

ExoGlow[™]-RNA EV Labeling Kit

Cat# EXOGR800A-1

User Manual

Store kit at +4^oC

Version 1 8/21/2017 A limited-use label license covers this product. By use of this product, you accept the terms and conditions outlined in the License and Warranty Statement contained in this user manual.

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Product Description

SBI's ExoGlow-RNA[™] EV Labeling Kit (Cat #EXOGR800A-1) is the latest generation of fluorescent labeling reagent to robustly and specifically label EV RNA cargo. Based on a highly specific polyT-fluorescent moiety conjugated to a stable peptidic backbone, the reagent specifically enters EVs and tightly binds mRNAs for fluorescence-based detection, while showing minimal background in an unbound state. As a result, the kit can be used for most applications that require visualization of labeled RNA cargo in EVs. With very low intrinsic background levels, these new dyes are well suited for demanding EV labeling studies that require high signal-to-noise ratios and specificity.

List of Components

Item	Volume	Storage Temperature
Incubation buffer	3 ml	4°C
RNA probe (100 μM)	100 µl	4 ⁰ C

*The kit is for 10 labeling reactions

Storage

The ExoGlow-RNA^M EV Labeling Kit is shipped at blue ice and should be **stored** at +4°C. Properly stored kits are stable for 12 months from the date received.

General Information

The reaction size is based on using 50-100 μ g of exosomes. The excitation and emission properties of ExoGlow-RNA are 485nm and 537nm, respectively.

Protect RNA probe from light.

If you decide to remove free probe before applying labeled exosomes on cells, we recommend PD Mini Trap or PD SpinTrap G-25 buffer exchange column (GE Healthcare, Cat# 28-9180-07 or 28-9180-04).

Protocol for labeling of exosomes

To label exosomes with ExoGlow RNA probe:

- 1. Add 50 μg-100 μg of exosomes into 300 μl of incubation buffer*
- 2. Add 3 μ M-10 μ M (final concentration) of ExoGlow RNA probe to the mixture. The exact amount of the probe required should be adjusted per experiment and cell type.
- 3. Incubate for 1 hr at 37°C in dark.

! Protect the tubes from light.

- 4. Remove free probe from labeled exosomes (OPTIONAL). We recommend using PD mini trap or PD SpinTrap G-25 buffer exchange column.
- 5. Add labeled exosomes to the cells in the media containing serum**. If you removed free probe you can use serum-free media.
- 6. Incubate cells for 24 h at 37° C in a CO₂ incubator and visualize by fluorescent microscope.

*The amount of exosomes, buffer and dye is enough to label 100 μ g of exosomes and label 100,000 cells in 6-well dish.

**If you are culturing the cells in the media that contain serum, you can skip free probe removal as serum significantly inhibits free RNA probe transfection into the cells. In this case ~99% of the probe will be delivered to the cells by labeled exosomes.

Example Data and Applications



Figure 1. ExoGlow-RNA enables clear visualization of labeled EVs being internalized by target cells. We labeled HEK293T EVs with ExoGlow-RNA and followed uptake by HEK293T cells. The fluorescence signal localizes to perinuclear and cytoplasmic regions, as would be expected for mRNA.



Figure 2. ExoGlow-RNA specifically labels mRNA. We used ExoGlow-RNA to label total RNA isolated from HEK293T cells, and then specifically depleted mRNA using polyT magnetic beads. The almost complete loss of signal in the mRNA-depleted sample demonstrates the specificity of ExoGlow-RNA dye for mRNA.

Technical Support

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

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

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Licensing and Warranty Statement

Limited Use License

Use of the ExoGlow-RNA EV 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.

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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.

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