Optimized Tools and Protocols for Fast and Standardized Fluorescent Analysis of EVs

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Introduction

Fluorescent nanoparticle tracking analysis (F-NTA) enables powerful characterization and molecular profiling of extracellular vesicles (EVs) [1,2]. Yet, reliable measurements require optimized antibody labelling, robust positive controls, and suitable standards for quantitative analysis. Here, we present a ready-to-use antibody labelling protocol by Particle Metrix optimized for efficient EV labelling without the need for washing away the excess dye. Complementing this workflow, HansaBioMed's superior EV standards provide reliable positive controls for antibody labelling, while advanced fluorescent bead standards enable fully quantitative fluorescence measurements. Together, these solutions simplify FNTA workflows and support reproducible, sensitive, and quantitative EV characterization.

Materials and Methods

HansaBioMed's TetraEVs derived from HEK293 cells engineered to overexpress CD63 and regular HEK293-derived EVs were labelled with F-NTA CD63 Detection Antibody 488 (Particle Metrix) according to the product data sheet (Figure 1) [3]. Antibody-only sample was prepared as a blank control. After 1 hour incubation, samples were diluted with PBS up to 2 mL and measured on ZetaView Evolution. HansaBioMed's fluorescent beads (four brightness levels) were used to standardize the fluorescence intensity signal into FITC equivalent reference fluorophore (ERF) units.

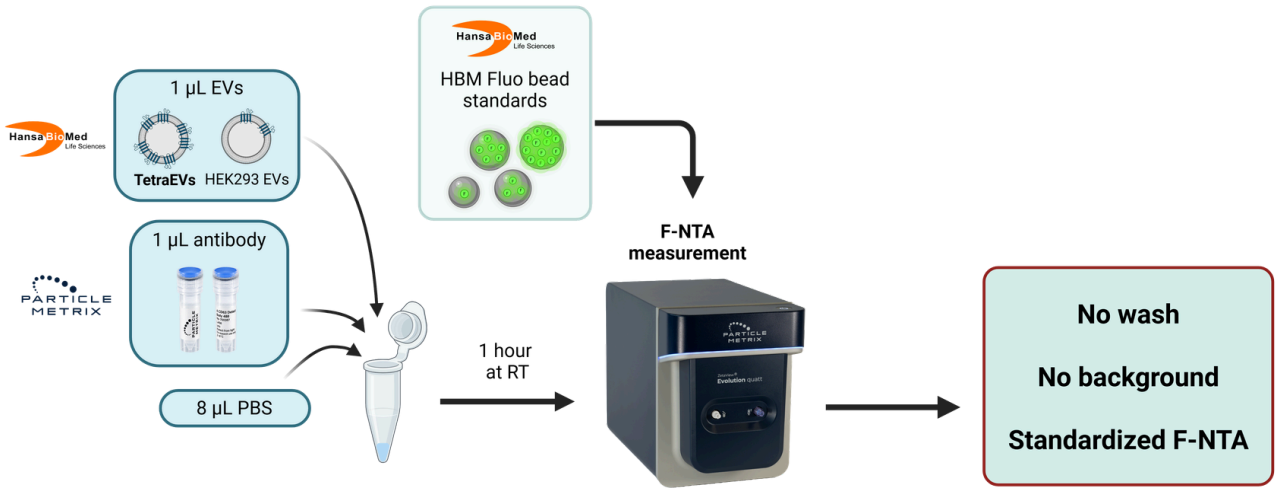

Figure 1: F-NTA workflow

Results

HansaBioMed's fluorescent beads showed high linearity on F-NTA and arbitrary median fluorescence intensity (MFI) units were successfully converted into standardized FITC ERF units (Figure 2).

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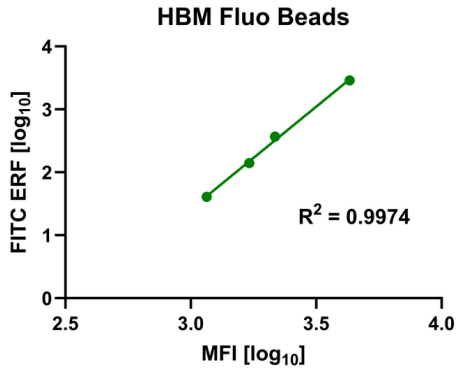

HBM Fluo Beads	MFI [log10]	FITC ERF	FITC ERF [log10]
P1	3.634	2883	3.460
P2	3.336	369	2.567
P3	3.234	141	2.148
P4	3.064	41	1.613

Figure 2: HansaBioMed's fluorescent bead standards measured on F-NTA.

TetraEVs yielded ~60-fold higher labelling efficiency and ~5-fold higher CD63 expression than regular HEK293 EVs, as measured by F-NTA, with >80% of EVs being CD63-positive (Figure 3).

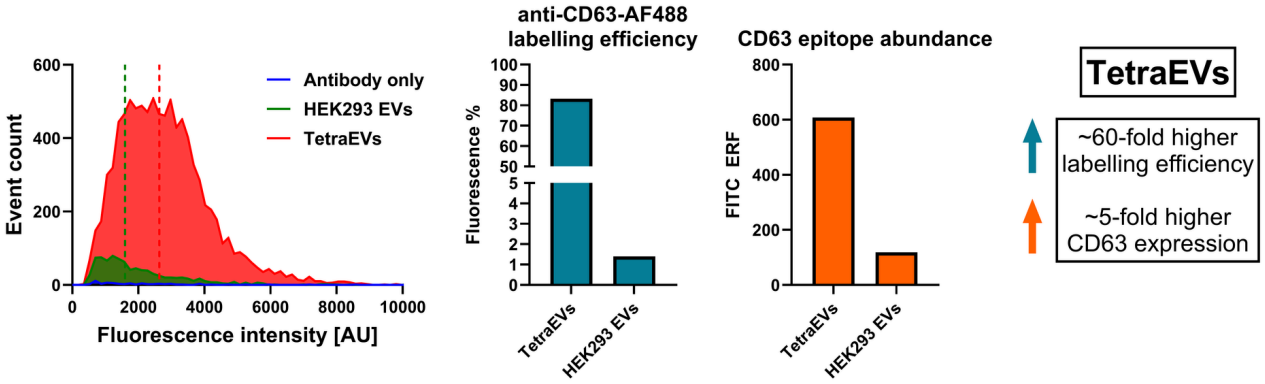

Figure 3: TetraEVs and regular HEK293 EVs labelled with CD63 detection antibody. Dotted lines represent median values.

Conclusion

- HansaBioMed's fluorescent bead standards enable advanced fluorescence analysis and quantification of biomarker expression on a single-particle level in standardized FITC ERF units.
- The optimized labelling protocol using the Particle Metrix CD63 detection antibody enables quick and direct biomarker profiling of EVs without the need to remove the excess dye.
- HansaBioMed's TetraEVs exhibit markedly higher CD63 expression compared with regular HEK293 EVs, making them a superior positive control for EV labelling protocols.

References

[1] Mladenović, D., Brealey, J., Peacock, B., Koort, K., & Zarovni, N. (2025). Quantitative fluorescent nanoparticle tracking analysis and nano-flow cytometry enable advanced characterization of single extracellular vesicles. Journal of Extracellular Biology, 4, e70031. https://doi.org/10.1002/jex2.70031

[2] Hendrix, A., Lippens, L., Pinheiro, C., Théry, C., Martin-Jaular, L., Lötvall, J., Lässer, C., Hill, A. F., & Witwer, K. W. (2023). Extracellular vesicle analysis. Nature Reviews Methods Primer, 3, 56. https://doi.org/10.1038/s43586-023-00240-z

[3] Particle Metrix protocol for F-NTA CD9/CD63/CD81 Detection Antibody 488 (#700387) https://cdn.shopify.com/s/files/1/0828/9203/0282/files/Antibodies_488.pdf?v=1741603704

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