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Product Information

ExoBrite™ WGA EV Staining Kits

Catalog Number: See Table 1 on page 2.

Kit Contents

Component	Full Size 500 labelings	Trial Size 100 labelings
ExoBrite™ WGA EV Stain	Component A 1 x 500 labelings	Component A 1 x 100 labelings
ExoBrite™ 1X PBS Solution	99879 1 x 1 mL	99879 1 x 1 mL

Storage and Handling

Store the kit at -20°C upon arrival and protect from light. Product is stable for at least 6 months from date of receipt when stored as recommended.

Reconstitution

To prepare 500X ExoBrite™ WGA EV Stain solution, reconstitute the lyophilized ExoBrite™ WGA EV Stain (Component A) using either 500 uL (full size kits) or 100 uL (trial size kits) of 1X ExoBrite™ PBS Solution. Pipet gently up and down to mix. The 500X stain solution can be stored protected from light for up to 6 months at -20°C.

Spectral Properties

See Table 1.

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. Biotium developed ExoBrite™ WGA EV Stains for fluorescent labeling and detection of EVs and exosomes by flow cytometry. Other potential applications include fluorescence microscopy and other fluorescence detection platforms such as NTA.

ExoBrite™ WGA EV Stains are uniquely formulated conjugates of wheat germ agglutinin (WGA), a carbohydrate-binding lectin with high affinity for N-acetylglucosamine moieties of glycoproteins. WGA conjugates are often used for labeling cell membranes as well as gram-positive bacteria. WGA conjugates have also been used to detect EVs due to the presence of glycoproteins on EV membranes. ExoBrite™ WGA EV Stains were designed to overcome some of the challenges of detecting isolated EVs, particularly in flow cytometry. For example, tetraspanin antibodies commonly used to stain EVs can have varying signal and coverage depending on the EV source. Conversely, ExoBrite™ WGA EV Stains show bright staining of EVs derived from a broad range of sources. See Table 2 on page 2 for a list of validated EV sources. ExoBrite™ WGA EV Stains are less prone to aggregation than hydrophobic membrane dyes and do not bind non-specifically to polystyrene beads, allowing them to be used to stain bead-bound EVs.

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

Biotium also offers other conjugates optimized for bright and sensitive staining of EVs and exosomes. This includes ExoBrite™ CTB EV Stains (cholera toxin B conjugates) and ExoBrite™ Annexin V EV Stains (see Related Products).

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 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.
- 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 instrument noise. Dilute the stained samples in filtered PBS if necessary.
- 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™ WGA EV Stains

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

- ExoBrite™ WGA EV Stains 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™ WGA EV Stains 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.
- The concentration of EVs in a sample can vary depending on the isolation method, and can be difficult to determine. If your EV preparation is very concentrated, testing several dilutions may be helpful.
- Individual exosomes and 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™ WGA EV Stains have not been validated for labeling EVs for cellular uptake. It may be necessary to remove unbound stain (by ultrafiltration, for example) before attempting to apply ExoBrite™ WGA-labeled EVs to cells.
- EVs can be imaged by super-resolution microscopy. Please see Table 1 for a list of compatible super-resolution applications for each ExoBrite™ dye. For imaging EVs by STORM, we also recommend our ExoBrite™ STORM CTB EV Staining Kits (see Related Products).
- ExoBrite™ WGA EV Stains have been found to label EVs derived from every cell line tested (See Table 2), but may not stain EVs from every source.
- ExoBrite™ WGA EV Stains tend to have a higher level of aggregation than our other ExoBrite™ stains. If you are experiencing issues with aggregates, we recommend trying ExoBrite™ Annexin EV Stains or ExoBrite™ CTB EV Stains instead.
- While we have found that staining with 1X ExoBrite™ WGA EV Stain gives a bright signal and low background under our typical staining conditions, the dye concentration may need optimization for different samples and detection systems.
- ExoBrite™ WGA EV 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 Exosomes" under Experimental Protocols).

Table 1. ExoBrite™ WGA EV Staining Kits

Cat. No.	Size	Product Name	Ex/Em (nm)	Laser Line(s) (nm)	Detection Channel	Other Compatible Applications
30123	500 labeling reactions	ExoBrite™ 410/450 WGA EV Staining Kit	416/452	405	Pacific Blue™	SIM, STED
30123-T	100 labeling reactions					
30124	500 labeling reactions	ExoBrite™ 490/515 WGA EV Staining Kit	490/516	488	FITC	STED, STORM, TIRF
30124-T	100 labeling reactions					
30125	500 labeling reactions	ExoBrite™ 560/585 WGA EV Staining Kit	562/584	532 or 561	PE	SIM, STED, STORM
30125-T	100 labeling reactions					
30126	500 labeling reactions	ExoBrite™ 640/660 WGA EV Staining Kit	642/663	633-640	APC	---
30126-T	100 labeling reactions					

Table 2. Validated EV Sources for ExoBrite™ WGA EV Stains

Staining validated for EVs derived from the following cell lines
MCF-7, J774, U-2 OS, Jurkat, HeLa, Raji, CHO, U937, A547

Experimental Protocols

Note: Before beginning, please read "Considerations for Staining with ExoBrite™ WGA EV Stains" on previous page.

Staining of purified EVs

This protocol was developed for staining purified EVs with ExoBrite™ WGA EV Stains for detection by flow cytometry.

1. Isolate or purify EVs or exosomes using the procedure of your choice.
2. Aliquot 50 uL of EVs into FACS tubes or microcentrifuge tubes.
3. Prepare 1X ExoBrite™ staining solution by diluting the 500X stock solution 1:500 in 1X PBS (e.g., add 2 uL ExoBrite™ stain to 1 mL PBS).

   Notes:
   a. The 1X ExoBrite™ staining solution should be used the day of preparation.
   b. The concentration of ExoBrite™ stain can be optimized by the user.
4. In addition to the ExoBrite™-stained EV samples, 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™ Stain
   c. EVs alone (no stain)
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.

Antibody co-staining of purified EVs

This protocol was developed for staining purified EVs with both ExoBrite™ WGA EV 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 1-3 in the "Staining Purified EVs" protocol. In addition to the antibody and ExoBrite™ 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™ Stain
   c. Buffer plus antibody

   EV controls
   a. Unstained EVs
   b. Single-stain ExoBrite™ 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™:
   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 Purified EVs" protocol.
4. Sequential incubation of antibodies and ExoBrite™:
   a. Add fluorescent antibody conjugate to the samples at the desired concentration. For example, to the 50 uL EV sample, add 0.05 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.
   b. Incubate at room temperature for 30 minutes, protected from light.
   c. Add 450 uL of 1X ExoBrite™ staining solution to each sample tube. Remember to also add the staining solution to the "buffer plus ExoBrite™" control and the ExoBrite™ single-stain control tubes.
   d. Continue to steps 6-7 in the "Staining Purified EVs" protocol.

Staining of bead-bound EVs

This protocol was developed for EVs bound to magnetic antibody capture beads, stained with ExoBrite™ WGA EV Stains, 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 the following control tubes:
   a. Beads alone (no EVs or stain)
   b. Beads plus ExoBrite™ Stain (no EVs)
3. Prepare 10X ExoBrite™ staining solution by diluting the 500X stock solution 1:50 in 1X PBS (e.g., add 2 uL ExoBrite™ stain to 100 uL PBS).

   Note: The 10X ExoBrite™ staining solution should be used the day of preparation.
4. Place the tubes with beads on a magnet for 1 minute, remove and discard the supernatant.
5. Remove the tubes with beads from the magnet and suspend in 50 uL of 10X ExoBrite™ staining solution. Remember to also add the staining solution to the "beads plus ExoBrite™" control.
6. Incubate at room temperature for 30 minutes, protected from light.
7. Place the tubes on a magnet for 1 minute, remove and discard the supernatant.
8. Remove the tubes from the magnet and add 100 uL of sterile-filtered PBS and gently pipet up and down to resuspend.
9. Place the tubes on a magnet for 1 minute, remove and discard the supernatant.
10. Remove the tubes from the magnet, add 500 uL of sterile-filtered PBS and gently pipet up and down to resuspend.
11. Run the samples on a flow cytometer.

Related Products

Cat. No.	Product
30111-30114	ExoBrite™ CTB EV Staining Kits
30119-30122	ExoBrite™ Annexin EV Staining Kits
30115-30118	ExoBrite™ STORM CTB EV Staining Kits
P003-410	ExoBrite™ 410/450 CD9 Flow Antibody
P003-490	ExoBrite™ 490/515 CD9 Flow Antibody
P003-560	ExoBrite™ 560/585 CD9 Flow Antibody
P003-650	ExoBrite™ 650/665 CD9 Flow Antibody
P003-RPE	ExoBrite™ R-PE CD9 Flow Antibody
P004-410	ExoBrite™ 410/450 CD63 Flow Antibody
P004-490	ExoBrite™ 490/515 CD63 Flow Antibody
P004-560	ExoBrite™ 560/585 CD63 Flow Antibody
P004-RPE	ExoBrite™ R-PE CD63 Flow Antibody
P005-410	ExoBrite™ 410/450 CD81 Flow Antibody
P005-490	ExoBrite™ 490/515 CD81 Flow Antibody
P005-560	ExoBrite™ 560/585 CD81 Flow Antibody
P005-RPE	ExoBrite™ R-PE CD81 Flow Antibody
P008-410	ExoBrite™ 410/450 IgG1 Isotype Control Flow Antibody
P008-490	ExoBrite™ 490/515 IgG1 Isotype Control Flow Antibody
P008-560	ExoBrite™ 560/585 IgG1 Isotype Control Flow Antibody
P008-650	ExoBrite™ 650/665 IgG1 Isotype Control Flow Antibody
P008-RPE	ExoBrite™ R-PE IgG1 Isotype Control Flow Antibody
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

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