SEC COLUMN · QUICK GUIDE

Apex MM · Apex 4B · Apex 6B

Size Exclusion Chromatography · Revision 5, June 2026

everest biolabs

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

▲ Column specification

PARAMETER	DESCRIPTION	VALUE
Sample volume	Volume of sample added into the column	**0.5–1 mL**
Fraction volume	Volume of eluent collected in a single fraction	**0.5 mL**
Discard volume *	Buffer added after sample before particles (e.g. EVs) start eluting	**2.0 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 † A 2.0 mL discard volume assumes a 0.5 mL sample volume.

▲ Materials needed

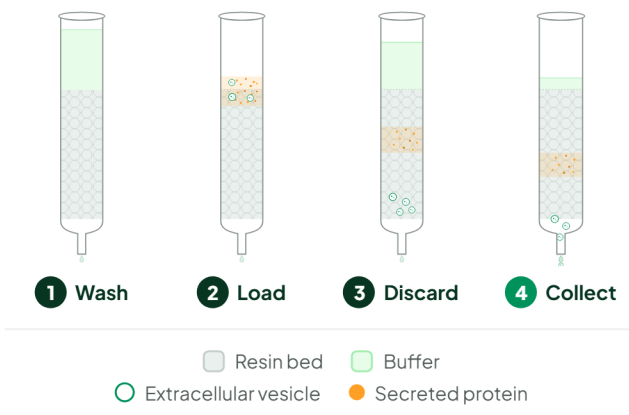
- Elution buffer (e.g. PBS)
- Ascent or Summit instrument, or column holder
- 2.0 mL or 1.5 mL tubes (e.g. Eppendorf Protein LoBind)

▲ Warnings & precautions

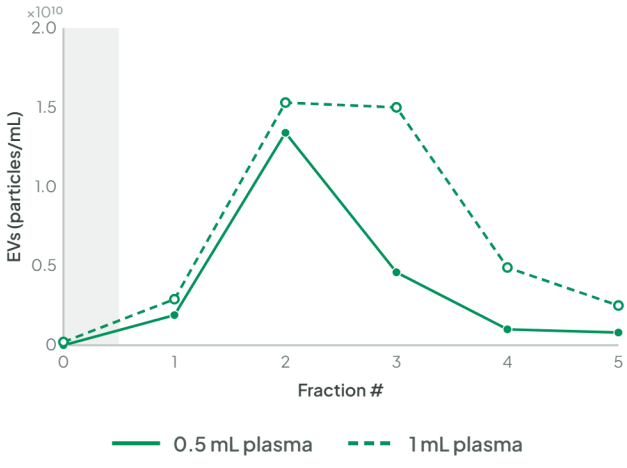
The column storage buffer contains **Sodium Azide** or **Proclin™ 200**. Avoid ingestion and contact with eyes, skin, and mucous membranes.

▲ EV elution

Elution process



Elution profile



EVs elute immediately after the discard volume; collect fractions 1–3. The profile shows EV concentration (Atlas EV ELISA kit) for **0.5 mL plasma (solid)** and **1 mL plasma (dashed)**; the shaded band marks the sample + discard volume.

▲ Sample & buffer preparation

- Centrifuge samples at 2,500 g for 10 minutes to remove cell debris or aggregates.
- Filter all buffers with a 0.22 µm filter and degas before use.
- Bring column and elution buffer to room temperature.

▲ Manual procedure

Note: columns must be at room temperature.

For the best results, use the **Ascent** or **Summit** instrument for washing and fraction collection.

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 with 18 mL of elution buffer (e.g. PBS); wait for dripping to stop.
5. Gently add 0.5–1 mL sample to the column; wait for dripping to stop.
6. Add the Discard volume of elution buffer (1.5 mL for a 1 mL sample; 2.0 mL for a 0.5 mL sample); wait for dripping to stop. Sample volume + Discard volume = Void volume of 2.5 mL.
7. Add Fraction volumes (0.5 mL elution buffer) and collect each in a separate tube; wait for dripping to stop.
8. EVs typically elute in fractions **1, 2, and 3** (fraction 1 starts after the Discard volume). Pool fractions 1–3 (0.5 mL each, 1.5 mL total) to isolate EVs.

▲ Post-run column wash, storage & reuse

Apex 4B / 6B — REUSE PROTOCOL

1. Flush columns with **4 mL of 0.5 M sodium hydroxide**, followed by **18 mL** of elution buffer (e.g. PBS).
2. Add another **18 mL** (as described previously) at the beginning of the next run.

Apex MM — REUSE PROTOCOL

1. Flush the column with **8 mL of 0.5 M sodium hydroxide + 15% isopropanol**, followed by **36 mL** (2 wash volumes) of elution buffer.
2. Add another **18 mL** (as described previously) at the beginning of the next run.

Long-term storage

Room temperature
Store in buffer with **0.05% sodium azide in PBS** or **20% ethanol in DI water**.

2–8 °C
Store columns in buffer, with or without bactericide.