MemBrite™ Fix Cell Surface Staining Kits

Catalog Number: See Table 1

Kit Contents

<table>
<thead>
<tr>
<th>Component</th>
<th>Trial size kit</th>
<th>Regular size kit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100 labeling reactions*</td>
<td>500 labeling reactions*</td>
</tr>
<tr>
<td>MemBrite™ Fix Dye Component A</td>
<td>1 vial**</td>
<td>Component A 5 vials**</td>
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<tr>
<td>MemBrite™ Fix Pre-Staining Solution, 1000X</td>
<td>99847-20uL</td>
<td>99847-100uL</td>
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<tr>
<td>MemBrite™ Fix Anhydrous DMSO</td>
<td>99953</td>
<td>150 uL</td>
</tr>
<tr>
<td>MemBrite™ Fix-ST</td>
<td>99953</td>
<td>150 uL</td>
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</tbody>
</table>

*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 at 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 staining 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™ Fix dyes do not associate with lipids in membranes, and consequently have lower cytoplasmic background after detergent permeabilization compared to CellBrite™ Fix.

Cova lent 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. CellBrite™ Fix and MemBrite™ Fix react with proteins differently, so in cases where CellBrite™ Fix interferes with subsequent immunostaining, MemBrite™ Fix may be a suitable alternative.

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

<table>
<thead>
<tr>
<th>Catalog number</th>
<th>Size</th>
<th>Dye Abs/Em (nm)</th>
<th>Specialized Applications</th>
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</thead>
<tbody>
<tr>
<td>30092-T</td>
<td>100 reactions</td>
<td>MemBrite™ Fix 405/430</td>
<td>SIM</td>
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<tr>
<td>30092</td>
<td>500 reactions</td>
<td>MemBrite™ Fix 480/515</td>
<td>STED, TIRF, 2-photon microscopy</td>
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<tr>
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<td>100 reactions</td>
<td>MemBrite™ Fix 543/560</td>
<td>N/A</td>
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<td>30093</td>
<td>500 reactions</td>
<td>MemBrite™ Fix 568/580</td>
<td>STORM, SIM, TIRF</td>
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<tr>
<td>30095</td>
<td>500 reactions</td>
<td>MemBrite™ Fix 594/615</td>
<td>2-photon microscopy</td>
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<tr>
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<td>100 reactions</td>
<td>MemBrite™ Fix 600/610</td>
<td>FLImP, SIM, TIRF</td>
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<td>MemBrite™ Fix 640/660</td>
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<td>100 reactions</td>
<td>MemBrite™ Fix 660/680</td>
<td>N/A</td>
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<td>MemBrite™ Fix 680/700</td>
<td>STORM, Single-molecule imaging, STED, 2-photon microscopy</td>
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<td>MemBrite™ Fix-ST 650/665</td>
<td>STORM</td>
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<td>MemBrite™ Fix-ST 667/685</td>
<td>STORM</td>
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</table>

FLImP: Fluorophore localization imaging with photobleaching; SIM: Structured illumination microscopy; STED: Stimulated emission depletion; STORM: Stochastic 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.
## Protocol Overview

1. Remove medium from cells and add Pre-Staining Solution diluted in buffer
   - 5 minutes at 37°C

2. Remove Pre-Staining buffer and add MemBrite™ Fix Dye diluted in buffer
   - 5 minutes at 37°C
   - or 30 minutes at 4°C

3. Rinse cells with buffer and image, or fix/permeabilize cells for IF staining

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

For cell surface staining, live cells must be stained before fixation. Staining cells after fixation will result in intracellular staining. Dead cells also will show bright intracellular staining.

For pre-treatment and staining steps, do not use buffer or medium containing amino acids, serum, or proteins such as BSA, because these will interfere with labeling. PBS, HBSS, or other protein-free buffer may be used. For adherent cells, we generally use HBSS with calcium and magnesium to maintain cell morphology.

1. Dilute the Pre-Staining Solution in buffer 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 before adding the buffer is optional but not required.
3. Incubate the cells in 1X Pre-Staining Solution for 5 minutes at 37°C. Longer incubation times up to 20 minutes will not negatively affect the staining 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.
   - Note: A rinse step is not needed after removing the Pre-Staining Solution and before adding dye solution.
6. Rinse cells twice with buffer or medium. If fixation is not required, cells can be imaged immediately.
   - Note: If labeling was done at 4°C, use pre-chilled buffer for the rinse step.
   - Note: Cells also can be returned to culture after labeling. Dye internalization will occur over time as membrane proteins undergo constitutive endocytosis and turnover. MCF-7 cells cultured after MemBrite™ Fix labeling showed a combination of intracellular vesicle and surface staining up to 48 hours after labeling, with no obvious toxicity for up to one week after labeling.
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.

#### Staining of cells in suspension

Cells can be stained in suspension at 10^6-10^7 cells in 100 uL following the protocol above. Pellet the cells by centrifugation and remove the supernatant in between each change of solution.

#### Staining of bacteria and yeast

MemBrite™ Fix dyes 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.

#### 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.

**Changes in dye localization over time in live cells**

MemBrite™ Fix dyes mainly stain the plasma membrane immediately after staining. However, dye localization in live cells changes over time. If live cells are cultured after staining, the labeled membrane will be internalized, so staining will gradually change from cell surface to intracellular vesicles, usually becoming mostly intracellular after about 24 hours.

Because they react with membrane proteins, staining with MemBrite™ Fix dyes is less stable than original CellBrite™ dyes (which are also internalized after staining, but can be used to track cells for weeks). Internalized MemBrite™ Fix fluorescence is usually detectable for up to 48 hours after staining.

For stable, long-term imaging of cell morphology, our ViaFluor® SE Cell Proliferation dyes (see related products) may be a more suitable alternative than membrane stains. These non-toxic dyes uniformly and covalently label live cells through the cytoplasm. The dyes can be used to trace cells for days to weeks. Because the labeling is covalent, staining doesn’t transfer between cells and can be fixed and permeabilized. Visit www.biotium.com to see our Tech Tip: Using ViaFluor® SE Stains for Cell Tracing and Co-Culture.

#### Staining of dead cells

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 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. 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™ dyes, which do not show such dramatic differences in signal between live and dead cells.
MemBrite™ Fix Cell Surface Staining Kits

PSF006

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

MemBrite™ Fix 405/430

Absorption

325 375 425 475 525

Emission

400 450 500 550 600

Wavelength (nm)

MemBrite™ Fix 488/515

Absorption

400 450 500 550 600 650

Emission

500 550 600 650 700

Wavelength (nm)

MemBrite™ Fix 543/560

Absorption

450 500 550 600 650

Emission

550 600 650 700 750

Wavelength (nm)

MemBrite™ Fix 568/580

Absorption

450 500 550 600 650

Emission

550 600 650 700 750

Wavelength (nm)

MemBrite™ Fix 543/560

Absorption

450 500 550 600 650

Emission

550 600 650 700 750

Wavelength (nm)

MemBrite™ Fix 640/660

Absorption

450 500 550 600 650

Emission

550 600 650 700 750

Wavelength (nm)

MemBrite™ Fix 594/615

Absorption

550 600 650 700 750

Emission

600 650 700 750 800

Wavelength (nm)

MemBrite™ Fix 680/700

Absorption

550 600 650 700 750

Emission

600 650 700 750 800

Wavelength (nm)

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

MemBrite™ Fix-ST 667/685

Absorption

550 600 650 700 750

Emission

600 650 700 750 800

Wavelength (nm)

MemBrite™ Fix-ST 650/665

Absorption

550 600 650 700 750

Emission

600 650 700 750 800

Wavelength (nm)

MemBrite™ Fix-ST 681/698

Absorption

550 600 650 700 750

Emission

600 650 700 750 800

Wavelength (nm)

MemBrite™ Fix-ST 677/685

Absorption

550 600 650 700 750

Emission

600 650 700 750 800

Wavelength (nm)

MemBrite™ Fix-ST 755/777

Absorption

550 600 650 700 750

Emission

600 650 700 750 800

Wavelength (nm)

MemBrite™ Fix-ST 650/665

Absorption

550 600 650 700 750

Emission

600 650 700 750 800

Wavelength (nm)

MemBrite™ Fix-ST 681/698

Absorption

550 600 650 700 750

Emission

600 650 700 750 800

Wavelength (nm)

MemBrite™ Fix-ST 755/777

Absorption

550 600 650 700 750

Emission

600 650 700 750 800

Wavelength (nm)

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

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