

Exo-Glow™ Tracking Labels

Fluorescently label exosome RNA and protein cargo

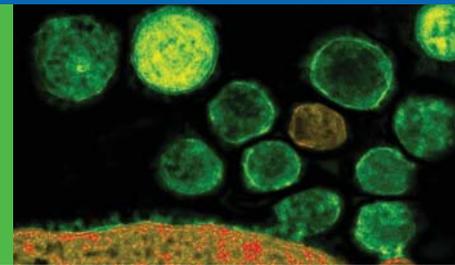
Exosomes are 60 - 180 nm membrane vesicles secreted by most cell types *in vivo* and *in vitro* and contain distinct subsets of RNAs and proteins depending upon the cell type from which they are secreted, making them useful for biomarker discovery and functional characterization. Exosomes are nano-sized shuttles that transport signalling RNAs and proteins to other cells.

The Exo-Glow kits allow you to fluorescently-label isolated exosomes to track cellular interaction and uptake. You can fluorescently label exosome internal RNA Red or internal exosome proteins Green for tracking. Isolated exosomes are incubated with either the 10x Exo-Red (for RNAs) or 10x Exo-Green (for proteins) labeling stain. The Exo-Red stain is based on an Acridine Orange (AO) chemistry. AO is membrane permeable and fluorescently-labels single-stranded RNAs inside of exosomes. These Red exoRNAs can then be monitored for delivery into target cells via the exosomes using fluorescent microscopy. 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 exosome esterases known to be 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 you to track exoProtein transfer into target cells using fluorescent microscopy.

View captured exosomes on magnetic beads

Exosomes from HEK293 cells grown in Exo-FBS exosome-depleted media supplement with standard DMEM were isolated using ExoQuick-TC. The exosome pellet was resuspended in 1 ml 1x PBS and contained an exosome protein content of 1 ug/ul. About 500 ul of this exosome suspension was labeled with 50 ul of either 10x Exo-Red or Exo-Green for 10 minutes at 37°C. The exosomes were re-isolated using the addition of 100 ul ExoQuick-TC (included in kits) and precipitation at 5°C for 30 minutes on ice or 4°C. The labeled exosome pellets were resuspended in 500 ul 1x PBS. CD63-coupled magnetic beads from SBI's Exo-Flow IP kit (cat# EXOFLOW32A-CD63). Briefly, 50 ul of CD63 magnetic beads were incubated with 100 ul 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 ul 1x wash buffer once. Then, 100 ul 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.

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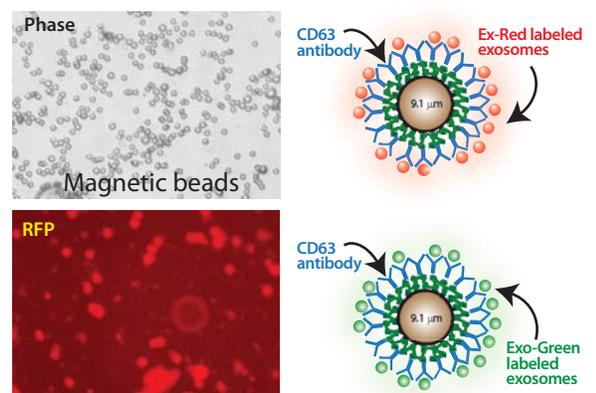
Highlights

- Label exoRNAs with Red fluorescent dye
- Monitor exoRNA delivery using fluorescent microscopy
- Label internal exosome proteins green
- Track exosome protein delivery using fluorescent microscopy
- Use labeled exosomes to follow cellular interaction and uptake *in vivo*

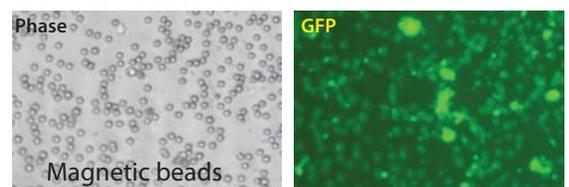
Description	Cat#	Size
Exo-Red exosome RNA fluorescent label	EXOR100A-1	20 rxns
Exo-Green exosome protein fluorescent label	EXOG200A-1	20 rxns
Exo-Red + Exo-Green exosome cargo fluorescent label Combo	EXOC300A-1	10 rxns each

Each Exo-Glow reaction (rxn) equates to labeling 1.5×10^8 exosomes.

Exo-Red labeled exosomes bound to CD63 beads



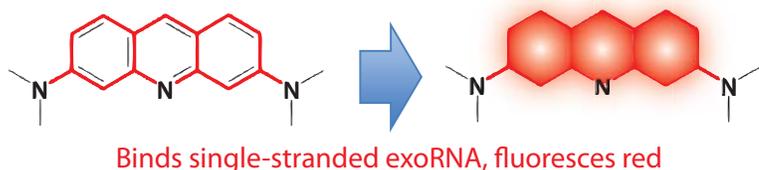
Exo-Green labeled exosomes bound to CD63 beads



Exo-Glow™ Tracking Labels

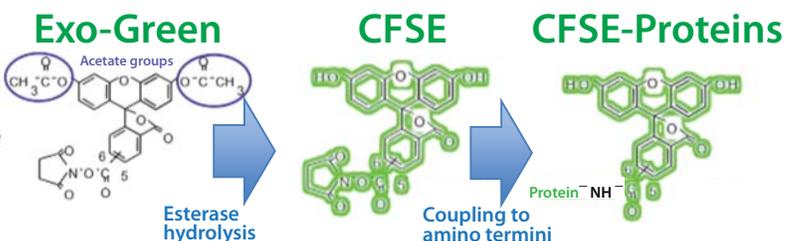
How the exosome labels work

Label internal exosome RNAs red



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

Label internal exosome proteins green



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 with DNA by intercalation and RNA by electrostatic attractions. 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 (green). When Exo-Red associates with RNA, the excitation maximum shifts to 460 nm (blue) and the emission maximum shifts to 650 nm (red).

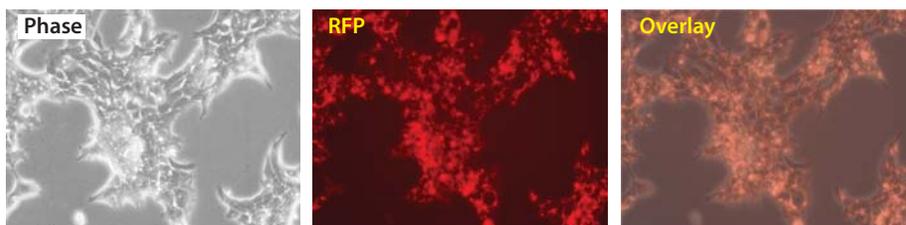
The Exo-Green stain is based on Carboxyfluorescein succinimidyl diacetate ester (CFSE) chemistry. CFSE is membrane permeable. When CFSE enters exosomes it is hydrolyzed by esterases known to be within exosomes that remove the diacetate residues. This activates the CFSE to fluoresce green and is then coupled to the amino ends of proteins, making them fluoresce green. Exo-Green has an excitation maximum at 494 nm and an emission maximum at 521 nm (green).

Monitor labeled exosome intraction and uptake in cells

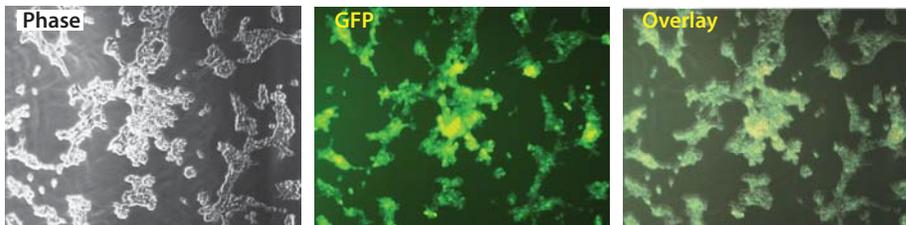
Human exosomes labeled with either Exo-Red or Exo-Green dyes were added to target HEK 293 cells in culture. The cells were plated at about 1×10^5 cells in a standard 6 well culture plate. Approximately 100 μ l of the labeled exosome suspension was added per 6 well with cells and allowed to interact/uptake for the time indicated in the data shown to the right.

Add labeled exosomes to cells in culture and monitor exosome cargo delivery in real-time.

Exo-Red labeled exosomes on cells (2 hours later): track RNA delivery



Exo-Green labeled exosomes on cells (2 days later): track protein delivery



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