ExoGlow-NTA™ Fluorescent Labeling Kit (for Malvern NanoSight)

Cat# EXONTA200A-1

User Manual

Store kit at +4^{0}C

Version 2
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Product Description

SBI's ExoGlow-NTA™ Fluorescent Labeling Kit (for Malvern NanoSight) (Cat #EXONTA200A-1) enables highly specific quantitation of extracellular vesicles (EVs) from a wide variety of biofluids and isolation protocols. Compatible with Malvern's NanoSight range of NanoTracking Analysis (NTA) instruments (equipped with 488nm laser) 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 four components: 1) Labeling dye 2) Standards 3) Reaction Buffer, and 4) Size exclusion columns. 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.

List of Components

Item	Volume	Storage Temperature
Reaction Buffer	160 µl	4^0C
Labeling Dye	60 µl	4^0C
Standards	3 µl	4^0C
Size exclusion column	13	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.

Protocol for sample labeling:

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

2. For each Sample and Standard, add 4 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	4 µl	4 µl
Exosomes (1-100 µg)	X µl	-
Standards	-	1 µl
Water or PBSx1 (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.

Protocol to remove free dye:

1. Mix the medium inside the column by vortexing.
2. Loosen the cap and twist off the bottom closure.
3. Place column into a collection tube and remove the storage solution by centrifugation at 800 x g for 1 min.
4. To wash the column, add 400 µL filtered PBS and centrifuge at 800 x g for 1 min.
5. Discard flow-through and replace the collection tube.
6. Repeats steps 4 and 5 four more times.
7. Replace the used collection tube with a new clean 1.5 ml eppendorf tube (not provided) for sample collection.
8. Adjust the volume of the sample to 140 µL with 1x1XPBS. Apply 140-180 µL of your sample slowly in the middle of the packed bed of the column. Recommended sample volume is 140 µL.
9. Centrifuge at 800 x g for 1 minute to collect labeled EVs.
10. The samples are ready for fluorescent NTA analysis.

We recommend optimizing your instrument settings with labeled standards for fNTA analysis. The following setting parameters are provided as suggested starting points for instrument optimization:

Instrument	Camera	Camera level	Slide shutter	Slide gain	Shutter/ms	Frames rate/fps	Syringe pump speed
LM 10/ NS 300	CMOS	11-16	890-1,300	146-512	22.25-32.5	24.98	100

Example Data and Applications

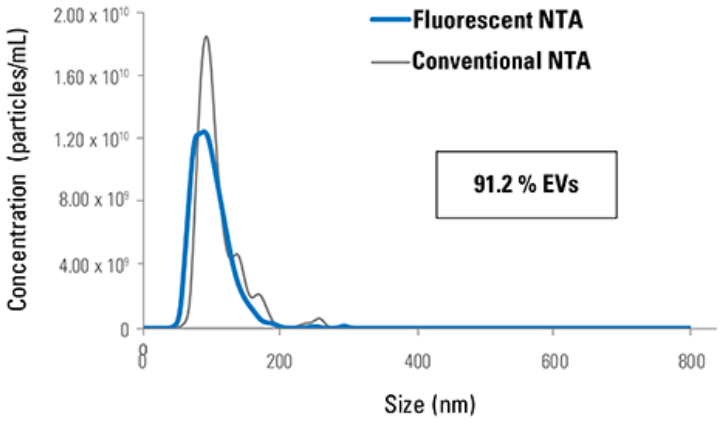

Figure 1. ExoGlow-NTA-labeled liposomes deliver consistent NTA data whether in light scattering or fluorescent mode. The high concordance of NTA and fluorescent NTA data collected from the ExoGlow-NTA Kit internal standards (ExoGlow-NTA-labeled synthetic liposomes) demonstrates the labeling efficiency of the ExoGlow-NTA Dye and accuracy of the fluorescent NTA method for characterizing EVs. ***Data collected using NanoSight LM10 instrument equipped with 488nm laser.***

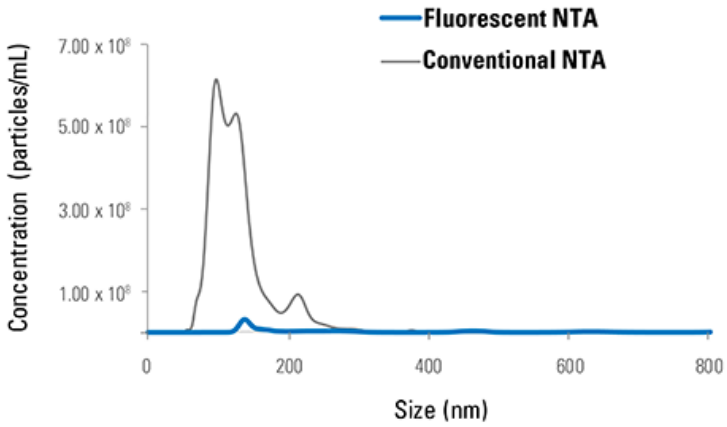

Figure 2. ExoGlow-NTA delivers undetectable background signal. When analyzing the ExoGlow-NTA dye alone in PBS, conventional NTA picks up background particles in the absence of EVs, while fluorescent NTA of the ExoGlow-NTA dye alone shows bias-free undetectable autofluorescence. ***Data collected using NanoSight LM10 instrument equipped with 488nm laser.***

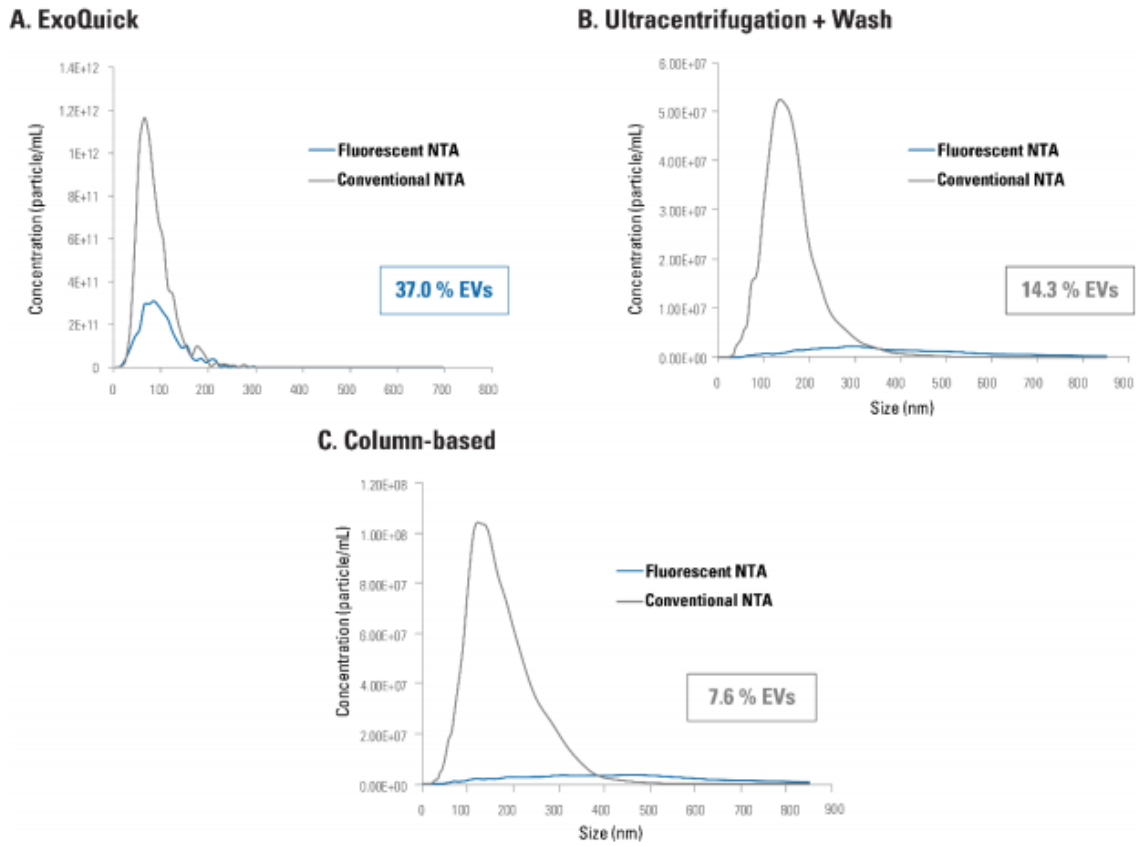

Figure 3. ExoGlow-NTA demonstrates that conventional NTA overestimates EV concentration in samples irrespective of EV isolation method. Representative data comparing conventional NTA and fluorescent NTA for EVs isolated using (A) ExoQuick, (B) ultracentrifugation and wash, or (C) column-based isolation, shows just how much of the conventional NTA signal is due to non-EV particles. ***Data collected using NanoSight LM10 instrument equipped with 488nm laser.***

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