Revised: January 19, 2023

Product Information

ExoBrite™ Flow Antibody Conjugates

Product List

Product	Catalog no.	Unit size
ExoBrite™ 410/450 CD9 Flow Antibody	P003-410-125	25 tests
	P003-410-500	100 tests
ExoBrite™ 490/515 CD9 Flow Antibody	P003-490-125	25 tests
	P003-490-500	100 tests
ExoBrite™ 560/585 CD9 Flow Antibody	P003-560-125	25 tests
	P003-560-500	100 tests
ExoBrite™ 410/450 CD63 Flow Antibody	P004-410-125	25 tests
	P004-410-500	100 tests
ExoBrite™ 490/515 CD63 Flow Antibody	P004-490-125	25 tests
	P004-490-500	100 tests
ExoBrite™ 560/585 CD63 Flow Antibody	P004-560-125	25 tests
	P004-560-500	100 tests
ExoBrite™ 410/450 CD81 Flow Antibody	P005-410-125	25 tests
	P005-410-500	100 tests
ExoBrite™ 490/515 CD81 Flow Antibody	P005-490-125	25 tests
	P005-490-500	100 tests
ExoBrite™ 560/585 CD81 Flow Antibody	P005-560-125	25 tests
	P005-560-500	100 tests
ExoBrite™ 410/450 IgG1 Isotype Control Flow Antibody	P008-410-125	25 tests
	P008-410-500	100 tests
ExoBrite™ 490/515 IgG1 Isotype Control Flow Antibody	P008-490-125	25 tests
	P008-490-500	100 tests
ExoBrite™ 560/585 IgG1 Isotype Control Flow Antibody	P008-560-125	25 tests
	P008-560-500	100 tests

Storage and Handling

Store at 4°C, protected from light. Product is stable for at least 24 months from date of receipt when stored as recommended.

Note: Storage of the antibody for more than a day at final working dilution is not recommended.

Spectral Properties

See Table 2.

Product Description

Extracellular vesicles (EVs), including exosomes, are lipid-bound vesicles that are released from cells. EVs display specific surface proteins and can carry nucleic acids and other cargo, allowing them to transfer biological information between cells in different parts of the body. Therefore EVs are increasingly studied for their potential use in drug delivery and medical diagnostic applications. The most common proteins used as EV markers are CD9, CD63, and CD81, members of the tetraspanin family. Tetraspanins are plasma membrane proteins with many proposed functions, including activation and sorting of other membrane proteins. They are also thought to play a role in the targeting of proteins to multivesicular bodies (MVBs) and EVs. These tetraspanins are broadly expressed on many cell types and can therefore be detected on many types of EVs, but their expression levels vary depending on the cell type of origin.

ExoBrite™ Flow Antibody Conjugates are validated by Biotium for optimal detection of EV markers CD9, CD63, and CD81 in purified or bead-bound EVs by flow cytometry. ExoBrite™ fluorophores 410/450, 490/515, and 560/585 offer exceptional brightness and signal-to-noise over alternative fluorophores (see Table 2. on page 3 for detection settings of each ExoBrite™ conjugate).

ExoBrite™ Isotype Control Flow Antibodies are offered as a negative control for non-specific antibody binding. The isotype controls have no known reactivity with any target in human cells, and have the same isotype as the tetraspanin antibodies.

Biotium also offers ExoBrite™ EV Membrane Staining Kits for detection of isolated EVs by flow cytometry. ExoBrite™ EV Membrane Staining Kits may also be combined with antibody staining, for multi-parameter analysis (see Related Products).

Table 1. Antibody Attributes

Antibody	Target	Host species	Species reactivity	Target MW	Target localization	Isotype	Entrez gene ID	SwissProt	Unigene	Synonyms
ExoBrite™ CD9 Flow Antibody	CD9	Mouse	Human, Baboon, Bovine, Cynomolgus monkey, Dog, Horse, Rabbit, Non-human primates, Sheep	24 kDa	Exosomes/EVs, Plasma membrane	IgG1, kappa	928	P21926	114286	Tspan-29, MRP-1
ExoBrite™ CD63 Flow Antibody	CD63	Mouse	Human, Baboon, Cynomolgus monkey, Non-human primates	26 kDa (core protein); 30-60 kDa (glycosylated)	Exosomes/EVs, Lysosomes, Plasma membrane, Membrane/vesicular, Multivesicular bodies	IgG1, kappa	967	P08962	445570	Tspan-30, LAMP-3, gp55
ExoBrite™ CD81 Flow Antibody	CD81	Mouse	Human, Baboon, Cynomolgus monkey, Non-human primates	26 kDa	Exosomes/EVs, Plasma membrane	IgG1, kappa	975	P60033	54457	Tspan-28, TAPA-1
ExoBrite™ IgG1 Isotype Control Flow Antibody	-	Mouse	-	-	-	IgG1, kappa	-	-	-	-

Considerations for Detecting EVs by Flow Cytometry

- Obtaining a clean EV prep is crucial for obtaining robust signal and proper interpretation of results. While there are several EV isolation methods, we have found that size exclusion chromatography (SEC) is an accessible and easy-to-use method that yields a relatively pure population of EVs. For a comparison of exosome isolation methods and protocols for exosome isolation and staining, see the following tech tips:
  Tech Tip: Exosome Isolation and Staining Protocols
  Tech Tip: Fluorescent Detection of Exosomes by Flow Cytometry
- EVs are extremely small vesicles (~30-150 nm in diameter), a size which is near or below the size 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. EVs that are bound to affinity beads are large enough to detect on any instrument.
- If it is an option on your flow cytometer, we recommend using the 405 nm laser instead of the 488 nm laser for the SSC channel, for improved sensitivity for small particles.
- For best results, buffers used for suspending and staining EVs should be filtered through a 0.2 um filter to remove particulates.
- Use a low flow rate to keep the event rate and abort rate low. This will result in reduced instrument noise. Dilute the stained samples in filtered PBS if necessary.

Considerations for ExoBrite™ Flow Antibody Conjugates

The following are general considerations for using ExoBrite™ Flow Antibodies to stain EVs. See Experimental Protocols for step-by-step instructions for use.

- ExoBrite™ Flow Antibody Conjugates have been validated in flow cytometry on the CytoFLEX LX from Beckman Coulter. Results on other instruments may vary based on the instruments size detection limit and other parameters.
- ExoBrite™ Flow Antibody Conjugates have been validated for staining EVs isolated using several different methods, including PEG precipitation, size exclusion chromatography, and affinity bead isolation. Staining results may vary depending on the EV isolation method used.

Experimental Protocols

Note: Before beginning, please read "Considerations for ExoBrite™ Flow Antibody Conjugates" section.

Antibody staining of purified EVs

This protocol was developed for staining purified EVs with ExoBrite™ Flow Antibody Conjugates for detection by flow cytometry.

1. Isolate or purify EVs using the procedure of your choice.
2. Aliquot 100 uL of EV sample into FACS tubes or microcentrifuge tubes. We recommend primary antibody and isotype controls for EV samples, as well as primary antibody controls with buffer alone.

   Buffer controls
   a. Buffer alone (no EVs, no antibody)
   b. Buffer plus ExoBrite™ Flow Antibody

   EV controls
   a. Unstained EVs
   b. Single-stain ExoBrite™ Flow Antibody
   c. Single-stain ExoBrite™ Isotype Control
3. Add 5 uL of ExoBrite™ antibody to each 100 uL sample. Remember to also add the antibody to the buffer plus ExoBrite™ Flow Antibody control and the antibody single-stain control tubes.
4. Incubate at room temperature for 30 minutes, protected from light.
5. Run the samples on a flow cytometer. For tips for flow cytometry detection of purified EVs read "Considerations for Detecting EVs by Flow Cytometry". See Table 2 for recommended detection settings for ExoBrite™ Flow Antibody Conjugates.

Antibody staining of bead-bound EVs

This protocol was developed for EVs bound to magnetic antibody capture beads, stained with ExoBrite™ Flow Antibody Conjugates and detected by flow cytometry.

1. Prepare EVs bound to the magnetic capture beads of your choice, according to the manufacturer's recommended procedure.
2. Prepare sample tubes and the following control tubes:

   Beads controls (no EVs)
   a. Beads alone
   b. Beads plus ExoBrite™ Flow Antibody

   Bead-bound EV controls
   a. Unstained bead-bound EVs
   b. Single-stain ExoBrite™ Flow Antibody
   c. Single-stain ExoBrite™ Isotype Control
3. Place the tubes with beads on a magnet for 1 minute, remove and discard the supernatant.

4. Remove the tubes from the magnet and suspend in 100 uL of PBS. Add 5 uL of ExoBrite™ antibody to each sample, including applicable controls.
5. Incubate at room temperature for 30 minutes, protected from light.
6. Place the tubes on a magnet for 1 minute, remove and discard the supernatant.
7. Remove the tubes from the magnet, add 100 uL of 0.2 um-filtered PBS and gently pipet up and down to resuspend.

   Note: If the beads are not completely recovered from the buffer after 1 minute on the magnet, leave the tubes on the magnet for a longer time (up to 4 minutes). We have found that briefly centrifuging tubes to collect the contents near the bottom before placing them on the magnet can improve bead recovery.
8. Place the tubes on a magnet for 1 minute, remove and discard the supernatant.
9. Remove the tubes from the magnet, add 500-900 uL of 0.2 um-filtered PBS and gently pipet up and down to resuspend.
10. Run the samples on a flow cytometer. See Table 2 for recommended detection settings for ExoBrite™ Flow Antibody Conjugates.

Table 2. Detection Settings for ExoBrite™ Flow Antibodies

Conjugate	Ex/Em	Laser Line(s) (nm)	Detection Channel
ExoBrite™ 410/450	416/452 nm	405	Pacific Blue™
ExoBrite™ 490/515	490/516 nm	488	FITC
ExoBrite™ 560/585	562/584 nm	532 or 561	PE

Related Products

Catalog number	Product
30111-30114	ExoBrite™ EV Membrane Staining Kits
P003-680	ExoBrite™ 680/700 CD9 Western Antibody
P003-770	ExoBrite™ 770/800 CD9 Western Antibody
P004-680	ExoBrite™ 680/700 CD63 Western Antibody
P004-770	ExoBrite™ 770/800 CD63 Western Antibody
P006-680	ExoBrite™ 680/700 CD81 Western Antibody
P006-770	ExoBrite™ 770/800 CD81 Western Antibody
P007-770	ExoBrite™ 770/800 Calnexin Western Antibody

Please visit our website at www.biotium.com for more information on our products for EV detection and western blotting including EV stains and antibodies for flow cytometry, western blot blocking buffers, and total protein stains.