Standardizing F-NTA Measurements

Evaluation of a Four-Wavelength Instrument for bio-marker detection on cell-line EVs

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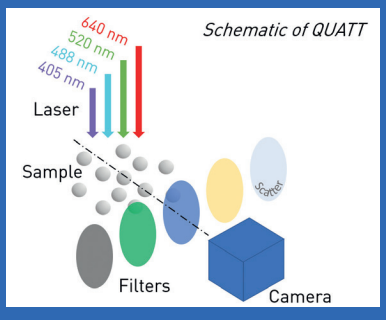
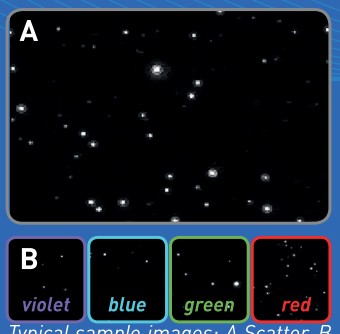
Introduction

Nanoparticle Tracking Analysis (NTA) has emerged as a critical-path technology for characterizing EVs and viruses. In combination with fluorescence detection, F-NTA enables the user to perform biomarker detection on the single particle level, thus enhancing population-specific EV concentration measurements. Classic NTA instruments are equipped with one laser, requiring phenotyping in sequence. Multi-fluorescence detection of four biomarkers on one sample by NTA is shown for the first time.





Typical sample images: A Scatter, B fluorescence channels

Material and Methods

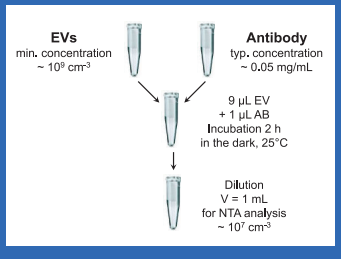
NTA ZetaView(R) QUATT with 405, 488, 520 and 640 nm laser and long pass filters

HCT116-EVs (HansaBioMed LTD) were reconstituted in water by vortexing for 30 s

MSC-EVs were purified by PEG precipitation followed by ultracentrifugation

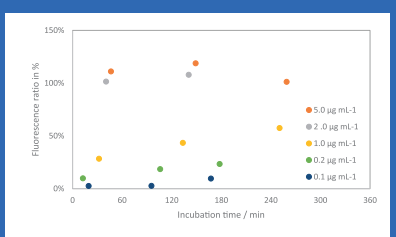
Antibodies: phenotyping was performed with direct labelled CD9, CD41, CD63 and CD81 antibodies

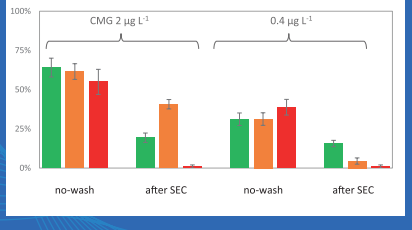
Membrane dyes: Cell Mask® Green (CMG), Orange (CMO) and Deep Red (CMDR)



Labeling Protocol

Influence of concentration and incubation time of membrane dyes CMG.

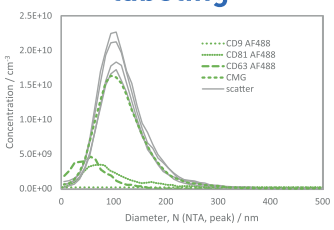


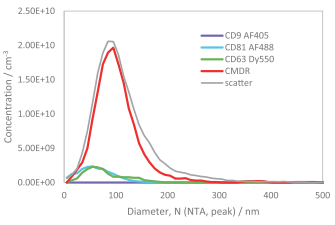


Comparison of no-wash strategy and removal of unbound dye by SEC.

3 Antibodies + Membrane dye

Single labeling vs. **Four-fold labeling QUATT**





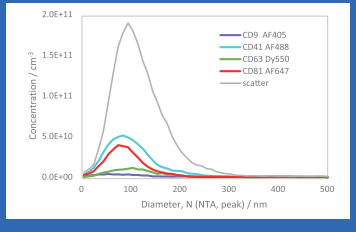
4 Samples
8 Measurements
~40 µL Incubation volume

1 Sample
5 Measurements
~10 µL Incubation volume

Sequence of three single labeling experiments CD9/63/81 and CMDR to monitor vesicle concentration.

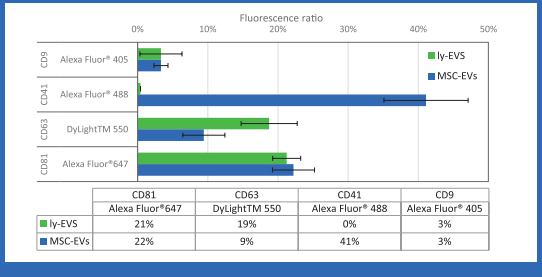
Four-fold labeling of one sample. The fluorescent populations conjugated with antibodies show sizes below 100 nm.

4 Antibodies



Four-fold labeling with CD9/41/63/83 on one sample.

Comparison of phenotype of lyophilized and MSC EVs. CD41 is present in platelet derived EVs.



	CD81 Alexa Fluor®647	CD63 DyLightTM 550	CD41 Alexa Fluor® 488	CD9 Alexa Fluor® 405
ly-EVS	21%	19%	0%	3%
MSC-EVs	22%	9%	41%	3%

Conclusions

- NTA scatter measures **total particle concentration**, NTA fluorescence results in bio-specific concentration. Both are needed to estimate **sample purity.**
- Multi-wavelength F-NTA **reduces total measurement time, minimizes the amount of sample** required, and improves experimental reproducibility.
- Control of the EV-to-antibody-dye ratio improves the F-NTA detection yield. Parameters such as antibody purity should be monitored

Acknowledgment

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