Revised: November 3, 2021

Product Information

CellBrite® Steady Membrane Labeling Kits

Catalog Number: See Table 1

Kit Contents

Component	100 labelings*	500 labelings*
CellBrite® Steady Membrane Dye 1000X in DMSO	Component A 20 uL	Component A 100 uL
CellBrite® Steady Enhancer 1000X in DMSO	99855-20 uL 20 uL	99855-100 uL 100 uL

*Kit sizes are based on 200 uL labeling volume, actual number of labelings may vary based on culture chamber size/staining volume.

Storage and Handling

Store at 4°C. Protect dye component from light. Product may also be stored at room temperature, protected from light, without affecting performance. Products are stable for at least 12 months from date of receipt when stored as recommended.

No information is available concerning potential hazards of CellBrite® Steady Membrane Dyes or CellBrite® Steady Enhancer. Exercise universal laboratory safety precautions during handling, and dispose as hazardous chemical waste.

Spectral Properties

See Table 1 and Figure 1.

Product Description

CellBrite® Steady Membrane Dyes are unique fluorescent probes for imaging cell surface membranes of living cells for up to several days in culture. Unlike other membrane dyes and cell surface stains that are rapidly lost from the cell surface by endocytosis, CellBrite® Steady dyes equilibrate between intracellular compartments and the plasma membrane. Cells retain both surface and intracellular staining over time in culture. Intracellular staining can be reduced or eliminated with the use of CellBrite® Steady Enhancer, enabling selective imaging of cell outlines and boundaries over the course of several hours to days.

The dyes can be used to stain live cell membranes in complete culture medium with much more even and uniform staining of cells than traditional lipophilic carbocyanine dyes like DiI and DiO. The dyes are non-toxic and do not covalently modify amino acids on cell surface proteins, reducing the potential for masking of antibody binding sites or other biological effects. Washing after staining is optional for confocal imaging of CellBrite® Steady staining, but washing is required for epifluorescence imaging.

CellBrite® Steady Enhancer is an optional reagent that can be used to mask intracellular fluorescence of CellBrite® Steady Membrane Dyes, for more selective visualization of cell boundaries. Enhancer may be incubated together with membrane dyes or added after staining to minimize any potential effect of Enhancer on cells. See Considerations for Using CellBrite® Steady Enhancer and Table 2: Overview of Staining Protocols for more information.

CellBrite® Steady dyes are available in colors from blue to near-infrared. CellBrite® Steady 550, 650, and 685 fluorophores are compatible with super-resolution imaging by STORM.

Considerations for Staining with CellBrite® Steady Dyes

- CellBrite® Steady Membrane Dyes are intended for use as live cell stains. In cells that are fixed before staining, the dyes only stain intracellular structures.
- Dye concentration may require optimization for different cell types and imaging systems.
- Staining can be performed in complete cell culture medium with serum. For short-term experiments, staining cells in buffer such as HBSS or PBS may give higher signal. For adherent cells, we use HBSS with Ca^{++}/Mg^{++} to maintain cell attachment and morphology. Staining in buffer is not recommended for incubation times longer than 30 minutes.
- After 30 minutes of incubation, the dye will localize to the cell surface. Over time, dye will equilibrate between the cell surface (plasma membrane) and intracellular organelles (endosomes and lysosomes). Dye is retained on the cell surface for 48 hours or longer, but intracellular staining becomes more prominent with longer incubation times.
- CellBrite® Steady Membrane Dyes have low cytotoxicity in immortalized cell lines, and can be incubated continuously with cells in culture. Alternatively, the dye solution can be removed and cells returned to fresh culture medium. Plasma membrane staining will be retained after dye is removed, but signal will become lower over time compared to staining by continuous dye incubation.
- Washing after staining is optional for imaging by confocal microscopy, but is required for imaging by epifluorescence. For dyes with fluorescence in the visible range (405, 488, and 550), stained cells may or may not be visible through the microscope eyepieces without washing, so you may need to use brightfield or another marker to focus on cells initially for confocal imaging.
- The dyes can be incubated with other live cells stains such as NucSpot® Live Nuclear Stains or MitoView™ Mitochondrial Dyes (see Related Products). We recommend first staining cells with each dye separately to optimize staining.
- CellBrite® Steady Membrane Dyes transfer readily between cells and are not recommended for labeling cells for transplantation or co-culture experiments.
- CellBrite® Steady staining is retained immediately after fixation with formaldehyde, but the dyes redistribute over time after fixation to stain intracellular structures. If cell must be fixed before imaging, we recommend imaging as soon as possible after fixation. Staining does not tolerate fixation with solvents such as methanol, permeabilization with detergent, or antifade mounting media with glycerol. For fixable cell surface staining, we recommend our CellBrite® Fix Membrane Stains and MemBrite® Fix Cell Surface Stains (see Related Products).

Considerations for Using CellBrite® Steady Enhancer

- CellBrite® Steady Enhancer is an optional accessory reagent that can be used to mask intracellular fluorescence of CellBrite® Steady Membrane Dyes, for clearer imaging of cell boundaries at time points beyond 30 minutes.
- For long-term staining experiments, Enhancer can be co-incubated with dyes. Alternatively, cells can be treated with Enhancer at the end of the experiment to minimize toxicity. For more information, see Table 2: Overview of Staining Protocols.
- For CellBrite® Steady 405, 488, and 550, Enhancer is most effective when used as a post-staining treatment. For CellBrite® Steady 650 and 685, Enhancer is equally effective with post-staining or co-incubation protocols and can mask intracellular signal for 24 hours or longer, depending on the cell type.
- Enhancer may be toxic to cells, especially at higher concentrations. A titration should be performed to find the lowest effective concentration of Enhancer. We recommend testing concentrations from 0.2X to 1X for co-incubation with dye.
- Enhancer may mask fluorescence from endosome tracers or lysosome stains.
- Enhancer does not tolerate fixation. If Enhancer-treated cells are fixed after staining, masking will be lost and intracellular fluorescence will return.

CellBrite® Steady Membrane Dye Staining Protocols

Note: Before beginning, please read Considerations for Staining with CellBrite® Steady dyes (page 1), and see Table 2: Overview of Staining Protocols.

Method 1: Staining by Medium Exchange

1. Dilute CellBrite® Steady dye to 1X in cell culture medium or buffer and vortex to mix. For example, add 1 uL dye to 1 mL of medium or buffer.

   Note: We recommend staining in medium for incubation times longer than 30 minutes.

2. Remove medium from cells and add medium or buffer containing dye.
3. Incubate cells at 37°C for 30 minutes or longer, up to several days depending on the cell type.

   Note: Staining solution can be removed after 30 minutes and replaced with fresh medium for continued cell culture. While the dye localization will be similar to continuous incubation with dye, the signal will be lower over time.

   Note: Enhancer is recommended when staining cells for several hours or days (see CellBrite® Steady Enhancer Protocols).

4. Washing before imaging is optional for confocal imaging, but is required for epifluorescence microscopy. To wash, remove dye solution, rinse twice with fresh medium, and replace with fresh medium.
5. Image fluorescence.

Method 2: Staining by Direct Addition of 10X Dye
This method can be more convenient and less disruptive to cells than medium exchange, with similar staining results.

Note: Do not add undiluted 1000X dye directly to cells because this will result in localized high dye concentration that could cause uneven staining or toxicity.

1. Dilute the dye to 10X concentration in cell culture medium. For example, make 10X dye by adding 1 uL dye to 100 uL medium.
2. Add 1/10 volume of 10X dye to the culture medium already on the cells and pipette gently up and down to mix. For example, add 20 uL of 10X dye to cells in 200 uL culture medium. Scale volumes as needed for your culture vessel.
3. Incubate cells at 37°C for 30 minutes or longer, up to several days depending on the cell type.

   Note: Staining solution can be removed after 30 minutes and replaced with fresh medium for continued cell culture. While the dye localization will be similar to continuous incubation with dye, the signal will be lower over time.

   Note: Enhancer is recommended when staining cells for several hours or days (see CellBrite® Steady Enhancer Protocols).

4. Washing before imaging is optional for confocal imaging, but is required for epifluorescence microscopy. To wash, remove dye solution, rinse twice with fresh medium, and replace with fresh medium.
5. Image fluorescence.

CellBrite® Steady Enhancer Protocols

Note: Before beginning, please read Considerations for Using CellBrite® Steady Enhancer (page 1), and see Table 2: Overview of Staining Protocols.

Note: Do not add undiluted 1000X Enhancer directly to cells because this will result in localized high Enhancer concentration that could cause uneven staining or toxicity.

Co-Incubation with Dye & Enhancer

1. Prepare dye staining solution as described for Method 1 or Method 2.
2. Dilute Enhancer into dye solution:
   For Method 1: Dilute Enhancer into dye solution at 1X concentration. For example, add 1 uL Enhancer to 1 mL of 1X dye solution.
   For Method 2: Dilute Enhancer into dye solution at 10X concentration. For example, add 1 uL Enhancer to 100 uL of 10X dye solution.
3. Add dye solution with Enhancer to cells and stain as described for Method 1 or Method 2.

Post-Staining Treatment with Enhancer

1. Stain cells with CellBrite® Steady dye according to Method 1 or Method 2.
2. Dilute CellBrite® Steady Enhancer to 10X concentration in culture medium. For example, add 1 uL of Enhancer to 100 uL medium.
3. Add 1/10 volume of 10X Enhancer to the culture medium already on the cells and pipette gently up and down to mix. For example, add 20 uL of 10X Enhancer to cells in 200 uL culture medium. Scale volumes as needed for your culture vessel.
4. Incubate cells at 37°C for 30 minutes.
5. Washing before imaging is optional for confocal imaging, but is required for epifluorescence microscopy. To wash, remove dye solution, rinse twice with fresh medium, and replace with fresh medium.
6. Image fluorescence.

Table 1. CellBrite® Steady Membrane Labeling Kits

Catalog no.	Size	Product Name	Dye Ex/Em*
30105-T	100 labeling reactions	CellBrite® Steady 405 Membrane Labeling Kit	406/428 nm
30105	500 labeling reactions		
30106-T	100 labeling reactions	CellBrite® Steady 488 Membrane Labeling Kit	505/529 nm
30106	500 labeling reactions		
30107-T	100 labeling reactions	CellBrite® Steady 550 Membrane Labeling Kit	562/579 nm
30107	500 labeling reactions		
30108-T	100 labeling reactions	CellBrite® Steady 650 Membrane Labeling Kit	656/676 nm
30108	500 labeling reactions		
30109-T	100 labeling reactions	CellBrite® Steady 685 Membrane Labeling Kit	686/708 nm
30109	500 labeling reactions		

* In MeOH; see Figure 1.

Table 2. Overview of Staining Protocols

Protocol	Dye Staining Alone	Co-Incubation with Dye & Enhancer	Post-Staining Treatment with Enhancer After Dye
No-wash staining for confocal microscopy	1. Stain with dye 2. Image	1. Stain with dye + Enhancer 2. Image	1. Stain with dye 2. Add Enhancer and incubate 30 min. 3. Image
Wash after staining for epifluorescence microscopy	1. Stain with dye 2. Rinse cells 3. Image	1. Stain with dye + Enhancer 2. Rinse cells 3. Image	1. Stain with dye 2. Add Enhancer and incubate 30 min. 3. Rinse cells 4. Image
Recommended for	• Short term imaging (30 minutes) • Long-term imaging together with lysosomal stains or endocytic tracers	• Long term imaging (hours to days) • Most effective with CellBrite® Steady 650 & 685	• Long-term imaging (hours to days) of sensitive cell lines (reduces potential for toxicity) • More effective than co-incubation for CellBrite® 405, 488, or 500

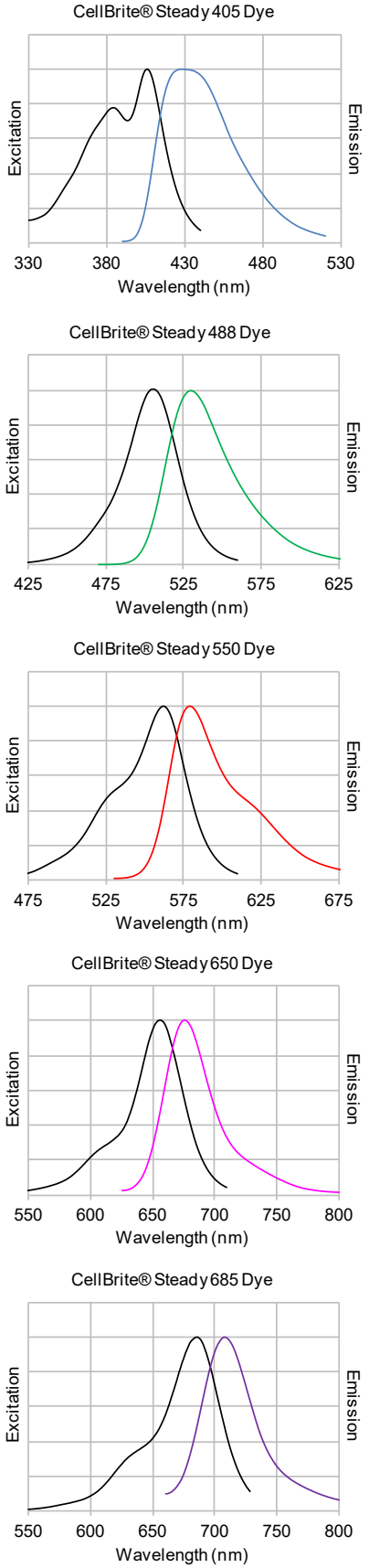

Figure 1. Excitation/emission spectra of CellBrite® Steady dyes in methanol.

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
70070	MitoView™ 405 Mitochondrial Stain
70054	MitoView™ Green Mitochondrial Stain
70055	MitoView™ 633 Mitochondrial Stain
70068	MitoView™ 720 Mitochondrial Stain
70075	MitoView™ 650
70082	MitoView™ Fix 640
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
70058	LysoView™ 633, 10 vials
70059... 70086	LysoView™ Lysosome Stains, 1000X in DMSO
70060	“Light-on” LysoView™ 555
30021-30024	CellBrite® Cytoplasmic Membrane Dyes
30070, 30077-30079	CellBrite® NIR Cytoplasmic Membrane Stain
70065	LipidSpot™ 488 Lipid Droplet Stain
70069	LipidSpot™ 610 Lipid Droplet Stain
30088-30090	CellBrite® Fix Membrane Stains
30092-30104	MemBrite® Fix Cell Surface Staining Kits
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative

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