# MemGlow NR12A Membrane Polarity Probe

V. 6.0

**Cat. # MG07 (4 nmoles)**

Upon arrival store at 4°C (desiccated)

See below for storage after reconstitution

## Background

MemGlow NR12A is a solvatochromic photostable plasma membrane-targeting dye. Upon binding with a plasma membrane in a predominant liquid ordered ($L_O$) phase NR12A exhibits a 45-50 nm wavelength shift relative to liquid disordered ($L_d$) phase enabling investigators to examine the nanoscale distribution of local chemical polarity in plasma membranes. NR12A can also be used to label the plasma membrane for conventional fluorescence microscopy imaging.

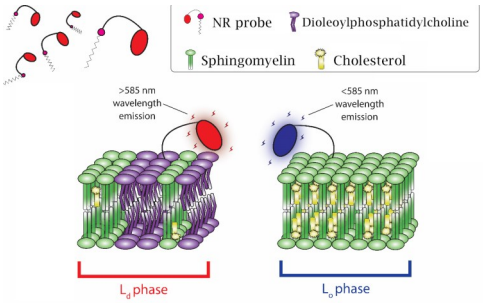


**Figure 1.** Diagram of NR12A's lipid order-dependent emission.

## Material

The absorption max of NR12A is 554 nm, with an emission spectra of 635 nm, an extinction coefficient of 45,000 $M^{-1}.cm^{-1}$, and can be visualized using a Cy 3.5 filter set or other suitable filter sets. NR12A is supplied as a lyophilized pellet. NR12A is a lipid binding dye and appropriate PPE should be worn at all times. Dispose of NR12A 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. NR12A should be reconstituted with 200 µl of anhydrous DMSO to create a 20 µM stock solution for cell 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.

## Important Technical Notes

A. NR12A was purpose-engineered for super resolution techniques. It is not intended for use in conventional microscopy; however, it can be used with good results.

B. Diluted solutions of NR12A in aqueous media should be used as soon as possible as it may precipitate.

C. Serum and proteins will reduce NR12A staining efficiency. When possible NR12A staining should take place in the absence of serum. Optimally, the imaging cell media is serum-free media, reduced serum media.

D. NR12A is generally non-toxic to live cells but morphological changes 12 hours after no-wash applications can cause cell shrinkage. For repeated imaging, follow cell labeling with a wash using cell media.

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

F. NR12A will work with a range of concentrations. Investigators should empirically determine the best concentration for their application. An initial concentration range of 10-200 nM is recommended.

G. NR12A has 10x higher affinity for the plasma membrane versus NR4A which was designed for SR-PAINT applications.

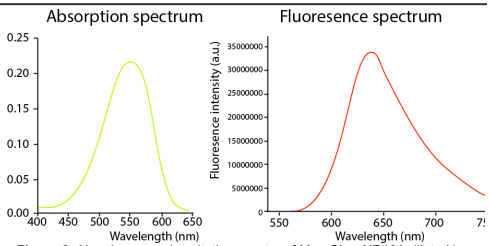


**Figure 2.** Absorbance and excitation spectra of MemGlow NR12A diluted in DMSO to 4.8 µM. Absorbance peak detected at 555 nm with peak emission at 637 nm.

**Note.** 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. NR12A (Cat. # MG07).
2. Semi-confluent HEK293 cells grown in a chamber slide.
3. Imaging medias: PBS, serum-free media or reduced serum media.

## Equipment

1. Fluorescent microscope with a Cy 3.5 excitation filter at 555 +/-20 nm and emission filter at 637 +/-20 nm for NR12A.
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.


**Phone:** (303) 322.2254 **Fax:** (303) 322.2257
**Customer Service:** cserve@cytoskeleton.com
**Technical Support:** tservice@cytoskeleton.com

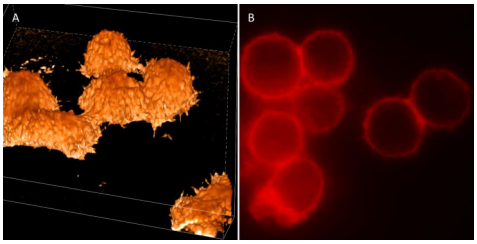


**Figure 3. A)** A 3D stacked image of KB cells stained with 20 nM NR12A (false colored orange). **B)** Widefield fluorescent imaging of live HeLa S1 cells labeled with 10 nM NR12A. HeLa S1 cells were imaged with a TRITC filter set, neutral density filter, a digital CCD camera, and 100x oil objective (false colored red).

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 NR12A stock in 1 mL imaging media to create a 100 nM working solution and mix thoroughly. Work quickly as the probes will begin to aggregate over time reducing labeling efficiency.
4. Replace the cell media with diluted probe solution ensuring cells are submerged. Incubate cells in MemGlow™ solution for 10 minutes at room temperature.
5. For SR-Paint applications the cells are ready to image. For general microscopy applications no washing step is required prior to imaging, but can be performed if desired with imaging media.

**Product Citations/Related Products**

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

1. Kucherak, O. A. et al. Switchable nile red-based probe for cholesterol and lipid order at the outer leaflet of biomembranes. J. Am. Chem. Soc. 132, 4907–4916 (2010).

2. Danylchuk, D. I., Moon, S., Xu, K. & Klymchenko, A. S. Switchable Solvatochromic Probes for Live-Cell Super-resolution Imaging of Plasma Membrane Organization. Angew. Chemie - Int. Ed. 58, 14920–14924 (2019).