# MemGlow 400™ Pro12A Blue Plasma-membrane Probe and Lipid Order/Disorder Probe

V. 1.2

**Cat. # MG09 (16 nmoles)**

**Lot# 011**

Upon arrival store in the dark at 4°C (desiccated)

## 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[1]. MemGlow 400™ has been validated with multiple microscopy techniques including epifluorescent (widefield), confocal, 2-photon, and TIRF[2]. 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].

Pro12A is an analogue of commonly used dye Laurdan, which was designed for specific labelling of cell plasma membrane outer leaflet. Owing to its solvatochromism, Pro12A can also be used to visualize lipid organization of cell membranes. Upon binding with a plasma membrane in a predominant liquid ordered ($L_O$) phase Pro12A exhibits a 45-50 nm wavelength shift relative to liquid disordered ($L_d$) phase (Fig.3) enabling investigators to examine the nanoscale distribution of local chemical polarity in plasma membranes.

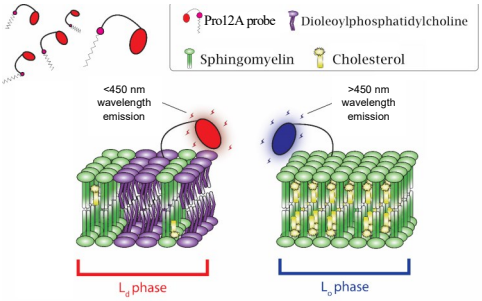


**Figure 1.** Mechanism of action of MemGlow 400 Pro12A.

## Material

The absorption max of Pro12A is 379 nm with an extinction coefficient of 20,000 $M^{-1}cm^{-1}$. The probe has multiple emission maxima depending on the environment, see important Technical notes below. Pro12A is supplied as a lyophilized pellet. It is a lipid binding dye and therefore appropriate PPE should be worn at all times. Dispose of Pro12A 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. Pro12A should be reconstituted with 40 µl of anhydrous DMSO to create a 400 µM (500x) stock solution for cell imaging. After reconstitution the solution should be stored at -20°C where it is stable for 6 months. Once reconstituted, allow product to warm to room temperature before opening tube.

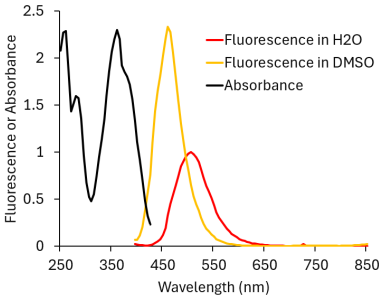


**Figure 2.** Excitation and emission spectra of MemGlow 400 Pro12A diluted to 5.0 µM. Absorbance peak detected at 363 nm, and peak emissions at 454 nm in DMSO, or 506 nm in water. Note lack of fluorescence above 650 nm.

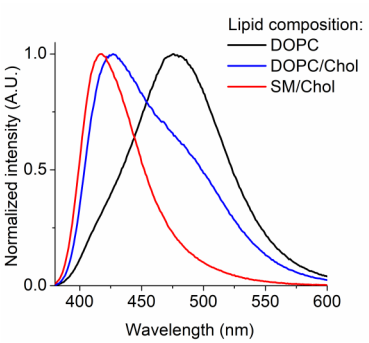


**Figure 3.** Fluorescence spectra of Pro12A in DOPC (ordered), DOPC/cholesterol (disordered) and SM/cholesterol (disordered) phases (ref. 2).

## Important Technical Notes

A. Pro12A was purpose-engineered for confocal and two photon excitation techniques. It is not also useful for epifluorescence where good results can be obtained (see Fig.4).

B. The probe has multiple emission maxima depending on the environment, see important Technical notes below. e.g. 415 nm in DOPC/Chol, 454 nm in DMSO, 476 nm in DOPC, and 506 nm in $H_2O$. For Generalized Polarization (GP) studies, the following wavelengths are used, 446 ± 23 nm(disordered) and at 499 ± 23 nm (ordered), where


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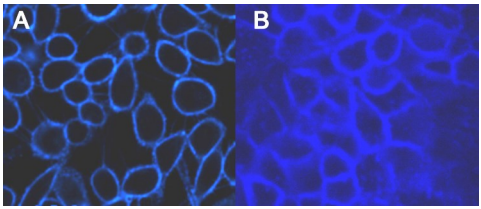


**Figure 3. A)** Confocal imaging of live CHO cells labeled with 800 nM Pro12A, and imaged with a Zeiss LSM 780 confocal microscope using a 405 nm laser and 410-600 nm emission. **B)** Widefield epi-fluorescent imaging of live A431 cells labeled with 800 nM Pro12A, and imaged with a Dapi filter set.

GP = I(440)-I(490)/I(440)+I(490), where I = intensity of the signal. In epifluorescence the probe can be visualized using a Dapi filter set or other suitable filter sets.

C. Diluted solutions of Pro12A in aqueous media should be used as soon as possible, as it may precipitate.

D. Serum and proteins will reduce Pro12A staining efficiency. When possible, Pro12A staining should take place in the absence of serum (PBS or serum free media).

E. Pro12A is generally non-toxic to live cells. For repeated imaging following cell labeling we do not recommend washing cells due to the weak binding properties of the Pro12A probe; however, optimization of probe concentration for longer studies may be required.

F. Pro12A will work with a range of concentrations. Investigators should empirically determine the best concentration for their application. An initial concentration of 800 nM is recommended.

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

**Reagents**

1. MemGlow 400 Pro12A (Cat. # MG09).
2. Semi-confluent A431 cells grown in a chamber slide.
3. Imaging medias: PBS, serum-free media or reduced serum media.

**Equipment**

1. Epifluorescent microscope with a Dapi filter set; 360 +/-20 nm excitation and 450 +/-20 nm for emission.
2. For measuring lipid order or two color imaging a spectral scanning attachment should be used.

**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 wash twice with PBS or serum free media and. Do not allow the cells to dry.
3. Prepare the probe solution by diluting 2 µl of 400 µM MemGlow Pro12A stock in 1.0 mL PBS or serum-free media to create a 800 nM working solution and mix thoroughly. Work quickly as the probes will begin to aggregate over time reducing labeling efficiency.
4. Replace PBS or serum-free media of the cell culture, as added in step 2, with diluted probe solution ensuring cells are submerged. Incubate cells for 10 minutes at room temperature.
5. Note that over longer incubation time, the Pro12A will gradually accumulate inside endosomes. Therefore, to avoid intracellular fluorescence, the imaging should be done as soon as the sample is prepared.
6. For general microscopy applications no washing step is required prior to imaging, but can be performed if desired with imaging media.

**Application 2:** Labeling extracellular vesicles.

**Reagents**

1. MemGlow 400 Pro12A (Cat. # MG09).
2. $10^5$-$10^7$ EVs/ml in PBS.
3. Imaging medias: PBS or serum-free media.

**Equipment**

1. Confocal or epifluorescent microscope with a 63x or 100x oil objective Dapi filter set; 360 +/-20 nm and emission filter at 450 +/-20 nm for Pro12A.

**Method:**

1. Prepare EVs with your laboratory protocol with the final step in non-serum media or PBS.
2. Incubate EV solution with Pro12A at 800 nM (final concentration) in PBS for 10 minutes at room temperature in the dark.
3. Centrifuge for 1 h at 100,000 xg at 4°C.
4. Resuspend pellet in 50 µl of PBS.
5. Mount 1 µl of vesicles on to a poly-lysine coated coverslip, and mount with 4 µl of mounting media on to a glass slide.
6. Shelf life is 24 h at 4°C.

**References:**

1. Collot, M, Ashokkumar, P, Anton, H, Boutant, E, Faklaris, O, Galli, T, Me´ly E, Danglot, L, & Klymchenko AS. 2019. MemBright: A Family of Fluorescent Membrane Probes for Advanced Cellular Imaging and Neuroscience. Cell Chemical Biology 26, 1–15. https://doi.org/10.1016/j.chembiol.2019.01.009

2. Danylchuk DI, Sezgin E, Chabert P, & Klymchenko, AS. 2020. Redesigning solvatochromic probe Laurdan for imaging lipid order selectively in cell plasma membranes. Anal. Chem. 92, 14798-14805. https://dx.doi.org/10.1021/acs.analchem.0c03559

3. Hyenne et al. 2019. Studying the Fate of Tumor Extracellular Vesicles at High Spatiotemporal Resolution Using the Zebrafish Embryo. Jo. Devel. Cell, 48(4), p.554-572. https://doi.org/10.1016/j.devcel.2019.01.014

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