

Product Information

CellBrite™ Fix Membrane Stains

Kit Contents

Component	Trial size (1 vial*)	Set of 5 vials*
CellBrite™ Fix Membrane Dye	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, 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 up to one month at -20°C, protected from light and moisture. Anhydrous DMSO can be stored at 4°C or -20°C.

Spectral Properties

Catalog number	Size	Name	Abs/Em*
30090-T	Trial size (1 vial)	CellBrite™ Fix 488	480/513 nm
30090	Set of 5 vials		
30088-T	Trial size (1 vial)	CellBrite™ Fix 555	542/571 nm
30088	Set of 5 vials		
30089-T	Trial size (1 vial)	CellBrite™ Fix 640	638/667 nm
30089	Set of 5 vials		

*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. Membrane dyes like DiO, Dil, Vybrant® membrane dyes, CellMask™, or PKH dyes can be fixed with formaldehyde. But they are not compatible with detergent permeabilization or methanol fixation, because 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. Unlike lectins such as WGA, which bind specific targets that may vary between cell types, CellBrite™ Fix dyes are general membrane stains.

CellBrite™ Fix Membrane Stains are fluorogenic dyes that rapidly accumulate in the plasma membrane, where they react covalently with the cell surface. 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. CellBrite™ Fix dyes 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 Dil. CellBrite™ Fix dyes are non-toxic and do not readily transfer between cells. They also can be used to stain yeast and bacteria (gram-positive or gram-negative).

CellBrite™ Fix 488 has green fluorescence, CellBrite™ Fix 555 has visible red fluorescence, and CellBrite™ Fix 640 has far-red fluorescence (see Figure 1).

Assay 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 PBS, HBSS, or other buffer. For adherent cells, we usually use HBSS with calcium and magnesium to maintain cellular adhesion and morphology.

Note: do not use buffer or medium containing Tris, amino acids, serum, or proteins such as BSA, because these will interfere with labeling.

Note: For surface membrane staining, live cells must be stained before fixation. Staining cells after fixation results in intracellular staining with minimal surface staining. Dead cells also will show bright intracellular staining.

2. Prepare staining solution by diluting CellBrite™ Fix Membrane 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 and surface selectivity.

3. Add staining solution to cells and incubate at 37°C for 15 minutes.

Note: 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 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.

Note: 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 without washing.
5. To fix cells, wash twice with buffer and fix according to your usual protocol. We usually fix with 4% paraformaldehyde in 1X PBS 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 dyes 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.

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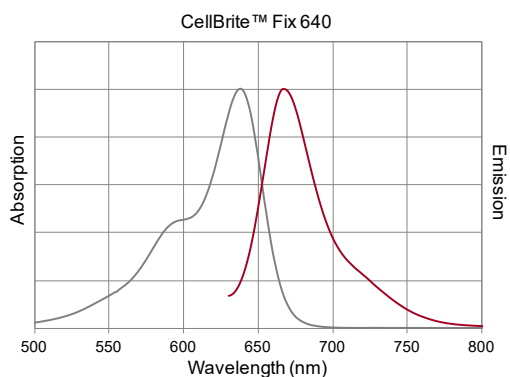
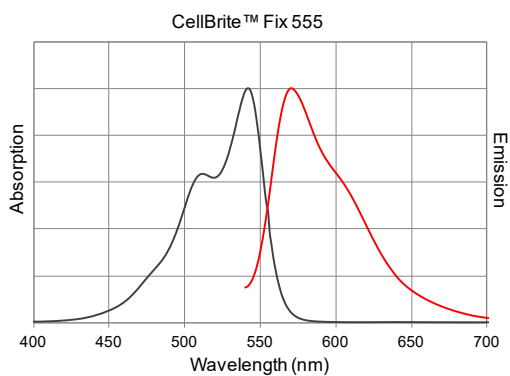
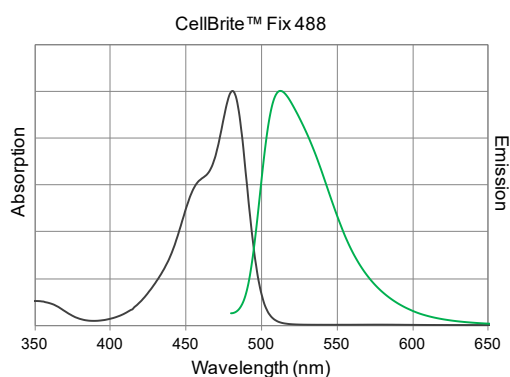


Figure 1. Normalized absorption and emission spectra of CellBrite™ Fix dyes in water.

Related Products

Catalog number	Product
70064	ViaFluor™ 405 Live Cell Microtubule Stain
70062	ViaFluor™ 488 Live Cell Microtubule Stain
70063	ViaFluor™ 647 Live Cell Microtubule Stain
70066	LysoView™ 405 Lysosome Stain
70067	LysoView™ 488 Lysosome Stain
70061	LysoView™ 540 Lysosome Stain
70058	LysoView™ 633 Lysosome Stain
70059	LysoView™ 650 Lysosome Stain
70070	MitoView™ 405 Mitochondrial Stain
70054	MitoView™ Green Mitochondrial Stain
70055	MitoView™ 633 Mitochondrial Stain
70068	MitoView™ 720 Mitochondrial Stain
40083	NucSpot™ 470 Nuclear Stain 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
30024	CellBrite™ Blue Cytoplasmic Membrane Staining Kit
30021	CellBrite™ Green Cytoplasmic Membrane Stain
30022	CellBrite™ Orange Cytoplasmic Membrane Stain
30023	CellBrite™ Red Cytoplasmic Membrane Stain
30070	CellBrite™ NIR 680 Cytoplasmic Membrane Stain
30077	CellBrite™ NIR 750 Cytoplasmic Membrane Stain
30078	CellBrite™ NIR 770 Cytoplasmic Membrane Stain
30079	CellBrite™ NIR 790 Cytoplasmic Membrane Stain
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative
22020	10X Phosphate-Buffered Saline (PBS)
90082	Anhydrous DMSO

Please visit our website at www.biotium.com for information on our life science research products including fluorescent CF™ dye labeled primary and secondary antibodies, lectins, toxins, and other probes and kits for live cell imaging and real-time apoptosis detection.

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