Detection of nucleic acid cargo in EVs by using Fluorescence Nanoparticle Tracking Analysis

While the characterization of extracellular vesicles (EVs) was mainly focused on proteins and lipids in the past [1-2], it is apparent that interest in the study of nucleic acid cargo in EVs is now increasing. The use of fluorescence nanoparticle tracking analysis (F-NTA) enables the detection of membrane-associated nucleic acids from EVs.

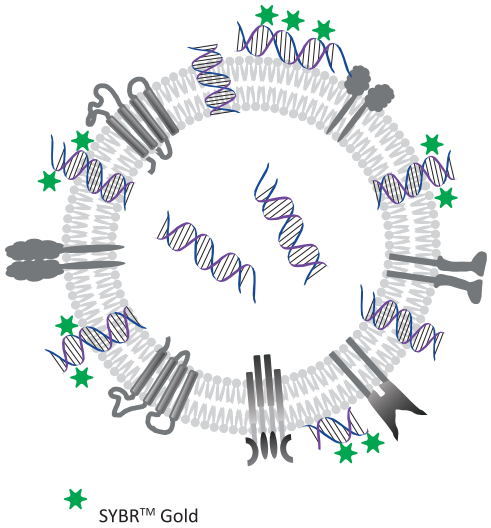

Figure 1.: EV-membrane-associated nucleic acids labeled with SYBR™ Gold.

These experiments used the nucleic acid dye SYBR™ Gold (#S11494, Thermo Fisher Scientific), which has shown a sensitivity of >10x more than ethidium bromide, for the detection of DNA and RNA in denaturing urea, glyoxal and formaldehyde gel [3].

The specificity of the experiment was achieved by a nuclease control aimed at specifically degrading EV-associated nucleic acids after labeling them. However, the use of Benzonase (#70746-3, Novagen) did not allow differentiation between DNA and RNA degradation in this experiment.

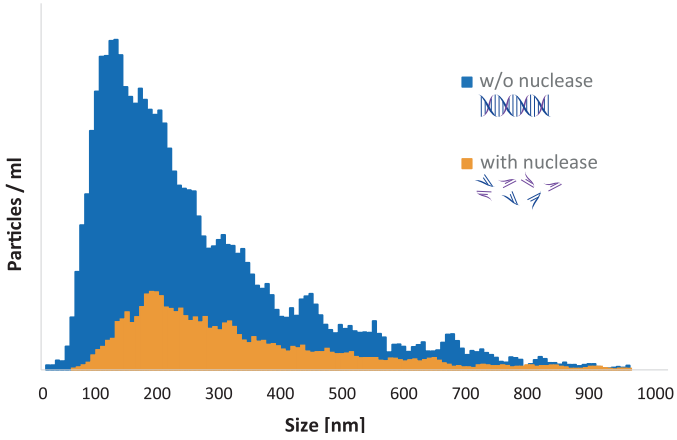

Figure 2.: Particle size distribution of SYBR™ Gold labelled EVs incubated with and without a nuclease prior NTA measurement.

Table 1: Summary of parameters and results of SYBR™ Gold labeled EVs incubated with and without nuclease.

Parameters & Results	Fluorescence λ=488F500nm w/o Nuclease	Fluorescence λ=488F500nm with Nuclease
Detection		
# Positions	11	11
# Cycles	1	1
Camera Parameters		
# Frames	30	30
Sensitivity	95	95
Shutter	200	200
Tracking Parameters		
Tracelength	12	12
Framerate	30 fps	30 fps
Min Area	0	0
Max Area	1000	1000
Min Particle Size	1	1
Max Particle Size	1000	1000
Low Bleach	Yes	Yes
Data Results		
Size (Peak)	82.6	116,5
X50	125.4	140
# Traced Particles	1286	393
Concentration	$3x10^7$	$8x10^6$

Conclusions:

The reduction of the fluorescent signal after incubation with Benzonase shows that SYBR™ Gold can be used as a specific stain for nucleic acid detection by F-NTA.

It can be assumed that the SYBR™-Gold signal remaining after nuclease treatment is due to nucleic acids in the EV lumen that cannot be degraded.

Therefore, SYBR™-Gold-activated nucleic acid detection can be used for selective measurement of membrane-associated nucleic acids due to the nuclease treatment.

References:

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   Minimal experimental requirements for definition of extracellular vesicles and their functions: a position statement from the International Society for Extracellular Vesicles
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   Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines
   J Extracell Vesicles **2018**; Nov 23;7(1):1535750

3. **Truma et al.:**
   Characterization of SYBR Gold nucleic acid gel stain: a dye optimized for use with 300-nm ultraviolet transilluminators
   Anal Biochem **1999**; Mar 15;268(2):278-88

Head Office

Particle Metrix GmbH
Wildmoos 4
D-82266 Inning / Germany

+49-8143-99172-0
info@particle-metrix.de

US Office

Particle Metrix Inc.
Mebane, NC 27302 / USA

+1-919-667-6960
usa@particle-metrix.com

Worldwide Distributors