

ExoGlow[™]-Protein EV Labeling Kit

Cat # EXOGP100A-1 Cat # EXOGP300A-1 Cat # EXOGP400A-1

User Manual

Storage: See individual components

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-Protein[™] EV Labeling Kit (Cat #EXOGPXXXA-1) is the latest generation of fluorescent labeling reagents to robustly and specifically label EV protein cargo. Based on an enzymatic conversion of the native, non-fluorescent dye to an activated fluorescent moiety when taken up by EVs, the ExoGlow-Protein dye can be used for most applications that require visualization of labeled EVs such as EV uptake studies. The kit comes in three different colors (Blue, Green, and Red), each spectrally separated for use in multi-EV labeling experiments. 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
Labeling Dye	2 x10 μL	-20ºC
ExoQuick-TC	3.5 mL	RT

*The kit is for 20 individual labeling reactions

Storage

The ExoGlow-Protein Kit is shipped on Blue Ice and the components should be stored at recommended temperatures. Properly stored kits are stable for 6 months from the date received.

General Information

The reaction size is based on 200-500 μg of total protein (as measured by Qubit or BCA assay) in the sample. **Protect Iabeling dye from light.**

Additional Notes

Spectral information for ExoGlow-Protein dyes:

	Excitation	Emission	Laser Line
Blue	403nm	454nm	405nm
Green	511nm	525nm	488nm
Red	573nm	588nm	561nm

Protocol for ExoGlow-Protein:

- 1. Resuspend 200-500 μg protein equivalent of EVs in 500 μL PBS.
- 2. Add 1 μL of the 500X labeling dye to the EV preparation
- 3. Incubate at 37°C with shaking (350 rpm) for 20 minutes
- 4. Add 167 µL ExoQuick-TC to the solution and incubate at 4°C from 2 hrs to overnight.
- 5. Spin the Eppendorf tube at 10,000 rpm for 10 minutes.
- 6. Carefully aspirate the supernatant from the corner of the tube.
- 7. Resuspend the EV pellet in PBS and proceed with downstream applications.

Note: For general uptake studies, we typically use 100 μ g of labeled protein equivalent of EVs to treat ~100,000 cells in a single well of a 12-well plate. These conditions are for HEK293T cells, and due to the biological heterogeneity of cells and differences in uptake efficiencies of EVs across cells, we recommend optimization of the delivery amount for your target cells of interest.

Example Data and Applications



Figure 1. ExoGlow-Protein enables clear visualization of labeled EVs being internalized by target cells. We labeled HEK293T EVs with ExoGlow-Protein and followed uptake by HEK293T cells. The punctate fluorescence signal shows internalization of labeled EVs by the target cells.



Figure 2. Punctate staining pattern is EV-specific. The punctate staining pattern seen (top panels) is due to ExoGlow-Protein-labeled EVs. When ExoGlow-Protein is added directly to target HEK293T cells (bottom panels), only diffuse, non-specific staining is seen.



Figure 3. ExoGlow-Protein signal is stable for up to at least 7 days after administration of labeled EVs to cells.

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:

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-Protein 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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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.

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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
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