MemGlow™ 590: Fluorogenic Membrane Probe

V. 1.1

A MEMBRIGHT™ Family probe

Cat. # MG03-02 (2 nmoles)

Lot #:

Upon arrival store at 4°C (desiccated)

See below for storage after reconstitution

Background

The MemGlow™ product line consists of bright & non toxic live cell membrane probes. MemGlow™ fluorogenic probes exhibit ideal microscopy characteristics including high specificity, low background, and simple application. MemGlow™ 590 has been validated with multiple microscopy techniques including epifluorescent (widefield), confocal, 2-photon, and TIRF[1]. MemGlow™ has been confirmed to work in fixed cells, fixed tissue, live cells, and other phospholipid membranes such as extracellular vesicles including exosomes[1-3].

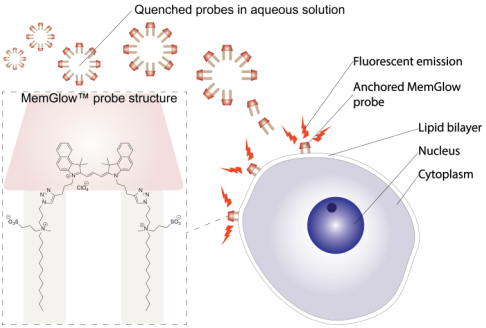

Figure 1. Turn-on mechanism of MemGlow™ probes. MemGlow™ probes are self-quenched nanoparticles until integration with the plasma membrane enables their excitation.

Material

As measured in DMSO, the absorption max of MemGlow™ 590 is 595 nm, with an emission spectra of 613 nm, an extinction coefficient of 120x10^3, and can be visualized using a Cy3.5 filter set or other suitable filter sets including TRITC. MemGlow™ 590 is supplied as a lyophilized pellet. Avoid contact with MemGlow™ by wearing appropriate PPE and dispose of according to local regulations and policies.

Storage and Reconstitution

The lyophilized product is stable at 4°C (<10% humidity) for 6 months and should be protected from light. To reconstitute, briefly centrifuge to collect the product at the bottom of the tube. MemGlow™ should be reconstituted with 100 µl of anhydrous DMSO to create a 20 µM stock solution for cell imaging or with 10 µl of anhydrous DMSO to create a 200 µM stock suitable for tissue or small organism imaging. After reconstitution the solution should be stored at -20°C where it is stable for 3 months. Once reconstituted, allow product to warm to room temperature before opening tube.

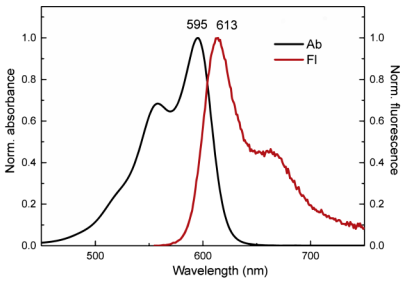

Figure 2. Absorbance and excitation spectra of MemGlow™ 590 diluted in DMSO. Absorbance peak detected at 595 nm and emission peak detected at 613 nm.

Important Technical Notes

A. Diluted solutions of MemGlow™ in aqueous media must be used immediately (<20 sec), as MemGlow™ will precipitate and/or bind to tube walls.

B. Serum can reduce MemGlow™ staining efficiency. When possible MemGlow™ staining should take place in the absence of serum. Optimally, the imaging cell media is serum-free media, reduced serum media, or PBS. In lieu of serum removal, the concentration of MemGlow™ should be increased.

C. Samples incubating in MemGlow™ solution should be protected from light.

D. MemGlow™ is non-toxic and live cells can be returned to normal cell media following labeling, and relabeled after 3-4 days.

E. The localization of MemGlow™ to lipid bilayers is easy to achieve with this product; however, differences in cell morphology and microscope technology, e.g., confocal vs. epifluorescence, will influence the visualization of MemGlow™.

F. When co-labeling with antibodies that require permeabilization limit the concentration of Triton-X to 0.1%.

G. MemGlow™ is fully compatible with 4% paraformaldehyde (PFA); however, 4% PFA partially permeabilizes the cell membrane so internalization of probes should be expected.

H. For tissues and small organisms an initial labeling concentration of 2 µM is recommended. For cell culture an initial labeling concentration of 20-200 nM is recommended depending on application (Table 1).

I. Homogeneity of tissue labeling can be optimized with a longer incubation at 4°C rather than relatively brief incubations at room temperature; however, both approaches can label plasma membranes.

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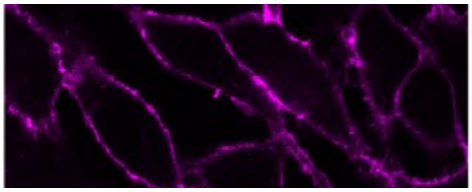

Figure 3. Laser scanning confocal imaging of live KB cells labeled with MemGlow™ 590. Laser excitation was set to 561 nm with emission collected between 567 and 750 nm.

	Live cells	Fixed cells	Tissue or small organisms
Epifluorescent working solution (nM)	100	100	2000
Confocal working solution (nM)	20	20	2000

Table 1. Recommended initial concentrations. Optimal conditions for efficient labeling should be determined for each cell line and application.

Application 1: Labeling the plasma membrane of live cells in culture.

Reagents
1. MemGlow™ 590 (Cat. # MG03).
2. Semi-confluent Tib-71 or HEK293 cells grown in a chamber slide.
3. Imaging medias: PBS, serum-free media or reduced serum media.

Equipment
1. Fluorescent microscope with a Cy3.5 excitation filter at 580 +/-20 nm and emission filter at 620 +/-20 nm for MemGlow™ 590.
2. Digital camera.

Method
1. Cells should be seeded onto imaging-appropriate glass or plastics and grown according to cell line requirements to semi-confluency.
2. Remove any cell culture media from your cells and replace with the media used for imaging (e.g., serum-free media). Do not allow the cells to dry.
3. Prepare the probe solution by diluting 5 µl of 20 µM MemGlow™ stock in 1 mL imaging media to create a 100 nM working solution or and mix thoroughly. Work quickly (<20 secs) as the probes will begin to aggregate reducing labeling efficiency.
4. Add diluted probe solution to cells by replacing the cell media with diluted probe solution until covered. Incubate cells in MemGlow™ solution for 10 minutes at room temperature. 37°C incubation can be used but will accelerate endocytosis of probes.
5. No washing step is required prior to imaging, but can be performed if desired with imaging media.
6. Proceed with imaging.

Application 2: Labeling the plasma membrane of fixed cells in culture.

Reagents
1. MemGlow™ 590 (Cat. # MG03).
2. Semi-confluent Tib71 or HEK293 cells grown on acid-washed coverslips.
3. Phosphate-buffered saline (PBS, 20 mM potassium phosphate pH 7.4, 150 mM NaCl) .
4. Fixative solution (4.0 % paraformaldehyde in PBS).
5. Glass microscope slide.
6. Coverslip sealing solution (clear nail polish).
7. EMS Fluoro-Gel mounting media (Cat. # 17985-10)

Equipment
1. Fluorescent microscope with a Cy3.5 excitation filter at 580 +/-20 nm and emission filter at 620 +/-20 nm for MemGlow™ 590.
2. Digital camera.

Method
1. Cells should be seeded onto imaging-appropriate glass or plastics and grown according to cell line requirements to semi-confluency.
2. Remove cell media and wash cells 1X-2X with PBS.
3. Fix cells for 10-15 minutes at room temperature with 4% paraformaldehyde (PFA).
4. Remove excess PFA by washing cells with PBS 3X.
5. (Optional) If co-labeling, permeabilization can be performed at this point. Add 0.1% Triton-X 100 in PBS followed by the primary and secondary antibody protocol according to supplier.
6. Prepare the probe solution by diluting 5 µl of 20 µM MemGlow™ stock in 1 mL imaging media to create a 100 nM working solution or and mix thoroughly. Work quickly (<20 secs) as the probes will begin to aggregate reducing labeling efficiency.
7. Incubate cells in MemGlow™ solution for 10 minutes at room temperature.
8. Remove MemGlow™ solution and wash cells with PBS 1X-2X.
9. If desired place mounting media onto microscope slide.
10. Apply cover slip cell-side down onto mounting media or microscope slide.
11. If desired apply coverslip sealing solution according to manufacturers directions.
12. Proceed with imaging.

Product Citations/Related Products
1. Collot, M. et al. MemBright: A Family of Fluorescent Membrane Probes for Advanced Cellular Imaging and Neuroscience. Cell Chem. Biol. 26, 600-614.e7 (2019).
2. Hyenne, V. et al. Studying the Fate of Tumor Extracellular Vesicles at High Spatiotemporal Resolution Using the Zebrafish Embryo. Dev. Cell 48, 554-572.e7 (2019)
3. Collot, M., Boutant, E., Lehmann, M. & Klymchenko, A. S. BODIPY with Tuned Amphiphilicity as a Fluorogenic Plasma Membrane Probe. Bioconjug. Chem. 30, 192–199 (2019).

For the latest protocols, citations and related products please visit www.cytoskeleton.com/memglow.