Apex

SEC Column Quick Guide

Revision 3 – May 2, 2025

The Apex size exclusion column (SEC) purifies extracellular vesicles (EVs) from biological fluids such as plasma, serum, urine, cell culture media, or cerebrospinal fluid (CSF).

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 *Volume of the columns, which is the volume between resin particles*	2.5 mL
Column resin bed volume *Volume occupied by the SEC resin*	8.75 mL
Wash volume *Volume of buffer added into column before sample*	17 mL

* Discard Volume = Void Volume – Sample Volume
† Discard Volume of 2 mL assumes a Sample Volume of 0.5 mL

Materials needed:

- Column elution buffer (e.g. PBS)
- Ascent Instrument or column holder
- 2.0 mL or 1.5 mL tubes (e.g. Eppendorf Protein LoBind)

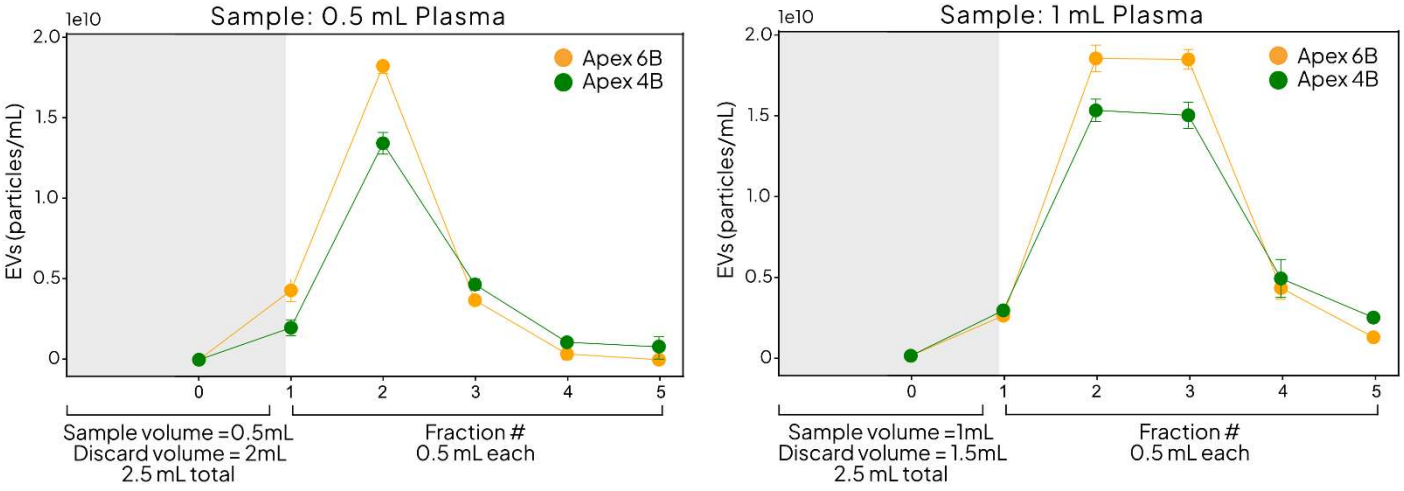
For the best results, we recommend using the Ascent instrument for fraction collection.

Warnings and precautions:

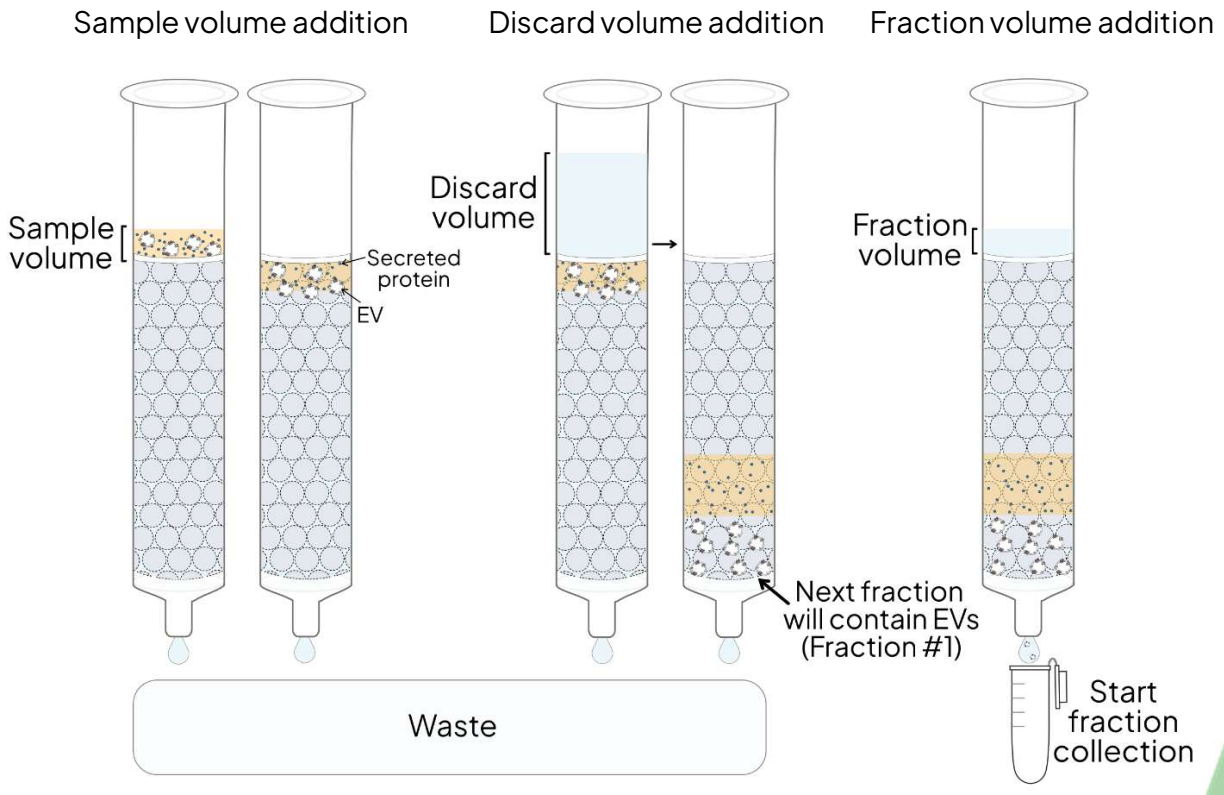
The column storage buffer contains ProClin 200® or Sodium Azide, which may cause an allergic skin reaction. Avoid ingestion and contact with eyes, skin, and mucous membranes.

EV elution profile:

This plot shows the typical EV concentration of fractions from Apex 6B and Apex 4B columns. The Ascent instrument was used to collect fractions from 0.5 mL (left) or 1 mL (right) human plasma sample. 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 buffer 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 17mL of PBS buffer. Wait for the column to stop dripping before proceeding.
    - If using another buffer, wash with at least two wash volumes.
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 - 2 mL) 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 and HSA ELISA kits should be used to optimize EV yield and purity.

Post-run column wash and storage:

- Flush column with 4 mL of 0.5M Sodium Hydroxide followed by 17 mL of elution buffer (e.g. PBS).
- For long-term storage at room temperature, use buffer with 0.05% Sodium Azide , 0.05% of ProClin 200, or 20% Ethanol in PBS.
- If bactericide is not used, store columns at 2-8^{0} C.

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