Product Information

MemBrite® Fix Cell Surface Staining Kits

Kit Contents

Component	100 Labelings*	500 Labelings*
MemBrite® Fix Stain (Component A)	1 vial**	5 vials**
MemBrite® Fix Pre-Staining Solution, 1000X	99847-20uL 1 x 20 uL	99847-100uL 1 x 100 uL
Anhydrous DMSO	99953 1 x 150 uL	99953 1 x 150 uL

*Kit sizes are based on a 200 uL labeling volume; actual number of reactions may vary based on sample size.
**Each stain vial makes 20 uL of 1000X stain solution after reconstitution in DMSO.

Storage and Handling

Store MemBrite® Fix Stain and MemBrite® Fix Pre-Staining Solution 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 MemBrite® Fix Stain 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. Reconstituted dye solution can be aliquoted and stored at -20°C, desiccated and protected from light, for at least 1 month.

Product Description

MemBrite® Fix Cell Surface Staining Kits are designed for covalent staining of the surface of live cells. Unlike traditional membrane dyes, like DiO, DiI, Vybrant®, CellMask™, or PKH dyes, MemBrite® Fix stains can withstand formaldehyde fixation, alcohol fixation, and detergent permeabilization. Because of this, MemBrite® Fix stains provide a convenient method for visualizing the cell surface in multi-color immunofluorescence staining experiments. Unlike lectins, such as WGA, which bind specific targets that may vary between cell types, MemBrite® Fix stains react widely with cell surface proteins. MemBrite® Fix staining is rapid and non-toxic to cells, and because MemBrite® Fix stains are highly water soluble, they stain cells much more evenly than lipophilic membrane stains. The kits can also be used to stain yeast and gram-positive bacteria, but not gram-negative bacteria.

MemBrite® Fix stains belong to Biotium's line of novel reactive cell surface stains that include CellBrite® Fix Membrane Stains. CellBrite® Fix Membrane Stains are fluorogenic dyes that rapidly accumulate in the plasma membrane, where they react covalently with the cell surface. CellBrite® Fix stains require only a single staining step compared to MemBrite® Fix staining, which is a two-step protocol. On the other hand, MemBrite® Fix stains are available with a wider selection of colors, some of which have been validated in specialized applications, such as super-resolution imaging. MemBrite® Fix stains do not associate with lipids in membranes, and consequently, have lower cytoplasmic background after detergent permeabilization compared to CellBrite® Fix.

Selecting a MemBrite® Fix Stain

Several MemBrite® Fix stains have been validated in super-resolution imaging applications or 2-photon microscopy (Table 1). MemBrite® Fix-ST stains are recommended for super-resolution imaging by STORM. MemBrite® Fix or MemBrite® Fix-ST stains can be used for standard microscopy applications, however, MemBrite® Fix stains are generally more photostable than MemBrite® Fix-ST stains.

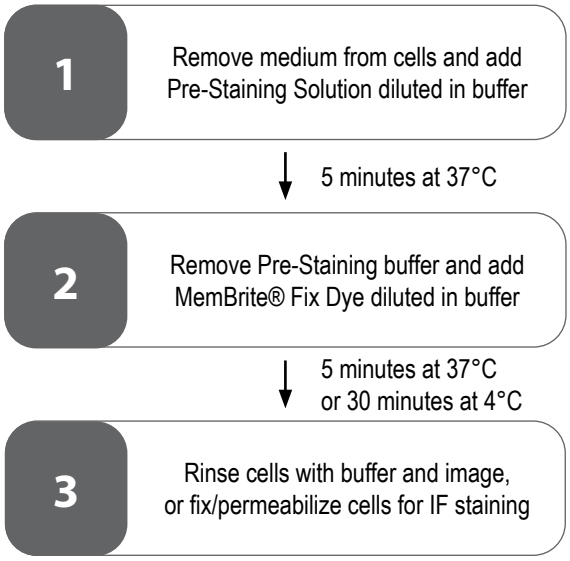
Considerations for Staining

The following are general considerations for using MemBrite® Fix stains. See Staining Protocols for step-by-step instructions for use.

- MemBrite® 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).
- MemBrite® 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.
- Treatment of cells with Pre-Staining Solution is required for efficient staining.
- While we do not expect MemBrite® Fix stains to react with poly-L-lysine coated surfaces, we have seen high background with these types of plates and with uncoated cell culture surfaces. To circumvent this issue, we recommend imaging cells by confocal microscopy to reduce out-of-plane background fluorescence. The stains will react with surfaces treated with collagen, gelatin, fibronectin, or other extracellular matrix protein coatings. See Tips for Imaging MemBrite® Fix Staining.

- MemBrite® 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 MemBrite® Fix Staining.
- MemBrite® Fix 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 MemBrite® Fix stains are usually detectable for up to 48 hours after staining, though this may vary by cell type. For long-term cell surface imaging in live cells, see our CellBrite® Steady Membrane Staining Kits (see Related Products).
- MemBrite® Fix stains can be used to stain yeast or gram-positive bacteria, but not gram-negative bacteria. Stain concentration, staining temperature, and staining time may need to be optimized for different organisms.
- 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 CellBrite® Fix Membrane Stains (see Related Products), which are covalent cell surface stains that react with proteins by a different chemistry than MemBrite® Fix. CellBrite® Fix may be a suitable alternative in cases where MemBrite® 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.

Protocol Overview



See detailed staining protocols on the next page.

Table 1. MemBrite® Fix Stains

Cat. No.	Dye	Ex/Em (nm)	Detection Channel	Specialized Applications
30092-T, 30092	MemBrite® Fix 405/430	411/431	Pacific Blue®	SIM, TIRF
30093-T, 30093	MemBrite® Fix 488/515	490/516	GFP or FITC	STED, TIRF, 2-photon microscopy
30094-T, 30094	MemBrite® Fix 543/560	543/563	PE	N/A
30095-T, 30095	MemBrite® Fix 568/580	562/584	Cy®3	STORM, SIM, TIRF
30096-T, 30096	MemBrite® Fix 594/615	593/615	Texas Red®	2-photon microscopy
30097-T, 30097	MemBrite® Fix 640/660	642/663	APC	FLImp, SIM, TIRF
30098-T, 30098	MemBrite® Fix 660/680	662/682	Cy®5	N/A
30099-T, 30099	MemBrite® Fix 680/700	680/701	Cy®5	STORM†, Single-molecule imaging, STED, 2-photon microscopy
30101-T, 30101	MemBrite® Fix-ST 650/665	652/669	APC	STORM
30102-T, 30102	MemBrite® Fix-ST 667/685	667/685	Cy®5	STORM
30103-T, 30103	MemBrite® Fix-ST 681/698	681/698	Cy®5	STORM†, Single-molecule imaging
30104-T, 30104	MemBrite® Fix-ST 755/777	755/779	Cy®7	STORM

FLImp: fluorophore localization imaging with photobleaching; SIM structured illumination microscopy; STED: stimulated emission depletion; STORM: stochastical optical reconstruction microscopy; TIRF: total internal reflection fluorescence

†MemBrite® Fix-ST 681/698 is reported to have better performance in STORM imaging than MemBrite® Fix 680/700.

Tips for Imaging MemBrite® 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 MemBrite® 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. Alternatively, if staining of cell membranes after fixation is an option for your workflow, consider using one of our CytoLiner™ Fixed Cell Membrane Stains (see Related Products).

Staining Protocols

Mammalian cell staining

1. Dilute the Pre-Staining Solution in a protein- and amine-free buffer, such as PBS or HBSS, to a final concentration of 1X. For example, add 1 uL of 1000X Pre-Staining Solution to 1 mL of buffer. Diluted Pre-Staining Solution should be prepared fresh on the day of use.
2. Remove culture medium from the cells and add enough 1X Pre-Staining Solution in buffer to completely cover the cells. Washing the cells with buffer before adding 1X Pre-Staining Solution is optional.

   Note: Cells can be stained in suspension at 10^5-10^6 cells in 100 uL. Pellet the cells by centrifugation and remove the supernatant in between each change of solution.
3. Incubate the cells in 1X Pre-Staining Solution for 5 minutes at 37°C. Incubation times up to 20 minutes will not negatively affect the reaction.
4. Prepare stain solution by diluting the 1000X MemBrite® Fix stock solution in buffer to a final concentration of 1X. For example, add 1 uL of 1000X staining solution to 1 mL of buffer. Staining solution should be prepared fresh immediately before use.

   Note: Stain concentration may need to be optimized for brightness.
5. Remove the Pre-Staining Solution from the cells. Add enough stain solution from step 4 to cover the cells and incubate at 37°C for 5 minutes. Longer staining times can be used, but more of the stain will be internalized. If fixation is required, fix cells immediately after 5 minutes to minimize dye internalization (see step 7).

   Notes:
   a. A rinse step is not needed after removing the Pre-Staining Solution and before adding the stain solution.
   b. Performing stain incubation at 37°C results in strong surface staining with a small amount of intracellular staining due to stain internalization. Alternatively, staining can be performed at 4°C for 30 minutes with pre-chilled staining solution to minimize stain internalization.
6. Rinse cells twice with buffer or medium. If fixation is not required, cells can be imaged immediately.

   Notes:
   a. If labeling was done at 4°C, use pre-chilled buffer for the rinse step.
   b. Cells can be returned to growth medium for continued culture, but staining will internalize over time (see Considerations for Staining).
7. To fix cells, add your preferred fixative after rinsing with buffer. We usually fix with 4% paraformaldehyde in PBS (Cat. No. 22023) for 20 minutes at room temperature or 4°C, or pre-chilled methanol for 5 minutes at -20°C.
8. To permeabilize cells after formaldehyde fixation, 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.
9. After fixation/permeabilization, you can perform immunofluorescence staining according to your preferred protocol.

Frequently Asked Questions

Question	Answer
What is the difference between CellBrite® Fix, MemBrite® Fix, CytoLiner™, CellBrite® Cytoplasmic Membrane Stains, and CellBrite® NIR?	CellBrite® Fix and MemBrite® Fix are covalent stains that can be fixed and permeabilized for IF staining. Both stains are fluorogenic reactive 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 CytoLiner™, 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 more even staining. CytoLiner™ tolerates mild permeabilization before staining and is suitable for antibody co-staining. CellBrite® Cytoplasmic Membrane Stains are ready-to-use solutions of classic lipophilic dyes DiO, DiI, and DiD. CellBrite® NIR Dyes are CellBrite® Dyes with near-infrared (NIR) fluorescence compatible with small animal NIR imaging systems. While suitable for membrane staining, these dyes have issues with internalization and staining consistency between experiments. We recommend using Biotium's other membrane stains that were developed for bright and consistent results for a variety of workflows. 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.
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. For long-term cell surface staining of live cells that will not be fixed, we recommend our CellBrite® Steady Membrane Stains (see Related Products).
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.
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
30088-30090	CellBrite® Fix Membrane Stains
30131-30135	CytoLiner™ Fixed Cell 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
29060...29137	CF® Dye PNA Lectin (*Arachis hypogaea*)
29096...29132	*Lycopersicon Esculentum* (Tomato) Lectin (LEL, TL) Conjugates
29108...29133	*Ulex Europaeus* Agglutinin (UEA I) Conjugates
29114...29134	*Phaseolus Vulgaris* Leucoagglutinin (PHA-L) Conjugates
29096...29131	*Datura Stramonium* Lectin (DSL) Conjugates
29120...29135	*Sambucus Nigra* Lectin (SNA, EBL) Conjugates
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.