MitoBrilliant™ Protocol

Next-generation fluorescent stains for the localization and tracking of mitochondria

Product	Unit Size	Abs/Em (nm)	Extinction Coefficient (M^{-1} cm^{-1})	$\Delta\psi_m$ Dependent?	Stain pre-fixed cells?
MitoBrilliant™ 646 **Cat. No. 7700**	5x50 µg	655/668	125,000	No*	Yes
MitoBrilliant™ Live 646 **Cat. No. 7417**	5x50 µg	648/662	127,800	Yes	No
MitoBrilliant™ Live 549 **Cat. No. 7693**	5x50 µg	550/568	100,000	Yes	No

** In live-cell staining, the mitochondrial membrane potential ($\Delta\psi_m$) helps to drive initial recruitment of the dye into mitochondria. After staining, localization of the dye becomes insensitive to $\Delta\psi_m$ changes. In pre-fixed cell staining, MitoBrilliant™ 646 can localize and be retained in mitochondria without $\Delta\psi_m$. For more information on MitoBrilliant™ including application data, please refer to the Product Guide.*

Storage and Handling

Store at -20°C and protect from light. We recommend that stock solutions, once prepared, are stored aliquoted in tightly sealed vials at -20°C or below and used within 1 month.

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Reconstitution and Stock Solution Preparation

Prepare a 1mM stock solution by adding high quality, anhydrous DMSO to one vial of MitoBrilliant™ dye.

Product	Molecular Weight	Quantity per vial	Recommended reconstitution
MitoBrilliant™ 646 **Cat. No. 7700**	493.6	50 µg (101 nmoles)	Add 101 µL DMSO for a 1 mM stock solution
MitoBrilliant™ Live 646 **Cat. No. 7417**	445.1	50 µg (112 nmoles)	Add 112 µL DMSO for a 1 mM stock solution
MitoBrilliant™ Live 549 **Cat. No. 7693**	417.0	50 µg (120 nmoles)	Add 120 µL DMSO for a 1 mM stock solution

Staining Live Cells

1. Dilute the 1mM DMSO stock solution using a warm (37 °C) buffer or growth medium and apply to live cells at a final working concentration between 50 to 200 nM. We recommend optimizing the final concentration used for individual experiments. Aqueous working solutions should be prepared and used on the same day.

2. Incubate for 30 to 60 minutes at 37°C prior to imaging (longer incubation times may give brighter staining). A washing step prior to imaging is not required but it is recommended: rinse the cells with 1x PBS and apply fresh media prior to imaging.

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Staining Cells in Suspension for Flow Cytometry

1. After gentle centrifugation, carefully resuspend the cell pellet in a prewarmed (37°C) buffer or growth medium containing 50-200 nM MitoBrilliant™ at a cell concentration of 1 x 10^6 per mL.

2. Incubate for 30-60 minutes at 37°C in the dark.

3. Re-pellet the cells buffer by gentle centrifugation, then resuspend cells in fresh pre-warmed medium ready for flow cytometry.

Staining Fixed Cells - ***Only Applicable to: MitoBrilliant™ 646 (Cat. No. 7700)***

MitoBrilliant™ 646 can be applied at either the pre-fixation or post-fixation stages of sample preparation. For more application information, please refer to the *Product Guide*.

- Dilute the 1 mM DMSO stock solution to a final working concentration between 50 to 200 nM. We recommend optimizing the final concentration used for individual experiments. Aqueous working solutions should be prepared and used on the same day.

- Appropriate fixation methods are critical for preserving cellular structure and achieving optimal staining. For HeLa cells, we recommend fixing with freshly prepared pre-warmed buffer or growth medium containing 4% paraformaldehyde at 37 °C for 10 to 20 minutes. Optimization might be required for different model systems. After fixation, rinse the cells several times in PBS.

- Permeabilization step (optional). Incubate fixed cells for 10 minutes in PBS containing 0.05% Triton® X-100.

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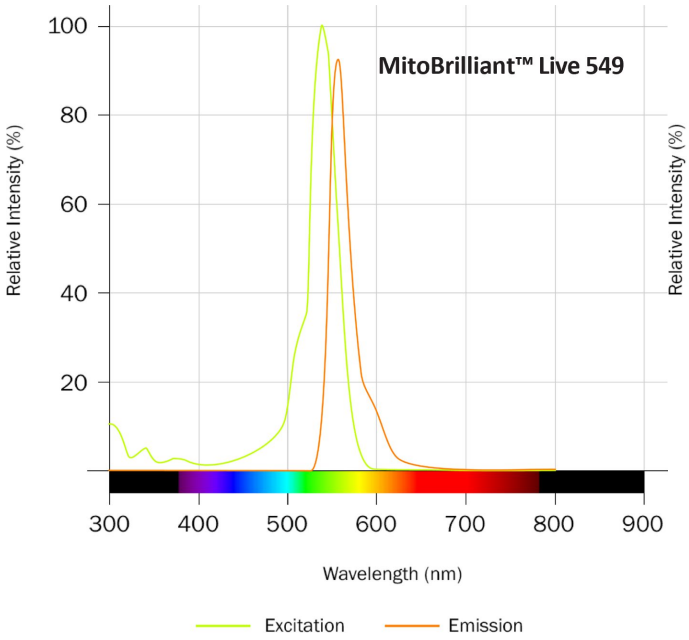

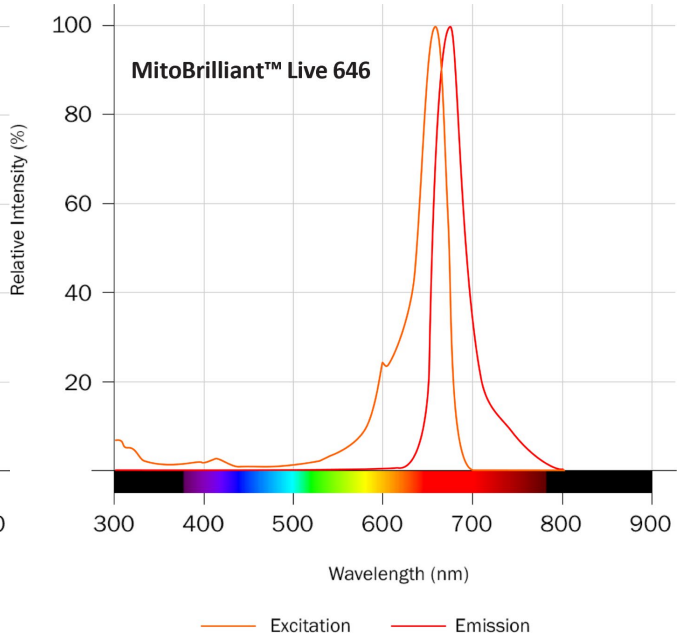

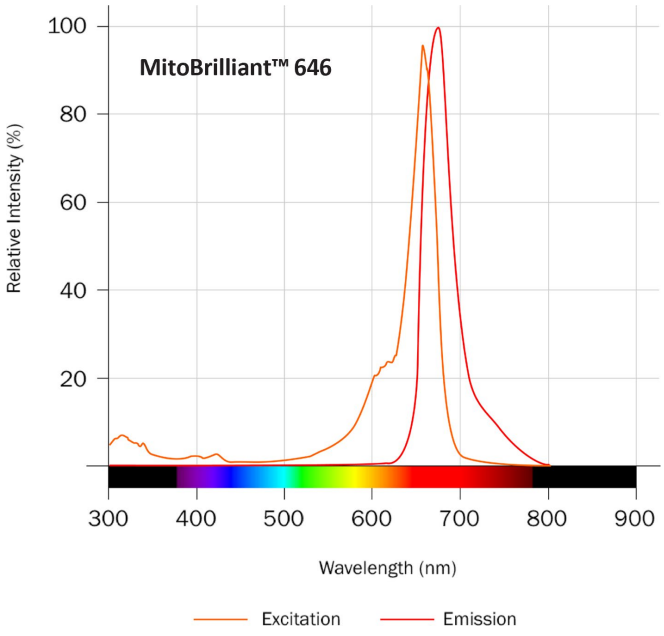

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