ZetaView® Scanning NTA

# Purity check of Extracellular Vesicle preparations by Particle Metrix ZetaView® Nanoparticle Tracking Analysis

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## Abstract

Over the past decade Nanoparticle Tracking Analysis (NTA) has emerged as an essential characterization tool for Extracellular Vesicle and Exosome research (Konoshenko et al., 2018, Giebel & Helmbrecht, 2017, Soo et al., 2012). In combination with fluorescence detection (f-NTA) the technology enables the user to perform purity testing with unspecific membrane dyes as well as phenotyping with specific biomarkers. A fast and reliable method to analyze the purity of EV samples after purification is described using membrane stains and the Particle Metrix ZetaView® instrument.

## Introduction

Exosomes are very small (30 to 150 nm) membrane encompassed Extracellular Vesicles (EV) which are secreted by almost all biological cells (Raposo et al. 2013, Yanez-Mo et al. 2015). They play a crucial role in a huge amount of cellular processes such as (I) maintaining the homeostasis (Baixauli et al. 2014), (II) cell-to-cell communication (Mathivanan et al. 2010), (III) the metastasis of tumors (Becker *et al.* 2016), (IV) inflammatory processes (Console *et al.* 2019) and many (V) other patho-physiological activities (Record et al. 2014). Furthermore, secretion of exosomes and membrane vesicles was also demonstrated in plants (Stanly et al., 2016) and bacterial systems (Deatherage & Cookson, 2012). Due to almost ubiquitous presence of Extracellular Vesicles and their important functions, EVs and exosomes are currently investigated in many laboratories worldwide. Among other technologies like Western Blotting, Elisa or electron microscopy, Nanoparticle Tracking Analysis (NTA) is a widely used and state-of-the-art technology to characterize EVs (Rupert et al., 2017). Unfortunately, NTA scatter technology alone is not enough to discriminate between biological vesicles like EVs or liposomes and inorganic particles such as ultra-fine bubbles, salt precipitates or protein aggregates. Here we report a fast and reliable method to specifically stain and measure biological material with unspecific, lipophilic membrane dyes and the Particle Metrix ZetaView® Instrument (Meerbusch, Germany) allowing the differentiation of membranous particles from non-membranous particles. The unique scanning technology of the instrument combines very short acquisition times of 0.5 to 1 seconds for low fluorescence bleaching with an unrivalled statistical relevance of the data.

## Methods

Lyophilized exosomes derived from a HTC116 cell line (HansaBioMed, Estonia) have been resuspended with ultra-pure water (Carl Roth, Germany) at 1µg/µl as described in the manufacturer's manual. The unspecific, lipophilic membrane dye Cell Mask© Deep Red (CMDR, Thermo Fisher, USA) was diluted to a final concentration of 1:1.000 also in ultra-pure water (Carl Roth, Germany). 9 µg EVs were thoroughly mixed with 1 µl pre-diluted CMDR and incubated for 2 hours at room temperature

in the dark. The staining mix was then diluted 1:1000 with 1 X PBS (Thermo Fisher, USA) to accomplish a total vesicle concentration of about 5.0 x $10^7$ particles/ml. The sample was measured for size and concentration in scatter mode (488 nm laser) as well as in fluorescence mode (640 nm laser) with Particle Metrix ZetaView QUATT® and the ZetaView® software version 8.05.10 with anti-bleach technology (Particle Metrix, Germany). The purity was calculated using the following formula:

$$purity = \frac{conc.\ fluorescence\ mode}{conc.\ scatter\ mode}$$

## Results & Conclusions

For downstream experiments, analysis of the concentration and purity of Extracellular Vesicles isolated by various purification methods is of great interest. As shown by other analysis technologies, the yield and purity of EVs varies significantly between different isolation methods (Corso *et al.*, 2017, Takov *et al.*, 2019). Unfortunately, the well-established scatter based NTA technology is not able to discriminate between different components of those preparations. Here we report a fast and easy technique to analyse the purity of EV samples using unspecific, lipophilic membrane dyes such as Cell Mask© Deep Red (CMDR, Thermo Fisher, USA) followed by an analysis with fluorescent Nanoparticle Tracking Analysis (f-NTA). As described in Methods we labelled a commercially available EV sample with CMDR and analysed the concentration ratio between total particle count in scatter vs. fluorescence positive membranous particles in the red fluorescence channel. (see Figure 1). The data clearly demonstrate that the commercial EV sample has a purity of about 80%.

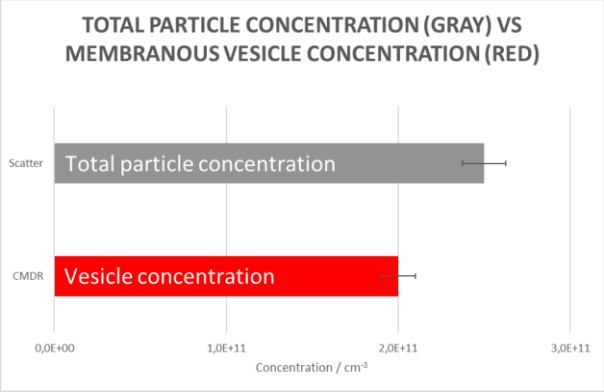


*Figure 1: Comparison between the total particle concentration (gray) and the biological vesicle concentration (red).*

Simultaneously performed size measurements showed that the membranous vesicles have a significantly smaller mean diameter (95,6 nm) than the total particles (110,3 nm). A very likely explanation for this shift is that bigger non-vesicular particles (e.g. nanobubbles, salt principates or protein aggregates) influence the size distribution and therefore also the mean diameter to a bigger size. This can also be observed in the size distribution in Figure 2.

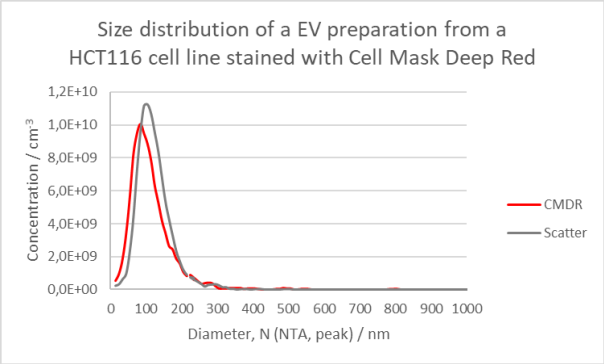


*Figure 2: Size distribution of an EV preparation from a HCT cell line in scatter mode (gray) and stained with Cell Mask© Deep Red (red)*

These results show that unspecific labelling with lipophilic membrane dyes like Cell Mask© Deep Red identify and discriminate Extracellular Vesicles from other, non-vesicular nanoparticles. Similar results can be obtained when using other unspecific membrane dyes like Cell Mask© Green, Cell Mask© Orange, DiL or DiO (data not shown). A broad range of dyes is available, and the method can be applied to all ZetaView® instruments regardless of the excitation laser / emission filter combination utilized.

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