Lumiprobe
Life science solutions

Staining Cell Membrane with MemBlaze® Dyes

MemBlaze® is a new generation of lipophilic fluorogenic probes designed for selective staining of plasma membranes in live and fixed cells. In an aqueous environment, they form self-quenching non-fluorescent nanoparticles. Upon incorporation into the lipid bilayer, these aggregates disassemble, allowing the probe to begin fluorescing brightly (the so-called fluorogenic "turn-on" effect).

Due to low membrane permeability, MemBlaze® staining remains localized in the plasma membrane and does not accumulate in the cytoplasm, providing clear and stable visualization of cell morphology.

MemBlaze® dyes provide bright, high-contrast visualization of cell boundaries, with high photostability and intense fluorescence. They are non-toxic to cells at concentrations up to 1 μM. All this makes them ideal markers for long-term live-cell imaging and time-lapse experiments.

Before you begin

- All incubations with MemBlaze® dyes should be carried out in the dark.
- MemBlaze® dyes are highly hydrophobic; therefore, working solutions should preferably be prepared in glass vials. Do not use vials with hydrophobic coating under any circumstances!
- The presence of serum in the medium reduces staining efficiency. Use serum-free media whenever possible. If serum removal is impossible, increase the probe concentration.
- Divalent cations (Ca^{2+}, Mg^{2+}) can promote dye precipitation; therefore, it is recommended to use buffers that do not contain them.
- The working solution diluted in an aqueous medium must be used immediately after preparation.
- The dyes are suitable for staining cells fixed with 4% paraformaldehyde (PFA); however, maximum signal intensity is achieved when staining live cells followed by fixation.
- Methanol, acetone, and other organic solvents extract lipids and therefore reduce membrane staining efficiency.
- PFA partially permeabilizes the membrane; therefore, some probe internalization may be observed when staining fixed cells.
- Long-term incubation of live cells with the probe, or culturing cells after staining, will result in internalization of the dye due to normal cell metabolism.
- For short-term imaging (up to 90 minutes), MemBlaze® 488 and MemBlaze® 560 are preferable. For longer (several hours) imaging, the dyes with additional benzene rings – MemBlaze® 560 and MemBlaze® 640 are preferable because they remain in the membrane longer.

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1. Preparation of stock solutions

20 µM MemBlaze® (for cell cultures):

1. Add 100 µL DMSO to a vial containing **2 nmol** of lyophilized dye.
   Add 500 µL DMSO to a vial containing **10 nmol** of lyophilized dye.
2. Vortex for 1 minute.
3. Excess solution can be aliquoted and stored at -20 °C for up to 3 months. Allow the frozen vial to warm to room temperature before opening. Avoid repeated freeze/thaw cycles.

200 µM MemBlaze® (for tissues or small organisms):

1. Add 10 µL DMSO to a vial containing **2 nmol** of lyophilized dye.
   Add 50 µL DMSO to a vial containing **10 nmol** of lyophilized dye.
2. Vortex for 1 minute.
3. Excess solution can be aliquoted and stored at -20 °C for up to 3 months. Allow the frozen vial to warm to room temperature before opening. Avoid repeated freeze/thaw cycles.

1% Tween® 20

1. Add 5 µL Tween® 20 to 495 µL deionized water.
2. Vortex for 1 minute.

2. Preparation of working solutions

Important! Use only tubes **without** hydrophobic coating or glass vials. Working solutions must be used immediately after preparation.

Below are recommended working solution concentrations for different applications. Exact concentrations depend on the cell/tissue type and experimental design and should be determined empirically.

Sample Type	Epifluorescence microscopy	Confocal microscopy
Live cells	100 nM	20 nM
Fixed cells	100 nM	20 nM
Tissue or small organisms	2 µM	2 µM

Staining buffer

1. The working dye solution is prepared in serum-free medium, HBSS, or PBS containing 0.005% Tween® 20.

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2. To prepare 1 mL of staining buffer, add 5 µL of 1% Tween® 20 to 995 µL of medium, HBSS, or PBS.
3. Vortex.

20 nM working solution

1. To prepare 1 mL of working solution, add 1 µL of 20 µM MemBlaze® to 999 µL of staining buffer.
2. Mix thoroughly and use immediately.

100 nM working solution

1. To prepare 1 mL of working solution, add 5 µL of 20 µM MemBlaze® to 995 µL of staining buffer.
2. Mix thoroughly and use immediately.

2 µM working solution

1. To prepare 1 mL of working solution, add 20 µL of 200 µM MemBlaze® to 980 µL of staining buffer.
2. Mix thoroughly and use immediately.

3. Staining live cells in suspension

1. Centrifuge cells at 1000–1500 rpm for 5 minutes.
2. Remove the supernatant and gently resuspend the cells in pre-warmed (37 °C) staining buffer.
3. Repeat centrifugation and washing twice.
4. Suspend cells at a density of 1×10^6/ mL in **20 nM** working dye solution.
5. Incubate for 10 minutes at room temperature (incubation at 37 °C accelerates probe endocytosis).
6. Cells can be visualized without prior washing.

4. Staining live adherent cells

1. Grow cells on coverslips or in an imaging chamber.
2. Remove the culture medium.
3. Wash cells 3 times with pre-warmed (37 °C) staining buffer.
4. Replace the buffer with **20 nM** working dye solution. Use a volume sufficient to cover the cells completely.
5. Incubate for 10 minutes at room temperature (incubation at 37 °C accelerates probe endocytosis).
6. Cells can be visualized without prior washing.

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5. Fixation of live cells after staining

- Best results are achieved by staining live cells BEFORE fixation.
- Cells stained with MemBlaze® dyes should be fixed with 2–4% PFA.
- Fixation with methanol, acetone, or other organic solvents extracts lipids, leading to poor cell staining.
- Glutaraldehyde is not recommended for cell fixation because even low concentrations (0.5%) can increase autofluorescence and affect the interpretation of subsequent immunocytochemical analysis.

6. Staining fixed cells

1. Fix cells with PFA.
2. Wash cells several times with PBS.
3. Replace the buffer with **100 nM** working dye solution.
4. Incubate for 10 minutes at room temperature in the dark.
5. Wash cells 3 times with PBS.
6. Visualize cells in PBS. Cells can be mounted in mounting media with minor loss (about 10%) of signal intensity.

7. Staining freshly isolated tissue

1. Wash freshly isolated tissue in PBS at 4 °C.
2. Cut into 1 mm thick sections.
3. Place sections in **2–5 µM** MemBlaze® working solution. Incubate for 1 to 24 hours at 4 °C (or 3 hours at room temperature).
4. Wash sections 3 times with PBS at room temperature.
5. Visualize sections in PBS. Sections can be mounted in mounting media with minor loss (about 10%) of signal intensity.

Important! When staining brain tissue with MemBlaze® dyes (especially 560, 640), the signal intensity in neurons is significantly brighter than in glial cells. It should be considered when planning experiments.

8. Combination with immunocytochemistry

1. Perform cell staining with MemBlaze® dye.
2. Fix cells with 4% PFA.
3. Permeabilize with 0.1% Triton® X-100 in PBS for 5 minutes at room temperature. This method better preserves plasma membrane staining than digitonin or saponin permeabilization. Using higher concentrations of Triton® X-100 is not recommended.

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4. Perform standard immunocytochemical protocol.
5. If necessary, mount cells using a mounting medium.

9. Detection

Microscopy:

Specimens stained with MemBlaze® 488, 560, and 640 can be detected using a fluorescence microscope with standard filter sets (FITC, TRITC, Cy5, respectively). For confocal and two-photon microscopy, filter selection is based on the spectral properties of the dyes:

Dye	Excitation max, nm	Emission max, nm	Channel color
MemBlaze® 488	498	506	Green
MemBlaze® 560	553	567	Orange-red
MemBlaze® 640	648	669	Far-red
MemBlaze® 700	684	710	Near-infrared

Flow cytometry:

Cells labeled with MemBlaze® 488, 560, and 640 can be analyzed using standard flow cytometer detection channels FL1, FL2, and FL3, respectively.

Sources:

[1] Bioconjugate Chem. 2019, 30, 1, 192–199. [2] Cell Chem. Biol. 2019, 26, 4, 600–614.

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