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Produktinformation

- Forschungsprodukte & Biochemikalien
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- Diagnostik & molekulare Diagnostik
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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Product Information

ExoBrite™ Annexin EV Staining Kits

Catalog Number: See Table 1 below.

Kit Contents

Component	Full Size 500 labelings	Trial Size 100 labelings
ExoBrite™ Annexin EV Stain	Component A 1 x 500 uL	Component A 1 x 100 uL
50X Annexin Binding Buffer	99878 5 x 1 mL	99878 1 x 1 mL

Storage and Handling

Store the kit at 4°C upon arrival and protect from light. Do not freeze. Product is stable for at least 6 months from date of receipt when stored as recommended.

Spectral Properties

See Table 1.

Product Description

ExoBrite™ Annexin EV Stains are uniquely formulated conjugates for staining the outer membrane of extracellular vesicles (EVs). Annexin V is a 35-36 kDa protein that binds phosphatidylserine (PS) in a calcium-dependent fashion. Annexin V conjugates are often used for detecting apoptotic cells that express PS on the outer leaflet of the plasma membrane. EV membranes commonly display PS on their outer membrane surface, allowing them to be labeled by fluorescent Annexin V conjugates.

ExoBrite™ Annexin EV Stains were designed to overcome some of the challenges of detecting isolated EVs, particularly in flow cytometry. For example, lipophilic membrane dyes commonly used to stain EVs can form aggregates of a similar size as exosomes or EVs, thus confounding analysis. Conversely, ExoBrite™ Annexin EV Stains are specially formulated to minimize aggregation in flow cytometry, allowing EVs to be identified with bright staining with minimal background. In addition, ExoBrite™ 490/515, 560/585, and 650/665 Annexin EV Stains are validated for detection by fluorescence nanoparticle tracking analysis (fNTA). ExoBrite™ Annexin EV Stains offer broad coverage of EVs isolated from different sources. Please visit the product page for a complete 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™ Annexin 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 offers other stains optimized for bright and sensitive staining of EVs and exosomes. This includes ExoBrite™ True EV Membrane Stains which are novel lipophilic membrane dyes as a superior method for pan-EV labeling. ExoBrite™ CTB EV Stains (cholera toxin B conjugates) and ExoBrite™ WGA EV Stains (wheat germ agglutinin conjugates) are also available (see Related Products).

Table 1. ExoBrite™ Annexin EV Staining Kits

Cat. No.	Size	Product Name	Ex/Em (nm)	Laser Line(s) (nm)	Detection Channel	Compatible Applications
30119	500 labeling reactions	ExoBrite™ 410/450 Annexin EV Staining Kit	416/452	405	Pacific Blue™	Flow, SIM, STED
30119-T	100 labeling reactions					
30120	500 labeling reactions	ExoBrite™ 490/515 Annexin EV Staining Kit	490/516	488	FITC	Flow, fNTA, STED, STORM, TIRF
30120-T	100 labeling reactions					
30121	500 labeling reactions	ExoBrite™ 560/585 Annexin EV Staining Kit	562/584	532 or 561	PE	Flow, fNTA, SIM, STED, STORM
30121-T	100 labeling reactions					
30122	500 labeling reactions	ExoBrite™ 650/665 Annexin EV Staining Kit	652/668	633-640	APC	Flow, fNTA, STORM
30122-T	100 labeling reactions					

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 EVs with ExoBrite™ Annexin 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™ Annexin 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™ Annexin 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.
- We do not recommend using ExoBrite™ 410/450 or ExoBrite™ 490/515 Annexin EV Stains to stain bead-bound EVs. For bead-bound EVs we recommend using ExoBrite™ 560/585 or ExoBrite™ 650/665 Annexin EV Stains, as well as ExoBrite™ CTB EV Stains or ExoBrite™ WGA EV Stains (see Related Products).
- 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™ Annexin EV 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™ Annexin-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™ Annexin EV Stains have been found to label EVs derived from every cell line tested, but may not stain EVs from every source. Please visit the product page for a complete list of validated EV sources.
- While we have found that staining with 1X ExoBrite™ Annexin 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™ Annexin 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).
- 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 beginning, please read "Considerations for Staining EVs with ExoBrite™ Annexin EV Stains".

Staining of purified EVs

This protocol was developed for staining purified EVs with ExoBrite™ Annexin 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 in 1X Binding Buffer as follows. Scale volumes proportionally to prepare 500 uL of staining solution for each sample and control to be tested. Mix well by gentle vortexing.

   490 uL dH_2O
   10 uL 50X Annexin Binding Buffer
   1 uL ExoBrite™ Annexin Stain

   Notes:
   a. The 1X ExoBrite™ staining solution should be used the day of preparation.
   b. Binding Buffer is required for Annexin binding.
   c. 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™
   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 2.

Antibody co-staining of purified EVs

This protocol was developed for staining purified EVs with both ExoBrite™ Annexin 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™
   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 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" controland 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™ Annexin EV Stains, and detected by flow cytometry.

Note: We recommend using ExoBrite™ 560/585 Annexin EV Stain or ExoBrite™ 650/665 Annexin EV Stain for bead-bound EVs (see Considerations for Staining With ExoBrite™ Annexin EV 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. Prepare 10X ExoBrite™ staining solution as shown below. Scale volumes proportionally to prepare 50 uL of 10X ExoBrite staining solution for each sample to be tested:

   48 uL dH_2O
   1 uL 50X Annexin Binding Buffer
   1 uL ExoBrite™ Annexin EV Stain
   Mix well by gentle vortexing.

   Notes:
   a. The 10X ExoBrite™ staining solution should be used the day of preparation.

   b. Binding Buffer is required for Annexin binding.

4. Prepare additional 1X Annexin Binding Buffer by diluting the 50X Annexin Binding Buffer in dH_2O at 1:50. For example, add 10 uL of 50X Annexin Binding Buffer to 490 uL of dH_2O and vortex to mix well. Scale volumes proportionally as needed.

   Notes:
   a. You will need 500 uL of 1X Annexin Binding Buffer per sample for step 10. If performing the optional wash in step 9, you will need to prepare 600 uL of 1X Annexin Binding Buffer per sample.

   b. 1X Annexin Binding Buffer can be stored at 4°C.

5. Place the tubes with beads on a magnet for 1 minute, remove and discard the supernatant.

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. Optional: Wash samples by adding 100 uL of 1X Annexin Binding Buffer and gently pipet up and down to resuspend. Place the tubes on a magnet for 1 minute, then remove and discard the supernatant.

10. Remove the tubes from the magnet and add 500 uL of 1X Annexin Binding Buffer to each tube. Gently pipette up and down to resuspend the beads.

11. Run the samples on a flow cytometer.

Related Products

Cat. No.	Product
30129... 30137	ExoBrite™ True EV Membrane Stains
30111-30114	ExoBrite™ CTB 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

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