Reliable Single-EV Analysis: The PICO Assay with TetraEV Standards for High-Reproducibility Research

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Introduction

The field of extracellular vesicle (EV) research is challenged by the scarcity of reliable, stable controls and devices that can deliver reproducible results, regardless of variations across measurement days or operators. This application note directly addresses these critical limitations. The TetraEV products by HansaBioMed are developed to overexpress CD63 marker, thus providing robust stability and control essential for rigorous EV research. At the same time, the innovative PICO assay offers a unique method for characterizing and quantifying single EVs by their surface markers. This powerful combination delivers the exceptional, urgently required reproducibility in EV research, eliminating the need for complex reference standards.

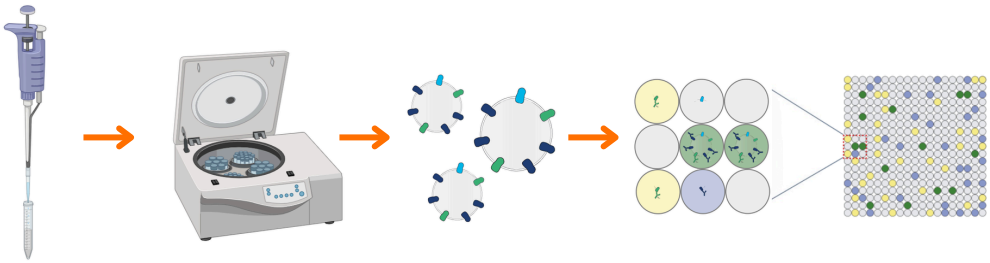

Figure 1: Experimental workflow consisting of TetraEVs reconstitution with MilliQ water followed by PICO assay

Materials and Methods

For manufacturing of TetraEVs, HEK293 cells were transfected to overexpress CD63. Then, HEK293 cells medium was subjected to sequential filtration by TFF-MV (SKU: HBM-TFF-MV) and TFF-EVs-S (SKU: HBM-TFF-EVs-S). Finally, the concentrated TetraEVs sample was further purified by size exclusion chromatography columns (SKU: HBM-PEV-5). Isolated and purified EVs are characterized by NTA for size and concentration measurements and by ELISA for marker assessment.

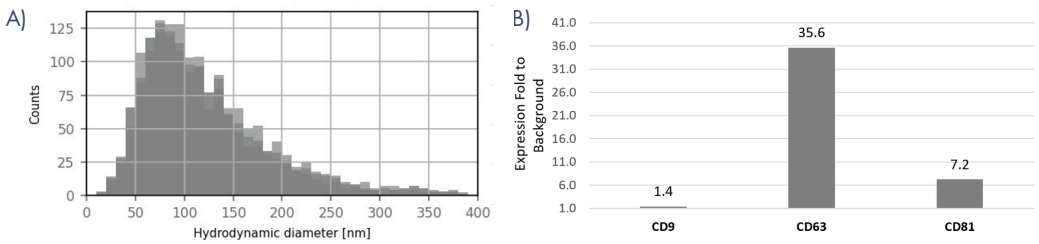

Figure 2: A) Size distribution profile of TetraEVs, measured by NTA, B) ELISA-based surface marker assessment of TetraEVs

The PICO assay was performed using the AMC Kit and EV-LABs (CD9 and CD63 DNA-labeled antibodies) from PICO BioScience. After overnight incubation with 2 µl of EVs at given concentrations, the samples were diluted, and the DNA labels were amplified by dPCR. Absolute quantification of EVs was achieved by processing the dPCR data with the PIQuant software (PICO BioScience).

Results

TetraEVs-CD63 were resuspended at 1 µg/µl and diluted to the indicated concentrations. Relative quantification (couplex counts) shows that, after roughly a 100-fold dilution (13.9 µg/µl), both endogenous (CD9+) and overexpressed (CD63+) markers are still detectable.

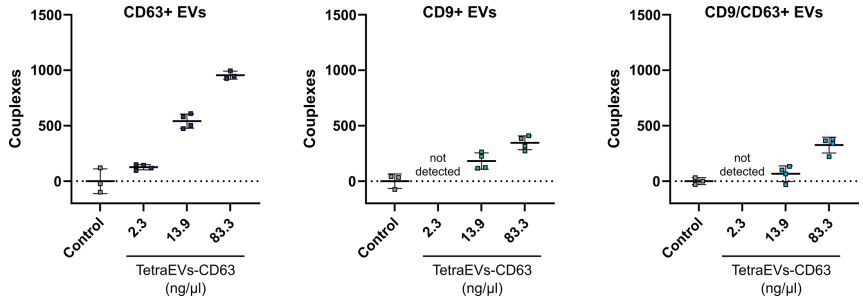

Figure 3: PICO assay of reconstituted TetraEVs-CD63 for the detection of CD9, CD63 and CD9/CD63+ vesicles

Compared against parental HEK293 EVs, PICO detected a high increase in the number of CD63+ vesicles, while the number of CD9+ vesicles remained stable.

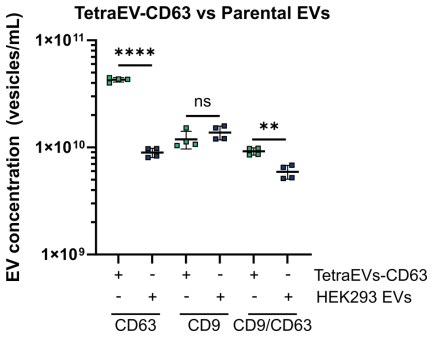

Figure 4: Comparison of tetraspanin profiles in TetraEVs-CD63 vs HEK293 EVs using the PICO assay

When tested in different operating conditions and after frozen storage, both the TetraEVs-CD63 and HEK293 EVs showed great reproducibility.

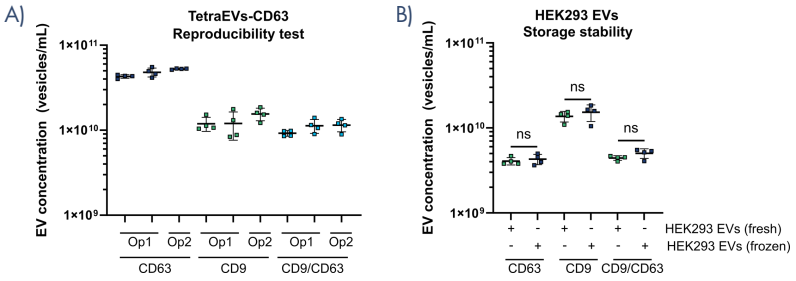

Figure 5: A) Reproducibility of the TetraEVs-CD63 in a series of PICO experiments performed by two operators on three different dPCR devices and three different days. B) Comparison of the number of HEK293 EVs before and after frozen storage.

Conclusion

The TetraEVs-CD63 are specifically engineered to overexpress the canonical marker CD63. Quantification via the PICO assay, which requires no external calibration, confirmed a robust five-fold increase in CD63+ populations while ensuring other markers, such as CD9, remained unaffected.

Setting a new benchmark for reliability, the PICO assay delivers unmatched reproducibility across different dPCR platforms, operators, and experimental days. Combined with the high stability of TetraEVs-CD63, this establishes a premier standard for EV research. Given the assay's exceptional sensitivity (requiring <30 ng per run), a single 30 µg vial provides a reliable positive control for hundreds of PICO experiments.

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