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Produktinformation

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- Diagnostik & molekulare Diagnostik
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See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Product Information

ExoBrite™ STORM CTB EV Staining Kits

Catalog Number: See Table 1

Kit Contents

Component	Full Size 500 labelings	Trial Size 100 labelings
ExoBrite™ STORM CTB EV Stain	Component A 5 vials	Component A 1 vial
ExoBrite™ Reconstitution Solution	99858 1 mL	99858 1 mL

Storage and Handling

Store Component A at -20°C upon arrival and protect from light. The ExoBrite™ Reconstitution Solution can be stored at 4°C or -20°C. Product is stable for at least 6 months from date of receipt when stored as recommended.

Reconstitution

To prepare 500X ExoBrite™ stain solution, dissolve one vial of Component A in 100 uL of ExoBrite™ Reconstitution Solution. Pipet gently up and down to mix. The 500X solution can be stored protected from light for up to 6 months at 4°C.

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

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.

ExoBrite™ STORM CTB EV Stains are unique fluorescent dyes conjugated to cholera toxin subunit B (CTB), which binds to GM1 gangliosides that are commonly found on the surface of mammalian lipid rafts and EVs. While some lipophilic dyes traditionally used to stain EV membranes can confound analysis by forming aggregates of a similar size as EVs, ExoBrite™ STORM CTB EV Stains show little to no background from aggregation.

Super-resolution microscopy techniques such as direct stochastic optical reconstruction microscopy (dSTORM) push beyond the diffraction limit of traditional light microscopy, allowing single-molecule resolution of subcellular structures, such as the proteins found on EVs. ExoBrite™ STORM CTB EV Stains were developed for STORM imaging of EVs and incorporate a selection of our STORM-validated CF® Dyes. ExoBrite™ 560/585 CTB EV Stain and ExoBrite™ STORM CF®647 CTB EV Stain have been validated for dSTORM on the ONI Nanoimager together with fluorescent tetraspanin antibodies, allowing single-EV characterization studies.

ExoBrite™ staining can be combined with antibody staining (for example, antibodies against tetraspanin proteins CD9, CD63, & CD81), for multi-parameter analysis. Biotium also offers a selection of validated ExoBrite™ antibodies optimized for detection of EVs by flow cytometry or western blot (see Related Products).

While workflows currently vary widely for the staining and imaging of EVs using STORM, the general staining protocol given here should be appropriate for use with the ONI Nanoimager® and the types of workflows in the references section at the top of the next column.

References

1. Biochim Biophys Acta, 1865, 12 (2018); 2. J Extracell Vesicles, 11, 3, (2022); 3. JoVE 174 (2021).

Considerations for Staining With ExoBrite™ STORM CTB EV Stains

The following are general considerations for using ExoBrite™ CTB EV Stains. See Procedures for Staining Purified EVs for a detailed staining protocol.

- For best results, buffers used for suspending and staining EVs should be filtered through a 0.2 um filter to remove particulates.
- Washing, EV capture or mounting, and image acquisition steps incorporated into a workflow that includes the ExoBrite™ staining protocol should be selected based on platform-specific protocols.
- ExoBrite™ STORM 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™-labeled EVs to cells.
- ExoBrite™ STORM CTB EV Stains are validated for staining EVs from various sources but may not work on EVs from other sources. See Table 2 on page 2 or visit the product page for staining performance for EVs from a variety of sources that were confirmed by Biotium or customer data.
- ExoBrite™ STORM CTB 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 exosome isolation method used.
- While we have found that staining with 1X ExoBrite™ STORM CTB EV Stain gives a bright signal and low background under our typical staining conditions, we have also seen excellent results at concentrations between 1X and 100X. The dye concentration may require optimization for different samples and detection systems.
- ExoBrite™ STORM CTB EV Stains can be used for co-staining with appropriate fluorescently labeled primary antibodies or in protocols for staining bead-bound EVs.

Procedure for Staining Purified EVs

This is a general protocol for staining purified exosomes in solution with ExoBrite™ STORM CTB EV Stains, and may be adapted for different imaging workflows. Also see “Considerations for Staining EVs with ExoBrite™ STORM CTB EV Stains” above.

1. Isolate or purify EVs or exosomes using the procedure of your choice.
2. Aliquot 50 uL of EVs into 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).

   Note: The concentration of ExoBrite™ stain can be optimized by the user; we find that concentrations ranging from 1X to 100X give good signal.
4. In addition to the ExoBrite™-stained exosome samples, it is often helpful to include several control samples at this point in the protocol. For example, an appropriate negative control for the EVs (such as a mock purification, or the buffer used to suspend the EVs), ExoBrite™ alone in buffer, or unstained EVs. Different controls may also be necessary when co-staining with antibodies, for example, samples using single stains.
5. Add 450 uL of ExoBrite™ staining solution to each tube containing 50 uL sample. Remember to also add the staining solution to the “buffer plus ExoBrite™” control, if that control is included.
6. Incubate at room temperature for 30 minutes, protected from light.
7. Proceed with your protocol for STORM detection of EVs.

Table 1. ExoBrite™ STORM CTB EV Staining Kits

Cat. No.	Size	Product Name	Ex/Em (nm)	Laser Line(s) (nm)	Detection Channel
30115	500 labeling reactions	ExoBrite™ STORM CF®505 CTB EV Staining Kit	505/519	488	FITC
30115-T	100 labeling reactions				
30116	500 labeling reactions	ExoBrite™ STORM CF®583R CTB EV Staining Kit	583/609	555 or 561	Rhodamine or Texas Red®
30116-T	100 labeling reactions				
30117	500 labeling reactions	ExoBrite™ STORM CF®647 CTB EV Staining Kit	652/668	633-640	Cy®5
30117-T	100 labeling reactions				
30118	500 labeling reactions	ExoBrite™ STORM CF®680 CTB EV Staining Kit	681/698	633-640	Cy®5.5
30118-T	100 labeling reactions				

Table 2. Validated EV Sources for ExoBrite™ STORM CTB EV Stains

EV Source	Biotium Data	Customer Reported
A549 cells	High	---
CHO cells	Low	---
hASC (human adipose stem cells)	---	Low
HeLa cells	Low	---
HUVEC (human umbilical vein endothelial cells)	---	Low
J774 cells	High	---
Jurkat cells	High	---
MCF-7 cells	High	---
Plasma	---	High
Raji cells	High	---
Skeletal myoblasts	---	High
U-2 OS cells	Low	---
U937 cells	Low	---

Value of “High” or “Low” indicates relative coverage of EVs based on Biotium’s internal data or customer reported data.

Related Products

Cat. No.	Product
30111-30114	ExoBrite™ CTB EV Staining Kits
30119-30122	ExoBrite™ Annexin EV Staining Kits
30123-30126	ExoBrite™ WGA 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-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
92550-92557	Mix-n-Stain™ STORM CF® Dye Antibody Labeling Kits
30092...30104	MemBrite® Fix Cell Surface Staining Kits

Please visit our website at www.biotium.com for information on our life science research products, including fluorescent CF® Dye antibody conjugates and reactive dyes, fluorescent probes, and kits for cell biology research.