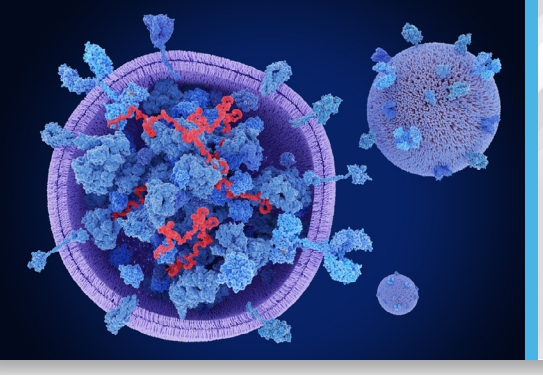


Detect EVs, Not Dye Aggregates

Discover ExoBrite™ EV Stains & Antibodies

ExoBrite™ True EV Membrane Stains - The Best Choice for Pan-EV Labeling

Membrane dyes are frequently used to detect EVs by labeling EV membranes. However, commonly used carbocyanine dyes such as PKH, DiO, and DiI have poor solubility and thus produce poor staining or form aggregates that can be mistaken for EVs. Other membrane dyes, such as di-8-ANEPPS, simply don't generate a signal that is robust or bright enough for efficient detection.

ExoBrite™ True EV Membrane Stains are novel lipophilic dyes designed to address these challenges by offering bright signal and clear differentiation of EVs from non-specific particles by flow cytometry. The stains are offered in four colors (Fig. 1) and can be used with antibodies for multi-parameter analysis. Flow cytometry and fluorescence nanoparticle tracking analysis (fNTA) demonstrated ExoBrite™ 515/540 to be a superior choice over PKH67 with higher (near-complete) pan-EV staining (Fig. 2).

Features of ExoBrite™ True EV Membrane Stains

- Lipophilic membrane dyes designed for pan-EV labeling
- Superior alternatives to PKH, DiO, DiI, DiD, and ANEPPS
- Near-complete coverage of EVs in a sample
- Stain EVs from all sources tested (see table, next page)
- Compatible with antibody co-staining
- Available for Pacific Blue™, FITC, PE, and APC channels

ExoBrite™ True EV Membrane Stains

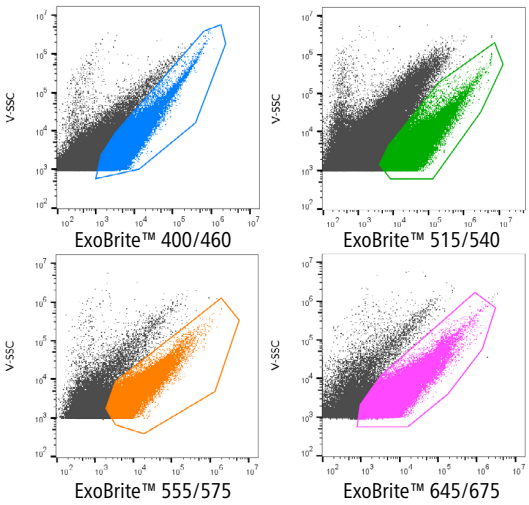
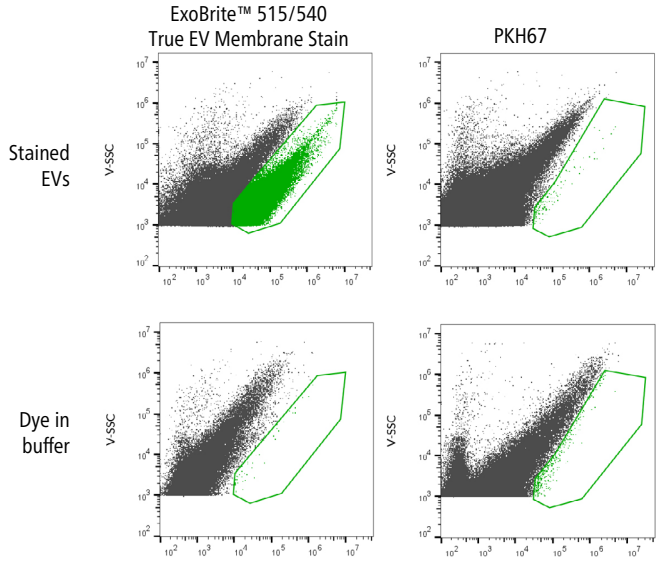


Figure 1. SEC-purified, MCF-7 cell-derived EVs stained with ExoBrite™ True EV Membrane Stains. Samples were run on a CytoFLEX® LX flow cytometer in the Pacific Blue™, FITC, PE, and APC channels.

ExoBrite™ 515/540 True vs. PKH67 in flow



ExoBrite™ 515/540 True vs. PKH67 in fNTA

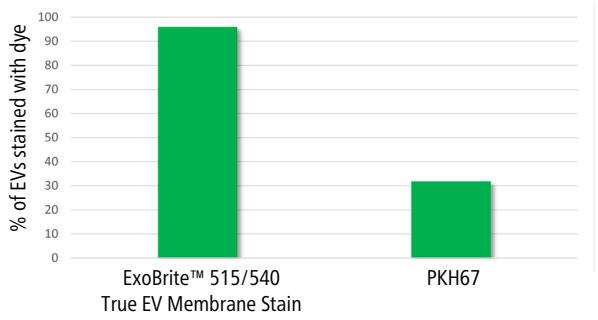


Figure 2. Flow cytometry and fNTA analysis of SEC-purified, MCF-7-derived EVs stained with ExoBrite™ 515/540 True EV Membrane Stain and PKH67. Top: Analysis on a CytoFLEX™ LX flow cytometer shows ExoBrite™ 515/540 True EV Membrane Stain was able to differentiate EVs from background particles, unlike PKH67. Bottom: In ZetaView® fNTA, ExoBrite™ 515/540 True EV Membrane Stain showed nearly complete coverage of EVs, much higher than PKH67.

biotium

ExoBrite™ EV Surface Stains

Optimized Annexin V, WGA, & CTB conjugates designed for bright staining of EVs

ExoBrite™ EV surface stains are fluorescent protein conjugates that bind to specific molecules commonly found on EV surfaces. The stains are available as Annexin V, wheat germ agglutinin (WGA), or cholera toxin B (CTB) conjugates, and were developed specifically for bright and sensitive staining of isolated EVs by flow cytometry. The conjugates display much lower background aggregation than most other EV stains and are suitable for antibody co-staining (Fig. 3 and Fig. 4). Biotium also developed ExoBrite™ STORM CTB EV Stains for examining EV morphology in fine detail with stochastic optical reconstruction microscopy (STORM) imaging (see table on page 4). ExoBrite™ EV surface stains have been found to label EVs derived from several tested cell lines, but may not stain EVs from every source. See the ExoBrite™ Stain Compatibility with EVs from Different Sources table below.

Features of ExoBrite™ EV Surface Stains

- Optimized conjugates of Annexin V, WGA, and CTB for EV labeling
- Bright staining of purified EVs with minimal dye aggregation
- Compatible with antibody co-staining
- Options for staining bead-bound EVs
- Available for Pacific Blue™, FITC, PE, and APC channels

ExoBrite™ Stain Compatibility with EVs from Different Sources

EV Source	ExoBrite™ True EV Membrane Stains	ExoBrite™ CTB Stains	ExoBrite™ WGA Stains	ExoBrite™ Annexin Stains
A549 cells	Yes	Yes	Yes	Yes
CHO cells	Yes	No	Yes	Yes
hASC[2]	ND	No[1]	ND	ND
HeLa cells	Yes	No	Yes	Yes
HUVEC[3]	ND	No[1]	ND	ND
J774 cells	Yes	Yes	Yes	Yes
Jurkat cells	Yes	Yes	Yes	Yes
MCF-7 cells	Yes	Yes	Yes	Yes
Plasma	ND	No	ND	Yes
Raji cells	ND	Yes	Yes	Yes
RAW 264.7	Yes	ND	ND	ND
Serum	ND	No	ND	Yes
Skeletal myoblasts	ND	Yes[1]	ND	ND
THP-1	Yes	ND	ND	ND
U2OS cells	Yes	No	Yes	Yes
U937 cells	Yes	No	Yes	Yes

[1]Customer-reported data
[2]Human adipose stem cells
[3]Human umbilical vein endothelial cells
Entry of "Yes" or "No" indicates coverage of EVs based on Biotium's internal data or customer-reported data. Value of "ND" indicates no data.
Scan QR code to view the latest information on our ExoBrite™ EV Stains

ExoBrite™ EV Surface Stain Comparison

ExoBrite™ EV Surface Stain	Pros	Cons
ExoBrite™ Annexin EV Staining Kits	• Broad compatibility with EVs from different sources • Validated for flow and fNTA • Low background aggregates	• May not stain every EV in a sample • Not recommended for bead-bound EVs
ExoBrite™ WGA EV Staining Kits	• Broad compatibility with EVs from different sources • Can be used with bead-bound EVs	• May not stain every EV in a sample • Not recommended for fNTA
ExoBrite™ CTB EV Staining Kits	• Validated for flow and fNTA • Extremely low background	• May not stain every EV in a sample • Does not stain EVs from every source

Strong Overlap with Tetraspanin-Positive EVs

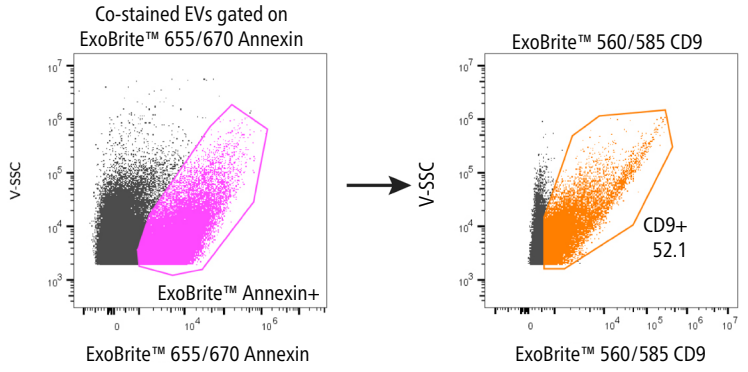


Figure 3. SEC-purified, HeLa cell-derived EVs were stained first with ExoBrite™ 560/585 CD9 Flow Antibody, followed by ExoBrite™ 655/670 Annexin EV Stain. EVs were detected on a CytoFLEX LX flow cytometer. Gating on Annexin-positive particles, ~50% were also positive for CD9.

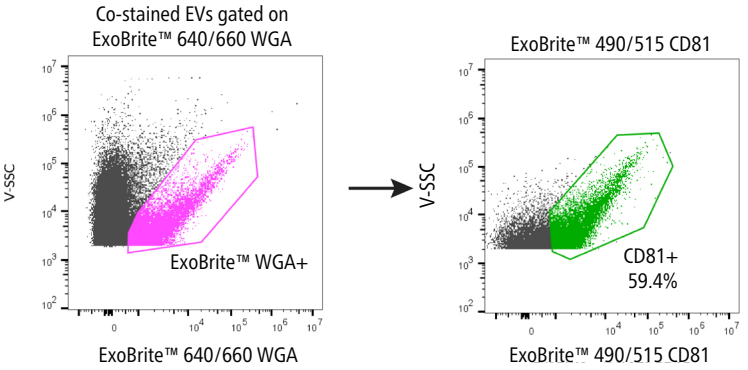


Figure 4. SEC-purified, MCF-7 cell-derived EVs were stained first with ExoBrite™ 490/515 CD81 Flow Antibody, followed by ExoBrite™ 640/660 WGA EV Stain. EVs were detected on a CytoFLEX LX flow cytometer. Gating on WGA-positive particles, ~60% were also positive for CD81.

ExoBrite™ Flow Antibody Conjugates

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

The tetraspanin family members CD9, CD63, and CD81 are widely used markers for EVs. While other companies may sell tetraspanin antibodies, few are validated for detection of EVs by flow cytometry. ExoBrite™ Flow Antibody Conjugates were curated and validated for flow cytometry to offer bright signal and low background for EV markers on purified and bead-bound EVs.

Features of ExoBrite™ Flow Antibody Conjugates

- Antibody conjugates against human and mouse tetraspanins
- Validated for staining purified and bead-bound EVs
- ExoBrite™ Isotype Control Flow Antibody available
- Options available for Pacific Blue™, FITC, PE, and APC channels

ExoBrite™ Western Antibody Conjugates

Validated for detection of EV markers in extracts

ExoBrite™ Western Antibodies were developed to offer bright signal and low background for EV markers CD9, CD63, and CD81 by near-IR fluorescent western blot. The antibodies are available with the near-IR dyes ExoBrite™ 680/700 and ExoBrite™ 770/800 for optimal signal-to-noise in near-IR fluorescent western detection. ExoBrite™ Calnexin Antibody Conjugates are offered as a cytoplasmic target control for EV purity.

Features of ExoBrite™ Western Antibody Conjugates

- Validated for near-IR western blot of EV extracts
- Negative control ExoBrite™ Calnexin Western Antibody available
- Available in 2 near-IR colors for the LI-COR® Odyssey and other near-IR imagers

ExoBrite™ EV Total RNA Isolation Kit

Efficient total RNA extraction from purified EVs

The ExoBrite™ EV Total RNA Isolation Kit is an optimized and easy-to-use kit for isolating total RNA, including mRNA and miRNA, from purified EVs. The isolated EV RNA can then be used for downstream analysis such as qPCR or RNAseq.

Features of ExoBrite™ EV Total RNA Isolation Kit

- Optimized for total RNA extraction from purified EVs
- Compatible with downstream applications such as qPCR or RNAseq
- Simple column-based purification
- No phenol/chloroform or ethanol precipitation steps

Robust Detection of EVs by Flow

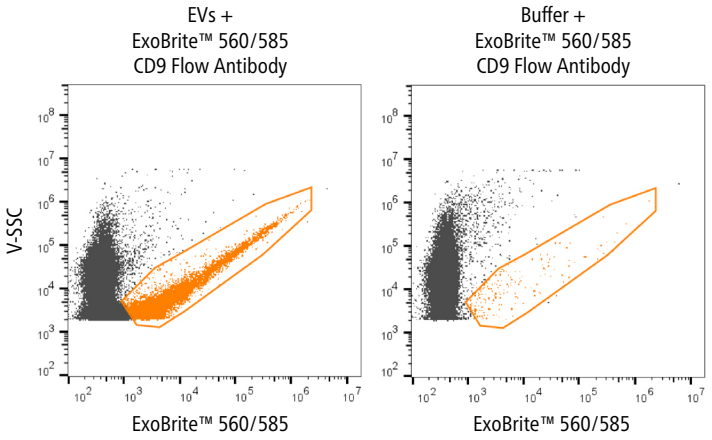


Figure 5. SEC-purified, MCF-7 cell-derived EVs were stained with ExoBrite™ CD9 560/585 Flow Antibody. Specific staining of EVs was seen (left), compared with the same antibody in buffer alone (right). EVs were detected on a CytoFLEX LX flow cytometer in the PE channel.

Conjugates for Near-IR WB of EV Extracts

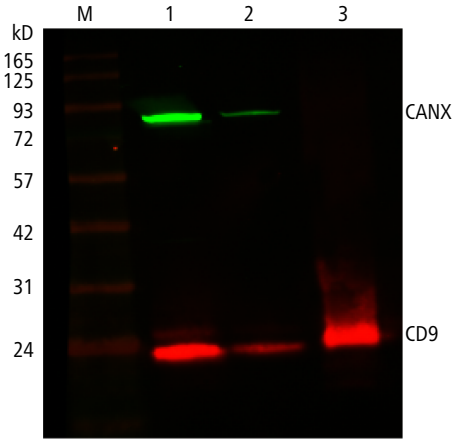


Figure 6. Western detection of human CD9 in MCF-7 cell and EV lysates using ExoBrite™ 680/700 CD9 Western Antibody (red) and ExoBrite™ 770/800 Calnexin Antibody (CANX; EV negative control) (green). Lane M: Protein marker. Lane 1: 10 ug cell lysate. Lane 2: 1 ug cell lysate. Lane 3: 1 ug EV lysate. The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

ExoBrite™ Streptavidin Magnetic Beads

Convenient & optimized capture of EVs

ExoBrite™ Streptavidin Magnetic Beads were developed to offer lower background and higher sensitivity for EV capture and fluorescence detection than similar products.

Features of ExoBrite™ Streptavidin Magnetic Beads

- Streptavidin-coated and magnetic polystyrene beads (4.5 um)
- Combine with a biotinylated antibody (not included) for convenient magnetic bead-based isolation of EVs
- Isolate EVs from biofluids without an overnight precipitation step
- Less autofluorescence than competitor beads
- Compatible with downstream EV analysis methods such as flow cytometry and western blot

Available Colors for ExoBrite™ EV Surface Stains & ExoBrite™ Flow Antibodies

Conjugates	Ex/Em (nm)	Laser Line(s) (nm)	Detection Channels	Applications
ExoBrite™ 410/450	416/452	405	Pacific Blue™	Flow
ExoBrite™ 490/515	490/516	488	FITC	Flow, fNTA
ExoBrite™ 560/585	562/584	532 or 561	PE	Flow, fNTA
ExoBrite™ 640/660	642/663	633-640	APC	fNTA (recommended), Flow
ExoBrite™ 650/665	652/668	633-640	APC	Flow (recommended), fNTA
ExoBrite™ R-PE	496, 546, 565/578	488, 532, or 561	PE	Flow

ExoBrite™ EV Surface Staining Kits

Product	Size	Cat. No.
ExoBrite™ Annexin EV Staining Kits	100 or 500 labelings	30119 - 30122
ExoBrite™ WGA EV Staining Kits	100 or 500 labelings	30123 - 30126
ExoBrite™ CTB EV Staining Kits	100 or 500 labelings	30111- 30114
ExoBrite™ EV Surface Stain Sampler Kit, Green	100 labelings	30127

ExoBrite™ Flow Antibodies

Product	Host	Target	Cat. No.
ExoBrite™ CD9 Flow Antibody	Mouse	Human CD9	P003
ExoBrite™ CD9 (Mouse) Flow Antibody	Rat	Mouse CD9	P018
ExoBrite™ CD63 Flow Antibody	Mouse	Human CD63	P004
ExoBrite™ CD63 (Mouse) Flow Antibody	Rat	Mouse CD63	P022
ExoBrite™ CD81 Flow Antibody	Mouse	Human CD81	P005
ExoBrite™ CD81 (Mouse/Rat) Flow Antibody	Hamster	Mouse or Rat CD63	P019
ExoBrite™ IgG1 Isotype Control Flow Antibody	Mouse	Negative control	P008

Available in 25 or 100 test sizes.

ExoBrite™ True EV Membrane Stains

Product	Ex/Em (nm)	Detection Channels	Cat. No.
ExoBrite™ 400/460 True EV Membrane Stain	402/460	Pacific Blue™	30136
ExoBrite™ 515/540 True EV Membrane Stain	515/542	FITC	30129
ExoBrite™ 555/575 True EV Membrane Stain	556/576	PE	30130
ExoBrite™ 645/675 True EV Membrane Stain	644/671	APC and Cy®5	30137

Available in 100 or 500 labeling sizes.

ExoBrite™ Western Antibodies

Product	Conjugates	Cat. No.
ExoBrite™ CD9 Western Antibody	ExoBrite™ 680/700	P003-680
	ExoBrite™ 770/800	P003-770
ExoBrite™ CD63 Western Antibody	ExoBrite™ 680/700	P004-680
	ExoBrite™ 770/800	P004-770
ExoBrite™ CD81 Western Antibody	ExoBrite™ 680/700	P006-680
	ExoBrite™ 770/800	P006-770
ExoBrite™ Calnexin Western Antibody	ExoBrite™ 770/800	P007-770

Available in 25 or 100 test sizes.

ExoBrite™ STORM CTB EV Staining Kits

Product	Ex/Em (nm)	Cat. No.
ExoBrite™ STORM CF®505 CTB EV Staining Kit	505/519	30115
ExoBrite™ STORM CF®583R CTB EV Staining Kit	583/609	30116
ExoBrite™ STORM CF®647 CTB EV Staining Kit	652/668	30117
ExoBrite™ STORM CF®680 CTB EV Staining Kit	681/698	30118

Available in 100 or 500 labeling sizes.

Other Products for EV Research

Product	Size	Cat. No.
ExoBrite™ Streptavidin Magnetic Beads	5 mL	28000
ExoBrite™ EV Total RNA Isolation Kit	50 preps	28001

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