APEX + ATLAS · WITH PARTICLE METRIX

Advancing plasma-derived EV analysis through high-purity isolation and particle-specific quantification

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INTRODUCTION

Everest Biolabs and Particle Metrix have partnered to establish a unified workflow for extracellular vesicle (EV) analysis, combining high-purity size-exclusion chromatography (SEC) isolation using Apex SEC columns with precise quantification via advanced nanoparticle tracking analysis (NTA).

A major challenge in EV research is that most particles in plasma and serum are not EVs, but lipoproteins and protein aggregates that overlap in size with vesicles. These contaminants can dominate total particle counts, obscure true EV signals, and lead to inaccurate conclusions about EV abundance or biomarker levels. High-purity EV isolation is therefore essential for quantitative, reproducible downstream analyses.

Apex SEC columns: optimizing purity and yield

Everest's Apex SEC columns (4B, 6B, and MM) enable scalable EV isolation across diverse biofluids. Each composition provides a distinct balance between yield and purity, letting researchers select the optimal configuration for their downstream application. The Apex MM column, in particular, features a multi-mode resin layer that combines SEC with additional interaction mechanisms to more effectively remove lipoproteins and plasma proteins, providing higher EV purity than conventional SEC columns, yielding cleaner fractions ideal for untargeted analysis such as proteomics.

Particle Metrix NTA: labeled and unlabeled tracking

The Particle Metrix ZetaView® Evolution platform enables parallel measurement of unlabeled (total) and fluorescently labeled particle populations. Using membrane dyes, the fraction of membrane-enclosed particles can be determined. Combined with the Particle Metrix F-NTA Tetraspanin Detection Kits, which simultaneously label the EV markers CD9, CD63, and CD81 — the proportion of true EVs within the total particle population can be quantified. These approaches enable both purity assessment (percentage of membranous particles or EVs) and precise determination of EV concentration (EVs/mL).

COMBINING SEC WITH NTA ANALYSIS

When EV fractions are analyzed using both total particle counts (unlabeled NTA) and EV-specific measurements (F-NTA or ELISA), the ratio between total and labeled particles serves as a sensitive indicator of purity (Figure 1). As isolation purity improves, this ratio approaches 100%, reflecting removal of non-EV particles and enrichment of genuine vesicles. A strong correlation is observed between F-NTA results and Atlas EV ELISA measurements, supporting the consistency and accuracy of the combined approach.

Figure 1

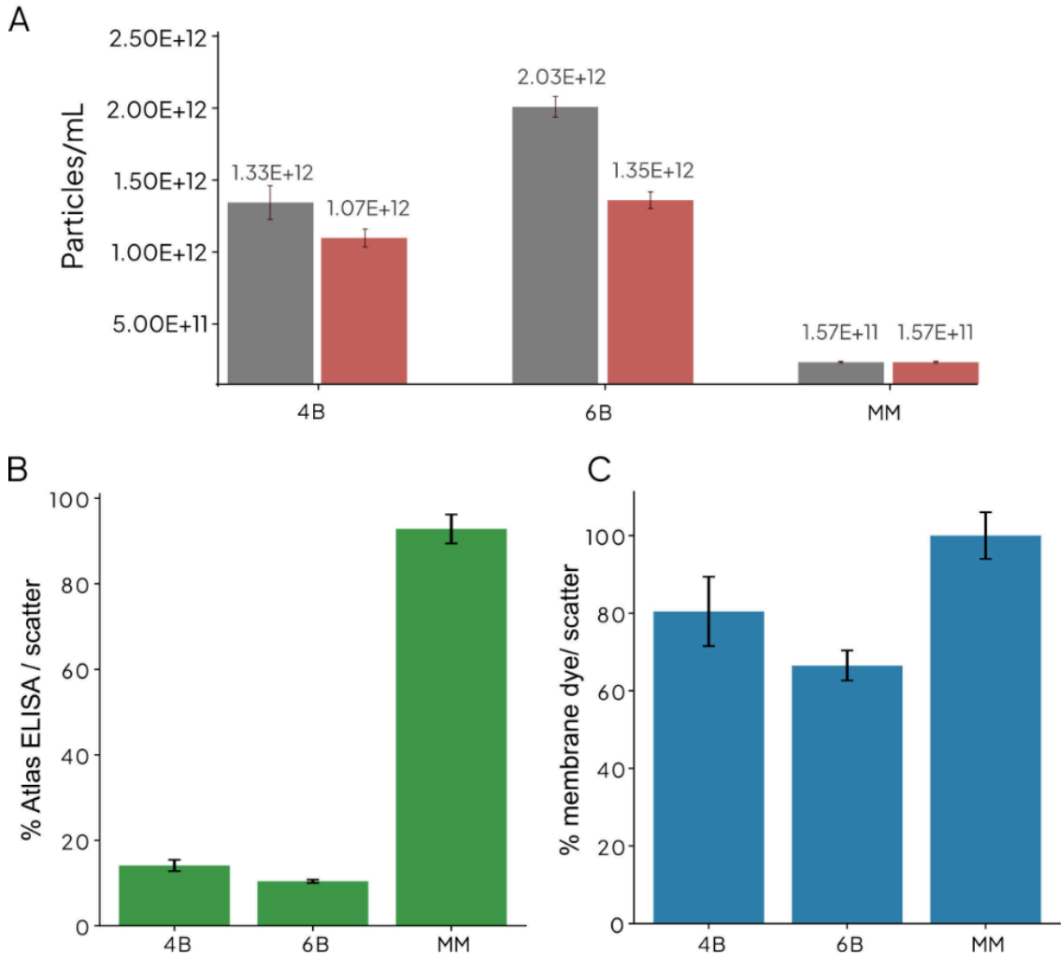

Figure 1. Comparison of EV purity across SEC columns. (A) Total particle concentration (NTA scatter, gray) and membrane dye–positive particle counts (red) for each Apex column type. (B) EV-specific Atlas ELISA signal as a percentage of total particle scatter. (C) Fraction of membrane dye–positive particles relative to total scatter (% membrane dye/scatter), indicating the proportion of lipid-bound vesicles. The Apex MM column achieved the highest EV purity, whereas Apex 6B provided greater total particle recovery but lower marker-specific enrichment.

Antibody labeling of low-purity samples such as plasma EVs is challenging: there is a trade-off between achieving sufficient labeling and avoiding background from excess unbound antibodies, often requiring extensive dilution that compromises accuracy. Performing the labeling step *prior* to SEC fractionation overcomes this (Figure 2). A higher antibody concentration during staining improves labeling efficiency; the subsequent SEC step removes unbound antibodies, preventing background fluorescence. This yields more tracked fluorescent particles, improving statistical robustness and giving brighter, faster, more accurate EV detection.

Figure 2

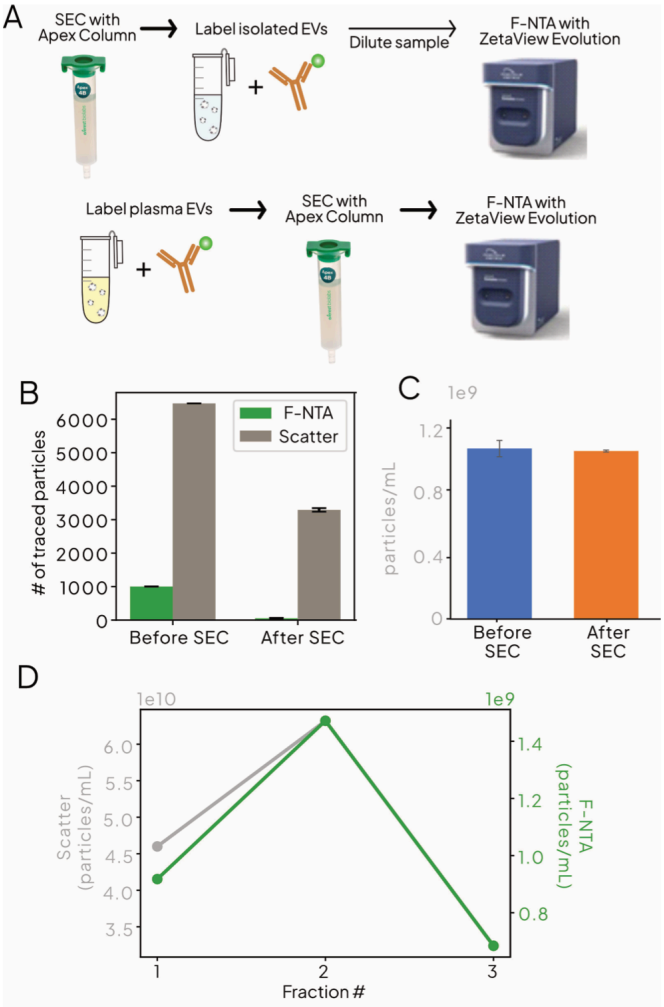

Figure 2. Fluorescent NTA (F-NTA) before and after SEC. (A) Workflow: EVs labeled either before or after SEC using Apex 6B columns, then analyzed by F-NTA with the ZetaView Evolution. (B) Total particle count (scatter) and fluorescent particle count (F-NTA) before and after SEC show that labeling plasma prior to SEC increases detectable fluorescent particles. (C) Improved statistical robustness (reduced error bars) with consistent outcome between pre- and post-SEC labeling. (D) Fraction-resolved scatter (gray) and F-NTA (green) showing correlation and the expected EV elution profile in Apex columns.

COMBINED WORKFLOW: EV-SPECIFIC INSIGHTS

Together, Apex SEC columns and the ZetaView® Evolution deliver complementary insights into EV biology. SEC efficiently separates EVs from contaminating particles, while the ZetaView® quantifies both total and EV-specific populations. The convergence of total and labeled particle counts after SEC highlights the purity of the isolation process, and pre-labeling EVs before SEC enhances sensitivity in fluorescence NTA.

This integrated workflow establishes a robust framework for high-precision EV characterization, linking isolation purity with quantitative, particle-specific analysis.

METHODS

Plasma fractionation using Apex SEC columns
Apex SEC columns were pre-equilibrated with 17 mL of 1× PBS. 0.5 mL of pooled plasma was loaded, followed by 2 mL of PBS as discard volume; 0.5 mL fractions were then collected. The SEC run was performed on the automated Ascent instrument.

Antibody staining of plasma EVs
For labeling prior to SEC, 2 mL of plasma was incubated 1 h with 10 µL each of CD9, CD63, and CD81 antibodies (Particle Metrix F-NTA EV Tetraspanin Detection Kit). SEC fractionation followed the manufacturer's recommendations using Everest Apex columns; the first three fractions were used for analysis on the ZetaView® Evolution. Post-SEC labeling followed the kit protocol.

Membrane staining of plasma EVs
1 µL of fraction 2 from the Apex SEC columns was incubated 1 h with 1 µL of CellMask™ Deep Red (Thermo Fisher Scientific), diluted 1:2000 in PBS.

NTA and F-NTA analysis
Measurements were performed on the ZetaView® Evolution quatt instrument using ZetaSphere Software. The PMX EV PAN Staining method settings were applied for all analyses.