ExoGlow™-NTA Fluorescent Labeling Kit

Cat # EXONTA100A-1

User Manual

Store kit at +4^{0}C

Version 1
5/16/2017

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Product Description

SBI's ExoGlow-NTA™ Fluorescent Labeling Kit (Cat #EXONTA100A-1) enables highly specific quantitation of extracellular vesicles (EVs) from a wide variety of biofluids and isolation protocols. Compatible with the popular NanoSight™ particle tracking platform to measure size and concentration of EVs, the ExoGlow-NTA kit leverages the fluorescent capabilities of the NanoSight 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) Internal Standards and 3) Reaction Buffer. Simply mix the dye with the reaction buffer, add 1-100ug of EVs (or protein equivalent), incubate for 30 minutes, and you are ready for fluorescent NTA analysis. The provided Internal Standards are size-controlled synthetic liposomes that provide a positive control for the NTA particle tracking as well as labeling using the ExoGlow-NTA kit.

***For fluorescent NTA analysis, the NanoSight NTA system (model LM10, NS300 or others) MUST be installed and properly calibrated with a 488nm laser (available separately). Please contact your local Malvern Instruments representative for additional information.**

Key Features and Benefits:

- Only kit commercially available for specifically labeling intact EVs for fluorescent NanoSight NTA 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 45 minutes

List of Components

Item	Volume	Storage Temperature
Reaction Buffer	240 µl	4^0C
Labeling Dye	40 µl	4^0C
Internal Standards	10 µl	4^0C

*The kit is for 10 individual labeling reactions

Storage

The ExoGlow-NTA 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 1-100 µg of total protein (as measured by Qubit or BCA assay) in the sample. We recommend mixing the Reaction Buffer well before usage. **Protect labeling dye from light.**

Additional Notes

The ExoGlow-NTA labeling dye is essentially non-fluorescent in water or 1XPBS and becomes strongly fluorescent upon binding of EVs. The absorption and fluorescence emission maxima of the dye in the bound state are about 465 nm and 635 nm, respectively.

Protocol for ExoGlow-NTA

1. Prepare the Labeling Reaction Buffer by adding 12 µl of Reaction Buffer and 2 µl of Labeling Dye into a sterile Eppendorf tube and mix well until dye is completely dissolved.

2. Add EVs (1-100 µg of protein) to the Labeling Reaction Buffer from step 1 and bring total volume of the reaction up to 50 µl with filtered water or 1xPBS (0.02um filtered recommended for best performance). To label Internal Standards, add 1 µl of the standards into Labeling Reaction Buffer.

Reagent	Sample Reaction	Internal Standard Reaction
Reaction Buffer	12 µl	12 µl
Labeling dye	2 µl	2 µl
EVs (1-100 µg)	X µl	-
Standards	-	1 µl
Filtered water or 1xPBS	Bring up to 50 µl	Bring up to 50 µl

3. Mix the samples well and incubate for 30 minutes at RT. The tubes do not need to be rotated during the incubation period. **Protect the tubes from light.**

4. The samples are ready for fluorescent NTA analysis.

Example Data and Applications

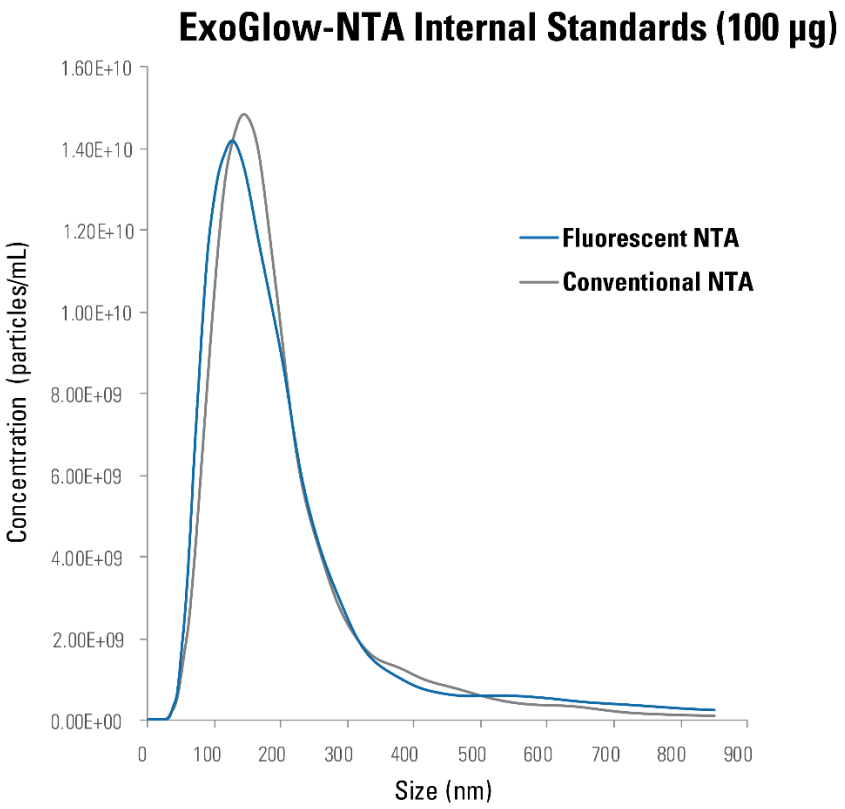

Figure 1. ExoGlow-NTA-labeled liposomes deliver consistent NanoSight NTA data whether in light scattering or fluorescent mode. The high concordance of NTA and fluorescent NTA data collected from the ExoGlow-NTA Kit internal standards (ExoGlow-NTA-labeled synthetic liposomes) demonstrates the accuracy of the fluorescent NTA method for characterizing EVs.

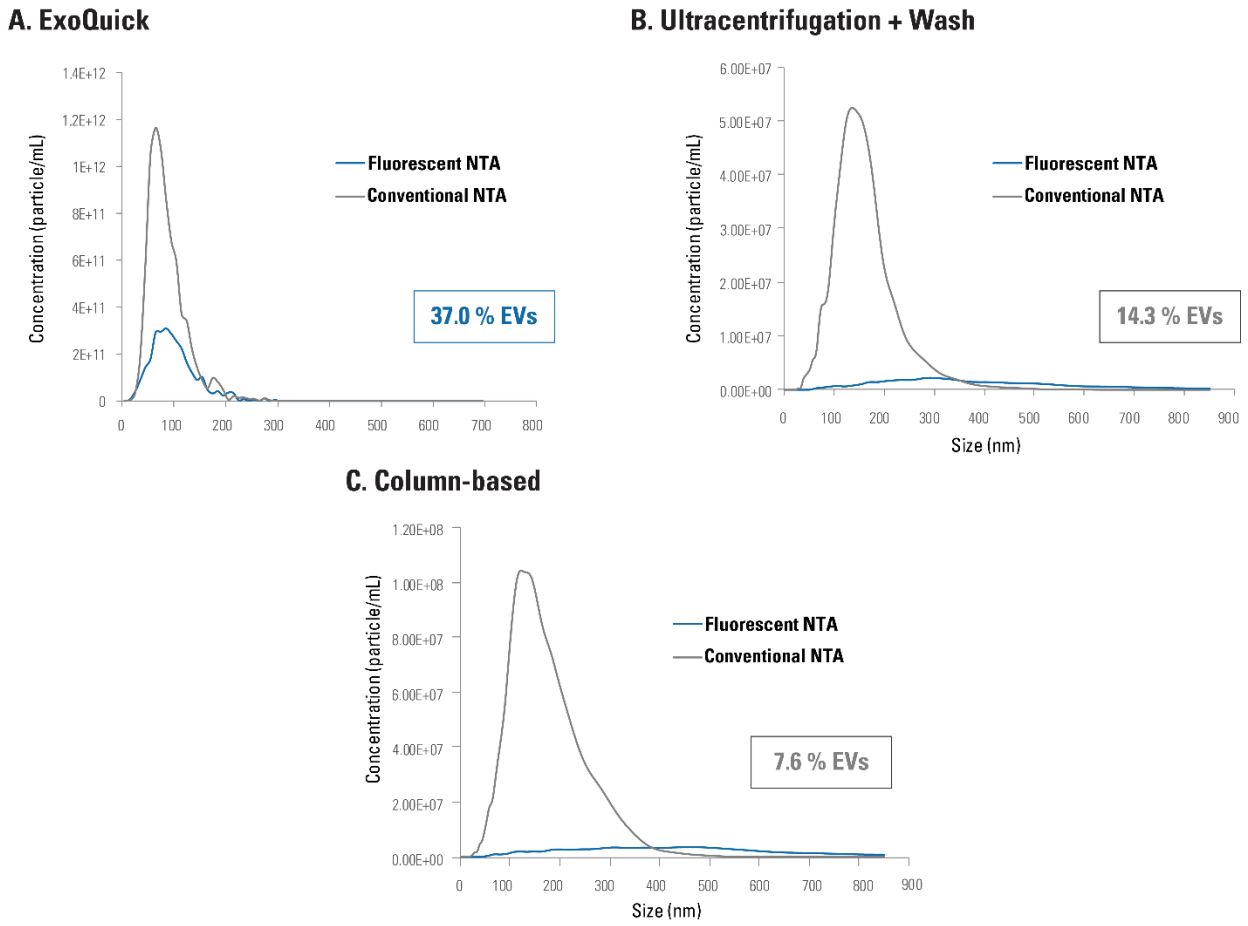

Figure 2. ExoGlow-NTA demonstrates that conventional NTA overestimates EV concentration in samples irrespective of EV isolation method. Representative data comparing conventional NTA and fluorescent NTA for EVs isolated using (A) ExoQuick (10 µg serum protein), (B) ultracentrifugation and wash (1 µg serum protein), or (C) column-based isolation (1 µg serum protein), shows how much of the conventional NTA signal is due to non-EV particles. Quantitation of the percentage of EVs delivered by each preparation method are shown on each plot, with the (A) ExoQuick prep consisting of 37.0% EVs, (B) ultracentrifugation plus wash prep consisting of 14.3% EVs, and (C) the column-based prep consisting of 7.6% EVs.

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

Licensing and Warranty Statement

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.

SBI has pending patent applications related to the Product. For information concerning licenses for commercial use, contact SBI.

Purchase of the product does not grant any rights or license for use other than those explicitly listed in this Licensing and Warranty Statement. Use of the Product for any use other than described expressly herein may be covered by patents or subject to rights other than those mentioned. SBI disclaims any and all responsibility for injury or damage which may be caused by the failure of the buyer or any other person to use the Product in accordance with the terms and conditions outlined herein.

Limited Warranty

SBI warrants that the Product meets the specifications described in this manual. If it is proven to the satisfaction of SBI that the Product fails to meet these specifications, SBI will replace the Product or provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to SBI within 30 days of receipt of the Product.

SBI's liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. SBI's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. SBI does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

SBI is committed to providing our customers with high-quality products. If you should have any questions or concerns about any SBI products, please contact us at (888) 266-5066.

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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