

Apex SEC plate

SEC Plate Quick Guide – Apex 4B, Apex 6B

Revision 3 – May 2, 2026

The Apex SEC plate purifies extracellular vesicles (EVs) from up to 24 samples from biological fluids such as plasma, serum, urine, cell culture media, or cerebrospinal fluid (CSF).

Plate specification:

Sample volume <i>Volume of sample added into the well</i>	0.5 - 1 mL
Fraction volume <i>Volume of eluent that is collected in a single fraction</i>	0.5 mL
Discard volume* <i>Volume of buffer added after sample before particles (e.g. EVs) start eluting</i>	2 mL [†]
Void volume <i>The mobile phase volume of the well</i>	2.5 mL
Well resin bed volume <i>Volume occupied by the SEC resin</i>	9 mL
Wash volume <i>Volume of buffer added into each well before sample</i>	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)
- Summit instrument or Multichannel, retractable pipette
- Waste reservoir
- 24 well Altitude collection plates

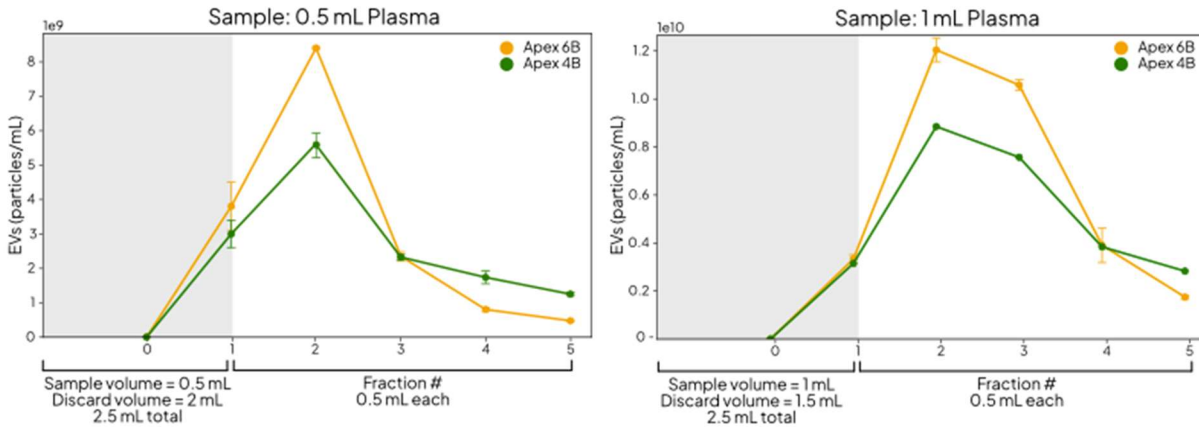
For the best results, we recommend using the Summit instrument for sample loading and fraction collection.

Warnings and precautions:

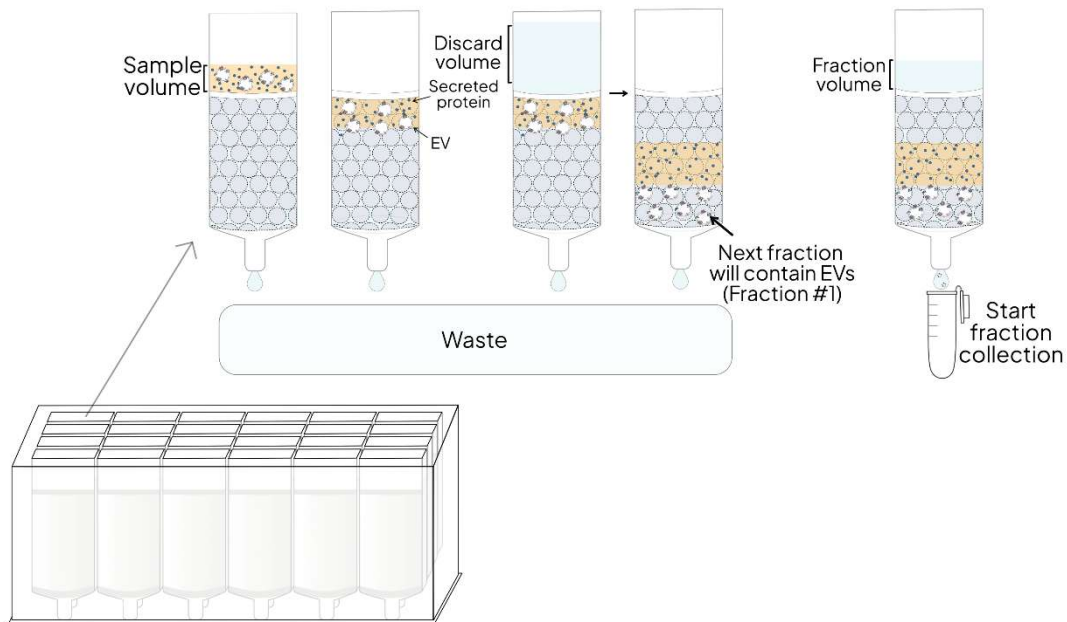
The plate storage buffer contains Sodium Azide, which may cause an allergic skin reaction. Avoid ingestion and contact with eyes, skin, and mucous membranes.

EV elution profile:

This plot shows the typical elution profile of EVs in fractions from wells in the SEC Plate. The Summit instrument was used to collect fractions from 0.5 mL (left) or 1 mL (right) 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. SEC plates and elution buffer should be at room temperature.

Manual Procedure

Note: Plates must be at room temperature.

1. Place the SEC plate over a liquid waste reservoir.
2. Using a multichannel pipette, wash each well with ≥ 18 mL of PBS. Add 4.5 mL per well, wait for full flow-through, and repeat 4x.
3. Wait for all wells to stop dripping before proceeding.
4. Sample Loading: Gently add 0.5–1 mL sample to each well. Wait for all the wells to stop dripping before proceeding.
5. Discard Volume Addition: Add the Discard volume (1.5 – 2 mL) of PBS to each well. Wait for all the wells to stop dripping before proceeding.
For 0.5 mL sample volume, add 2 mL discard volume; for 1 mL sample volume, add 1.5 mL discard volume
6. Carefully move the SEC plate on top of a clean Altitude collection plate.
7. Add 1.5 mL of PBS to each well to elute and pool the EV-rich fraction (fractions 1–3).
If collecting individual fractions, add 0.5 mL of PBS, wait for the wells to stop dripping, move the SEC plate on top of unused wells, and continue to the next fraction.
8. Wait for the plate to drain fully.

Typically, EVs elute in fractions 1, 2, and 3 when using the recommended protocol (with fraction 1 starting after Discard volume).

Everest Biolabs Atlas EV ELISA kit should be used to optimize EV yield.

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

- Flush wells with 4 mL of 0.5M Sodium Hydroxide followed by 18 mL of elution buffer (e.g. PBS), and an additional 18mL (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 wells at 2–8 $^{\circ}$ C.



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