Apex MM

SEC Column Quick Guide

Revision 2 – May 2, 2026

The Apex multi-mode (MM) column purifies extracellular vesicles (EVs) from biological fluids such as plasma, serum, urine, cell culture media, or cerebrospinal fluid (CSF). By combining SEC with a multi-mode resin that uses hydrophobic interaction and ion-exchange to selectively bind and remove lipoproteins and other soluble contaminants, Apex MM columns enhance EV purity.

Column specification:

Sample volume *Volume of sample added into the column*	0.5 - 1 mL
Fraction volume *Volume of eluent that is collected in a single fraction*	0.5 mL
Discard volume* *Volume of buffer added after sample before particles (e.g. EVs) start eluting*	2 mL†
Void volume *The mobile phase volume of the column*	2.5 mL
Column resin bed volume *Volume occupied by the SEC resin*	9 mL
Wash volume *Volume of buffer added into column before sample*	18 mL

* Discard Volume = Void Volume – Sample Volume

† Discard Volume of 2 mL assumes a Sample Volume of 0.5 mL

Materials needed:

- Elution buffer (e.g. PBS)
- Ascent or Summit instrument or column holder
- 2.0 mL or 1.5 mL tubes (e.g. Eppendorf Protein LoBind)

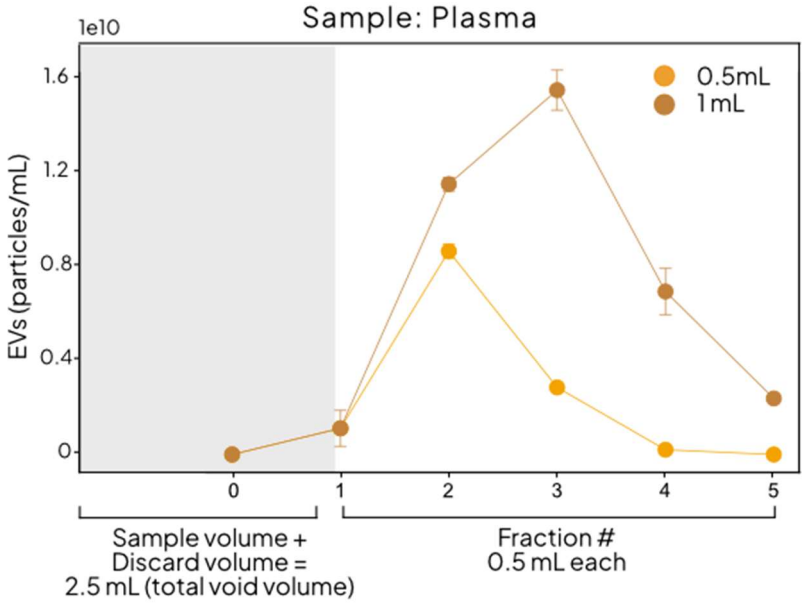
For the best results, we recommend using the Ascent or Summit instrument for fraction collection.

Warnings and precautions:

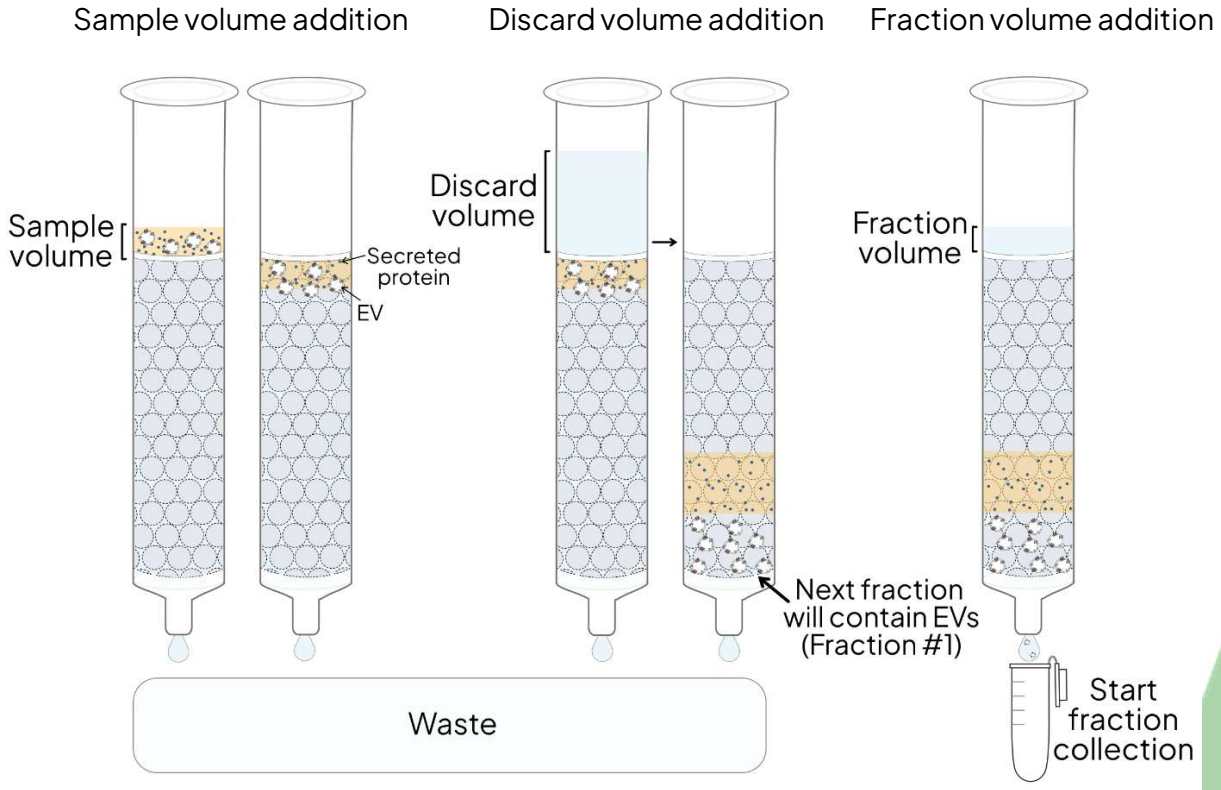
The column storage buffer contains Sodium Azide, which may cause an allergic skin reaction. Avoid ingestion and contact with eyes, skin, and mucous membranes.

EV elution profile:

This plot shows the typical elution profile of EVs in fractions from Apex MM columns. The Ascent instrument was used to collect fractions from 0.5 mL (light orange) or 1 mL (dark orange) human plasma samples. The Atlas EV ELISA kit was used to measure EV concentration.



EV elution process:



Sample and buffer preparation recommendations:

1. Samples should be centrifuged at 2,500 g for 10 minutes to remove any cell debris or aggregates.
2. All buffers should be filtered with a 0.22 µm filter and degassed before use.
3. Column and elution buffer should be at room temperature.

Manual procedure:

Note: Columns must be at room temperature.

1. Remove the top cap of the column.
2. Place the column on a column stand.
3. Remove the bottom cap.
4. Wash the column by adding 18 mL of PBS buffer. Wait for the column to stop dripping before proceeding.
    - If using another buffer, wash with the Wash volume.
5. Gently add 0.5-1 mL sample to the column. Wait for the column to stop dripping before proceeding.
6. Add the Discard volume (1.5 for 1 mL input sample, 2 mL for 0.5mL input sample) of PBS to the column. Wait for the column to stop dripping before proceeding.
7. Add individual Fraction volumes (0.5 mL) of PBS to columns and immediately collect in separate Eppendorf tubes. Wait for the column to stop dripping between each fraction.
8. Typically, EVs elute in fractions 1, 2, and 3 when using the recommended protocol (with fraction 1 starting after Discard volume).
9. Everest Biolabs Atlas EV ELISA kit should be used to optimize EV yield.

Post-run Column Wash, Storage, and Reuse Guidance:

- Reuse is possible but not recommended for critical applications, as performance (particularly purity) may decline after repeated use.
- To reuse columns, first flush column with 8 mL of 0.5M Sodium Hydroxide and 15% Isopropanol, followed by 36 mL (2 wash volumes) of elution buffer (e.g. PBS).
    - It is recommended to run long wash (36 mL) immediately after solvent flush, and additional 18 mL (as described previously) at the beginning of the next run.
- For long-term storage at room temperature, use buffer with 0.05% Sodium Azide, or 20% Ethanol in PBS.
- If bactericide is not used, store columns at 2-8^{0} C.

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