Apex

SEC Column Quick Guide

Revision 1 – November 11, 2024

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). Everest Biolabs Atlas EV and human serum albumin (HSA) ELISA kits can be used to optimize EV yield and purity during fraction collection.

Column Specification:

Column Volume	8.75 mL
Wash Volume	10 mL
Discard Volume*	1.5 mL
Sample Volume	0.5 mL
Fraction Size	0.5mL

* Discard volume 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 (ex. Eppendorf Protein LoBind)

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

Sample and Buffer Preparation Recommendations:

1. Samples should be centrifuged at 2,000 g for 10 minutes and filtered with a 0.45 µm filter before applying to the column to remove any cell debris or aggregates.
2. Elution buffer should be filtered with a 0.22 µm filter and degassed before use.
3. Column and elution buffer should be at the same 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 10mL 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 0.5mL sample to the column. Wait for the column to stop dripping before proceeding.

6. Add the discard volume (1.5mL) of PBS to the column. Wait for the column to stop dripping before proceeding.
7. Add individual fraction volumes (0.5mL) 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 2, 3, and 4 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 for each fraction.

Post-Run column wash and storage:

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

Warnings and precautions:
The column storage buffer contains ProClin 200®, 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 a 0.5mL Human plasma sample. The Atlas EV ELISA kit was used to measure EV concentration.