Revised: January 4, 2022

Product Information

CellBrite® Fix Membrane Stains

Kit Contents

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

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

Storage and Handling

Store at -20°C, desiccated and protected 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 up to 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

Cat. No.	Product	Ex/Em* (nm)
30090-T, 30090	CellBrite® Fix 488	480/513
30088-T, 30088	CellBrite® Fix 555	542/571
30089-T, 30089	CellBrite® Fix 640	638/667

*See Figure 1.

Product Description

CellBrite® Fix Membrane Stains are a new class of membrane dyes that can be used to stain the cell surface in live cells. PKH dyes or membrane dyes like DiO, DiI, Vybrant®, and CellMask™ can be fixed with formaldehyde. But they are not compatible with detergent permeabilization or methanol fixation, as these treatments extract 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 stains are general membrane stains.

CellBrite® Fix 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. They also can be used to stain yeast and bacteria (gram-positive or gram-negative).

Considerations for Staining with CellBrite® Fix Stains

The following are general considerations for using CellBrite® Fix 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.
- CellBrite® Fix stains react with proteins and amino acids. Therefore, staining must be done in protein- and amine-free buffers such as PBS or HBSS. For adherent cells, we typically use HBSS with calcium/magnesium to maintain cell adhesion and morphology.
- CellBrite® Fix stains will react 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 below.
- CellBrite® Fix stains react irreversibly with cellular proteins. In live cells, this occurs on the cell surface because the dyes can't penetrate the membrane. But they 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 on page 2.
- 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 stains 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 CellBrite® Fix 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.

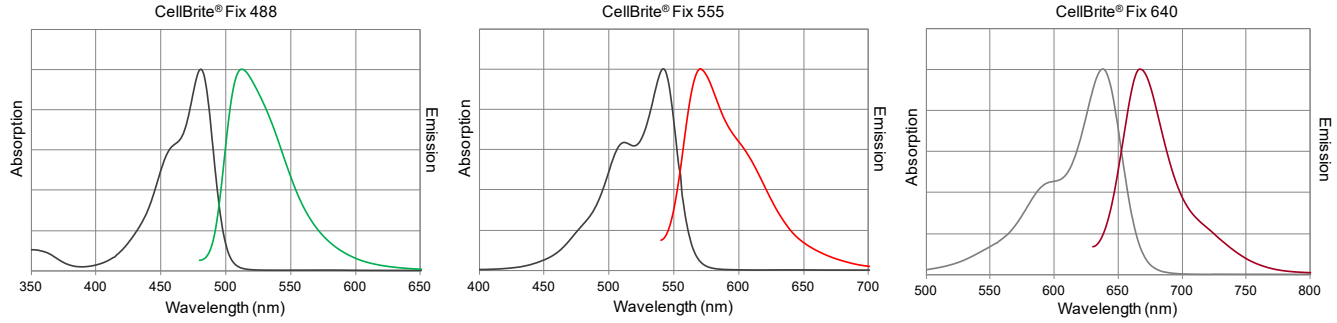

Figure 1. Normalized absorption and emission spectra of CellBrite® Fix dyes in water.

Tips for imaging CellBrite® 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. 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. 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

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 vortex or pipet up and down to ensure that all of the dye has dissolved. Once dissolved, the dye should be used within a few hours. Unused dye stock solution can be aliquoted and stored desiccated at -20°C for at least 1 month.

Mammalian cell staining

1. Wash cells with protein- and amine-free buffer such as PBS or HBSS.
2. Prepare staining solution by diluting CellBrite® Fix Membrane Stain 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.

   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. Staining also can be performed at room temperature or 4°C to inhibit dye internalization, but incubation time may need to be increased to allow the dye to react with the cell surface.

   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: Cells also 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 1X PBS (Cat. No. 22023) for 20 minutes at room temperature or 4°C. Cells also can be fixed with pre-chilled methanol for 5-10 minutes at -20°C. Methanol fixation may result in an increase in intracellular fluorescence.
6. To permeabilize cells, 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. Permeabilization may result in an increase in intracellular fluorescence.

Staining of bacteria and yeast

CellBrite® Fix stains can be used to stain yeast or bacteria (gram-positive and gram-negative), but a higher dye concentration may be needed. We recommend following the same general protocol above, 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.

Related Products

Cat. No.	Product
30070, 30077-30079	CellBrite® NIR Cytoplasmic Membrane Stains
30105-30109	CellBrite® Steady Membrane Staining Kits
30021-30023	CellBrite® Cytoplasmic Membrane Stains
30092-30099	MemBrite® Fix Cell Surface Staining Kits
30101-30104	MemBrite® Fix-ST Cell Surface Staining Kits for STORM
41033... 40085	NucSpot® Nuclear Stains for dead or fixed cells
40081	NucSpot® Live 488 Nuclear Stain for live or fixed cells
40082	NucSpot® Live 650 Nuclear Stain for live or fixed cells
40060	RedDot™1 Far-Red Nuclear Stain for live cells
40061	RedDot™2 Far-Red Nuclear Stain for dead or fixed cells
40046	Hoechst 33342, 10 mg/mL in water
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
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
40011... 40043	DAPI
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative

Please visit our website at www.biotium.com for information on our life science research products, including fluorescent CF® Dye antibody conjugates, Mix-n-Stain™ Antibody Labeling Kits, apoptosis reagents, and other probes and kits for cell biology research.