Lyophilized Fluorescent Exosomes

Fluorescently labelled Exosomes from 293F

REF	Σ	
ExoEGFPCD63 ExomScarletCD81 ExoBFPCD9	30 µg	RUO

1. INTRODUCTION

Exosomes are small extracellular vesicles that are released from cells upon fusion of an intermediate endocytic compartment, the multivesicular body (MVB) 1 , with the plasma membrane. They are thought to provide a means of intercellular communication (2,3) and of transmission of macromolecules between cells allowing the spread of proteins, lipids, mRNA, miRNA and DNA and as contributing factors in the development of several diseases. Exosomes can also modulate cancer microenvironment (4) and the immune response (5,6) .

Fluorescent exosomes are labelled by expressing a specific exosome membrane marker (such as CD63) fused to a fluorescent protein (i.e. EGFP) in 293F cells.

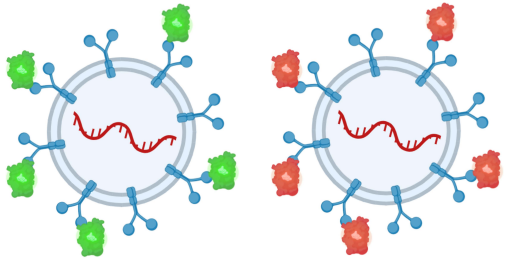


Figure 1. Representation of fluorescent extracellular vesicles. Exosomes are isolated from cell cultures that recombinantly express tetraspanins fused to fluorescent protein markers.

2. PRODUCT DESCRIPTION

Lyophilized fluorescent exosomes (~30 µg or 1×10^{10}) from 293F Human Embryonic Kidney cells.

- Tested application: Flow Cytometry (FCM), Nanoparticles Tracking Analysis (NTA, NanoSight), Nano Flow Cytometry (CytoFLEX nano).
- Species reactivity: Human.
- Presentation: Lyophilized.
- Fluorescent tags: EGFP (Ex: 488 nm, Em: 507 nm); mScarlet (Ex: 569 nm, Em: 597 nm), mTagBFP2 (Ex: 399 nm, Em: 454 nm).
- Reconstitution of Exosomes: For reconstitution, we recommend adding sterile, distilled water to achieve a final exosome concentration of 10^{11} exosomes/µL (e.g. add 30uL of dH O to a vial of 10^{10} lyophilized exosomes). After the addition of water, recap vial and briefly vortex making sure that the liquid has been evenly distributed and has covered the entire inside of the vial. After vortexing, make sure that the solution is collected at the bottom of the vial, if not centrifuge shortly the vial solution. Now the standard is ready to use.
- Application: Positive control for EV analysis (NTA, flow Cytometers, fluorescence microscopy), cell spike-in and in vitro tracking, EV uptake monitoring, assay and instrument calibration.

3. APPROPRIATE STORAGE AND HANDLING CONDITIONS

Lyophilized exosomes can be stored between 2 °C and 8 °C for up to 2 years without functional compromise.

Immunostep recommends storing small, single-use aliquots of reconstituted exosomes, at -20°C for up to one month or at -80°C for longer periods, preferably in locations in frost-free freezers without appreciable temperature fluctuation. This will minimize protein denaturation that can occur after multiple freeze/thaw cycles. Reconstituted exosomes, stored properly, are functionally guaranteed for up to six months from date of reconstitution. Any unfrozen and/ or unused exosome standard can be stored at 4°C for short term use (<1 week) and should not be re-frozen.

4. EVIDENCE OF DETERIORATION

Reagents should not be used if any evidence of deterioration is observed. For more information, please contact our technical service: tech@immunostep.com

5. BIOSAFETY LEVEL 1

Biosafety classification is based on 2000/54/EC Directive from the European Council. Customer has to ensure that their facilities comply with biosafety regulations for their own country.

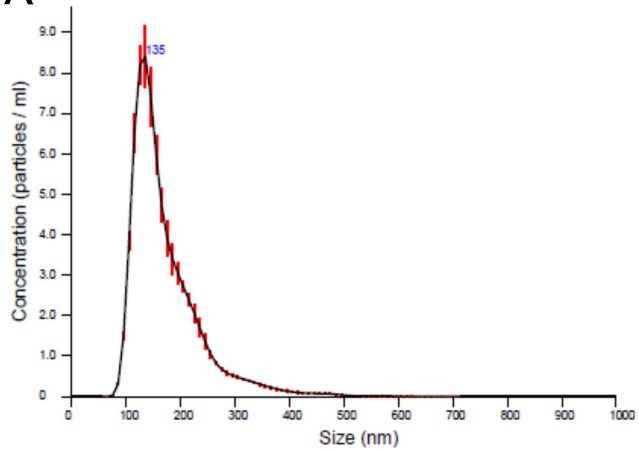
6. WARRANTY

Warranted only to conform to the quantity and contents stated on the label or in the product labelling at the time of delivery to the customer. Immunostep disclaims hereby other warranties. Immunostep's sole liability is limited to either the replacement of the products or refund of the purchase price.

7. PERFORMANCE DATA

All exosome standard batches have been Validated using FCM and NTA Analysis, additionally in order to compare the effects of lyophilization process we have compared all lyophilized batches with respect to fresh exosomes stored at -20°C.

A



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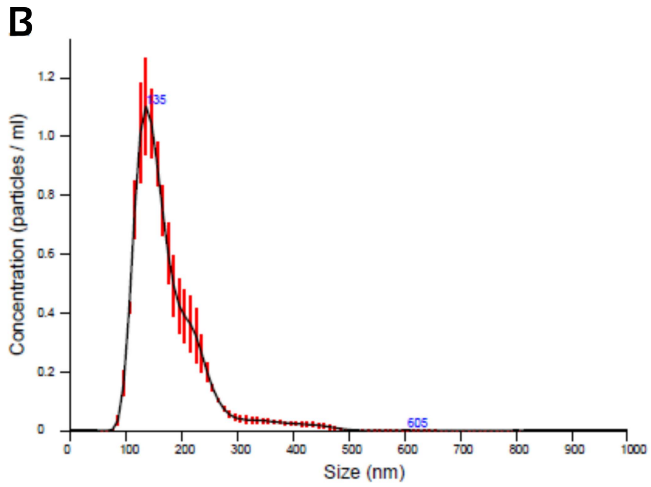

Figure 2. NTA Analysis (Nanosight NS300) of EGFP-CD61 (A) and mScarlet- CD81 (B) labelled exosomes. Analysis is conducted by diluting purified exosomes 1:1000 in PBS. Median particle size of purified fluorescent exosomes is 150 nm.

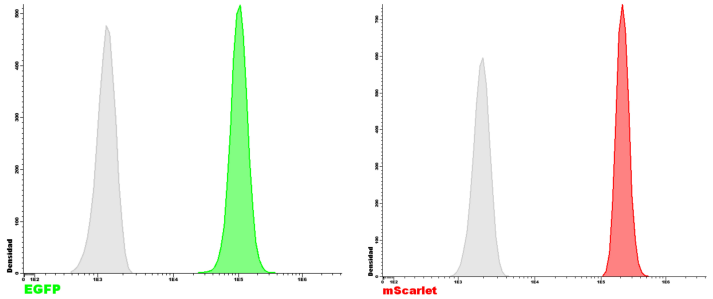

Figure 3. Fluorescently labelled exosomes expressing EGFP-CD63 or mScarlet-CD81 analyzed by flow cytometry. Fluorescent EGFP (green) or mScarlet (red) are separated from unlabeled exosomes Lyophilized exosomes from PC3). Exosomes (0.6 µg or ~1 x 108 exosomes) were incubated with anti-CD63 (clone TEA3/18) capture beads or anti-CD81 (clone M38) and acquiredon a 3-laser Aurora spectral flow cytometer (Cytek).

8. RELATED PRODUCTS

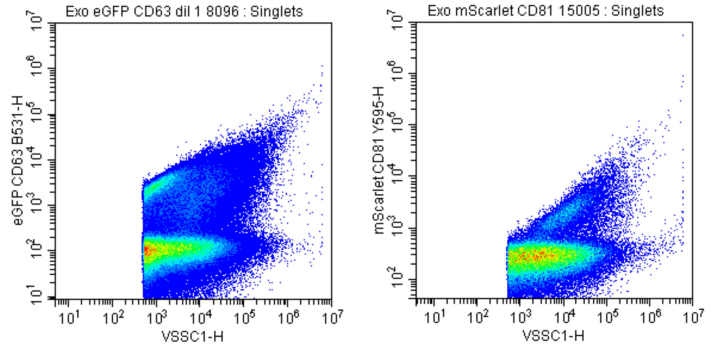

Figure 4. Exosomes labelled with EGFP-CD63 (left) and mScarlet-CD81 (right) analyzed with a Beckman Coulter Life Sciences CytoFLEX nano. EGFP or mScarlet positive exosomes are clearly differentiated from non-fluorescent exosomes.

9. ADDITIONAL INFORMATION

For research use only. Not for diagnostic use.

Not for resale. Immunostep will not be responsible of violations that may occur with the use of this product. Any use of this product other than the specified in this document is strictly prohibited.

Unless otherwise indicated by Immunostep by written authorization, this product is intended for research only and is not to be used for any other purpose, including without limitation, for human or animal diagnostic, therapeutic or commercial purposes.

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10. EXPLANATION OF SYMBOLS

	Fluorochrome
	Product reference
	Content for <n> analysis
	Regulatory Status
	Description
	Research Use Only
	Manufacturer

11. MANUFACTURED BY

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