APEX · MULTI-MODE

Why Apex MM columns outperform conventional SEC for isolating EVs from plasma or serum

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

Size-based EV isolation and its trade-offs

Extracellular vesicles (EVs) are essential to diagnostics and therapeutic research, serving as rich sources of biomarkers and key mediators of intercellular communication. Size-exclusion chromatography (SEC) is widely used for isolating EVs owing to its simplicity, reproducibility, and gentle purification conditions.

Sepharose CL-4B provides suitable pore sizes for efficient EV isolation from plasma or serum. Here, we examine the limitations of large- and small-pore resins for separating lipoproteins from EVs, and introduce Everest Biolabs' Apex MM columns, which overcome them. The Apex MM column leverages SEC and multi-mode resins to significantly enhance EV purity against lipoproteins while maintaining high yield and minimal size bias against small EVs.

The lipoprotein co-elution challenge

EVs typically range from 30 to 200 nm,[1] overlapping in size with various lipoproteins: HDL (~8–13 nm), LDL (~18–25 nm), and VLDL (~30–80 nm).[2] This overlap presents a considerable challenge, as traditional SEC columns can co-isolate these lipoproteins, compromising sample purity.

Smaller-pore resins (Sepharose CL-4B, ~35 nm) tend to increase co-elution of lipoproteins because of the restricted separation range, while larger-pore resins (Sepharose CL-2B, ~70 nm) may improve purity but reduce EV yield.[2,4]

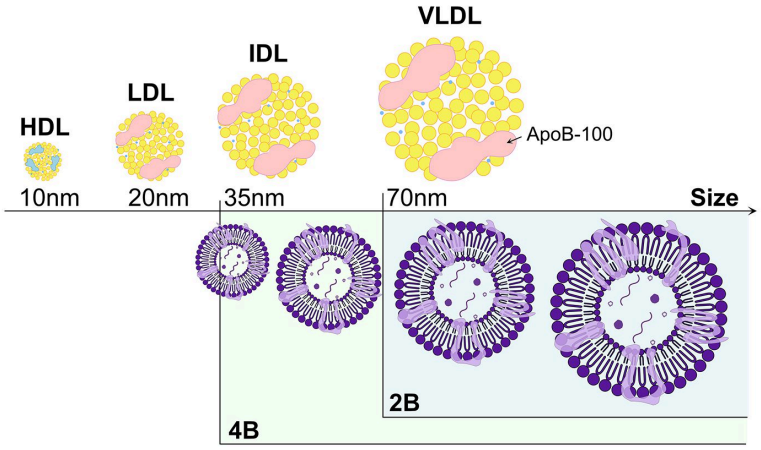

Figure 1. Lipoprotein depletion using SEC columns. Major plasma lipoproteins and their size overlap with EVs, relative to SEC resins with 35 nm (4B) and 70 nm (2B) cutoffs. The 2B resin more effectively separates EVs from lipoproteins, but at the cost of reduced EV yield and enrichment for larger EVs.

INTRODUCING APEX MULTI-MODE

Everest Biolabs developed Apex MM columns by combining a small-pore SEC resin with a multi-mode (MM) resin that uses hydrophobic interaction and ion-exchange to selectively bind and remove lipoproteins and other soluble contaminants, significantly enhancing EV purity. While EV yield from Apex MM is lower than from the Apex 4B column, it is higher than from the competitor qEV70 column. Importantly, Apex MM achieves the highest purity, measured by reduced levels of major protein contaminants: apolipoproteins (ApoA1 and ApoB-100) and human serum albumin (HSA). Apex MM columns are particularly advantageous for unbiased downstream analyses such as proteomics, whereas Apex 4B columns are better suited for targeted assays like ELISAs or Western blots.

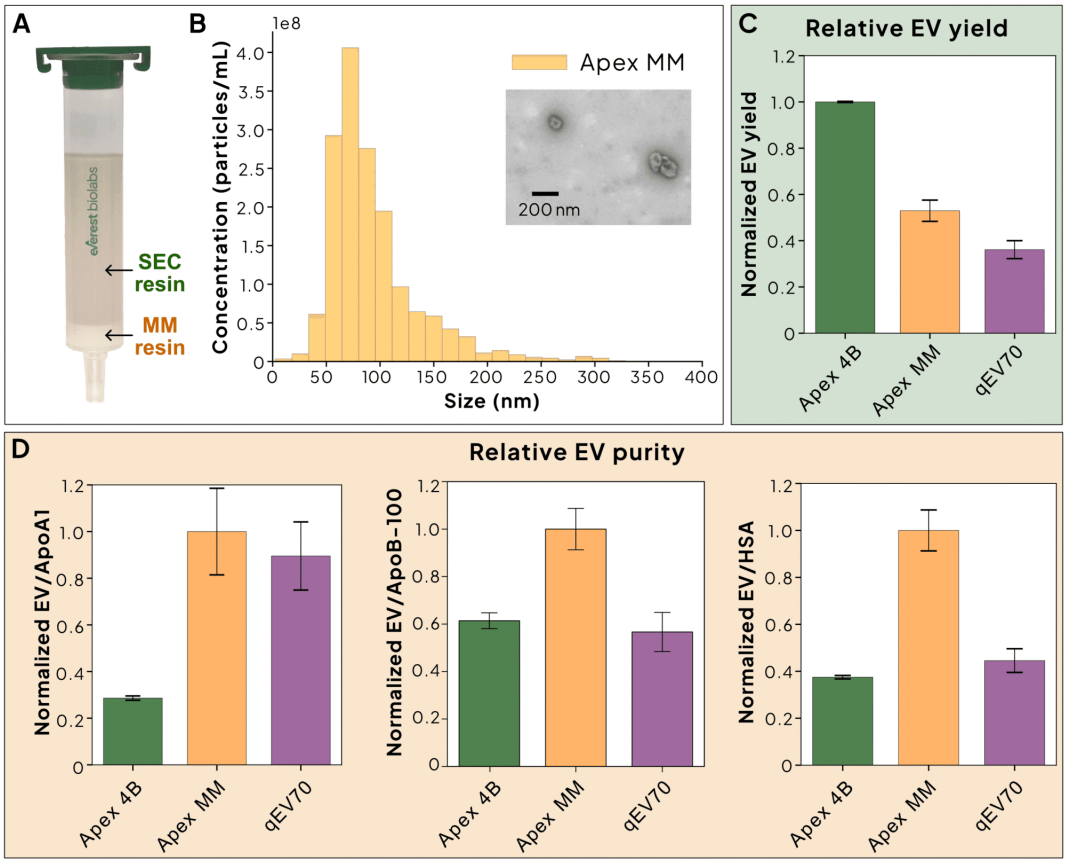

Figure 2. Performance of Apex MM columns. (A) Structure of a multi-mode column, with a secondary resin below the SEC layer to enhance lipoprotein and secreted-protein depletion. (B) Nanoparticle tracking analysis (NTA) of pooled EV fractions isolated from plasma using the Apex MM column, with a representative TEM image showing typical EV morphology. (C) Plasma processed using Apex 4B, Apex MM, and qEV70 columns; EV levels quantified by Atlas EV ELISA and normalized to the highest measured value. (D) ApoA1, ApoB-100, and HSA measured by ELISA; EV purity calculated by dividing EV level by each contaminant level, normalized to the highest column-specific ratio.

CHOOSING THE RIGHT COLUMN

Parameter	Apex MM	Apex 4B	qEV70
Resin composition	Cross-linked agarose + multi-mode	4% cross-linked agarose	Proprietary
Size cutoff	< 50 nm	~35 nm	~70 nm
Secreted-protein depletion	Very high	High	High
Lipoprotein depletion	Very high	Moderate	Very high
EV yield	Moderate	High	Low
Recommended applications	Untargeted: mass spec, RNA-seq, NTA, flow cytometry	Targeted protein assays (ELISA / Western)	—
Soluble-protein isolation	No, trapped in MM resin	Yes	No, small EVs elute with soluble proteins

Table 1. *Comparative analysis of SEC columns for isolating EVs from plasma or serum.*

SUMMARY

Everest Biolabs' Apex MM columns overcome limitations inherent in traditional SEC columns by effectively managing the trade-off between purity and yield. By providing superior EV purity, maintaining optimal yield without introducing size biases, and preserving EV functionality, Apex MM columns significantly enhance biomarker discovery and therapeutic research applications. The Apex MM columns are compatible with the Ascent instrument, allowing for the simultaneous running of 8 samples automatically.

REFERENCES

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4. Guo S, et al. Purification of exosomes — a comparison of methods and market analysis. *DiVA Portal*. 2021.