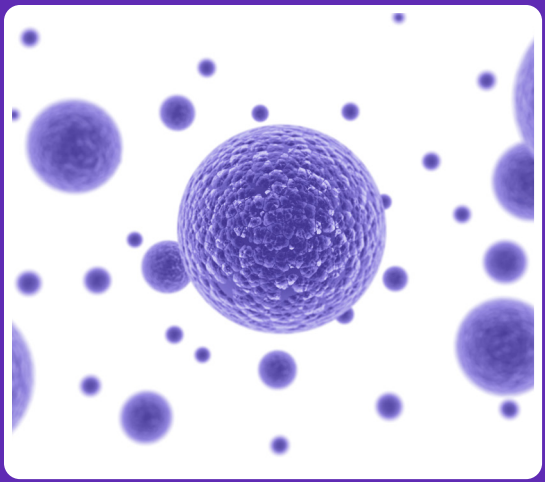


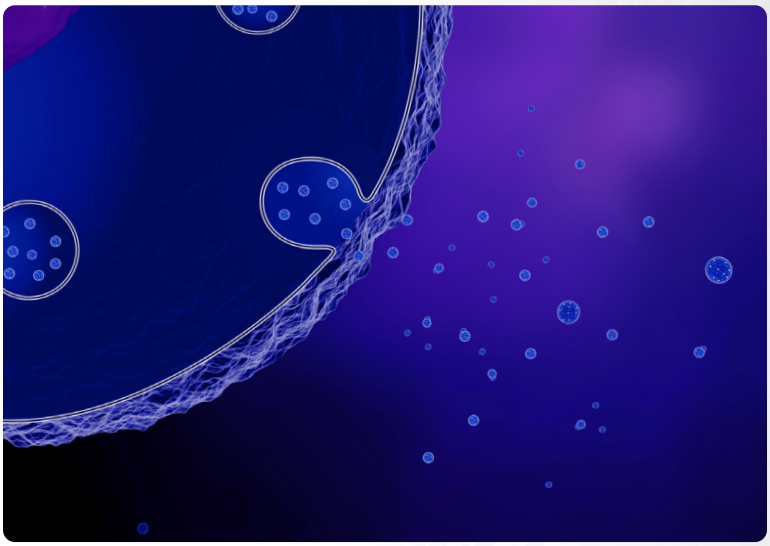
Extracellular Vesicle Stains You Can Trust

ExoBrite™ EV Stains & Antibodies

EV Analysis: Small Targets, Big Challenge

Analyzing extracellular vesicles (EVs) such as exosomes, poses many challenges. Their minute size near the detection limit of flow cytometry makes them difficult to distinguish from cell debris and other small particles. In addition, many commercially available dyes used for detection of EVs can form similarly sized aggregates, which is a frustrating problem for EV researchers.

Detection of EVs using antibodies targeting EV markers such as CD9, CD63, and CD81 is another option. However, commercially available antibodies for these markers are rarely validated for this application. Biotium scientists have developed unique solutions for EV detection with ExoBrite™ EV Membrane Stains, as well as validated ExoBrite™ Antibodies for flow cytometry and western blotting.



Validated Probes for Extracellular Vesicles

ExoBrite™ EV Membrane Stains

Detect EVs, not dye aggregates

Membrane dyes, common tools for labeling EVs, bind to the membrane that encases EVs. However, not all membrane dyes work equally well for EV staining. For example, classic carbocyanine dyes such as PKH, DiO, and DiI, have poor solubility and thus form aggregates that can be confused with EVs (see Fig. 2). Other membrane dyes that researchers have attempted to use for EV staining, such as di-8-ANEPPS, simply don't generate a signal that is robust or bright enough for efficient detection. To overcome these challenges, Biotium developed ExoBrite™ EV Membrane Stains, which provide bright staining of isolated EVs with minimal dye aggregation. For flexible panel design, ExoBrite™ EV Membrane Stains are available in four colors for the Pacific Blue™, FITC, PE, and APC channels (Fig. 1). ExoBrite™ stains are also available in four color options designed for super-resolution imaging by STORM (see next page).

ExoBrite™ EV Membrane Stain Features

- Validated for EV detection by flow cytometry
- Bright fluorescence and minimal dye aggregation
- Compatible with antibody co-staining
- Stain purified or bead-bound EVs
- Available in 4 colors

Available in 4 colors for flexible panel design

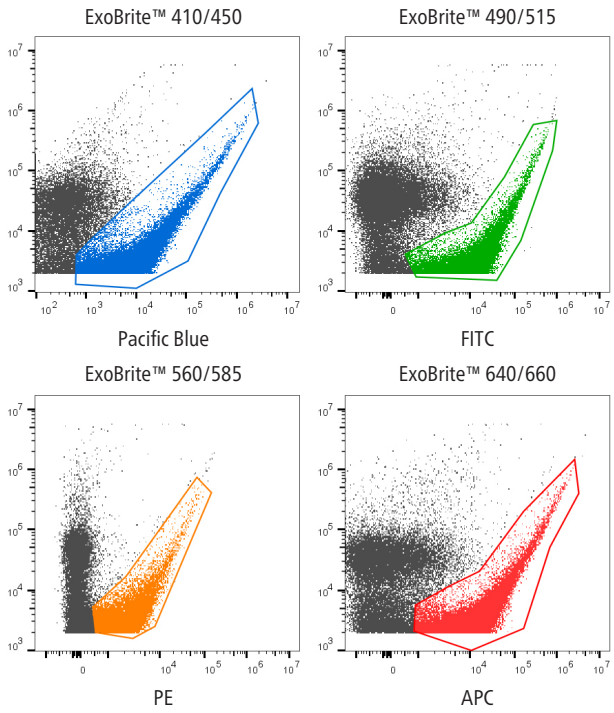


Figure 1. Size exclusion column-purified MCF-7-derived EVs were stained with ExoBrite™ EV Membrane Stains. EVs were detected on a CytoFLEX LX flow cytometer.

We tested more than 40 membrane stains to develop reagents that offer the best detection of EVs

After extensive research, we developed ExoBrite™ EV Membrane Stains to offer the best performance for detection of EVs by flow cytometry. Though widely used for EV staining, lipophilic dyes such as DiO form aggregated dye particles that are indistinguishable from EVs by flow cytometry scatter analysis. Other membrane stains, such as CellMask™, also show an unacceptable amount of dye aggregation which fall within the EV gate. ExoGlow™ dyes show some aggregates that can mostly be gated away from the true EVs, but can have lower percent positive staining. In our testing, ExoBrite™ EV Membrane Stains demonstrated the greatest degree of EV coverage as well as the lowest amount of dye aggregation (Fig. 2).

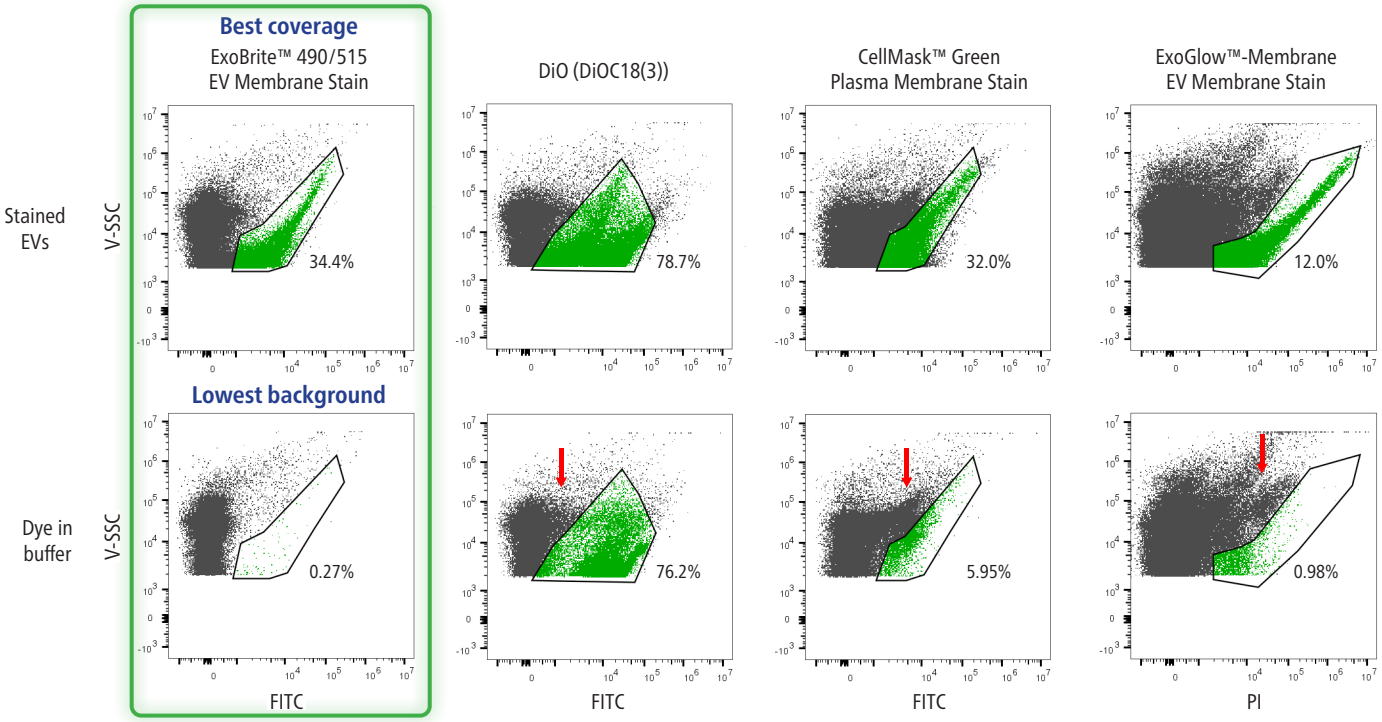


Figure 2. Size exclusion column-purified MCF-7 EVs were stained in PBS with the indicated dyes (top row). The positive population was gated in each EV sample relative to negative control samples containing dye added to filtered PBS (bottom row). The percentage of particles falling within the gate is shown. Red arrows indicate dye aggregates. EVs were detected on a CytoFLEX LX flow cytometer.

ExoBrite™ EV Membrane Stains

Compatible with antibody co-staining

ExoBrite™ EV Membrane Stains are hydrophilic membrane-binding probes that label a broad population of EVs. The stains are compatible with antibody co-staining, allowing users to quantify how many EVs express the protein of interest. For example, in the EVs that we tested (MCF-7-derived), the tetraspanin CD9 is highly and ubiquitously expressed. In our experiments, we found that co-staining with ExoBrite™ 560/585 EV Membrane Stain and CD9 (H19a)-CF®488A results in near-complete overlap of their positive populations (Fig. 3).

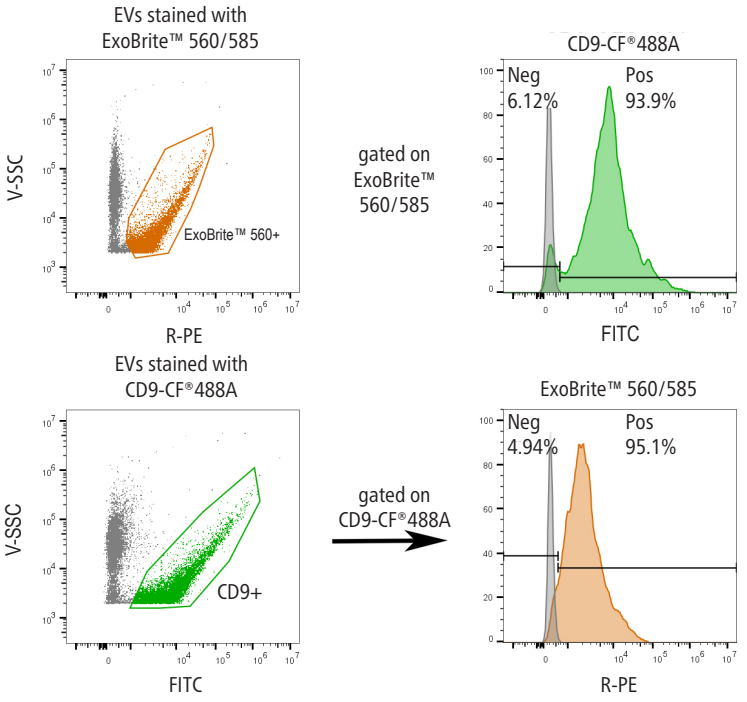


Figure 3. Size exclusion column-purified MCF-7-derived EVs were stained with ExoBrite™ 560/585 EV Membrane Stain and CD9 (H19a)-CF®488A. When gated on ExoBrite™ 560/585-positive particles, ~95% were also positive for CD9 (top row). Likewise, when gated on CD9-positive particles, ~ 95% were also positive for ExoBrite™ 560/585. EVs were detected on a CytoFLEX LX flow cytometer.

Suitable for purified or bead-bound EVs

ExoBrite™ EV Membrane Stains have been validated in flow cytometry for their ability to stain EVs derived from several different cultured cell lines, and isolated by several different methods, including size exclusion chromatography (SEC), polyethylene glycol (PEG) precipitation, and magnetic bead immunoprecipitation (IP). Also, unlike lipophilic membrane dyes, ExoBrite™ EV Membrane Stains don't bind non-specifically to polystyrene beads, allowing for their use with bead-bound EVs. In our testing, we observed near complete coverage of CD9 and CD81 positive bead-bound EVs when co-stained with ExoBrite™ Membrane Stains (Fig. 4).

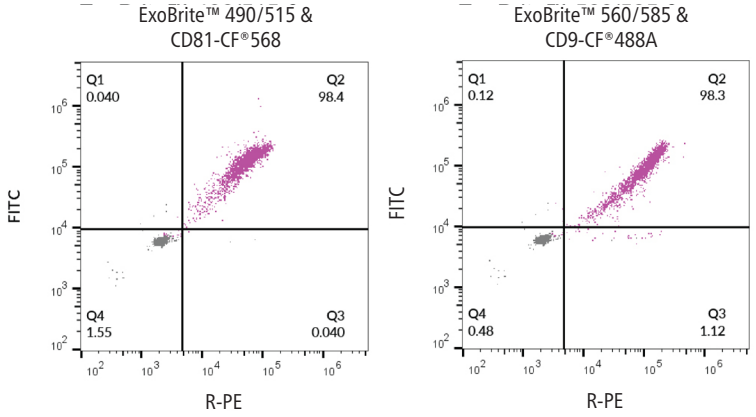


Figure 4. Flow cytometry of bead-bound EVs derived from MCF-7 cells co-stained with ExoBrite™ 490/515 and CD81-CF®568 (left) or ExoBrite™ 560/585 and CD9-CF®488A (right). Unstained EVs are gray and co-stained EVs are in pink. EVs were detected on a CytoFLEX LX flow cytometer in the FITC and R-PE channels.

Options for super-resolution imaging by STORM

ExoBrite™ STORM EV Membrane Stains are options developed specifically for super-resolution imaging of isolated EVs by stochastic optical reconstruction microscopy (STORM). The stains are coupled to a selection of four validated CF® Dyes for STORM. Visit our website for more information and to view a list of publications validating CF® Dyes as the industry-preferred dyes for STORM.

Ordering Information

Cat. #	Product	Ex/Em	Detection Channels	Size
30111-T, 30111	ExoBrite™ 410/450 EV Membrane Staining Kit	416/452 nm	Pacific Blue™	100 Labelings 500 Labelings
30112-T, 30112	ExoBrite™ 490/515 EV Membrane Staining Kit	490/516 nm	FITC	
30113-T, 30113	ExoBrite™ 560/585 EV Membrane Staining Kit	562/584 nm	PE	
30114-T, 30114	ExoBrite™ 640/660 EV Membrane Staining Kit	642/663 nm	APC	
30115-T, 30115	ExoBrite™ STORM CF®505 EV Membrane Staining Kit	505/519 nm	FITC	
30116-T, 30116	ExoBrite™ STORM CF®583R EV Membrane Staining Kit	585/609 nm	Cy®3 or Texas Red®	
30117-T, 30117	ExoBrite™ STORM CF®647 EV Membrane Staining Kit	652/668 nm	Cy®5	
30118-T, 30118	ExoBrite™ STORM CF®680 EV Membrane Staining Kit	681/698 nm	Cy®5.5	

ExoBrite™ Flow Antibody Conjugates

Validated for detection of EV markers in purified or bead-bound EVs

The tetraspanin family members CD9, CD63, and CD81 are widely used markers for EVs. While antibodies targeting these proteins are available from commercial suppliers, few are validated for detection of EVs. ExoBrite™ Flow Antibody Conjugates were curated and validated for flow cytometry to offer bright signal and low background for EV markers in purified and bead-bound EVs.

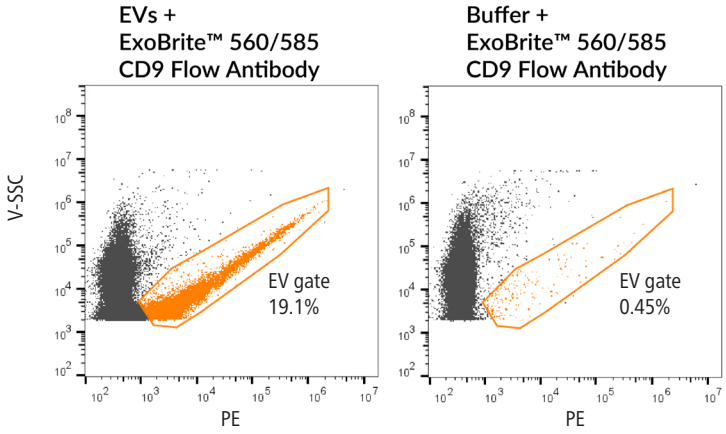


Figure 5. Size exclusion column-purified MCF-7-derived EVs were stained with ExoBrite™ CD9 560/585 Flow Antibody. Specific staining of EVs was seen (left), compared with the same antibody in buffer alone (right). EVs were detected on a CytoFLEX LX flow cytometer in the PE channel.

Features of ExoBrite™ Flow Antibody Conjugates

- Validated for detection of EV markers CD9, CD63, and CD81 in purified or bead-bound EVs by flow cytometry
- ExoBrite™ Isotype Control Flow Antibody available
- Available in 4 colors for Pacific Blue™, FITC, and PE channels

Cat. #	Product	Conjugates	Size
P003-410... P003-RPE	ExoBrite™ CD9 Flow Antibody	ExoBrite™ 410/450 ExoBrite™ 490/515 ExoBrite™ 560/585 R-PE	25 Tests 100 Tests
P004-410... P004-RPE	ExoBrite™ CD63 Flow Antibody		
P005-410... P005-RPE	ExoBrite™ CD81 Flow Antibody		
P008-410... P008-RPE	ExoBrite™ IgG1 Isotype Control Flow Antibody		

ExoBrite™ Western Antibody Conjugates

Validated for detection of EV markers in extracts

ExoBrite™ Western Antibodies were developed to offer bright signal and low background of EV markers CD9, CD63, and CD81 in EV extracts by near-IR fluorescent western blot. The antibodies are available with two near-infrared fluorescent dyes, ExoBrite™ 680/700 and ExoBrite™ 770/800, which offer greater signal-to-noise than dyes with visible light emission for western blotting. ExoBrite™ Western Antibodies are validated for EV extracts and demonstrate enrichment in the EV lysates relative to total lysates (Fig. 6).

Negative control calnexin antibody available

An ExoBrite™ Calnexin Western Antibody detects a resident protein of the endoplasmic reticulum that is not found in EVs. It is offered as a validated negative control to assess the purity of isolated EV extracts (Fig. 6).

ExoBrite™ 680/700 CD9 Antibody

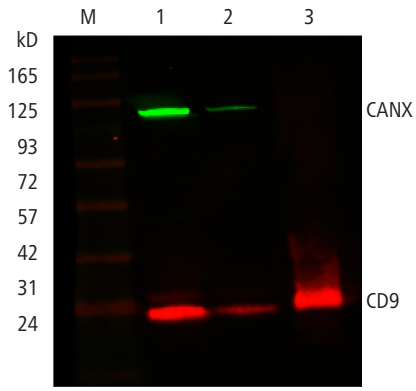


Figure 6. Western detection of human CD9 in MCF-7 cell and EV lysates using ExoBrite™ 680/700 CD9 Western Antibody and ExoBrite™ 770/800 Calnexin Antibody (CANX; EV negative control). Lane M: Protein marker. Lane 1: 10 ug cell lysate. Lane 2: 1 ug cell lysate. Lane 3: 1 ug EV lysate. The blots were imaged on a LI-COR Odyssey® infrared imaging system.

Features of ExoBrite™ Western Antibody Conjugates

- Validated for detection of EV markers CD9, CD63, and CD81 in EV extracts by near-IR western blotting
- Negative control ExoBrite™ Calnexin Western Antibody available
- Available in 2 near-infrared colors

Cat. #	Product	Conjugates	Size
P003-680, P003-770	ExoBrite™ CD9 Western Antibody	ExoBrite™ 680/700 ExoBrite™ 770/800	25 Tests 100 Tests
P004-680, P004-770	ExoBrite™ CD63 Western Antibody		
P006-680, P006-770	ExoBrite™ CD81 Western Antibody		
P007-770	ExoBrite™ Calnexin Western Antibody	ExoBrite™ 770/800	

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