Check videos of protocol, examples
of results and much more on:
idylle-labs.com/glowmito

A protocol designed in March 2023.

GlowMito

PROTOCOL

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1. The material you need

REAGENTS

- GlowMito stock solution, 1 mM in water
- DPBS 1X

CONSUMABLES

- Plates, dishes or coverslips (depending on your experiment)
- Pre-heated ~~cell~~ culture medium of your choice
- Your favorite cells

2. Storing the 1 mM GlowMito stock solution

- The 1 mM GlowMito stock solution can be stored at 4°C for one month. For long-term storage, aliquot to avoid repeated freeze/thaw cycles and store at -20°C for up to 6 months. Always store GlowMito protected from light.

3. Using GlowMito

- Seed cells in your culture vessel of choice and culture as desired (A).

GlowMito is a light-sensitive compound. The following steps should be carried out away from direct sunlight to avoid affecting GlowMito performance. Loosely cover tube racks with a piece of foil if the dye vials are going to be out for more than 30 minutes.

- When cells are ready for analysis, thaw one aliquot of 1 mM GlowMito stock solution and quickly vortex.
- In a separate vial, prepare your final GlowMito solution at 500 nM by diluting 1 mM GlowMito stock solution at a 1:2000 ratio in your culture medium of choice (B).
- Homogenize the solution by pipetting up and down or quickly vortexing.

In parallel, a "control" solution might be prepared by diluting water at a similar dilution factor in culture medium.

We recommend using 500 nM as a starting point to allow a fast internalization of the probe. The final GlowMito concentration might be adjusted depending on the cell type and experimental conditions used.

- Rinse the cells with DPBS 1X (C).
- Directly add to the cells the 500 nM GlowMito solution you just prepared in culture medium (D).
- Incubate for 30 minutes in an incubator set at 37°C, 5% CO_2.
- Immediately proceed to imaging (Ex: 542 nm/Em: 690 nm) or collect cells for downstream analysis.

For use in flow cytometry, we recommend using a 488 nm laser and a 655-730 nm emission filter.

How to use GlowMito in pictures