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Lieferung & Zahlungsart

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Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Product Information

ExoBrite™ EV Surface Stain Sampler Kit, Green

Catalog Number: 30127

Kit Contents

Component	Size
30120A-100uL: ExoBrite™ 490/515 Annexin EV Stain	1 x 100 uL
30124A-100: ExoBrite™ 490/515 WGA EV Stain	1 x 1 vial
30112A: ExoBrite™ 490/515 CTB EV Stain	1 x 1 vial
99878: 50X Annexin Binding Buffer	1 x 1 mL
99879: 1X ExoBrite™ PBS Solution	1 x 1 mL
99858: ExoBrite™ Reconstitution Solution	1 x 1 mL

Storage and Handling

Store ExoBrite™ 490/515 WGA EV Stain (Cat. No. 31024A-100) and ExoBrite™ 490/515 CTB EV Stain (Cat. No. 30112A) at -20°C, protected from light.

Store ExoBrite™ 490/515 Annexin EV Stain (Cat. No. 30120A-100uL) at 4°C, protected from light. Do not freeze.

ExoBrite™ Reconstitution Solution (Cat. No. 99858), 1X ExoBrite™ PBS Solution (Cat. No. 99879), and 50X Annexin Binding Buffer may be stored at either -20°C or 4°C.

Reconstitution

ExoBrite™ WGA and CTB conjugates are supplied as lyophilized solids and must be reconstituted into a 500X stock before use.

To prepare 500X ExoBrite™ 490/515 WGA EV Stain solution, reconstitute the vial of 30124A-100 using 100 uL of 1X ExoBrite™ PBS Solution (Cat. No. 99879). 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.

To prepare 500X ExoBrite™ 490/515 CTB EV Stain solution, reconstitute the vial of 30112A in 100 uL of ExoBrite™ Reconstitution Solution (Cat. No. 99858). Pipet gently up and down to mix. The 500X stain solution can be stored protected from light for up to 6 months at 4°C.

Note: ExoBrite™ Reconstitution Solution contains 0.05% sodium azide.

ExoBrite™ 490/515 Annexin EV Stain is supplied as a 500X stock solution and the 1X staining solution should be freshly prepared on the day of use (see “Staining of purified EVs” on page 2.

Note: ExoBrite™ Annexin EV Stains contain <0.1% sodium azide.

Table 1. ExoBrite™ 490/515 Spectral Properties

Ex/Em (nm)	Laser Line(s) (nm)	Detection Channel	Other Compatible Applications
490/516	488	FITC	STED, STORM, TIRF

Product Description

The ExoBrite™ EV Surface Stain Sampler Kit, Green was developed to offer each of Biotium’s ExoBrite™ EV Surface Stains (CTB, WGA, and Annexin V) for assessing which stain offers the best coverage for the EV samples of interest. ExoBrite™ EV Surface Stains are conjugates of probes for labeling EV membrane surface targets using Biotium’s unique fluorescent dyes for superior brightness and specificity. The stains were designed to overcome some of the challenges of EV detection, particularly in flow cytometry. For example, some lipophilic membrane dyes used to stain EVs can form aggregates of a similar size as exosomes or EVs, thus confounding analysis. ExoBrite™ EV stains have been formulated for bright and specific staining of EV surface targets with minimal aggregation in flow cytometry. In addition, ExoBrite™ EV Stains do not bind non-specifically to polystyrene beads, and therefore unlike hydrophobic membrane dyes, they can be used to stain bead-bound EVs.

A major issue for EV detection includes varying signal and coverage using tetraspanin antibody staining of EVs isolated from different cell types or biological fluids. This sampler kit includes three ExoBrite™ EV Surface Stains that bind membrane targets that may be found on the surface of EVs. ExoBrite™ CTB EV Stains are conjugates of cholera toxin subunit B (CTB), which binds to GM1 gangliosides that are found on the surface of mammalian lipid rafts and some EV populations. 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 frequently exposed on EV membranes. ExoBrite™ Annexin V is a calcium-dependent phospholipid-binding protein with high affinity for phosphatidyleserine (PS), which is exposed on the surface of apoptotic cells and also used as a marker for EV from a variety of sources.

Different ExoBrite™ EV stains may be optimal for detecting EVs from different biological sources and for different applications. Among these stains, ExoBrite™ CTB and Annexin EV stains show the lowest level aggregation in flow cytometry, allowing EVs that bind these probes to be identified with bright and specific staining. Conversely, ExoBrite™ WGA EV Stains and ExoBrite™ Annexin EV Stains offer broader coverage of EVs isolated from different sources compared to CTB.

EVs are often labeled with fluorescent antibodies targeting one or more of the tetraspanin proteins CD9, CD63, and CD81. ExoBrite™ EV Stains can be combined with antibody staining, for multi-parameter analysis (see Antibody costaining of purified EVs). Biotium offers a selection of fluorescent ExoBrite™ Flow Antibodies against CD9, CD63, and CD81 that are optimized for detection of free or bead-bound EVs by flow cytometry (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. Buffers provided by this kit are pre-filtered.

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

EV Source	ExoBrite™ CTB Stains	ExoBrite™ WGA Stains	ExoBrite™ Annexin Stains
A549 cells	Yes	Yes	Yes
CHO cells	No	Yes	Yes
hASC (human adipose stem cells)	No[1]	ND	ND
HeLa cells	No	Yes	Yes
HUVEC (human umbilical vein endothelial cells)	No[1]	ND	ND
J774 cells	Yes	Yes	Yes
Jurkat cells	Yes	Yes	Yes
MCF-7 cells	Yes	Yes	Yes
Plasma	No	ND	Yes
Raji cells	Yes	Yes	Yes
Serum	No	ND	Yes
Skeletal myoblasts	Yes[1]	ND	ND
U2OS cells	No	Yes	Yes
U937 cells	No	Yes	Yes

[1]Customer-reported data
Value of "Yes" or "No" indicates coverage of EVs based on Biotium's internal data or customer-reported data. Value of "ND" indicates no data. For the most up-to-date information on validated EV sources, please view the product page for each stain on our website.

Considerations for Staining With ExoBrite™ EV Surface Stains

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

- ExoBrite™ EV Surface 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™ EV Surface 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.
- We do not recommend using ExoBrite™ 490/515 Annexin EV Stain to stain bead-bound EVs. For bead-bound EVs we recommend using ExoBrite™ 560/585 Annexin EV Stain, ExoBrite™ 655/670 Annexin EV Stain, as well as 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™ EV Surface Stains have not been validated for labeling EVs for cellular uptake. It may be necessary to remove free stain by re-purifying ExoBrite™-labeled EVs before attempting to apply them to cells.
- EVs can be imaged by specialized microscopy techniques including super-resolution imaging. ExoBrite™ 490/515 is compatible with STED, STORM, and TIRF. For imaging EVs by STORM that are suitable for CTB staining, we recommend our ExoBrite™ STORM CTB EV Staining Kits (see Related Products).
- ExoBrite™ EV Surface Stains have been validated to label EVs from the cell lines listed in Table 2, but may not stain EVs from every source. ExoBrite™ WGA EV Stains and ExoBrite™ Annexin EV Stains offer broader coverage of EVs isolated from different sources compared to CTB.
- While we have found that staining with 1X ExoBrite™ 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™ EV Surface 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).
- Annexin binding to phosphatidylserine is calcium-dependent, and calcium must be included in staining buffers. The 50X Annexin Binding Buffer provides an optimal calcium concentration for Annexin binding when diluted to 1X working concentration.

Experimental Protocols

Note: Before you begin, please read "Considerations for Staining EVs with ExoBrite™ EV Surface Stains" on previous page.

Staining of purified EVs

This protocol was developed for staining purified EVs with each of the three ExoBrite™ EV Surface Stains separately for comparing the stains side by side in flow cytometry. Before you begin, see "Reconstitution" on page 1 for instructions on preparing WGA and CTB stock solutions.

1. Isolate or purify EVs using the procedure of your choice.
2. Aliquot 50 uL of EVs into three FACS tubes or microcentrifuge tubes.
3. 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™ for each stain
   c. EVs alone (no stain)
4. If using ExoBrite™ 490/515 WGA EV Stain and ExoBrite™ 490/515 CTB EV Stain or the first time, prepare a 500X stock solution as described in the "Reconstitution" section on page 1.
5. Separately, prepare 1X staining solutions of all ExoBrite™ EV Surface Stains as shown in Table 3.

   Scale volumes proportionally to prepare enough 1X staining solution of each ExoBrite™ EV Surface Stain to allocate 500 uL for each sample and control to be tested. Mix well by gentle vortexing.

   Notes:
   a. 1X staining solutions of ExoBrite™ EV Surface Stains should be used the day of preparation.
   b. After adding each component, gently vortex the tube to mix well.
   c. The concentration of all ExoBrite™ stains can be optimized by the user.
   d. Annexin Binding Buffer is required for binding of ExoBrite™ Annexin EV Stain to EVs.

Table 3. Preparation of 1X Staining Solutions

Component	ExoBrite™ 490/515 CTB Stain	ExoBrite™ 490/515 WGA Stain	ExoBrite™ 490/515 Annexin Stain
PBS	500 uL	500 uL	---
dH_2O	---	---	490 uL
50X Annexin Binding Buffer	---	---	10 uL
500X stock solution	1 uL	1 uL	1 uL

6. 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.
7. Incubate the samples at room temperature for 30 minutes, protected from light.
8. Run the samples on a flow cytometer detecting in the FITC channel. For tips for flow cytometry detection of purified EVs read "Considerations for Detecting EVs by Flow Cytometry" on page 1.
   a. For each ExoBrite™ stain, create a stained-EV gate using unstained EVs, stained EVs, and buffer + dye control samples.

b. In order to determine which ExoBrite™ stain has the best coverage of your EVs of interest, compare the % of particles in the stained-EV gate between each stain.

Antibody co-staining of purified EVs

This protocol was developed for staining purified EVs with both ExoBrite™ EV Surface Stains and ExoBrite™ Flow 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™
   c. Buffer plus antibody

   EV controls
   a. Unstained EVs
   b. Single-stain ExoBrite™
   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 7-8 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 7-8 in the "Staining purified EVs" protocol.

Staining bead-bound EVs

This protocol was developed for EVs bound to magnetic antibody capture beads, stained with ExoBrite™ EV Surface Stains, and detected by flow cytometry.

Note: We do not recommend using ExoBrite™ 490/515 Annexin EV Stain to stain bead-bound EVs (see Considerations for Staining With ExoBrite™ EV Surface Stains).

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™ (no EVs)

3. If using ExoBrite™ 490/515 WGA EV Stain and ExoBrite™ 490/515 CTB EV Stain or the first time, prepare a 500X stock solution as described in the "Reconstitution" section on page 1.

4. Separately, prepare 10X staining solutions of ExoBrite™ 490/515 WGA EV Stain and ExoBrite™ 490/515 CTB EV Stain by diluting in 1X PBS (e.g., add 2 uL of 500X stock solution to 100 uL PBS).

   Notes:
   a. 10X staining solutions of ExoBrite™ EV Surface Stains should be used the day of preparation.
   b. The concentration of all ExoBrite™ stains can be optimized by the user.

5. Place the tubes with beads on a magnet for 1 minute, remove and discard the supernatant. Be careful not to aspirate any beads in this step and in subsequent steps.

6. 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.

7. Incubate at room temperature for 30 minutes, protected from light.

8. Place the tubes on a magnet for 1 minute, remove and discard the supernatant.

9. Remove the tubes from the magnet and add 100 uL of sterile-filtered PBS and gently pipet up and down to resuspend.

10. Place the tubes on a magnet for 1 minute, remove and discard the supernatant.

11. Remove the tubes from the magnet, add 500 uL of sterile-filtered PBS and gently pipet up and down to resuspend.

12. Run the samples on a flow cytometer.

Related Products

Cat. No.	Product
30119-30122	ExoBrite™ Annexin EV Staining Kits
30123-30126	ExoBrite™ WGA EV Staining Kits
30111-30114	ExoBrite™ CTB EV Staining Kits
30115-30118	ExoBrite™ STORM CTB EV Staining Kits
28000	ExoBrite™ Streptavidin Magnetic Beads
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-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-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
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