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Product Information

MemBrite® Fix Cell Surface Staining Kits

Catalog Number: See Table 1

Kit Contents

Component	Trial size kit 100 labeling reactions*	Regular size kit 500 labeling reactions*
MemBrite® Fix Stain	Component A 1 vial**	Component A 5 vials**
MemBrite® Fix Pre-Staining Solution, 1000X	99847-20uL 20 uL	99847-100uL 100 uL
Anhydrous DMSO	99953 150 uL	99953 150 uL

*Kit sizes are based on 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 desiccated at -20°C and protect from light. Anhydrous DMSO can be stored desiccated at room temperature, 4°C, or -20°C. These products are stable for at least 12 months from date of receipt when stored as recommended. After reconstitution in anhydrous DMSO, the stain solution can be stored for at least one month at -20°C, protected from light and moisture.

Spectral Properties

MemBrite® Fix stains are named for their absorbance/emission maxima (Table 1). See Figures 1-2 (page 3) for dye spectra.

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 also can be used to stain yeast and gram-positive bacteria, but not gram-negative bacteria.

MemBrite® Fix Staining Kits 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.

Table 1. MemBrite® Fix Stains

Cat. No.	Dye Ex/Em (nm)	Specialized Applications
30092-T, 30092	MemBrite® Fix 405/430	SIM, exosome staining
30093-T, 30093	MemBrite® Fix 488/515	STED, TIRF, 2-photon microscopy
30094-T, 30094	MemBrite® Fix 543/560	N/A
30095-T, 30095	MemBrite® Fix 568/580	STORM, SIM, TIRF
30096-T, 30096	MemBrite® Fix 594/615	2-photon microscopy
30097-T, 30097	MemBrite® Fix 640/660	FLImP, SIM, TIRF
30098-T, 30098	MemBrite® Fix 660/680	N/A
30099-T, 30099	MemBrite® Fix 680/700	STORM†, Single-molecule imaging, STED, 2-photon microscopy
30101-T, 30101	MemBrite® Fix-ST 650/665	STORM
30102-T, 30102	MemBrite® Fix-ST 667/685	STORM
30103-T, 30103	MemBrite® Fix-ST 681/698	Single-molecule imaging, STORM†
30104-T, 30104	MemBrite® Fix-ST 755/777	STORM

FLImP: fluorophore localization imaging with photobleaching; SIM: structured illumination microscopy; STED: stimulated emission depletion; TORM: 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.

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. They will stain intracellular structures in fixed cells.
- MemBrite® Fix stains react with proteins and amino acids. Therefore, staining must be done in protein- and amine-free buffer such as PBS or HBSS. For adherent cells, we typically use HBSS with calcium/magnesium 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 below.
- MemBrite® Fix stains react irreversibly with cellular proteins. In live cells, this occurs on the cell surface, because the stains can't penetrate the membrane. But the stains do get inside dead cells, where there are many more targets for reaction. As a consequence, this product stains dead cells much more brightly than live cells. See tips for imaging below.
- MemBrite® Fix stains are designed to be fixed shortly after staining when they primarily localize to the plasma membrane/cell surface. Cells can be returned to growth medium and cultured after staining as well; however, stain 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 stain is 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).
- 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.
- MemBrite® Fix 405/430 has been validated for staining of isolated exosomes for analysis by flow cytometry. Treatment with Pre-Staining Solution is not required for exosome staining. For optimized membrane staining of exosomes and EVs we recommend ExoBrite™ EV Membrane Staining Kits (see Related Products).
- MemBrite® Fix stains can be used to stain yeast or gram-positive (but not gram-negative) bacteria. Stain concentration, staining temperature, and 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 stain concentration used for labeling should be optimized to use the lowest effective concentration. We also offer CellBrite® Fix (see Related Products), which are covalent cell surface stains that react with proteins by a different chemistry than MemBrite® Fix. In cases where MemBrite® Fix staining interferes with subsequent immunostaining for a particular epitope, CellBrite® Fix may be a suitable alternative.
- 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 stains for fixed cells, stains for long-term membrane staining in live cells, and membrane stains for super-resolution imaging.

Tips for Imaging MemBrite® Fix Staining

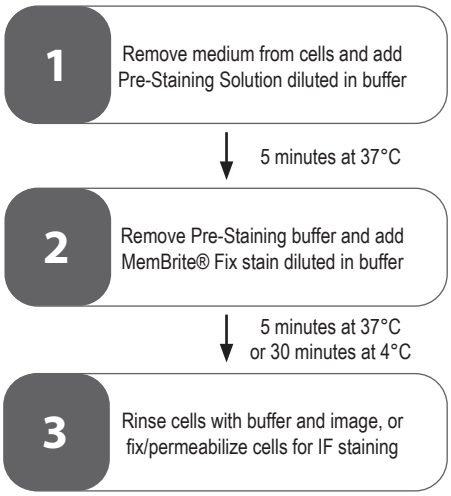
Confocal vs. epifluorescence microscopy

If you have access to a confocal microscope, we recommend using it to image membrane staining for the best results. 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 surface to avoid this background and image the cell outlines. Membrane stains can be imaged with a regular epifluorescence microscope, but the images will be more diffuse because fluorescence from membranes above and below the cell borders will be captured.

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 dead cell signal will likely be saturated under these settings. 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.

Protocol Overview



See detailed staining protocols below.

Staining Protocols

Stain reconstitution

Remove one vial of staining solution and the anhydrous DMSO from the freezer and bring to room temperature. To make 1000X stain stock solution, add 20 uL of anhydrous DMSO to the vial and mix well. Unused stain stock solution can be aliquoted and stored desiccated at -20°C for at least 1 month.

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 but not required.
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 MemBrite® Fix stain 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 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.

   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. Staining also 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. Permeabilization also can be performed at 4°C.
9. After fixation/permeabilization, you can perform immunofluorescence staining according to your preferred protocol.

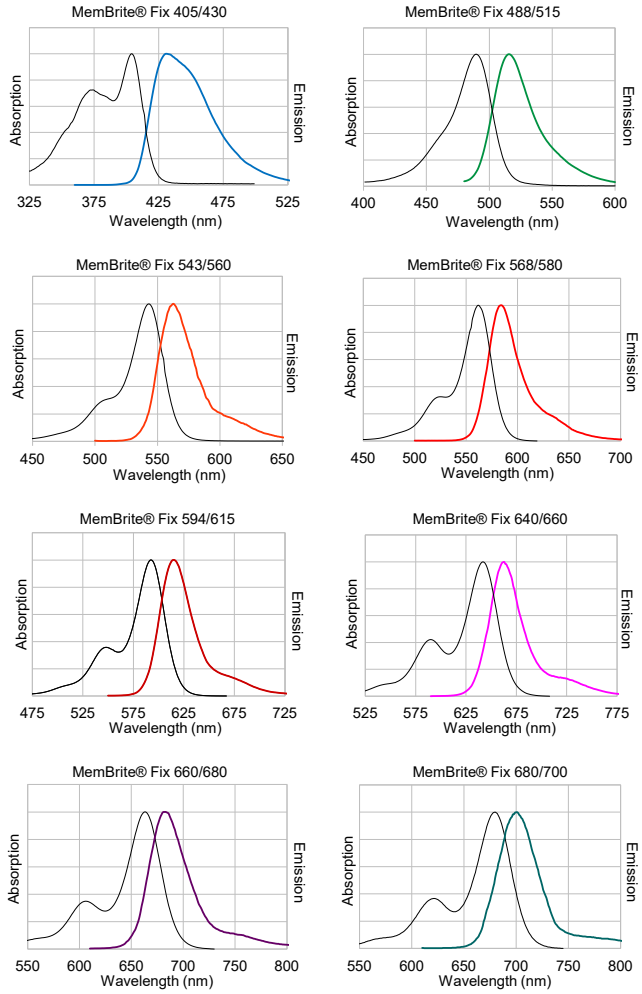


Figure 1. MemBrite® Fix stains absorbance and emission spectra.

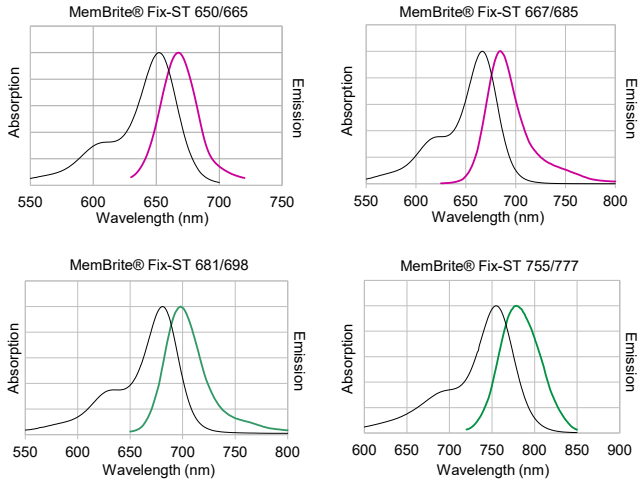


Figure 2. MemBrite® Fix-ST stains absorbance and emission spectra.

Related Products

Cat. No.	Product
30021-30023	CellBrite® Cytoplasmic Membrane Stains
30070, 30077-30079	CellBrite® NIR Cytoplasmic Membrane Stains
30088-30090	CellBrite® Fix Membrane Stains
30105-30109	CellBrite® Steady Membrane Staining Kits
30111-30114	ExoBrite™ EV Membrane Staining Kits
40083... 41038	NucSpot® Nuclear Stains
40081-40082	NucSpot® Live Cell Nuclear Stains
40060	RedDot™1 Far-Red Nuclear Stain
40061	RedDot™2 Far-Red Nuclear Stain
30068	ViaFluor® 405 SE Cell Proliferation Kit
30086	ViaFluor® 488 SE Cell Proliferation Kit
70065	LipidSpot™ 488 Lipid Droplet Stain
70069	LipidSpot™ 610 Lipid Droplet Stain
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative
23001	EverBrite™ Mounting Medium
23002	EverBrite™ Mounting Medium with DAPI
23008	Drop-n-Stain EverBrite™ Mounting Medium without DAPI
23009	Drop-n-Stain EverBrite™ Mounting Medium with DAPI
23003	EverBrite™ Hardset Mounting Medium
23004	EverBrite™ Hardset Mounting Medium with DAPI
23016	EverBrite™ Hardset Mounting Medium with NucSpot® 640
23017	EverBrite TrueBlack® Hardset Mounting Medium
23018	EverBrite TrueBlack® Hardset Mounting Medium with DAPI
23019	EverBrite TrueBlack® Hardset Mounting Medium with NucSpot® 640

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