# ExoFlow-ONE™ EV Labeling Kit for Flow Cytometry

**Cat# EXOFXXXA-1**

## User Manual

**Storage: See individual components**

Version 1
9/25/2017

## Contents

## Product Description

SBI's ExoFlow-ONE™ EV Labeling Kit for Flow Cytometry (Cat # EXOFXXXA-1) enables specific fluorescent-based detection of extracellular vesicles (EV) such as exosomes for use in Flow Cytometry applications. Using a proprietary dye formulation with high quantum efficiency (SBI's exclusive Gemstone dye collection), the kit enables efficient labeling of EV internal contents and detection approaching near single-vesicle resolution*, opening up new possibilities for characterizing EVs from a wide range of biofluids using flow-based approaches.

Each dye in the ExoFlow-ONE kit comes with enough labeling reagents to perform 25 reactions**, along with 1.5ml of reference size standards covering sizes of 110nm to 1300nm, with two of the standards being fluorescent. The standards (provided by Apogee Flow Systems) have been specially designed to produce a refractive index and scatter patterns similar to biological particles, allowing more accurate representation of particle size.

***Requires specialized flow cytometry imaging instrumentation (e.g. Amnis® ImageStreamX Mark II, Apogee Micro-PLUS) capable of high-resolution vesicle analysis**

****Each reaction is defined as 1 ul of working dye combined with 200 – 500 ug of EV protein resuspended in 500ul of 1X PBS**

## List of Components

| Item | Volume | Storage Temperature |
|---|---|---|
| ExoFlow-ONE dye [Sapphire Blue, Emerald Green, Citrine Yellow, Ruby Red, Garnet Far Red] | 25 µL | -20$^0$C |
| Size Reference Beads | 1.5 ml in Dropper Bottle | 4$^0$C |
| ExoQuick-TC reagent | 5 ml | RT |

## Storage

The ExoFlow-ONE™ kits are shipped on blue ice and should be **stored** accordingly to the labels in the kit. Properly stored kits are stable for 6 months from the date received.

## General Information

The reaction size is based on 200-500 µg of total protein (as measured by Qubit or BCA assay) in the sample. **Protect dyes from light.**

The spectral information and optimal laser for detection of ExoFlow-ONE dyes are shown below:

| | Excitation | Emission | Laser Line |
|---|---|---|---|
| Sapphire Blue | 403nm | 454nm | 405nm |
| Emerald Green | 511nm | 525nm | 488nm |
| Citrine Yellow | 542nm | 556nm | 532nm |
| Ruby Red | 573nm | 588nm | 561nm |
| Garnet Far Red | 628nm | 643nm | 633nm |

### Information on Size Reference Beads

The ApogeeMix contains 1.5mL of an aqueous mixture of beads containing diameters of 180nm, 240nm, 300nm, 590nm, 880nm and 1300nm diameter with refractive index η=1.43, and 110nm and 500nm green fluorescent (blue laser) beads with refractive index η=1.59. The intended use of these beads is to assess flow cytometer's light scatter and fluorescence performance in the instrument being tested, looking at both sensitivity and resolution as parameters. The below table contains typical data from the ApogeeMix analyzed on an A50-Micro flow cytometer (Apogee Flow Systems, FL1=Green fluorescence). The fluorescent beads may be used to assess the fluorescence sensitivity and to assess the performance of the flow cytometer's optics at a different refractive index.

Approximate particle concentrations for Lot # CAL0046:

| Particle Size (nm) | Approximate number per microlitre | Fluorescence from 488nm excitation |
|---|---|---|
| 110 | 8000 | Green |
| 180 | 23000 | None |
| 240 | 10000 | None |
| 300 | 9000 | None |
| 500 | 3600 | Green |
| 590 | 2700 | None |
| 880 | 3900 | None |
| 1300 | 3400 | None |
| | | |

*Courtesy of Apogee Flow Systems*

## Protocol for ExoFlow-ONE EV Labeling Kit

Recommendation: Mix the size reference beads prior to use by inverting the dropper bottle a few times. Use 1 drop in 500µL 1xPBS pre-filtered using a 0.02 µm filter

1. Resuspend 200-500µg protein equivalent of EVs in 500µL PBS pre-filtered using a 0.02 µm filter.

2. Add 1µL of the labeling dye to the exosome preparation and incubate at 37°C with shaking for 20 minutes.

3. Proceed with flow cytometric analysis of sample.

The labeled exosomes are now ready for flow cytometric analysis. ExoFlow-ONE dyes are membrane permeable and are fluorescently activated upon exosome internalization; residual free dyes in the buffer is not known to exhibit any detectable background. Should removal of the free dye be desirable, the following optional procedure is recommended (reagent included).

**Optional dye removal step:**

1. Add 167µL ExoQuick-TC to the solution and incubate at 4$^{o}$C for 2hrs.

2. Spin the Eppendorf tube at 10,000rpm for 10 minutes to pellet EVs.

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

4. Resuspend the exosome pellet in 500µL PBS pre-filtered using a 0.02 µm filter and proceed with flow cytometric analysis.

## Example Data and Applications

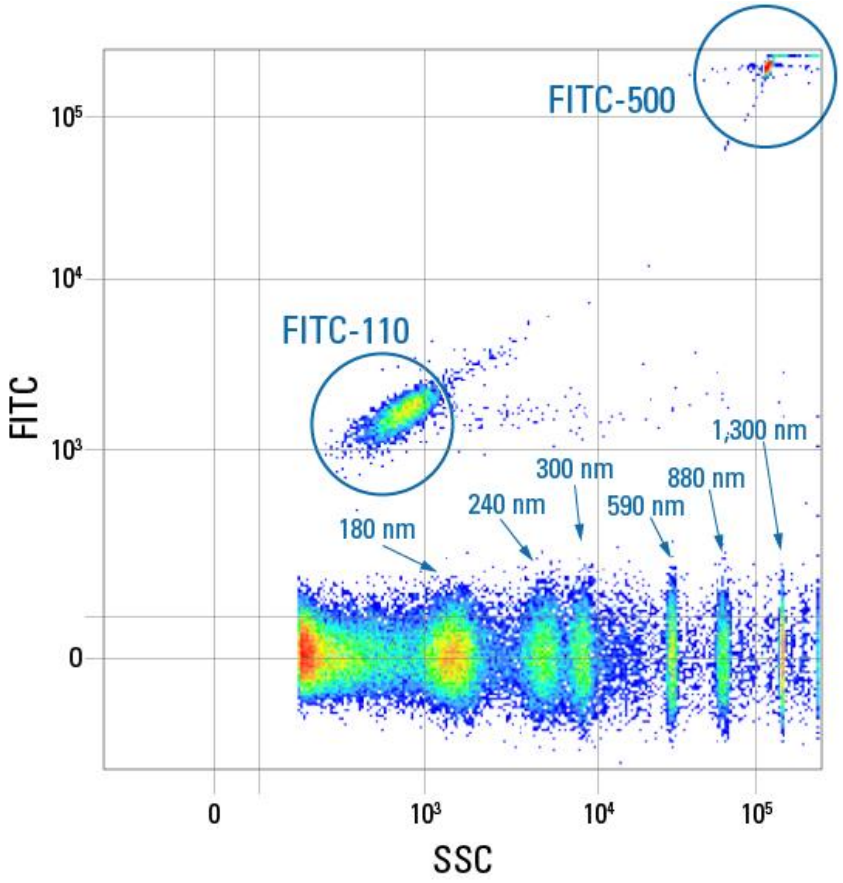


**Figure 1.** Analytical plot of FITC vs SSC for size reference bead mix in the ExoFlow-ONE kit, showing the SSC and FITC profiles for unlabeled beads of known sizes as well as two fluorescently-labeled beads (110nm and 500nm). Data collected on a BD® LSR II instrument, and beads are from Apogee Flow Systems.

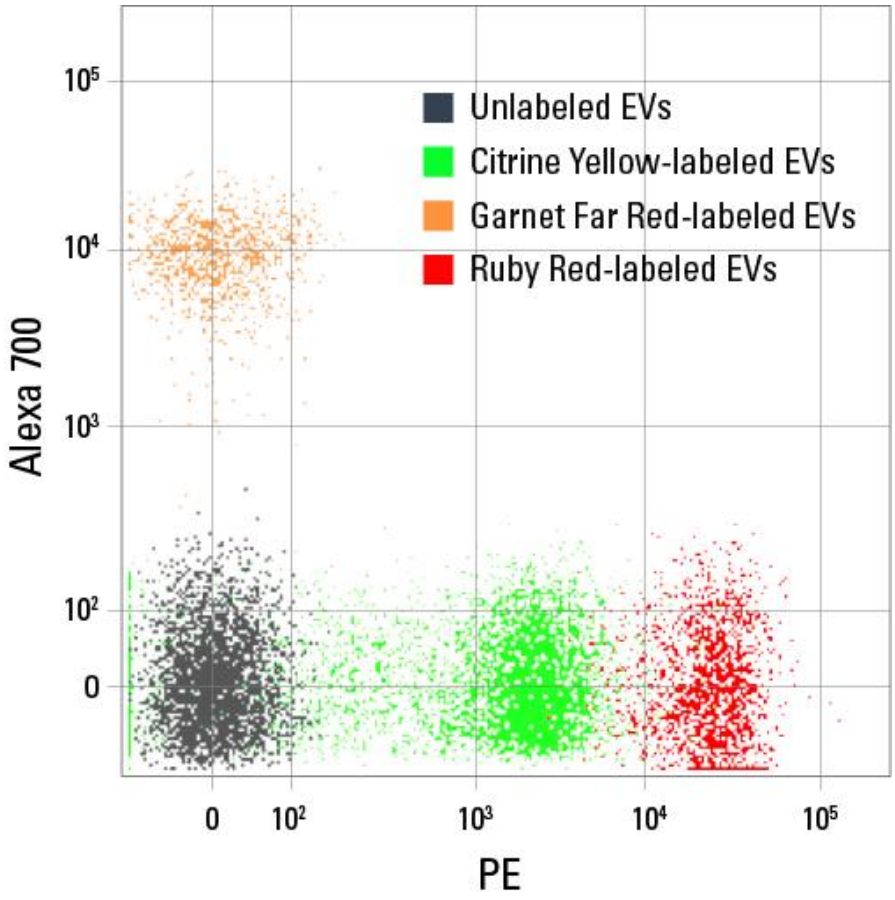


**Figure 2**. Analytical plot of PE and Alexa 700 showing significant spectral separation of ExoFlow-ONE dye-labeled HEK293T EVs from unlabeled EV population. Data collected on a BD® LSR II instrument.

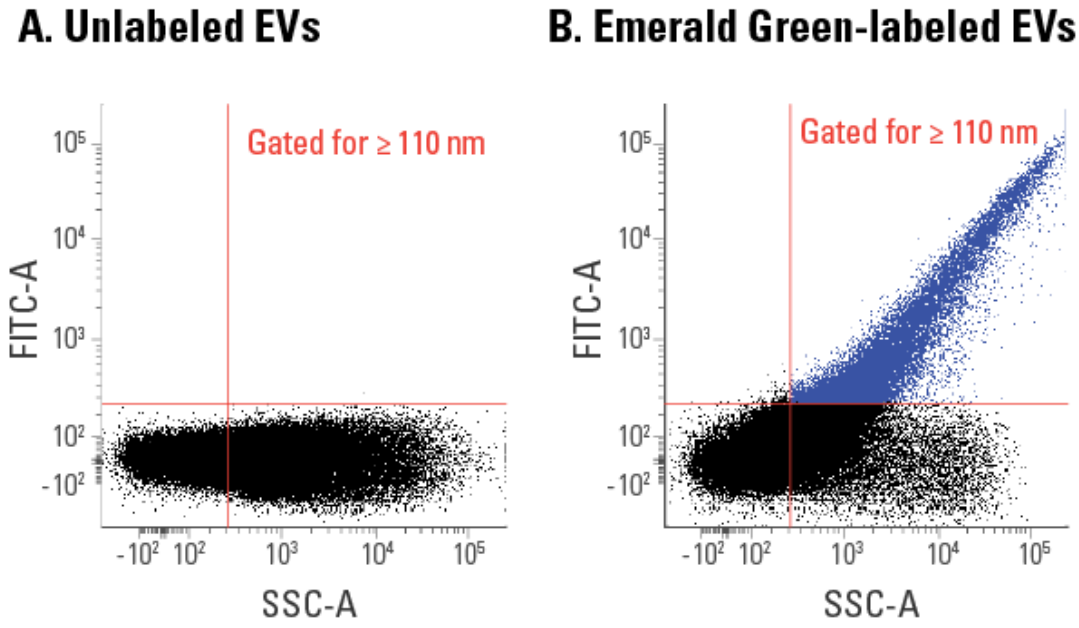

**Figure 3**. **ExoFlow-ONE labeled EVs can be robustly detected from background.** Both unlabeled (A) and ExoFlow-ONE Emerald Green-labeled EVs (B) from human serum were analyzed on a BD® LSR II Flow Cytometer, with size gate set for EVs >110nm based on reference bead signals. Labeled EVs can be cleanly detected and distinguished from the background, demonstrating the ability of ExoFlow-ONE labeled EVs to be analyzed via flow cytometry.

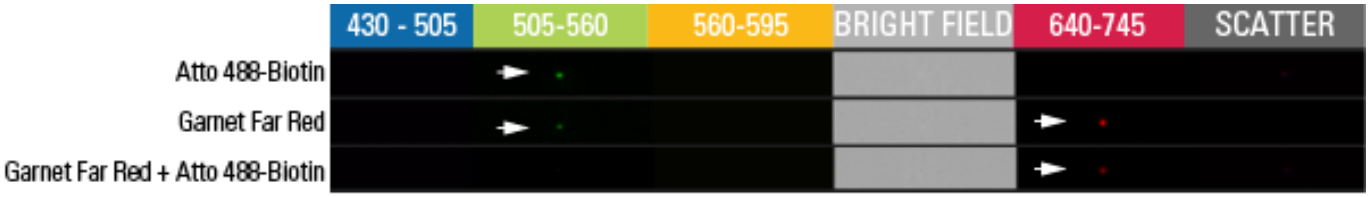


**Figure 4**. High-resolution detection of EVs using ExoFlow-ONE and conjugated dyes. Streptavidin-coated HEK293T EVs were captured and stained with either biotinylated Atto 488 dye, ExoFlow ONE Garnet Far Red dye, or combination of both dyes. Labeled EVs show correct signals at respective channels optimal for each dye, while bright field and scatter channels show no perceptible signal, indicating the observed event(s) are under the diffraction limit of detection. Data collected using an Amnis® ImageStream$^X$ Mark II Flow Cytometer.

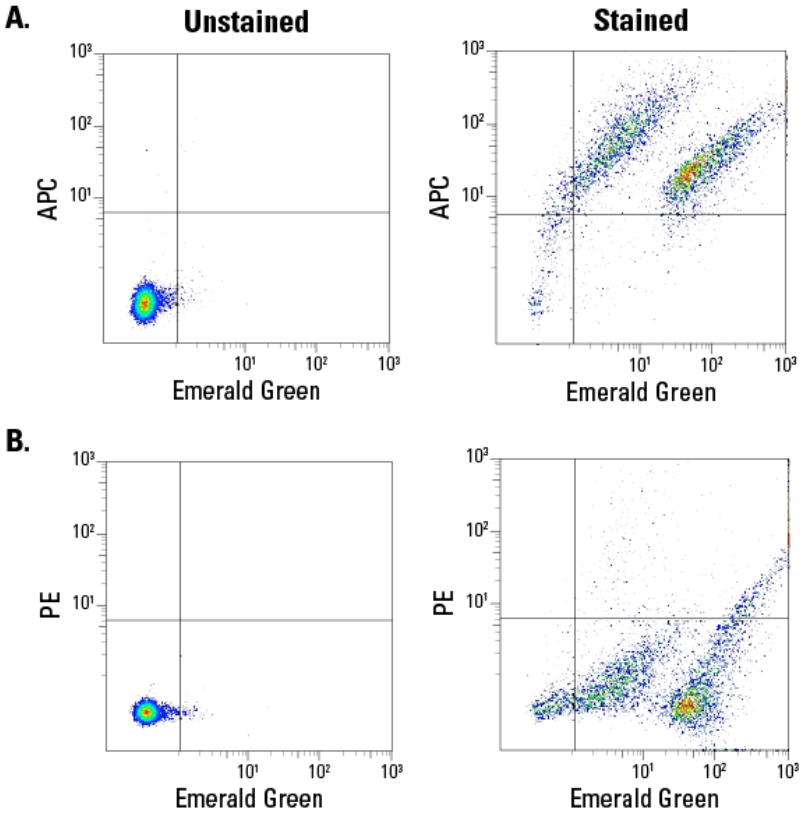

**Figure 5**. **ExoFlow-ONE dyes multiplexed with antibody-conjugated dyes are able to identify subpopulations of EVs.** Murine-derived EVs were isolated and co-stained with Emerald Green ExoFlow-ONE and an APC-conjugated antibody against a cell adhesion protein (A) or Emerald Green ExoFlow-ONE and a PE-conjugated antibody against a macrophage membrane protein (B). In panel A, right, two distinct populations of EVs can be seen (Emerald Green axis), each of which expresses a range of the cell adhesion protein (APC axis). In contrast, in panel B, right, while the two distinct populations of EVs can still be seen (Emerald Green axis) the EVs do not appear to express the macrophage-specific protein. Thus, ExoFlow-ONE dyes multiplexed with antibody-conjugated dyes can be used to easily detect subpopulations of EVs

## 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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Use of the ExoFLOW-ONE EV Labeling Kit for Flow Cytometry (*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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