: No liquid flows from the top of the disk

This may occur if the sample is too viscous or contains too many particles. Dilute the sample to an appropriate concentration before separation

Case 2: EVs are not detected in the eluted fractions

The particle concentration in the sample may be insufficient. Verify the particle concentration through characterization, as chromatography inherently dilutes the sample. You may concentrate either the original sample or the collected EV fractions prior to use.

Case 3: The disk inside the column is completely dried

A fully dried disk can impair separation performance. In such cases, discard the column and use a new one.

Case 4: Reusing the column

The column may be reused up to three times by following the cleaning procedure below:

1) Allow all residual PBS to flow through after completing the separation.
2) Fill the column up to the marked line with 0.025% Triton X-100 diluted in PBS, and let it flow through.
3) Once the Triton X-100 has fully passed and dripping stops, fill the column with an equal volume of DPBS.
4) When the DPBS has completely passed, close the bottom cap to complete the washing.
5) Add preservation solution above the top disk, close the top cap, and store the column vertically at 4 °C.

   * If reusing within 24 hours: use DPBS as the preservation solution.
   For long-term storage before reuse: use 20% Ethanol as the preservation solution