Universal Exosome Isolation Kit

Cat. No. : P-CA-503
Size : 3Tests

Product Description

This kit leverages the principle of size exclusion chromatography, facilitating the separation of exosomes based on the differential molecular sizes of their constituents. It provides several key advantages, including simplicity, exceptional purity, and high recovery rates, making it particularly ideal for exosome isolation from large-volume samples. The isolated exosomes are highly versatile, suitable for a wide range of applications, including Western blot (WB) analysis, nanoparticle tracking analysis (NTA), nanoparticle flow cytometry for particle size characterization, electron microscopy, omics research, and functional studies in both cellular and animal models.

Product Composition

Component	3 Tests	Storage Conditions
Exosome Purification Column (30 mL)	3 Tests	2-8°C, Shading Light
Column Adapter (30 mL)	3 Tests	2-30°C, Shading Light

Storage Conditions

The kit is shipped with ice packs. Store the exosome purification columns at 2-8°C. The product has a shelf life of 18 months.

Applicable Samples

This kit is primarily used for large sample volumes such as cell culture supernatants and urine. It is also suitable for serum, plasma, and other similar samples. For rare or precious samples, please consult our technical support team for guidance.

Required Instruments, Reagents, and Consumables (Not Included)

- High-speed refrigerated centrifuge
- Centrifuge tubes
- Waste liquid container
- Ultrafiltration tubes (MWCO: 50 kDa)
- Sterile PBS (prepared fresh, filtered through 0.2 μm filter, sonicated or vacuum degassed)
- 20% ethanol (prepared fresh, filtered through 0.2 μm filter, sonicated or vacuum degassed)
- Purification Column Stand

Protocol

1. Sample Processing

1) **Cell Removal:** Centrifuge the sample at 300 × g for 5 minutes at 4°C. Carefully transfer the supernatant to a new centrifuge tube.
   Note: This step can be skipped for cell-free samples.
2) **Removal of Cellular Debris:** Centrifuge the supernatant obtained from Step 1 at 2,000 × g for 10 minutes at 4°C. Transfer the supernatant to a new centrifuge tube.

3) **Removal of Large Particles:** Centrifuge the supernatant obtained from Step 2 at 14,000 × g for 30 minutes at 4°C. Carefully transfer the resulting supernatant to a new centrifuge tube.

2. Purification Column Preparation

1) **Column Installation and Equilibration:** Place the Exosome Purification Column (30 mL) onto the Purification Column Stand, positioning a waste container beneath it. Allow the column to equilibrate at room temperature for at least 30 minutes.
Note: Ensure the column is fully equilibrated to room temperature (18-25°C); deviations may affect exosome isolation efficiency.

2) **Air Removal Check:** Inspect the bottom of the column for any air bubbles. If the bottom of the column contains no air, skip this step. If air is present, invert the column, remove the bottom cap, and use a pipette or syringe to displace the air with water or 20% ethanol. Ensure the bottom cap is filled with the liquid before resealing and returning the column to the stand.

3) **Column Equilibration with PBS:**
- Remove the top cap first, then the bottom cap. Discard the storage buffer by either pouring it out or using a pipette.
- 6mL PBS was added to break the pressure difference between inside and outside the column.
- Attach the Column Adapter (30 mL) to the purification column. Equilibrate the column by adding 2 column volumes (60 mL) of PBS from the top until no liquid flows out from the bottom. Ensure the top frit remains moist throughout the washing process.
- Once the washing is complete, replace the bottom cap and disconnect the adapter. Add 3 mL of PBS into the column for standby use.

Important Notes:

a. Always open the top cap before removing the bottom cap to prevent air from entering the column, which could impact separation efficiency.

b. Ensure the top frit remains moist throughout the entire purification process, as dryness may affect exosome isolation performance.

c. When adding large volumes of liquid, connect the adapter to the column and add the liquid through the adapter.

d. It is recommended to freshly prepare PBS, filter it through a 0.2 μm membrane, or use commercially available sterile PBS to avoid microbial or particulate contamination. Ensure the PBS is equilibrated to room temperature before use to prevent bubble formation within the column, which may affect exosome separation efficiency.

3. Exosome Isolation

1) **Sample Loading:** Remove PBS from the purification column. Add 3 mL of the prepared sample to the top of the column. If the sample volume is less than 3 mL, dilute it with PBS to a final volume of 3 mL before loading. Remove the bottom cap and allow the sample to fully enter the column before adding PBS.
Additional Notes:

a. If the sample volume exceeds 3 mL, concentrate it to 3 mL using a 50 kDa ultrafiltration tube. Ensure the concentration factor does not exceed 20 ×. Refer to the ultrafiltration tube manual for detailed instructions.

b. For high viscosity samples, such as plasma, serum, or highly viscous pleural/ascetic fluid, dilute 1.5 mL of the sample with PBS to a total volume of 3 mL before loading.

c. Ensure the entire sample enters the column before adding PBS to prevent dilution, which could negatively impact the separation efficiency.

2) **Exosome Separation:**
   - Place a 1.5 mL centrifuge tube beneath the purification column.
   - Add 1 mL of PBS to the top of the column. Once 1 mL of eluate is collected, add another 1 mL of PBS and use a new 1.5 mL centrifuge tube to collect the next fraction.
   - Label the fractions sequentially according to the collection order.
3) **Exosome Collection:**
   - Collect fractions 8, 9, 10, 11 and 12, which contain the exosomes. Fractions 9, 10 and 11 typically have the highest exosome concentration.
   - The collected fractions can be directly analyzed for exosome particle count and protein concentration.
4) **Exosome Concentration:**
   - Measure the particle concentration and protein content of the collected exosome fractions. Depending on the requirements of downstream experiments, further concentration may be necessary.
   - If concentration is required, use a 50 kDa MWCO ultrafiltration tube and centrifuge at 4,000 × g until the desired volume is achieved.

4. Purification column maintenance:

1) **PBS Cleaning:** After collecting all fractions, reconnect the adapter to the purification column. Add at least 2 × the column volume (60 mL) of PBS from the top, allowing it to flow through until no liquid exits the column.
2) **Ethanol Cleaning:** Add 1.5 × the column volume (45 mL) of 20% ethanol to the column and allow it to flow through completely until no liquid remains.
3) **Storage Preparation:** Disconnect the adapter. Add 5 mL of 20% ethanol as a storage solution into the column. Secure the top cap, fill the bottom outlet cap with 20% ethanol, and seal it. Store the column upright at 4°C.

Product Advantage

1. **Wide Sample Volume Compatibility:** Suitable for sample volumes ranging from 0.1 to 3 mL.
2. **High Purity Exosome Isolation:** Isolated exosomes exhibit high purity, making them directly applicable for characterization, tracking studies, and functional research.
3. **Simple Operation:** The gravity-based column method eliminates the need for specialized purification equipment, reducing infrastructure requirements and processing time.
4. **High Recovery Rate:** Fraction selection allows for minimal protein contamination, ensuring that the isolated components are free from protein impurities.

Notes

1. New or previously used purification columns may exhibit small gaps between the top frit and the white agarose beads due to gel settling during storage and handling. This does not affect column performance. Simply press the top frit down to eliminate any gaps before use.
2. The purification column is stored in 20% ethanol. Prepare 20% ethanol freshly before use, filter it

through a 0.2 μm membrane, and degas it via ultrasonication or vacuum to prevent bubble formation, which can impair separation efficiency.

3. Prior to each use, wash the column with sterile PBS equilibrated to room temperature. Freshly prepare the PBS, filter it through a 0.2 μm membrane, and degas it to prevent bubble formation that could compromise performance.
4. Ensure no bubbles are present in the column before or during use. Inspect carefully to avoid any impact on experimental outcomes.
5. While the column can be reused, performance may decline with multiple uses. It is recommended to limit reuse to a maximum of 5 times
6. For exosome separation intended for NGS or other omics analyses, use a new purification column for each sample to avoid cross-contamination.