Exo-Glow™

Exosome Labeling Kits

Cat# EXOR100A-1

Cat# EXOG200A-1

Cat# EXOC300A-1

User Manual

Store kit at -20°C on receipt

Version 8
3/10/2017

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Product Description

The Exo-Glow kits allow researchers to fluorescently-label isolated exosomes to track cellular interaction and uptake. Exosomes are first enriched using SBI's ExoQuick product line or immunopurified using SBI's Exo-Flow IP kits. The isolated exosomes are then resuspended and incubated with either the Exo-Red or Exo-Green labeling reagents. Exo-Red fluorescently-labels nucleic acids, and Exo-Green labels proteins within exosomes. These labeled biomolecules can then be monitored for delivery into target cells via exosomes using fluorescent microscopy.

List of Components

Item	Catalog #	Contents/Kit	Reactions
Exo-Red Exosome RNA Fluorescent Label (10X dissolved in dH_2O)	EXOR100A-1	Exo-Red (10X)- 1mL ExoQuick-TC- 2mL	20 reactions
Exo-Green Exosome Protein Fluorescent Label (10X dissolved in DMSO)	EXOG200A-1	Exo-Green (10X)- 1mL ExoQuick-TC- 2mL	20 reactions
Exo-Red + Exo-Green Exosome Cargo Fluorescent Label Combo Pack	EXOC300A-1	Exo-Red (10X)- 500 µL Exo-Green (10X)- 500 µL ExoQuick-TC- 2mL	20 reactions (10 reactions of each label)

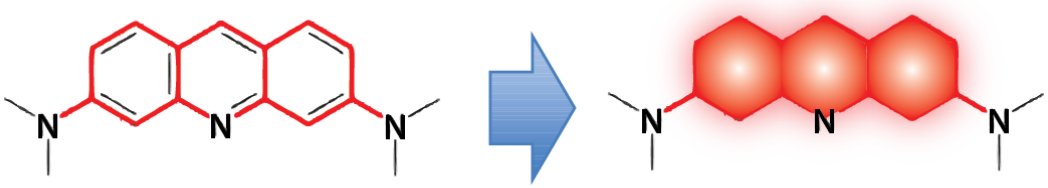
Storage

The Exo-Glow kits are shipped on dry ice and should be stored at -20°C. Freeze-thaw cycles of the reagents should be avoided. The ExoQuick-TC component can be stored at room temperature, 4°C or -20°C, however we recommend storing all components of the kit together at -20°C. The shelf life of the product is 1 year from the date received, if stored at -20°C.

General Information

The **Exo-Red** exosome label is based on acridine orange chemistry and is a nucleic acid-selective fluorescent cationic dye. It is cell-permeable, and interacts noncovalently with RNA by electrostatic attractions and DNA by intercalation. When Exo-Red associates with RNA, excitation occurs at 460 nm and emission maximum occurs at 650 nm, producing a red fluorescence. A majority of the nucleic acid content of exosomes is small RNA (miRNA, lncRNA, etc.), thus application of Exo-Red labels the internal exosomal RNAs and grants the ability to monitor RNA delivery to cells via exosomes. When bound to DNA, Exo-Red is very similar spectrally to fluorescein, with an excitation maximum at 502 nm and an emission maximum at 525 nm and will produce green fluorescence. When Exo-Red is used to label exosomes that are added to target cells, the cells will initially fluoresce red, for up to 2 hours. Within 2 hours, the Exo-Red will start to intercalate with intracellular dsDNA, and the fluorescence will shift to green.

Label internal exosome RNAs red



Binds single-stranded exoRNA, fluoresces red

The **Exo-Green** stain is based on carboxyfluorescein succinimidyl diacetate ester (CFSE) chemistry. CFSE is also membrane permeable. When CFSE enters exosomes it is hydrolyzed by esterases within exosomes that remove the diacetate residues. This activates the CFSE to fluoresce green and is then coupled to the amino ends of proteins. This approach allows researchers to track exosome protein transfer into target cells using fluorescent microscopy. The Exo-Green chemistry typically takes approximately 2 hours to clearly visualize via fluorescent microscopy, and will last until the reagent has been completely hydrolyzed by the cells. This may change due to the rate of cell division.

Label internal exosome proteins green

NOTE: Exosomes are too small (60 – 200 nm) to be detected by light microscopy. The Exo-Red and Exo-Green dyes allow visualization of the transfer of exosomal contents into target cells, but these reagents will not enable visualization of the exosomes directly. Visualization of labeled exosomes can be achieved by precipitating the exosomes using SBI's ExoFlow kits followed by FACS or fluorescent microscopy.

Due to the ability of Exo-Red to shift spectrally upon interaction with intracellular DNA, and the lability of RNA within cells, the Exo-Red and Exo-Green fluorescent labels are not intended for use as a simultaneous double-labeling technique. The Exo-Red should be visualized within 2 hours of application to target cells and the Exo-Green should be visualized after 2-4 hours of incubation between the exosomes and the target cells.

Protocol:

Protocol for labeling exosomes:

1. Add 50 µL 10x Exo-Red or Exo-Green to 500µL volume of resuspended exosome suspension in 1X PBS in a 1.5 mL eppendorf tube. You can typically add about 100-500 µg protein of isolated exosomes per Exo-Glow reaction. Protein isolation can be performed using NanoDrop or via a BCA assay. Exosomes do not need to be lysed for these measurements.
2. Mix well by flicking/inversion. Do not vortex.
3. Incubate the exosome solution at 37°C for 10 minutes (rotation not necessary).
4. To stop labeling reaction, add 100 µL of the ExoQuick-TC reagent to the labeled exosome sample suspension and mix by inverting 6 times.
5. Place the labeled exosome sample on ice (or at 4°C) for 30 minutes.
6. Centrifuge the sample for 3 minutes at 14,000 rpm in a microcentrifuge.
7. Remove the supernatant with excess label and resuspend the labeled exosome pellet in 500 µL 1x PBS.
8. The labeled exosomes are ready to be monitored for tracking.

Adding Exo-Glow labeled exosomes to cells:

1. Add at least 100 µL of labeled exosomes to approximately 1x10^5 cells per well in a 6-well culture plate (as an example). You can scale this ratio up or down depending upon your experimental requirements.
2. For Exo-Red, visualize with fluorescent microscopy for up to 2 hours. For Exo-Green, incubate for 2 to 24 hours, visualize with fluorescent microscopy using the following excitation/emission guidelines below.

Exosome Label	Excitation	Emission	Filter setting
Exo-Red	460 nm	650 nm (red)	Typical RFP filter set
Exo-Green	494 nm	521 nm (green)	Typical GFP filter set

Next Steps and Related Products

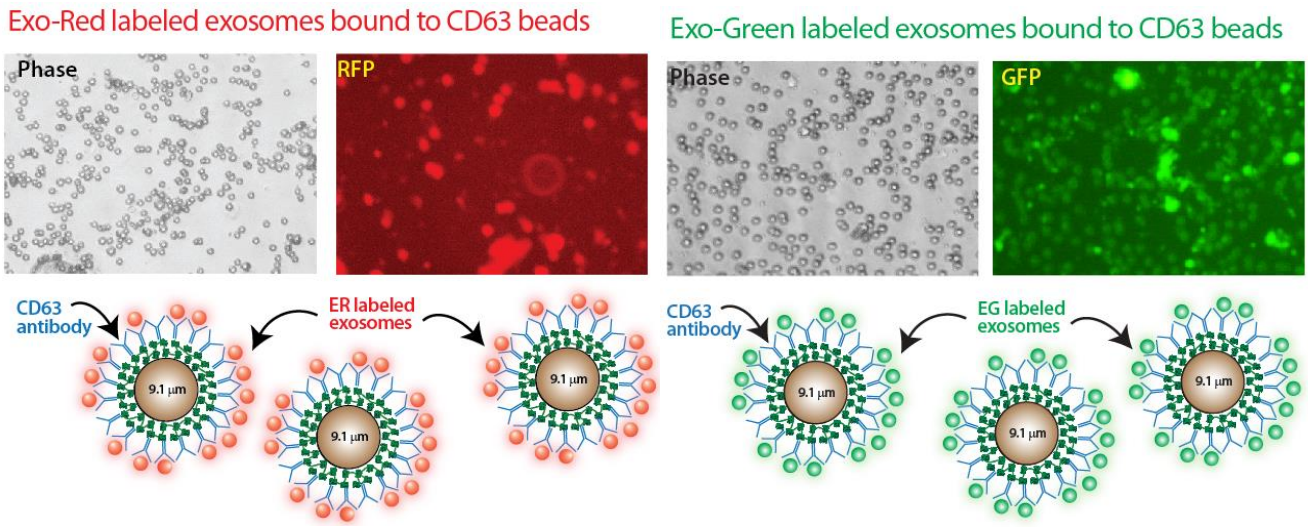
Application	Related Products	Website links
Precipitation of Exosomes from other biological fluids		
Exosome Isolation from Tissue Culture Media	ExoQuickTC	https://www.systembio.com/microrna-research/exoquick-exosomes/ordering
Exosome Isolation from Plasma	ExoQuick Plasma prep and Exosome precipitation kit	https://www.systembio.com/microrna-research/exoquick-exosomes/ordering
Protein Characterization of Exosomes		
Western blotting	Exosome antibodies	https://www.systembio.com/microrna-research/exosome-antibody/exoab
Antibody Arrays	ExoCheck Assays	https://www.systembio.com/microrna-research/exosome-antibody-arrays

ELISA	ExoELISA Kits	https://www.systembio.com/microrna-research/exosome-antibody/elisas
Quantification of Exosomes		
Quantification of exosomes	EXOCET Assays	https://www.systembio.com/microrna-research/exosome-antibody/exocet-assay
	EXOFLOW Kits	https://www.systembio.com/exosome-research/overview
RNA extraction from Exosomes		
RNA extraction and profiling	SeraMir kits	https://www.systembio.com/microrna-research/seramir-exosome-rna-profiling/overview

Example Data and Applications

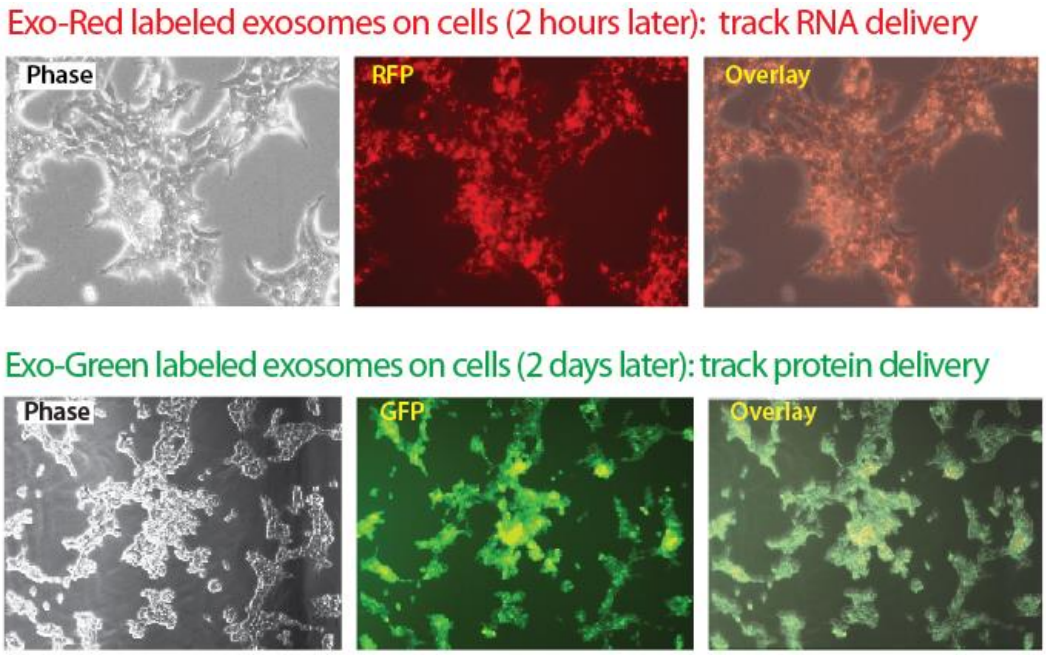
Exosomes were isolated from tissue culture medium from HEK293 cells grown in DMEM supplemented with Exo-FBS (exosome-depleted FBS). The cells were grown to 80-90% confluency in a 10 cm cell culture dish. The secreted exosomes were isolated as stated in the protocol for ExoQuick-TC. The exosome pellet was resuspended in 1 mL 1X PBS and contained an exosome protein content of 1 µg/µL. 500 µL of this exosome suspension was labeled with 50 µL of either 10x Exo-Red or Exo-Green for 10 minutes at 37°C. To stop the reaction, the exosomes were precipitated using the addition of 100 µL ExoQuick-TC. The labeled exosome pellets were resuspended in 500 µL 1X PBS.

50 µL of anti-CD63 magnetic beads from SBI's Exo-Flow IP kit (cat# EXOFLOW32A-CD63) were incubated with 100 µL of the labeled exosomes overnight at 4°C on a rotator. The following day, the bead/labeled exosomes were placed on a magnetic plate for 5 minutes and then washed with 100 µL 1x wash buffer once. Then, 100 µL 1x PBS was added to the IP well and placed on the magnetic rack for 2 minutes to position the beads at the bottom of the wells. The beads with captured, labeled exosomes were imaged using fluorescent microscopy.



Exo-Glow exosomes added to target cells

The labeled exosomes were added to target HEK 293 cells in culture. The cells were plated at about 1x10^5 cells in a standard 6 well culture plate. Approximately 100 µL of the labeled exosome suspension was added per 6 well with cells and allowed to interact/uptake for the time indicated in the following figures.



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 Exo-Glow products (*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.

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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