Revised: October 21, 2024

Product Information

ExoBrite™ True EV Membrane Stains

Storage and Handling

Store at 4°C upon arrival and protect from light. Product is stable for at least 12 months from date of receipt when stored as recommended.

Product Description

ExoBrite™ True EV Membrane Stains are novel lipophilic dyes designed to stain EVs for detection by flow cytometry, fNTA, and other applications. They are a significant improvement upon classic membrane dyes like PKH or DiO/DiI/DiD-type dyes for EV staining. They have higher (near-complete) coverage of EVs as determined by fNTA, much higher than competitor membrane dyes. They also have greatly improved solubility, reducing the amount of aggregation and allowing stained EVs to be readily differentiated from background particles, which is not usually possible with other membrane dyes. ExoBrite™ True EV Membrane Stains labeled all EV types tested to date. See Table 1 for a list of validated EV sources.

EVs are often labeled with fluorescent antibodies targeting one or more of the tetraspanin proteins CD9, CD63, and CD81. ExoBrite™ True EV Membrane staining can be combined with antibody staining, for multi-parameter analysis (see Experimental Protocols). Biotium offers a selection of fluorescent ExoBrite™ Flow Antibodies against CD9, CD63, and CD81 that are optimized for detection of EVs by flow cytometry (see Related Products).

Biotium also offers other fluorescent probes optimized for bright and sensitive staining of EVs. This includes ExoBrite™ Annexin EV Stains, ExoBrite™ CTB EV Stains (cholera toxin B conjugates), and ExoBrite™ WGA EV Stains (wheat germ agglutinin conjugates) (see Related Products).

For more suggestions and other protocols for working with EVs see our Tech Tips: Isolation and Staining of Extracellular Vesicles and Fluorescent Detection of EVs by Flow Cytometry.

Product List

Cat. No.	Unit Size	# Reactions	Product Name	Ex/Em (nm)	Laser Line(s) (nm)	Detection Channel	Compatible Applications
30136	100 uL	500	ExoBrite™ 400/460 True EV Membrane Stain	402/460	405	Pacific Blue®	Flow cytometry, fNTA
30136-T	500 uL	100					
30129	500 uL	500	ExoBrite™ 515/540 True EV Membrane Stain	515/542	488, 532	FITC	Flow cytometry, fNTA
30129-T	100 uL	100					
30130	500 uL	500	ExoBrite™ 555/575 True EV Membrane Stain	556/576	532, 561	PE	Flow cytometry, fNTA
30130-T	100 uL	100					
30137	100 uL	500	ExoBrite™ 645/675 True EV Membrane Stain	644/671	633, 640	APC	Flow cytometry, fNTA
30137-T	500 uL	100					

Table 1. Validated EV Sources for ExoBrite™ True EV Membrane Stains

Staining validated with EVs from the following cell lines
MCF-7, J774, U2OS, Jurkat, HeLa, CHO, U937, A549, THP-1, RAW 264.7

Table 2. Suggested Instrument Settings for fNTA using the ZetaView® x30

Dye	Laser	LP filter	Sensitivity	Shutter	Tracelength (nm)	nm/Class	Frames per position
ExoBrite™ 400/460	405	410	90	200	7	5	Low
ExoBrite™ 515/540	488	500	90	100	15	5	High
ExoBrite™ 555/575	520	550	90	100	15	5	High
ExoBrite™ 645/675	640	660	90	90	15	5	High

Note: Settings should be adjusted as needed depending on experimental conditions.

Considerations for Detecting EVs by Flow Cytometry

- EVs are extremely small vesicles (~30-150 nm in diameter), a size which is near or below the detection limit of some flow cytometers. We recommend determining the size detection limit of your instrument by running sizing beads (for example, ranging from 0.02-2 um) in SSC before attempting to detect purified EVs. We also recommend running sizing beads before each EV detection experiment and using them to set the SSC threshold.
- Consider using a 405 nm laser for the SSC instead of a 488 nm laser for improved sensitivity for small particles.
- Use a low flow rate to keep the event rate and abort rate low. This will result in reduced noise.
- For best results, buffers used for suspending and staining EVs should be filtered through a 0.2 um filter to remove particulates.

Considerations for Staining with ExoBrite™ True EV Membrane Stains

- ExoBrite™ True EV Membrane Stains have been validated in flow cytometry on the CytoFLEX LX from Beckman Coulter and in fNTA on the ZetaView® QUATT NTA System from Particle Metrix. Results on other instruments may vary based on the instrument's parameters.
- We do not recommend using ExoBrite™ True EV Membrane Stains to stain bead-bound EVs. For bead-bound EVs we recommend using ExoBrite™ CTB EV Stains or ExoBrite™ WGA EV Stains (see Related Products).
- Individual EVs are too small to be imaged by conventional fluorescence or confocal microscopy, but clusters of EVs taken up by cells may be visualized. ExoBrite™ True EV Membrane Stains have not been validated for labeling EVs for cellular uptake. It may be necessary to remove free stain (by ultrafiltration, for example) before attempting to apply ExoBrite™ True EV Membrane Stain-labeled EVs to cells.
- ExoBrite™ True EV Membrane Stains have not been validated for super-resolution applications. For imaging EVs by STORM, we recommend our ExoBrite™ STORM CTB EV Staining Kits (see Related Products).
- ExoBrite™ True EV Membrane Stains have been found to label EVs isolated from cell culture supernatant from every cell line tested at Biotium (see Table 1), but staining may vary for EVs from other biological fluids or sources.
- While we have found that staining with 1X ExoBrite™ True EV Membrane Stains give a bright signal and low background under our typical staining conditions, the dye concentration may need optimization for different samples and detection systems.
- ExoBrite™ True EV Membrane Stains can be used for co-staining with fluorescently labeled primary antibodies. Co-staining can be performed concurrently or sequentially (see "Antibody co-staining of purified EVs" under Experimental Protocols).

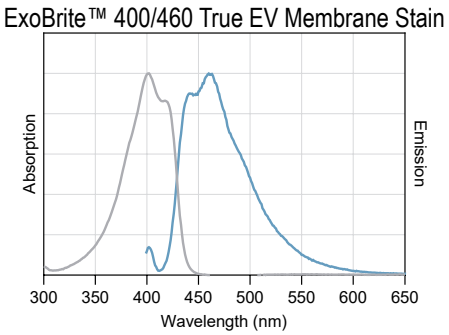

Figure 1. Absorption and emission spectra of ExoBrite™ 400/460 True EV Membrane Stain.

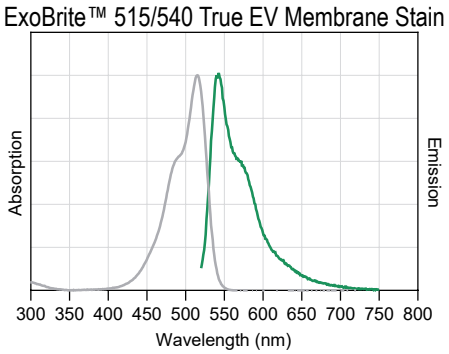

Figure 2. Absorption and emission spectra of ExoBrite™ 515/540 True EV Membrane Stain.

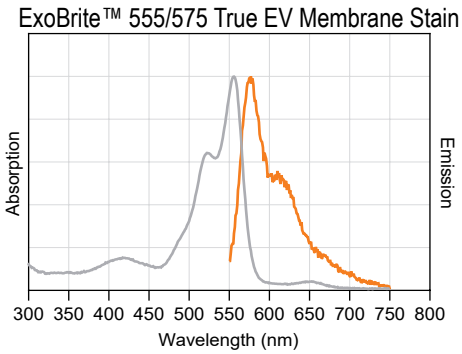

Figure 3. Absorption and emission spectra of ExoBrite™ 555/575 True EV Membrane Stain.

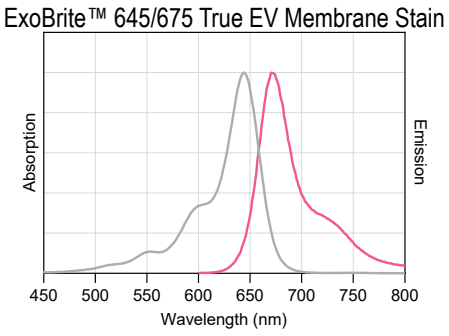

Figure 4. Absorption and emission spectra of ExoBrite™ 645/675 True EV Membrane Stain.

Experimental Protocols

Note: Before beginning, please read "Considerations for Staining with ExoBrite™ True EV Membrane Stains".

Staining of purified EVs

1. Isolate or purify EVs using the procedure of your choice.
2. Aliquot 50 uL of EVs into as many FACS tubes or microcentrifuge tubes as needed for samples and controls (see step 3).
3. In addition to the EVs stained with ExoBrite™ True EV Membrane Stain, it is helpful to include the following controls (the buffer should be an appropriate negative control for the EVs, such as a mock purification or the buffer used to suspend the EVs):
   a. Buffer alone (no EVs, no stain)
   b. Buffer plus ExoBrite™ True EV Membrane Stains
   c. EVs alone (no stain)
4. Prepare 1X ExoBrite™ True EV Membrane staining solution by diluting the 500X dye stock 1:500 in filtered PBS or other buffer of choice.

   Notes:
   a. The 1X ExoBrite™ True EV Membrane staining solution should be used the day of preparation.
   b. The working concentration of ExoBrite™ True EV Membrane Stain can be optimized by the user.
5. Add 450 uL of 1X ExoBrite™ staining solution to each tube containing 50 uL sample. Remember to also add the staining solution to the "buffer plus ExoBrite™" control.
6. Incubate at room temperature for 30 minutes, protected from light.
7. Run the samples on a flow cytometer. For tips for flow cytometry detection of purified EVs read "Considerations for Detecting EVs by Flow Cytometry" on page 1.

Analyzing stained EVs by fNTA

For analysis on an fNTA device, such as ZetaView®, follow steps 1-6 in the "Staining of purified EVs" protocol. Make sure to include the preparation of the mock sample (dye in buffer).

After step 6, prepare dilutions of the stained EVs and control dye in 5-10 mL filtered water. We recommend starting with a 1:100 dilution in water, but depending on your sample, the optimum dilution could be anywhere from 1:50-1:500. When running on the fNTA instrument, choose the particular dilution factor that falls within the recommended particle concentration range. It is desirable that for a particular concentration, the mock dye sample has fewer than 10% of the number of particles of the stained EV samples. See Table 2 on page 1 for suggested instrument settings on the ZetaView®. Settings should be adjusted as needed depending on experimental conditions.

Antibody co-staining of purified EVs

This protocol was developed for staining purified EVs with both ExoBrite™ True EV Membrane Stains and fluorescent antibodies, and detecting them by flow cytometry.

Note: Use labeled primary antibodies at the manufacturer's recommended concentration, or try staining in the range of 0.1-5 ug/mL. Either co-incubation or sequential incubations can be performed as described below.

1. Follow steps 1-4 in the "Staining of purified EVs" protocol. In addition to the antibody and ExoBrite™ True EV Membrane Stain co-stained EV samples, it is helpful to include the following controls (if using multiple antibodies, include "buffer plus antibody" and single-stain controls for each antibody).

   Buffer controls
   a. Buffer alone (no EVs, no stain)
   b. Buffer plus ExoBrite™ True EV Membrane Stain
   c. Buffer plus antibody

   EV controls
   a. Unstained EVs
   b. Single-stain ExoBrite™ True EV Membrane Stain
   c. Single-stain antibody
2. Choose whether to co-stain by co-incubation (proceed to step 3) or sequential incubation (proceed to step 4).
3. Co-incubation of antibodies and ExoBrite™ True EV Membrane Stain:
   a. Add 450 uL of 1X ExoBrite™ staining solution to each tube containing 50 uL of EVs. Remember to also add the staining solution to the "buffer plus ExoBrite™" control and the ExoBrite™ single-stain control tubes.
   b. Add fluorescent antibody conjugate to the samples at the desired concentration. For example, to the 500 uL staining reaction, add 0.5 ug antibody for 1 ug/mL. Remember to also add the antibody to the "buffer plus antibody" control and the antibody single-stain control tubes.
   c. Continue to steps 6-7 in the "Staining of purified EVs" protocol.
4. Sequential incubation of antibodies and ExoBrite™ True EV Membrane Stain:
   a. Add fluorescent antibody conjugate to the samples at the desired concentration. For example, to a 50 uL EV sample, add 0.05 ug antibody for 1 ug/mL. Remember to add the antibody to the "buffer plus antibody" control and the antibody single-stain control tubes.
   b. Incubate at room temperature for 30 minutes, protected from light.
   c. Add 450 uL of 1X ExoBrite™ True EV Membrane staining solution to each sample tube. Remember to also add the staining solution to the "buffer plus ExoBrite™" control and the ExoBrite™ True EV Membrane Stain single-stain control tubes.
   d. Continue to steps 6-7 in the "Staining of purified EVs" protocol.

Related Products

Cat. No.	Product
30111-30114	ExoBrite™ CTB EV Staining Kits
30119-30122	ExoBrite™ Annexin EV Staining Kits
30123-30126	ExoBrite™ WGA EV Staining Kits
30115-30118	ExoBrite™ STORM CTB EV Staining Kits
30127	ExoBrite™ EV Surface Stain Sampler Kit, Green
28000	ExoBrite™ Streptavidin Magnetic Beads
28001	ExoBrite™ EV Total RNA Isolation Kit
P003-410... P003-RPE	ExoBrite™ CD9 Flow Antibody
P018-410... P018-650	ExoBrite™ CD9 (Mouse) Flow Antibody
P004-410... P004-RPE	ExoBrite™ CD63 Flow Antibody
P022-410... P022-560	ExoBrite™ CD63 (Mouse) Flow Antibody
P005-410... P005-RPE	ExoBrite™ CD81 Flow Antibody
P019-410... P019-560	ExoBrite™ CD81 (Mouse/Rat) Flow Antibody
P008-410... P008-RPE	ExoBrite™ IgG1 Isotype Control Flow Antibody
P003-680... P003-770	ExoBrite™ CD9 Western Antibody
P004-680... P004-770	ExoBrite™ CD63 Western Antibody
P006-680... P006-770	ExoBrite™ CD81 Western Antibody
P007-770	ExoBrite™ 770/800 Calnexin Western Antibody

Please visit our website at www.biotium.com for information on our life science research products, including fluorescent CF® Dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, and kits for cell biology research.