ExoGlow-NTA™ Fluorescent Labeling Kit (for Particle Metrix ZetaView®)

Cat # EXONTA110A-1

User Manual

Store kit at +4^{0}C

Version 3
6/27/2018

Contents

Product Description

SBI's ExoGlow-NTA™ Fluorescent Labeling Kit (for Particle Metrix ZetaView®) (Cat #EXONTA110A-1) enables highly specific quantitation of extracellular vesicles (EVs) from a wide variety of biofluids and isolation protocols. Compatible with Particle Metrix® ZetaView Nano Tracking Analysis (NTA) platform to measure size and concentration of EVs, the ExoGlow-NTA kit leverages the fluorescent capabilities of the platform to specifically detect only labeled EVs in heterogeneous mixtures. The proprietary dye formulation in our ExoGlow-NTA kit binds specifically and efficiently to the membranes of intact EVs only, thus avoiding detection of protein aggregates, membrane fragments and other background particles and ensuring much more accurate EV particle counts than currently available methods.

The kits come with three components: 1) Labeling dye 2) Standards and 3) Reaction Buffer. Simply mix the dye with the reaction buffer, add 1-100ug of EVs (or protein equivalent), incubate for 3-5 minutes, and you are ready for fluorescent NTA analysis. The provided standard is a size-controlled synthetic liposome prep that provide a positive control for the NTA particle tracking as well as labeling using the ExoGlow-NTA kit.

Key Features and Benefits:

- Only kit commercially available for specifically labeling intact EVs for fluorescent Nano Tracking Analysis quantitation
- Proprietary dye binds EVs with high signal-to-noise ratio
- Validated using common EV isolation methods such as ExoQuick™, ultracentrifugation, and column-based methods
- Optimized protocol - sample to analysis in under 20 minutes

List of Components

Item	Volume	Storage Temperature
Reaction Buffer	160 µl	4^0C
Labeling Dye	30 µl	4^0C
Standard	3 µl	4^0C

*The kit is for 10 labeling reactions. We will provide 3 reactions for the Standard, as it will not be necessary to run the standard every time the NTA is run.

Storage

The kit is shipped on ice and should be **stored** at +4°C. Properly stored kits are stable for 6 months from the date received.

General Information

The reaction size is based on using 1-100 µg of total proteins in the sample.

We recommend to pre-warm Reaction Buffer for 3-5 min at 37°C and mix well before use. Protect labeling dye from light.

Recommended ZetaView hardware specifications for fluorescent NTA analysis:

Laser(s): 520nm, 488nm, or 405nm (520nm is preferred)

Camera: Requires CMOS camera (640 x 480 pixels)

Initial Suggested Instrument Settings (for running Standards):

Instrument	Camera	Sensitivity	Brightness	Min pixel size	Max pixel size	Shutter	Frames /sec	Trace length range	Video length
PMX 120 series	CMOS	90-95	25-30	5	1,000	100	30 or 60	8-12	Start with 1 sec.

Protocol for Labeling:

1. Pre-warm Reaction buffer for 3-5 minutes at 37°C and mix well.

2. For each Sample and Standard, add 2 µl of Labeling Dye into 12 µl of Reaction Buffer and mix well until dye is dissolved completely to make Labeling Reaction buffers.

3. Add exosomes (equivalent to 1-100 µg of protein) to the Sample from step 2 and bring total volume of the reaction up to 50 µl with water or 1xPBS. To label Standards, add 1 µl of the standards into Labeling Reaction buffer.

Compound	Sample Reaction	Standard Reaction
Reaction Buffer	12 µl	12 µl
Labeling dye	2 µl	2 µl
Exosomes (XXX µg)	X µl	-
Standards	-	1 µl
Water or 1xPBS (filtered)	Up to 50 µl	Up to 50 µl

3. Mix well by pipetting and incubate for 3-5 minutes at RT. The tubes do not need to be rotated during the incubation period.

   ! Protect the tubes from light.

4. Both Sample and Standards are ready for fluorescent NTA analysis.

Example Data and Applications

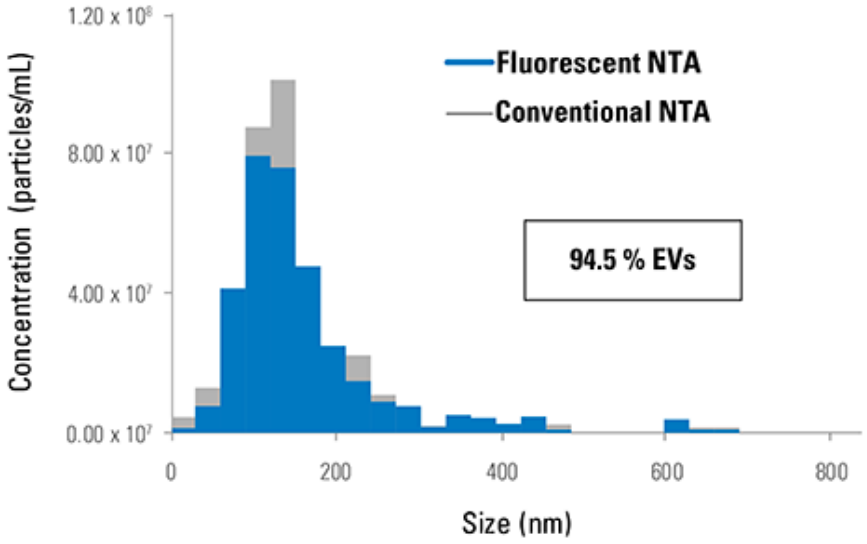

Figure 1. ExoGlow-NTA-labeled liposomes deliver consistent NTA data whether in light scattering or fluorescent mode. The high concordance of NTA and fluorescent NTA data collected from the ExoGlow-NTA Kit internal standards (ExoGlow-NTA-labeled synthetic liposomes) demonstrates the labeling efficiency of the ExoGlow-NTA Dye and accuracy of the fluorescent NTA method for characterizing EVs. ***Data collected using Particle Metrix PMX120 instrument equipped with 520nm laser.***

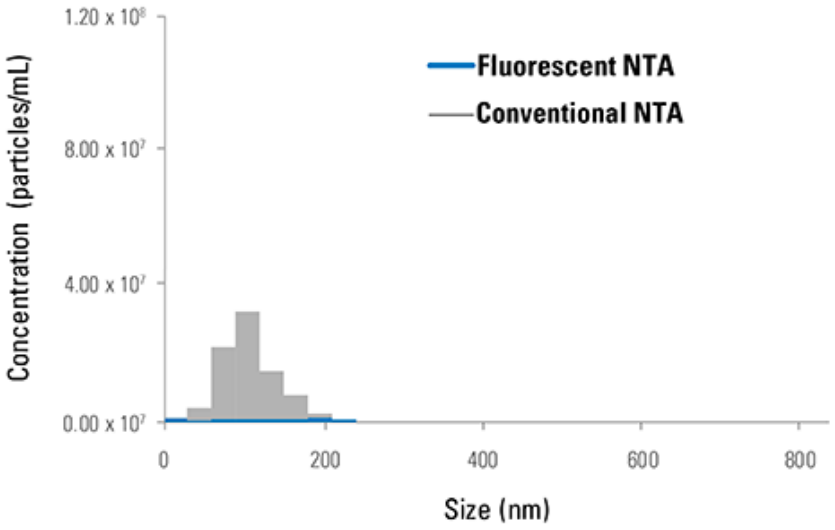

Figure 2. ExoGlow-NTA delivers undetectable background signal. When analyzing the ExoGlow-NTA dye alone in PBS, conventional NTA picks up background particles in the absence of EVs, while fluorescent NTA of the ExoGlow-NTA dye alone shows bias-free undetectable autofluorescence. ***Data collected using Particle Metrix PMX120 instrument equipped with 520nm laser.***

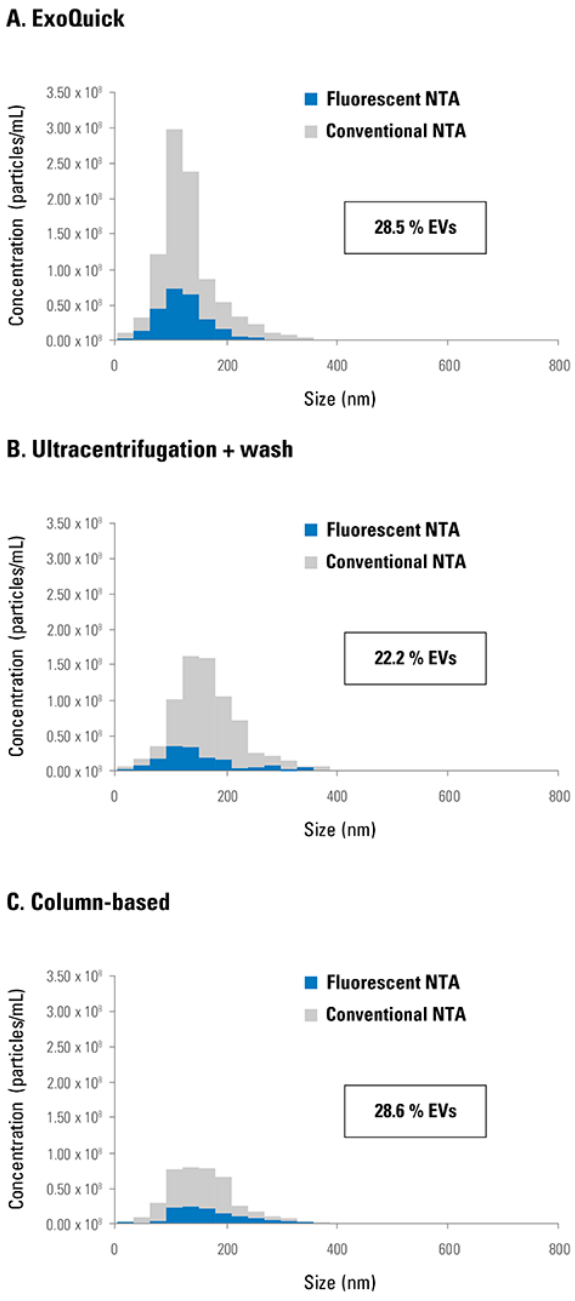

Figure 3. ExoGlow-NTA demonstrates that conventional NTA overestimates EV concentration in samples irrespective of EV isolation method. Representative data comparing conventional NTA and fluorescent NTA for EVs isolated using (A) ExoQuick, (B) ultracentrifugation and wash, or (C) column-based isolation, shows just how much of the conventional NTA signal is due to non-EV particles. ***Data collected using Particle Metrix PMX120 instrument equipped with 520nm laser.***

Technical Support

For more information about SBI products and to download manuals in PDF format, please visit our web site: **http://www.systembio.com**

For additional information or technical assistance, please call or email us at:

System Biosciences (SBI)
2438 Embarcadero Way
Palo Alto, CA 94303

Phone: (650) 968-2200
Toll-Free: (888) 266-5066
Fax (650) 968-2277

E-mail:
General Information: info@systembio.com
Technical Support: tech@systembio.com
Ordering Information: orders@systembio.com

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Limited Use License

Use of the ExoGlow-NTA Fluorescent Labeling Kit (*i.e.*, the "Product") is subject to the following terms and conditions. If the terms and conditions are not acceptable, return all components of the Product to System Biosciences (SBI) within 7 calendar days. Purchase and use of any part of the Product constitutes acceptance of the above terms.

The purchaser of the Product is granted a limited license to use the Product under the following terms and conditions:

- The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use.
- The Product may not be resold, modified for resale, or used to manufacture commercial products without prior written consent of SBI.
- This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

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System Biosciences (SBI)
2438 Embarcadero Way
Palo Alto, CA 94303

Phone: (650) 968-2200
Toll-Free: (888) 266-5066
Fax: (650) 968-2277

E-mail:
General Information: info@systembio.com
Technical Support: tech@systembio.com
Ordering Information: orders@systembio.com