APEX · SEC PRIMER

Why is SEC becoming the most widely used EV isolation technique?

Apex™ SEC columns are for research use only. Not for use in diagnostic procedures.

Size-exclusion chromatography (SEC) is a commonly used method for extracellular vesicle (EV) and exosome isolation from biological fluids. SEC is easy, reproducible, and provides a high yield of purified EVs that retain their functionality (Table 1). The MISEV2023 guidelines highlight SEC as a highly accessible size-based technique for separating EVs from non-vesicle-associated extracellular proteins.

These advantages are propelling SEC toward becoming one of the most widely used EV isolation techniques. Its main historical drawback: low throughput when performed manually — has, until recently, restricted its utility for biomarker discovery and diagnostic research.

METHOD COMPARISON

Isolation method	Reproducibility	Ease of use	Purity	Yield
SEC	+++	+++	++	+++
Precipitation	++	+++	+	+++
Ultracentrifugation	+	++	+	+
Density gradient	++	+	+++	+

Table 1. *Performance comparison of the most widely used EV and exosome purification techniques.*[1,2]

HOW DOES SEC PURIFY EVS?

SEC separates particles and biomolecules based on size. EVs range around 30–200 nm, while contaminant molecules and particles range from 7–68 nm.[3] In a SEC column, EVs are separated from smaller particles as they flow through porous Sepharose resin beads: most contaminants in biological fluids (albumin, immunoglobulins, HDL, and LDL) are smaller than the pore size, so they enter the pores, travel more slowly, and elute later (Figure 1). Sepharose resin is available with different agarose content, giving different pore sizes (Table 2), allowing columns to be optimized for different EV purity and yield.[3,4] Sepharose CL-6B and CL-4B allow EVs across the expected size range to elute in the void volume. The larger pore size of CL-2B is sometimes used to further deplete larger lipoproteins (IDL, VLDL); this added purity comes with significant EV loss that limits its use for low-abundance EV subpopulations.[5,6]

RESIN PORE SIZE & SELECTIVITY

The choice of Sepharose resin sets the balance between EV purity and yield. Figure 1 illustrates the separation principle and a typical EV elution profile, and Table 2 summarizes how agarose content determines pore size and exclusion limit across the CL-2B, CL-4B, and CL-6B resins used in EV isolation.

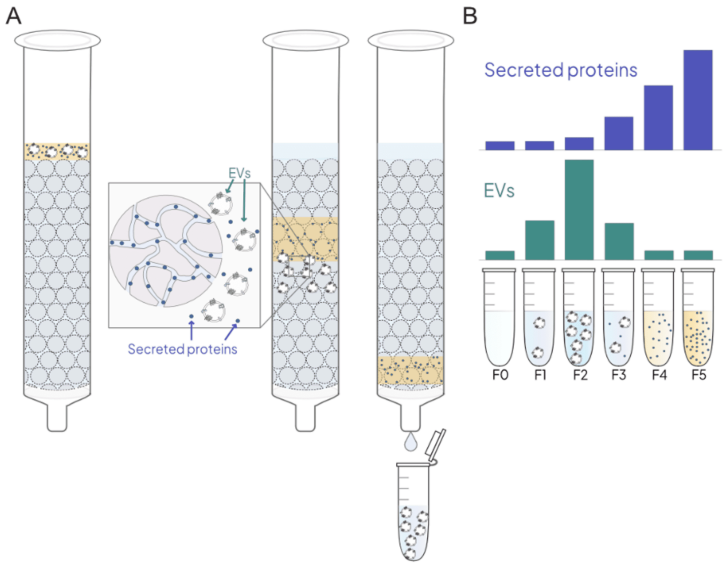

Figure 1. (A) Principle of size-exclusion chromatography. (B) Typical elution profile of EVs and secreted proteins after SEC fractionation.

Resin type	Agarose amount	Exclusion limit
Sepharose CL-2B	2%	75 nm
Sepharose CL-4B	4%	35 nm
Sepharose CL-6B	6%	20 nm

Table 2. *Specifications of cross-linked Sepharose resins.*

SPEED UP & STANDARDIZE EV ISOLATION

Everest Biolabs solutions bring reproducibility and throughput to SEC-based EV isolation:

- **Apex 6B column**: Sepharose CL-6B, optimized for the highest EV recovery. Ideal for targeted downstream analysis: immunoassays, label-based imaging and flow assays, Western blots, and marker-specific pull-downs.
- **Apex 4B column**: Sepharose CL-4B, optimized for the highest purity. Ideal for unbiased downstream analysis such as proteomics and RNA-seq.
- **Ascent instrument**: automated, high-throughput EV isolation with SEC columns, running up to 8 samples in parallel.

REFERENCES

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5. Ter-Ovanesyan D, Gilboa T, Budnik B, et al. Improved isolation of EVs by removal of both free proteins and lipoproteins. *eLife*. 2023.
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