

# Product Information

## ExoBrite™ Flow Antibody Conjugates

### ExoBrite™ Flow Anti-Human\* Tetraspanin Antibodies

Product	Cat. 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™ 640/660 CD9 Flow Antibody	P003-640-125	25 tests
	P003-640-500	100 tests
ExoBrite™ 650/665 CD9 Flow Antibody	P003-650-125	25 tests
	P003-650-500	100 tests
ExoBrite™ APC CD9 Flow Antibody	P003-APC-125	25 tests
	P003-APC-500	100 tests
ExoBrite™ R-PE CD9 Flow Antibody	P003-RPE-125	25 tests
	P003-RPE-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™ 640/660 CD63 Flow Antibody	P004-640-125	25 tests
	P004-640-500	100 tests
ExoBrite™ 650/665 CD63 Flow Antibody	P004-650-125	25 tests
	P004-650-500	100 tests
ExoBrite™ APC CD63 Flow Antibody	P004-APC-125	25 tests
	P004-APC-500	100 tests
ExoBrite™ R-PE CD63 Flow Antibody	P004-RPE-125	25 tests
	P004-RPE-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™ 640/660 CD81 Flow Antibody	P005-640-125	25 tests
	P005-640-500	100 tests
ExoBrite™ 650/665 CD81 Flow Antibody	P005-650-125	25 tests
	P005-650-500	100 tests

Product	Cat. No.	Unit Size
ExoBrite™ APC CD81 Flow Antibody	P005-APC-125	25 tests
	P005-APC-500	100 tests
ExoBrite™ R-PE CD81 Flow Antibody	P005-RPE-125	25 tests
	P005-RPE-500	100 tests

\*See Table 1 on page 3 for other species reactivity.

### ExoBrite™ Flow Anti-Mouse\* Tetraspanin Antibodies

Product	Cat. No.	Unit Size
ExoBrite™ 410/450 CD9 (Mouse) Flow Antibody	P018-410-125	25 tests
	P018-410-500	100 tests
ExoBrite™ 490/515 CD9 (Mouse) Flow Antibody	P018-490-125	25 tests
	P018-490-500	100 tests
ExoBrite™ 560/585 CD9 (Mouse) Flow Antibody	P018-560-125	25 tests
	P018-560-500	100 tests
ExoBrite™ 640/660 CD9 (Mouse) Flow Antibody	P018-640-125	25 tests
	P018-640-500	100 tests
ExoBrite™ 650/665 CD9 (Mouse) Flow Antibody	P018-650-125	25 tests
	P018-650-500	100 tests
ExoBrite™ 410/450 CD63 (Mouse) Flow Antibody	P022-410-125	25 tests
	P022-410-500	100 tests
ExoBrite™ 490/515 CD63 (Mouse) Flow Antibody	P022-490-125	25 tests
	P022-490-500	100 tests
ExoBrite™ 560/585 CD63 (Mouse) Flow Antibody	P022-560-125	25 tests
	P022-560-500	100 tests
ExoBrite™ 640/660 CD63 (Mouse) Flow Antibody	P022-640-125	25 tests
	P022-640-500	100 tests
ExoBrite™ 650/665 CD63 (Mouse) Flow Antibody	P022-650-125	25 tests
	P022-650-500	100 tests
ExoBrite™ 410/450 CD81 (Mouse/Rat) Flow Antibody	P019-410-125	25 tests
	P019-410-500	100 tests
ExoBrite™ 490/515 CD81 (Mouse/Rat) Flow Antibody	P019-490-125	25 tests
	P019-490-500	100 tests
ExoBrite™ 560/585 CD81 (Mouse/Rat) Flow Antibody	P019-560-125	25 tests
	P019-560-500	100 tests
ExoBrite™ 640/660 CD81 (Mouse/Rat) Flow Antibody	P019-640-125	25 tests
	P019-640-500	100 tests
ExoBrite™ 650/665 CD81 (Mouse/Rat) Flow Antibody	P019-650-125	25 tests
	P019-650-500	100 tests

\*See Table 1 on page 3 for other species reactivity.

## ExoBrite™ Flow Mouse Isotype Control Antibodies

Product	Cat. No.	Unit Size
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
ExoBrite™ 640/660 IgG1 Isotype Control Flow Antibody	P008-640-125	25 tests
	P008-640-500	100 tests
ExoBrite™ 650/665 IgG1 Isotype Control Flow Antibody	P008-650-125	25 tests
	P008-650-500	100 tests
ExoBrite™ R-PE IgG1 Isotype Control Flow Antibody	P008-RPE-125	25 tests
	P008-RPE-500	100 tests

## ExoBrite™ CD9/CD63/CD81 3-Color Antibody Cocktails

Product	Cat. No.	Unit Size
ExoBrite™ CD9/CD63/CD81 3-Color Antibody Cocktail (Blue/Green/Far-Red)	P028-125	125 uL
	P028-500	500 uL
ExoBrite™ CD9/CD63/CD81 3-Color Antibody Cocktail (Green/Orange/Far-Red)	P029-125	125 uL
	P029-500	500 uL

### 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.

### Product Description

ExoBrite™ Flow Antibody Conjugates were developed by Biotium for detection of the most commonly used EV markers, CD9, CD63, and CD81, on EVs by flow cytometry. The antibodies and dye labels were carefully selected and validated for robust detection of isolated EVs. In addition, the antibodies are provided in a proprietary buffer formulation for reduced antibody aggregation and brighter EV staining for optimal accuracy and signal-to-noise.

The antibodies are suitable for detection of purified or bead-bound EVs. They are available in different clones for both human and mouse tetraspanins. ExoBrite™ fluorophores 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 the ExoBrite™ anti-human antibodies. The isotype controls have no known reactivity with any target in human cells, and have the same isotype as the mouse anti-human ExoBrite™ antibodies.

Depending on the source of the EVs, CD9, CD63, and CD81 will be present at varying levels. The ExoBrite™ CD9/CD63/CD81 3-Color Antibody Cocktails were designed for fast, reproducible phenotyping of an EV sample by flow cytometry. The cocktails can be used on their own for tetraspanin profiling, or combined with other antibodies of interest for more multi-target analyses.

The ExoBrite™ CD9/CD63/CD81 3-Color Antibody Cocktails have been meticulously optimized for optimal brightness and signal-to-noise of each antibody conjugate. Each individual antibody in the mixture has been validated to have the same coverage as the single antibody, for accurate results. In addition, like all ExoBrite™ Flow antibodies, the ExoBrite™ cocktails are provided in a proprietary buffer formulation for reduced antibody aggregation and brighter EV staining for optimal accuracy and signal-to-noise.

Biotium also offers other products for EV research, including ExoBrite™ conjugates of CTB, WGA, and Annexin V, as well as ExoBrite™ True EV Membrane Stains for pan-EV labeling. See Related Products and visit our [technology page](#) for more information.

### 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 EV isolation methods and protocols for EV isolation and staining, see the following tech tips: [Tech Tip: Isolation and Staining of Extracellular Vesicles](#) [Tech Tip: Fluorescent Detection of EVs by Flow Cytometry](#)
- EVs are extremely small vesicles (~30-150 nm in diameter), 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. Sizing beads should be used to set the SSC threshold before each EV detection experiment. EVs that are bound to affinity beads are large enough to detect on any instrument.
- To improve the sensitivity for detecting small particles, we recommend using the 405 nm laser for the SSC channel if it is an option on your flow cytometer.
- For best results, buffers used for suspending and staining EVs should be filtered through a 0.2 um filter to remove particulates.
- To reduce instrument noise, use a low flow rate to keep the event rate and abort rate low. Dilute the stained samples in filtered PBS if necessary.

**Table 1. Antibody Attributes**

Antibody	Target	Host	Species Reactivity	Target MW	Isotype	Entrez Gene ID	SwissProt	Unigene	Synonyms	Target Localization
ExoBrite™ CD9 Flow Antibody	CD9	Mouse	Human, Baboon, Bovine, Cynomolgus monkey, Dog, Horse, Rabbit, Non-human primates, Sheep	24 kDa	IgG1, kappa	928	P21926	114286	Tspan-29, MRP-1	Exosomes/ EVs, Plasma membrane
ExoBrite™ CD9 (Mouse) Flow Antibody	CD9	Rat	Mouse	24 kDa	IgG2a, kappa	12527	P40240	-	DRAP-27, MRP-1, p-24	
ExoBrite™ CD63 Flow Antibody	CD63	Mouse	Human, Baboon, Cynomolgus monkey, Non-human primates	26 kDa (core protein); 30-60 kDa (glycosylated)	IgG1, kappa	967	P08962	445570	Tspan-30, LAMP-3, gp55	Exosomes/EVs, Lysosomes, Plasma membrane, Membrane/ vesicular, Multivesicular bodies
ExoBrite™ CD63 (Mouse) Flow Antibody	CD63	Rat	Mouse	53 kDa	IgG2a, kappa	12512	P41731	-	LIMP, LAMP-3, gp55, ME491	
ExoBrite™ CD81 Flow Antibody	CD81	Mouse	Human, Baboon, Cynomolgus monkey, Non-human primates, Mouse (low reactivity)	26 kDa	IgG1, kappa	975	P60033	54457	Tspan-28, TAPA-1	Exosomes/ EVs, Plasma membrane
ExoBrite™ CD81 (Mouse/Rat) Flow Antibody	CD81	Hamster	Mouse, Rat	26 kDa	IgG1, kappa	12520 (mouse), 25621 (rat)	P35762 (mouse), Q62745 (rat)	-	TAPA-1	
ExoBrite™ IgG1 Isotype Control Flow Antibody	-	Mouse	-	-	IgG1, kappa	-	-	-	-	-

**Table 2. Detection Settings for ExoBrite™ Flow Antibodies**

Conjugate	Ex/Em (nm)	Laser Line(s) (nm)	Detection Channel	Applications
ExoBrite™ 410/450	411/452	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	642/668	633-640	APC	fNTA (recommended)*, Flow
ExoBrite™ 650/665	652/668	633-640	APC	Flow*
ExoBrite™ APC	651/660	633-640	APC	Flow
ExoBrite™ R-PE	496, 546, 565/578	488, 532, or 561	PE	Flow

\*ExoBrite™ 640/660 and ExoBrite™ 650/665 are spectrally similar and detected in the same channels. However, ExoBrite™ 640/660 has enhanced photostability compared to ExoBrite™ 650/665, and therefore is recommended for fNTA where photobleaching can be a concern. ExoBrite™ 650/665 has high brightness that is optimal for flow cytometry, but may be more susceptible to photobleaching in fNTA.

**Table 3. Detection Settings for ExoBrite™ CD9/CD63/CD81 3-Color Antibody Cocktails**

Product	Antibody	Ex/Em (nm)	Laser Line(s) (nm)	Detection Channel
ExoBrite™ CD9/CD63/CD81 3-Color Antibody Cocktail (Blue/Green/Far-Red)	ExoBrite™ 410/450 CD9	411/452	405	Pacific Blue™
	ExoBrite™ 490/515 CD63	490/516	488	FITC
	ExoBrite™ 640/660 CD81	642/668	633-640	APC
ExoBrite™ CD9/CD63/CD81 3-Color Antibody Cocktail (Green/Orange/Far-Red)	ExoBrite™ 490/515 CD63	490/516	488	FITC
	ExoBrite™ 560/585 CD81	562/584	532 or 561	PE
	ExoBrite™ 640/660 CD9	642/668	633-640	APC

## 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 instrument's size detection limit and other parameters.
- ExoBrite™ 490/515, 560/585, and 640/660 Flow Antibody Conjugates were validated on a ZetaView® QUATT for detection by fluorescence nanoparticle tracking analysis (fNTA). See Table 2 on page 3 for validated applications.
- 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 the “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 or ExoBrite™ CD9/CD63/CD81 3-Color Antibody Cocktails for detection by flow cytometry.

1. Isolate or purify EVs using the procedure of your choice. Thaw EVs if they have been stored frozen.
2. Aliquot 100 uL of EV sample into FACS tubes or microcentrifuge tubes. We recommend setting up control tubes of antibody in buffer alone, as well as isotype controls (if available).
  - Buffer controls (no EVs)
    - a. Buffer alone (no EVs, no antibody)
    - b. Buffer plus ExoBrite™ antibody or cocktail
  - EV samples
    - a. Unstained EVs
    - b. ExoBrite™ antibody or cocktail
    - c. ExoBrite™ Isotype Control (if available)
3. Add ExoBrite™ antibody or cocktail to each 100 uL sample. For R-PE conjugates, APC conjugates, and cocktails, add 5 uL. For all other ExoBrite™ conjugates, add 1-5 uL. Remember to also add the antibody or cocktail to the buffer plus ExoBrite™ antibody or cocktail control.
4. Incubate at room temperature for 30 minutes, protected from light.
5. Add filtered PBS to the desired volume and 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 and Table 3 for recommended detection settings.

### Antibody staining of bead-bound EVs

This protocol was developed for EVs bound to magnetic antibody capture beads, stained with ExoBrite™ Flow Antibody Conjugates or ExoBrite™ CD9/CD63/CD81 3-Color Antibody Cocktails 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 (no EVs, no antibody)
    - b. Beads plus ExoBrite™ antibody or cocktail
  - Bead-bound EV samples
    - a. Unstained bead-bound EVs
    - b. ExoBrite™ antibody or cocktail
    - c. ExoBrite™ Isotype Control (if available)
3. Place the tubes with bead-bound EVs on a magnet for 1 minute, then remove and discard the supernatant.

**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.

4. Remove the tubes from the magnet. Add 300 uL of 0.2 um-filtered PBS and gently pipette up and down to resuspend.
5. Repeat steps 3-4 once.
6. Remove the tubes from the magnet and resuspend beads in 100 uL of PBS. Add ExoBrite™ antibody or cocktail to each sample, including applicable controls. For R-PE conjugates, APC conjugates, and cocktails, add 5 uL. For all other ExoBrite™ conjugates, add 1-5 uL.
7. Incubate at room temperature for 30 minutes, protected from light.
8. Place the tubes on the magnet for 1 minute, then discard the supernatant.
9. Remove the tubes from the magnet. Add 300 uL of PBS and gently pipette up and down to resuspend.
10. Place the tubes on the magnet for 1 minute, then discard the supernatant.
11. Remove the tubes from the magnet. Add 500-900 uL of PBS and transfer to flow tubes.
12. Run the samples on a flow cytometer. See Table 2 and Table 3 for recommended detection settings.

## Related Products

Cat. No.	Product
30129, 30137	ExoBrite™ True EV Membrane Stains
30111-30114	ExoBrite™ CTB EV Staining Kits
28002	ExoBrite™ EV Stain Enhancer
30123-30126	ExoBrite™ WGA EV Staining Kits
30119-30122	ExoBrite™ Annexin EV Staining Kits
30115-30118	ExoBrite™ STORM CTB EV Staining Kits
30127	ExoBrite™ EV Surface Stain Sampler Kit, Green
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
28000	ExoBrite™ Streptavidin Magnetic Beads
28001	ExoBrite™ EV Total RNA Isolation Kit

Please visit our website at [www.biotium.com](http://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.

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