ExoGlow™-Membrane EV Labeling Kit

Cat# EXOGM600A-1

User Manual

Store kit at +4^{0}C

Version 2
4/26/2018

Contents

Product Description

SBI's ExoGlow-Membrane™ EV Labeling Kit (Cat #EXOGM600A-1) is the latest generation of fluorescent labeling reagent to robustly and specifically label EV membranes. Based on a highly specific membrane sensor, the reagent specifically labels intact EVs 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 EVs for tracking studies. With very low intrinsic background levels, the dye is well suited for demanding EV labeling studies that require high signal-to-noise ratios and specificity.

List of Components

Item	Volume	Storage Temperature
Reaction Buffer	300 µl	4^0C
Labeling Dye	50 µl	4^0C

*The kit is for 25 labeling reactions

Storage

The ExoGlow-Membrane kit is shipped on ice and should be **stored** at +4°C. Properly stored kits are stable for 12 months from the date received.

General Information

1. The reaction size is based on using 50-100 µg of exosomes.

2. The ExoGlow-Membrane excitation and emission values in the bound state are 465nm and 635nm, respectively.

3. We recommend mixing Reaction Buffer well before the usage.

4. Protect labeling dye from light.

5. We recommend to remove free dye before applying labeled exosomes on the cells with PD MiniTrap or PD SpinTrap G-25 buffer exchange column (GE, Cat# 28-9180-07 or 28-9180-04).

6. Due to the nature of the sensor dye and its broad emission spectrum, the signal can be also detected in green channel. Therefore, we don't recommend this product for multiplexing experiments.

Protocol for Labeling:

1. Into 12 µl of Reaction Buffer add 2 µl of Labeling Dye and mix well until dye is dissolved completely to make Labeling Reaction buffer.

2. Add 50-100 µg exosomes to the Labeling reaction buffer from step 1.

3. Mix well the sample 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. Remove free unlabeled dye. We recommend buffer exchange column PD MiniTrap or PD SpinTrap G-25 from GE (not provided). A single 1 min spin at the recommended speed will be sufficient for dye removal.

5. Alternative protocol for free dye removal. If you have high background after PD SpinTrap G-25, the re-precipitation protocol with ExoQuick-TC is a better choice:

   - Add 35 µL of ExoQuick-TC to 100 µL of Sample and incubate at 4^{o}C from 30 min to overnight.

   - Spin the Eppendorf tube at 10,000 rpm for 10 minutes.

   - Carefully aspirate the supernatant from the corner of the tube.

   - Resuspend the EV pellet in PBS and proceed with downstream applications.

6. Labeled exosomes can be added to the cells or used for downstream applications.

Example Data and Applications

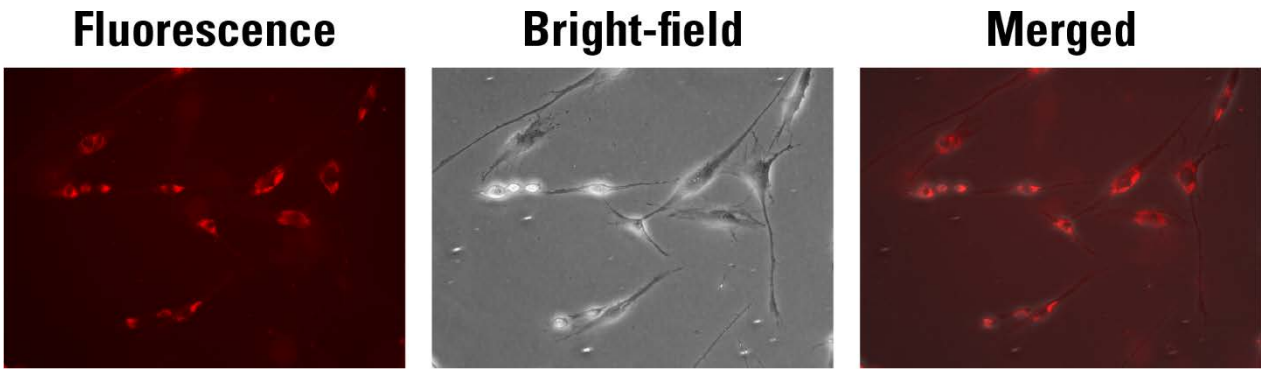

Figure 1. ExoGlow-Membrane enables clear visualization of labeled EVs being internalized by target cells. We labeled 50 µg of HEK293T EVs with ExoGlow-Membrane and followed uptake by HEK293T cells. The evenly distributed fluorescence signal shows internalization of labeled EVs by the target cells and the distribution of EV membranes to cellular membranes.

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:

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Licensing and Warranty Statement

Limited Use License

Use of the ExoGlow-Membrane 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.

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