ExoGlow™-Vivo EV Labeling Kit (Near IR)

Cat # EXOGV900A-1

User Manual

Storage: See individual components

Version 2
7/11/2018

Contents

Product Description

Visualizing extracellular vesicles (EVs) in cells and organisms has been technically difficult due to the high background from autofluorescence in the UV and visible areas of the spectrum and a lack of specificity from lipophilic dyes that are frequently used for this application. SBI's ExoGlow™-Vivo EV Labeling Kit (Near IR) (Cat #EXOGV900A-1) is the first reagent specifically designed to overcome these problems through the use of a proprietary, non-lipophilic dye that emits in the near infrared (NIR) range (excitation at 784 nm; emission at 806 nm). Delivering a level of specificity and sensitivity that takes the guesswork out of tracking EVs *in vivo*, ExoGlow-Vivo is ideal for EV biodistribution and kinetic studies needed to fully realize the value of EVs in basic research and translational applications.

List of Components

Item	Volume	Storage Temperature
Labeling Dye (lyophilized)	1 vial	-20^{0}C
ExoQuick-TC	2 mL	RT

*The kit is for 12 individual labeling reactions

Storage

The ExoGlow-Vivo EV Labeling Kit (Near IR) 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 250 µg of total protein (as measured by Qubit or BCA assay) in the sample. **Protect labeling dye from light.**

Additional Notes

ExoGlow-Vivo dye characteristics:

Max excitation wavelength: 784nm, Max emission wavelength: 806nm

Protocol for ExoGlow-Vivo Labeling:

Preparation of dye stock solution

1. Briefly spin down the tube and add 25µL anhydrous DMSO.

2. Vortex the tube for 5 seconds to completely dissolve the dye

3. Centrifuge the tube (brief pulse spin to max RCF) to collect the resuspended dye at the bottom at the tube.

ExoGlow-Vivo staining protocol

1. Add 2µL ExoGlow Vivo dye to a maximum of 250µg protein equivalent of EVs (as measured by Qubit or BCA assay) in 500µL 1xPBS.

2. Incubate the reaction for 45 mins to 1 hour at room temperature.

3. Purify the EVs from the labeling reaction using your preferred method, or use the method below using ExoQuick-TC reagent (provided in the kit):

ExoQuick-TC based EV recovery

1. Add 167µL ExoQuick-TC to the reaction (provided). Mix well by pipetting up and down several times. Do not vortex.

2. Incubate the reaction for 2 hr to O/N at 4^0C.

3. Spin the tube at 13,000 x g for 10 mins to recover the EVs.

4. Carefully discard the supernatant.

5. Resuspend the pellet in a sterile buffer of choice (e.g. 1xPBS) to desired volume. Suggested resuspension volume is 200-250µl.

6. Labeled EVs are ready for use.

Example Data and Applications

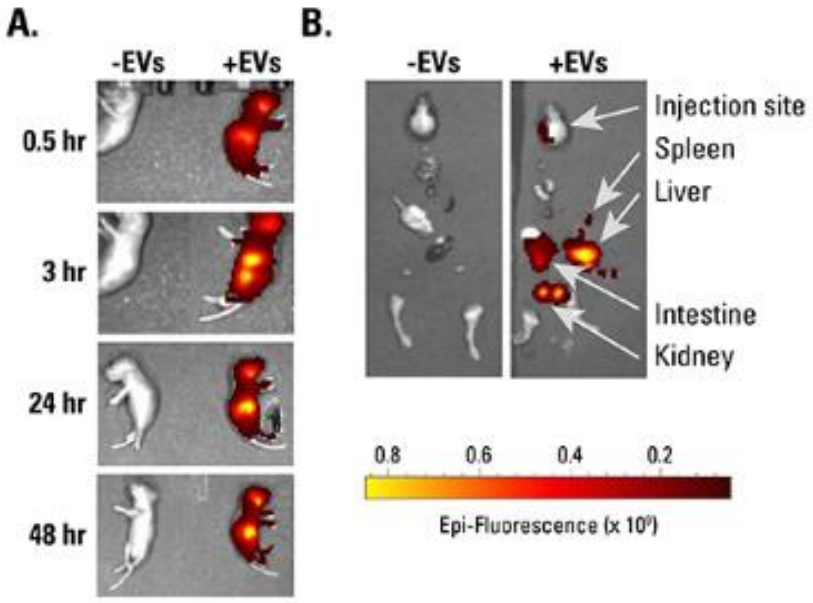


Figure 1. ExoGlow-Vivo labeled EVs show robust signal *in vivo*. A. HEK293-derived EVs isolated using ExoQuick-TC® were labeled with the ExoGlow-Vivo dye and administered intravenously via the superficial temporal vein into post-natal day-4 C57BL6 mice. Animals were imaged at various time points using IVIS® In Vivo Imaging System (PerkinElmer). **B**. Dissection after 24-hours shows the preferential accumulation of labeled EVs in the liver and kidneys. *Data courtesy of Gareth Willis, PhD., Harvard Medical School and Boston Children's Hospital*.

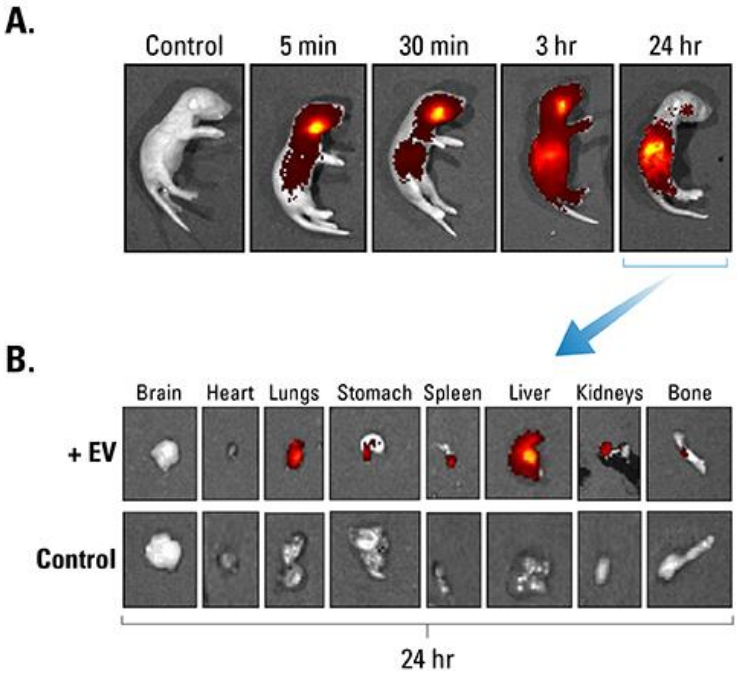
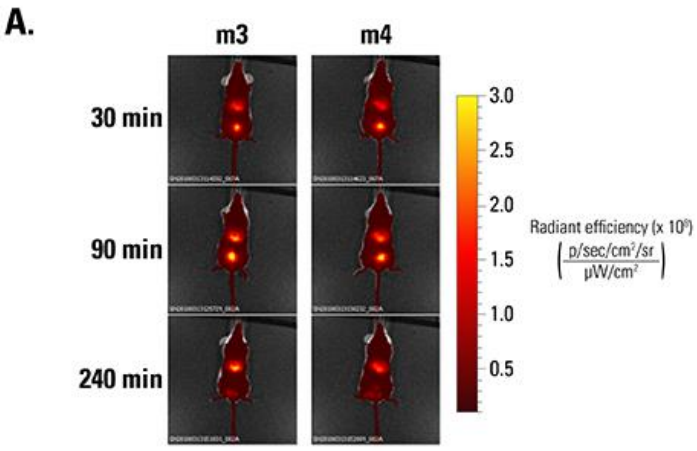


Figure 2. ExoGlow-Vivo dye delivers minimal background. **A.** Human mesenchymal stem cell-derived EVs were labeled with ExoGlow-Vivo dye and unbound dye removed via ultracentrifugation and a wash. EVs were administered intravenously via the superficial temporal vein into post-natal day-4 FVB mice. Animals were imaged at various time points using an IVIS® In Vivo Imaging System (PerkinElmer). Control refers to supernatant from wash step (*i.e.* free dye). **B.** Dissection after 24-hours shows the preferential accumulation of labeled EVs in specific organs and very low residual background signal from free dye.



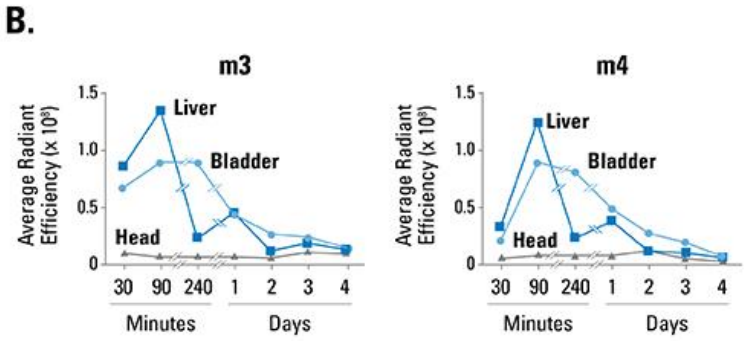


Figure 3. ExoGlow-Vivo enables kinetic analysis of EV persistence in living mice. A. Human PBMC-derived EVs were labeled with ExoGlow-Vivo and administered via tail vein injection into SCID mice (m3 and m4). Animals were imaged at various time points using an IVIS® In Vivo Imaging System (PerkinElmer). **B**. Plotting signal intensity in different organs (liver, bladder, head) as a function of time after injection shows that EVs are rapidly taken up by target organs within 90-minutes of injection and decline at different rates over time. *Data courtesy of Sam Noppen, Rega Institute KU Leuven, Belgium.*

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:

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-Vivo 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.

The ExoGlow-Vivo labeling dye is supplied under a license agreement with Biotium.

Purchase of the product does not grant any rights or license for use other than those explicitly listed in this Licensing and Warranty Statement. Use of the Product for any use other than described expressly herein may be covered by patents or subject to rights other than those mentioned. SBI disclaims any and all responsibility for injury or damage which may be caused by the failure of the buyer or any other person to use the Product in accordance with the terms and conditions outlined herein.

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.

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