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Product Information

CellBrite® Fix Membrane Stains

Kit Contents

Component	Trial Size	Full Size
CellBrite® Fix Membrane Stain (Component A)	1 vial*	5 vials*
99953: Anhydrous DMSO	1 x 150 uL	1 x 150 uL

*Each dye vial makes 20 uL of 1000X dye solution after reconstitution in DMSO.

Storage and Handling

Store CellBrite® Fix Membrane Stain at -20°C, desiccated and protected from light. Store DMSO at room temperature, 4°C, or -20°C, desiccated and protected from light. Products are stable for at least 12 months from date of receipt when stored as recommended.

To make 1000X dye stock solution, bring one vial of lyophilized CellBrite® Fix and the anhydrous DMSO to room temperature. Add 20 uL of anhydrous DMSO to the vial, and vortex or pipette up and down to ensure that all of the dye has dissolved. Once dissolved, the dye should be used within a few hours. Leftover dye solution can be aliquoted and stored desiccated at -20°C for at least 1 month.

Product Description

CellBrite® Fix Membrane Stains are a unique class of membrane dyes that covalently stain the cell surface in live cells. PKH dyes or membrane dyes like DiO, DiI, Vybrant®, and CellMask™ can be fixed with formaldehyde, but are not compatible with detergent permeabilization or methanol fixation, as these treatments extract the lipophilic dyes from membranes. In contrast, CellBrite® Fix Membrane Stains are unique in that their surface staining can withstand permeabilization and methanol fixation, allowing plasma membrane staining to be combined with intracellular immunofluorescence. This is accomplished by the rapid accumulation of these fluorogenic dyes in the plasma membrane, where they react covalently with cell surface proteins. As a result, surface staining is well retained after permeabilization or methanol fixation, with only a slight increase in intracellular fluorescence compared to formaldehyde fixation alone. Unlike lectins, such as WGA, which bind specific targets that may vary between cell types, CellBrite® Fix Membrane Stains are general membrane stains.

CellBrite® Fix Membrane Stains have better water solubility than classic lipophilic dyes, and as a result, they yield much more uniform staining compared to lipophilic carbocyanine dyes, like DiO and DiI. CellBrite® Fix staining has low toxicity and does not readily transfer between cells. The dyes have been validated for staining of isolated exosomes for analysis by flow cytometry, and they can also be used to stain yeast, gram-positive bacteria, or gram-negative bacteria.

Spectral Properties

Cat. No.	Dye	Ex/Em (nm)	Detection Channel
30090-T, 30090	CellBrite® Fix 488	480/513	FITC
30088-T, 30088	CellBrite® Fix 555	542/570	PE
30089-T, 30089	CellBrite® Fix 640	638/667	APC

Considerations for Staining with CellBrite® Fix Membrane Stains

The following are general considerations for using CellBrite® Fix Membrane Stains. See Staining Protocols for step-by-step instructions for use.

- CellBrite® Fix stains must be used on live cells. The dyes will stain intracellular structures in fixed cells. We recommend CytoLiner™ Fixed Cell Membrane Stains for staining formaldehyde-fixed cells (see Related Products).
- CellBrite® Fix reacts with proteins and amino acids. Staining must be done in protein- and amine-free buffers, such as PBS or HBSS. For adherent cells, we typically use HBSS with Ca^{2+}/Mg^{2+} to maintain cell adhesion and morphology.
- CellBrite® Fix reacts with plates coated with poly-L-lysine, collagen, gelatin, or other proteins, resulting in high background. The dyes tend to have high background on uncoated cell culture surfaces as well. Imaging cells by confocal microscopy can reduce interference from out-of-plane background fluorescence. See Tips for Imaging CellBrite® Fix Staining.
- CellBrite® Fix reacts irreversibly with cellular proteins. In live cells, this occurs on the cell surface because the dyes can't penetrate the membrane. However, the dyes do get inside dead cells, where there are many more targets for reaction. As a consequence, the dyes stain dead cells much more brightly than live cells. See Tips for Imaging CellBrite® Fix Staining.
- Cells can be stained in suspension at 10^5-10^6 cells in 100 uL following the protocol provided. Pellet the cells by centrifugation and remove the supernatant in between each change of solution.

- CellBrite® Fix Membrane Stains are designed to be fixed shortly after staining when they primarily localize to the plasma membrane. Alternatively, cells can be returned to growth medium and cultured after staining, though dye localization in live cells changes over time. Labeled membranes become internalized, so staining gradually changes from cell surface to intracellular vesicles, usually becoming mostly intracellular after about 24 hours. Internalized CellBrite® Fix Membrane Stains are usually detectable for up to 48 hours after staining, though this may vary by cell type.
- Covalent modification of cell surface protein epitopes may interfere with subsequent antibody binding. To reduce the chance of interference, the dye concentration used for labeling should be optimized to use the lowest effective concentration. We also offer MemBrite® Fix Membrane Stains (see Related Products), which are covalent cell surface stains that react with proteins by a different chemistry than CellBrite® Fix. MemBrite® Fix may be a suitable alternative in cases where CellBrite® Fix staining interferes with immunostaining for a particular epitope.
- See Related Products and visit our website to see our full selection of membrane and cell surface stains, including additional covalent surface stains with more color options, membrane dyes for fixed cells, dyes for long-term membrane staining in live cells, and membrane stains for super-resolution imaging.

Tips for Imaging CellBrite® Fix Staining

Confocal vs. epifluorescence microscopy

We recommend using a confocal microscope to image membrane staining for the best results. Confocal imaging screens out fluorescence from above and below the plane of focus, allowing very crisp imaging of cell boundaries. These stains tend to have high background on the surface of the culture substrate. While imaging cells by confocal microscopy can reduce interference from out-of-plane background fluorescence, it is usually necessary to focus at a level above the substrate to avoid this background and image the cell outlines. Compared to regular epifluorescence imaging, confocal is more sensitive and gives you more control over excitation power to limit photobleaching.

Membrane dyes can be imaged with a regular epifluorescence microscope, but the images will be more diffuse due to out-of-plane background fluorescence.

Staining of dead cells

When imaging CellBrite® Fix staining, do not focus on very bright, rounded-up, or shrunken dead cells. Instead, adjust the plane of focus and imaging settings to detect the live cell membrane staining. The signal from dead cells will likely be saturated. If the dead cell staining interferes with your imaging, try using high magnification and confocal imaging to exclude dead cells from the field of view. Or, try using one of our original CellBrite® Cytoplasmic Membrane Stains, which do not show dramatic differences in signal between live and dead cells (see Related Products).

Staining Protocols

Mammalian cell staining

1. Wash cells with protein- and amine-free buffer, such as PBS or HBSS.
2. Prepare staining solution by diluting the 1000X CellBrite® Fix stock solution in buffer to a final concentration of 1X. For example, add 1 uL of 1000X dye to 1 mL of buffer. Staining solution should be prepared fresh immediately before use.

   Note: Dye concentration may need to be optimized for brightness and surface selectivity.
3. Add staining solution to cells and incubate at 37°C for 15 minutes. If fixation is required, fix cells immediately after 15 minutes to minimize dye internalization (see step 5).

   Notes:

   a. Performing dye incubation at 37°C results in strong cell surface staining with a small amount of intracellular staining due to dye internalization. If dye internalization is a problem in your cell type when staining at 37°C, staining can be performed at room temperature or 4°C. Incubation times may need to be increased to achieve stable staining.

   b. Cells can be incubated with dye at 37°C for longer times without obvious toxicity. However, dye will be internalized and intracellular staining will increase over time.
4. If fixation is not required, cells can be imaged immediately. Washing is optional for confocal imaging, but may be required to reduce extracellular background when imaging by epifluorescence microscopy.

   Note: Alternatively, cells can be placed in growth medium for continued culture, but staining will be internalized over time (see Considerations for Staining).
5. To fix cells, wash twice with buffer and fix according to your usual protocol. We usually fix with 4% paraformaldehyde in PBS (Cat. No. 22023) for 20 minutes at room temperature or 4°C. Alternatively, cells can be fixed with pre-chilled methanol for 5-10 minutes at -20°C. Methanol fixation may result in an increase in intracellular fluorescence.

   Note: Fixation should be performed shortly after staining when the dye primarily localizes to the plasma membrane.
6. To permeabilize cells, rinse twice with PBS, then incubate with PBS containing 0.1% Triton® X-100 for 10 minutes at room temperature. Alternatively, permeabilization can be performed at 4°C. Permeabilization may result in an increase in intracellular fluorescence.

Staining of bacteria and yeast

CellBrite® Fix Membrane Stains can be used to stain yeast, gram-positive bacteria, or gram-negative bacteria, but a higher dye concentration may be needed. We recommend following the same general protocol for mammalian cells, but using 10X dye and optimizing the concentration as needed. Bacteria can be stained at room temperature. Yeast can rapidly internalize the dyes, so staining should be done at room temperature or 4°C to limit staining to the cell surface. Dead cells also may show bright intracellular staining.

Frequently Asked Questions

Question	Answer
What is the difference between CellBrite®, CellBrite® NIR, CellBrite® Fix, MemBrite® Fix, and CytoLiner™?	CellBrite® Cytoplasmic Membrane Stains are lipophilic dyes for simple, non-toxic, stable labeling of membranes in live or fixed cells. Cells can be fixed with formaldehyde before or after CellBrite® staining. The staining has poor tolerance for permeabilization after fixation, and cannot be used with methanol fixation. The dyes also do not stain bacteria or yeast. CellBrite® NIR Dyes are CellBrite® Dyes with near-infrared (NIR) fluorescence compatible with small animal NIR imaging systems. CellBrite® Fix and MemBrite® Fix are covalent stains that can be fixed and permeabilized for IF staining. Both stains are fluorogenic reactive membrane dyes that rapidly accumulate at the plasma membrane and react covalently with membrane proteins for stable labeling. Staining takes only 15 minutes in a single step with no wash. CellBrite® Fix stains mammalian cells, yeast, and bacteria. MemBrite® Fix Cell Surface Stains react with membrane proteins by a different chemistry than CellBrite® Fix. MemBrite® Fix requires a two-step staining protocol with washing, but offers a more extensive choice of dye colors than CellBrite® Fix. MemBrite® Fix also can be used to stain yeast. Unlike original CellBrite® Dyes and lectins, CellBrite® Fix and MemBrite® Fix cannot be used on cells that are already fixed. CytoLiner™ Fixed Cell Membrane Stains are novel lipophilic fluorescent dyes for selective staining of plasma membranes in formaldehyde-fixed cells. These dyes are uniquely engineered to be more soluble than other lipophilic dyes, resulting in less variability. CytoLiner™ tolerates mild permeabilization before staining and is suitable for antibody co-staining. To select a dye that's right for your application, see our Membrane and Cell Surface Stains Comparison, or download our Membrane & Surface Stains Brochure.
Can CellBrite® or MemBrite® Stains be used to stain exosomes/extracellular vesicles?	CellBrite® Cytoplasmic Membrane Dyes do not efficiently stain EVs, but CellBrite® Fix, MemBrite® Fix, CellBrite® Steady, and other stains have been used for this application. However, for optimal staining of EV membranes, we recommend our ExoBrite™ True EV Membrane Stains (see Related Products).
How stable are CellBrite® Fix and MemBrite® Fix membrane staining? Are the dyes toxic to cells?	Staining with the covalent stains CellBrite® Fix and MemBrite® Fix lasts up to 48 hours in tissue culture cells, though over time, all cell surface stains will be internalized and become intracellular as membranes turn over by endocytosis. In immortalized cells in culture, most of the surface staining becomes internalized over the course of about 24 hours for CellBrite® Fix and MemBrite® Fix stains. CellBrite® Fix and MemBrite® Fix were designed to be fixed shortly after staining when they primarily localize to the plasma membrane.
Can cells be fixed after CellBrite® Fix or MemBrite® Fix staining?	CellBrite® Fix and MemBrite® Fix stains covalently label the cell surface. They can withstand fixation and permeabilization, or fixation with alcohol after labeling of live cells. CellBrite® Fix and MemBrite® Fix cannot be used to label the plasma membranes of fixed cells or tissues (the dyes label the cytoplasm in fixed cells).
Can CellBrite® Fix or MemBrite® Fix be used to stain cells that are already fixed?	CellBrite® Fix and MemBrite® Fix cannot be used to stain the plasma membrane of fixed samples. These dyes will primarily stain intracellular structures in cells that are already fixed. For staining fixed cells, we recommend our CytoLiner™ Fixed Cell Membrane Stains (see Related Products).
Can CellBrite® Fix or MemBrite® Fix be used on tissue sections (FFPE or cryosections)?	CellBrite® Fix and MemBrite® Fix are recommended for use on live cells only. In fixed cells or sections, they will label intracellular structures.

Related Products

Cat. No.	Product
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative
30092... 30104	MemBrite® Fix Cell Surface Staining Kits
30131-30135	CytoLiner™ Fixed Cell Membrane Stains
30021-30024	CellBrite® Cytoplasmic Membrane Dyes
30070... 30079	CellBrite® NIR Cytoplasmic Membrane Stains
30105-30109	CellBrite® Steady Membrane Staining Kits
29021... 29128	Wheat Germ Agglutinin (WGA) Conjugates
29015... 29136	Concanavalin A (Con A) CF® Dye Conjugates
00068... 29127	Cholera Toxin Subunit B CF® Dye Conjugates
30129... 30137	ExoBrite™ True EV Membrane Stains
40081, 40082	NucSpot® Live Cell Nuclear Stains
41033... 41040	NucSpot® Nuclear Stains
40083	NucSpot® 470 Nuclear Stain
40085	NucSpot® Far-Red, 1000X in DMSO
40060	RedDot™1 Far-Red Nuclear Stain, 200X in Water
40061	RedDot™2 Far-Red Nuclear Stain, 200X in DMSO
32010	Live-or-Dye NucFix™ Red
70058... 70086	LysoView™ Dyes
70054... 70082	MitoView™ Mitochondrial Dyes
70082	MitoView™ Fix 640
70065, 70069	LipidSpot™ Lipid Droplet Stains
00027... 00064	Phalloidin Conjugates
30050... 30139	ViaFluor® SE Cell Proliferation Dyes
23001	EverBrite™ Mounting Medium
23002	EverBrite™ Mounting Medium with DAPI
23003	EverBrite™ Hardset Mounting Medium
23004	EverBrite™ Hardset Mounting Medium with DAPI
23008	Drop-n-Stain EverBrite™ Mounting Medium
23009	Drop-n-Stain EverBrite™ Mounting Medium with DAPI

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, Mix-n-Stain™ antibody labeling kits, apoptosis reagents, cell viability kits, and kits for cell biology research.

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