Exosome Isolation Kit

Catalog No.: abx705215

Size: 4 tests

Storage: Store Exosome Purification Column at 4 °C and Column Adapter at 2-30 °C. Store both components in the dark.

Introduction: Abbexa's Exosome Isolation Kit is a quick and convenient method for the isolation of exosomes from cell culture supernatants, urine and other biological fluids. Isolation is based on the principle of size exclusion chromatography wherein exosomes are separated based on the differential molecular weight of their constituents. High recovery rates and high purity of separated exosomes makes this kit ideal for use with large volume samples. The separated exosomes can be used in a wide array of downstream applications such as Western Blot (WB), nanoparticle tracking analysis (NTA) and omics research.

Kit Components

1. Exosome Purification Column: 4 tests
2. Column Adapter: 4 tests

Materials Required But Not Provided

1. Sterile filtered Phosphate-Buffered Saline (PBS)
2. Sterile filtered 20% Ethanol (EtOH)
3. Micropipette and pipette tips
4. Ultrafiltration tubes (MWCO: 50 kDa)
5. Centrifuge and centrifuge tubes
6. Waste liquid container
7. Purification Column Stand

For Reference Only

Protocol

A. Sample Preparation

Analyze immediately or store samples at 2-8°C (within 24 hrs). For long term storage, aliquot and store at -20°C or -80°C. Avoid multiple freeze-thaw cycles. Keep samples on ice during preparation. Equilibrate samples to room temperature prior to analysis.

- **Cell culture supernatants:** Centrifuge sample at 300 x g for 5 minutes at 4°C and collect supernatant into a centrifuge tube. To remove cellular debris, centrifuge the supernatant at 2000 x g for 10 minutes at 4°C and collect supernatant into a centrifuge tube. To remove large particles, centrifuge the supernatant at 14,000 x g for 30 minutes at 4°C and collect supernatant for purification.

B. Purification Column Preparation

1. Place the Exosome Purification Column onto the purification column stand and place a waste container below. Allow the column to equilibrate for at least 30 minutes at room temperature.
2. Inspect the bottom of the column for air bubbles. To remove air, invert the column, remove the bottom cap and displace with water or 20% EtOH using a micropipette. Ensure bottom cap is filled with liquid before resealing and return the column to the stand.
3. Remove the top cap then the bottom cap of the column. Discard storage buffer by pouring it out or using a micropipette.
4. Add 3 ml of PBS then attach the Column Adapter (10 ml) to the purification column.
5. Equilibrate the column by adding 20 ml of PBS. Repeat wash until no liquid exits the column, ensuring the top frit remains wet throughout.
6. Once washing is complete, replace the bottom cap and disconnect the adapter. Add 1 ml of PBS into the column.

Notes:

- Always open the top cap of the column before removing the bottom cap to prevent air from entering. Introduction of air can impact separation efficiency.
- Ensure top frit of column does not dry out during the purification process as this can impact exosome isolation.
- Add large volumes of liquid into the column using the adapter.
- Sterile filtered (0.2 µm membrane) PBS is recommended. Alternatively, commercially available sterile PBS can be used to avoid microbial or particulate contamination. Ensure PBS is equilibrated to room temperature before use to prevent bubble formation within the column.

C. Exosome Isolation

1. Remove PBS from the purification column and add 1 ml of prepared sample to the top of the column.
2. Remove the bottom cap and allow the sample to enter the column before adding PBS.
3. Place a 1.5 ml centrifuge tube under the purification column and add 500 µL of PBS to the column. Once the eluate is collected, repeat, using a new 1.5 ml centrifuge tube to collect the next fraction. Label the fractions sequentially.
4. Collect fractions 4, 5, 6, 7 and 8 and analyze for exosome particle count and protein concentration.
5. Depending on the requirements of downstream experiments, if concentration of purified sample is required use a 50 kDa MWCO ultrafiltration tube and centrifuge at 4,000 × g until the desired concentration is achieved.

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Notes:

- If sample volume is less than 1 ml, dilute with PBS to a final volume of 1 ml before loading. If sample volume exceeds 1 ml, concentrate the sample using a 50 kDa ultrafiltration tube. Ensure the concentration factor does not exceed 20 x.
- For high viscosity samples, dilute 500 µL of the sample with 500 µL of PBS before adding to the column.
- Ensure the entire sample enters the column before adding PBS to prevent dilution.

D. Purification Column Maintenance

1. After collecting all fractions, reconnect the adapter to the purification column. Add 20 ml of PBS from the top and repeat until no liquid exits the column.
2. Add 15 mL of 20% EtOH to the column and allow it to completely exit the column.
3. Disconnect the adapter. Add 3 ml of 20% EtOH as storage solution into the column. Secure the top cap and fill the bottom cap outlet with 20 % EtOH. Seal column and store upright at 4°C.

Notes:

- Purification columns can exhibit small gaps between the top frit and the white agarose beads due to settling of the gel during storage or handling. This does not affect column performance. Press down on the top frit to eliminate gaps before use.
- Prepare fresh 20% EtOH for use as storage solution. Sterile filtration (0.2 µm membrane) and degassing via ultrasonication or vacuum is recommended to prevent bubble formation.
- Prior to each use, wash the column with room temperature sterile PBS.
- Recurrent use of the column can lead to a decline in performance over time. It is recommended to use the column no more than 5 times.
- For exosome separation intended for Next Generation Sequencing (NGS) or other omics analysis, use a fresh purification column for each sample to avoid cross-contamination.

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.

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