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

Storage and Handling

Store at -20°C, desiccate, and protect from light. Product is stable for at least 12 months from date of receipt when stored as recommended. After reconstitution in anhydrous DMSO, the dye solution can be stored for at least one month at -20°C, protected from light and moisture. Anhydrous DMSO can be stored desiccated at room temperature, 4°C, or -20°C.

Spectral Properties

MemBrite® Fix dyes 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 covalently staining the surface of live cells. Unlike traditional membrane dyes like DiO, DiI, Vybrant® membrane dyes, CellMask™, or PKH dyes, MemBrite® Fix staining can withstand both formaldehyde fixation and detergent permeabilization, or alcohol fixation. 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 dyes react widely with cell surface proteins. MemBrite® Fix staining is rapid and non-toxic to cells, and because MemBrite® Fix dyes are highly water soluble, they stain cells much more evenly than lipophilic membrane dyes. MemBrite® Fix 405/430 has been validated for staining of isolated exosomes for flow cytometry analysis. 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 dyes are available with a wider selection of colors, some of which have been validated in specialized applications such as super resolution imaging. MemBrite® dyes do not associate with lipids in membranes, and consequently have lower cytoplasmic background after detergent permeabilization compared to CellBrite® Fix.

Selecting a MemBrite® Fix Dye

Several MemBrite® Fix dyes have been validated in super-resolution imaging applications or 2-photon microscopy (Table 1). MemBrite® Fix-ST dyes are recommended for super-resolution imaging by STORM.

MemBrite® Fix or MemBrite® Fix-ST dyes can be used for standard microscopy applications; however, MemBrite® Fix dyes are generally more photostable than MemBrite® Fix-ST dyes.

Table 1. MemBrite® Fix Dyes

Catalog number	Dye Abs/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; STORM: Stochastical optical reconstruction microscopy; TIRF: Total internal reflection fluorescence

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

Considerations for Staining with MemBrite® Fix Stains

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.
- 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.
- MemBrite® Fix stains can be used with poly-L-lysine coated surfaces. However, the stains will react with surfaces treated with collagen, gelatin, fibronectin, or other extracellular matrix protein coatings. The dyes tend to have higher 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 on page 2.

- MemBrite® Fix dyes react irreversibly with cellular proteins. In live cells, this occurs on the cell surface, because the dyes can't penetrate the membrane. But 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, below.
- MemBrite® Fix dyes are designed to be fixed shortly after staining, when they primarily localize to the plasma membrane/cell surface. Cells also can be returned to growth medium and cultured after staining, however, 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 dye 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. Dye 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 dye 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 dyes for fixed cells, dyes for long-term membrane staining in live cells, and membrane stains for super-resolution imaging.

Tips for imaging MemBrite® Fix staining

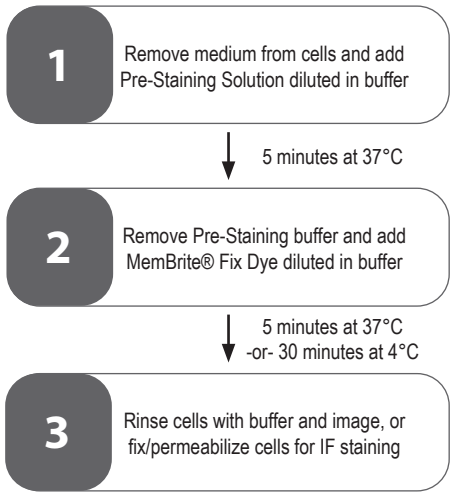
Confocal vs. epifluorescence microscopy

If you have access to a confocal microscope, we recommend using it to image membrane staining for the best results. Confocal imaging screens out fluorescence from above and below the plane of focus, allowing very crisp imaging of cell boundaries. 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 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

Dye reconstitution

Remove one vial of dye and the anhydrous DMSO from the freezer and bring to room temperature. To make 1000X dye stock solution, add 20 uL of anhydrous DMSO to the vial and mix well. Unused dye 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 dye solution by diluting MemBrite® Fix Dye 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.
5. Remove the Pre-Staining Solution from the cells. Add enough dye solution to cover the cells and incubate at 37°C for 5 minutes. Longer staining times can be used, but more dye will be internalized.

   Notes:
   a. A rinse step is not needed after removing the Pre-Staining Solution and before adding dye solution.
   b. Performing dye incubation at 37°C results in strong surface staining, with a small amount of intracellular staining due to dye internalization. Staining also can be performed at 4°C for 30 minutes with pre-chilled staining solution to prevent dye 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 also can be returned to growth medium for continued culture, but staining will be internalized 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 (catalog 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.

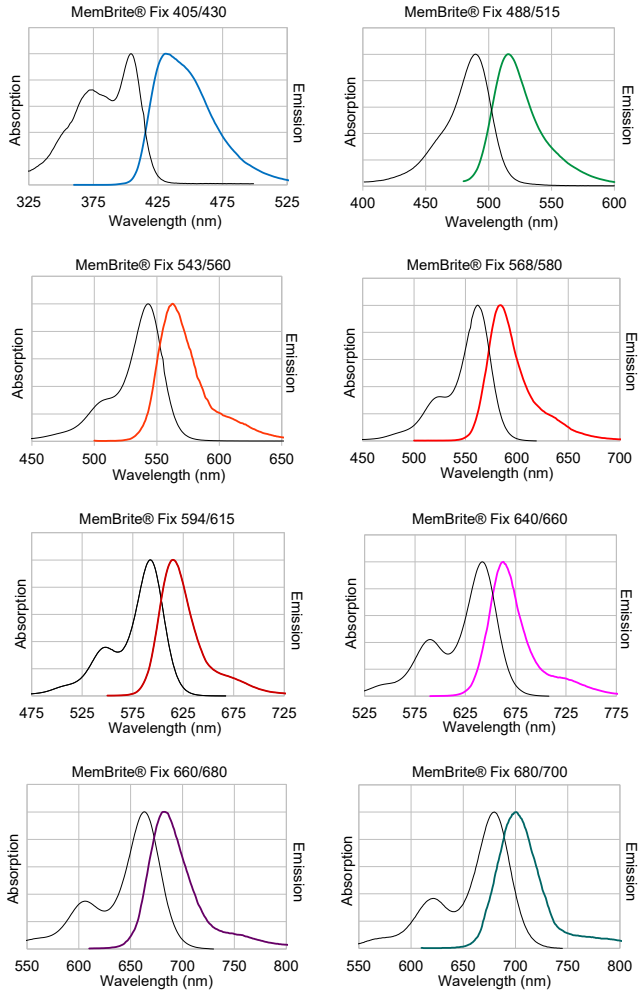


Figure 1. MemBrite® Fix dyes absorbance and emission spectra.

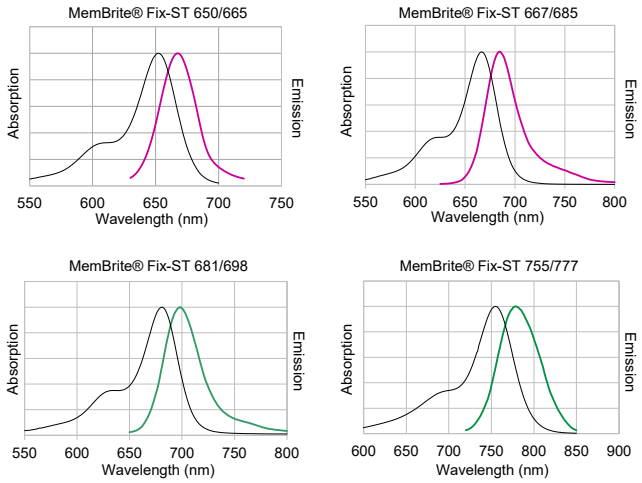


Figure 2. MemBrite® Fix-ST dyes absorbance and emission spectra.

Related Products

Catalog number	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
30112	ExoBrite™ 490/515 EV Membrane Staining Kit
30113	ExoBrite™ 560/585 EV Membrane Staining Kit
40083	NucSpot® 470
40081	NucSpot® Live 488
40082	NucSpot® Live 650
40085	NucSpot® Far-Red
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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