MitoSpy™ Green FM

Catalog# / Size	424805 / 5 x 50 µg 424806 / 20 x 50 µg
Regulatory Status	RUO
Other Names	Mitochondrial labeling
Description	MitoSpy™ mitochondrial localization probes are cell-permeant, fluorogenic chemical reagents that are used for labeling mitochondria of living cells. MitoSpy™ Green FM's attraction to the mitochondria is not based on membrane potential and thus can be used to measure mitochondrial mass of individual cells in flow cytometry.

Product Details

Verified Reactivity	Human, Mouse, Rat, All Species
Molecular Mass	671.88 g/mol
Preparation	The stock solution for MitoSpy™ Green FM is prepared by dissolving the lyophilized probe in dimethyl sulfoxide (DMSO) to make a final concentration of 1 mM by adding 74 µl of DMSO to each vial.
Storage & Handling	Store MitoSpy™ Green FM at -20°C.
Application	ICC - *Quality tested*
Recommended Usage	Each lot of this reagent is quality control tested by immunocytochemistry staining. For immunocytochemistry microscopy, a concentration range of 50nM to 500nM is recommended. It is recommended that the reagent be titrated for optimal performance for each application.
Application Notes	MitoSpy™ Green FM is excited at 490nm and emits at 516 nm. 1. Prior to reconstitution, spin down the vial of lyophilized reagent in a microcentrofuge to ensure the reagent is at the bottom of the vial. 2. Reconstitute MitoSpy™ Green FM to a 1 mM concentration with DMSO by adding 74 µl DMSO to an individual vial of lyophilized material. Protect the stock solution from light and keep frozen for storage. 3. Prepare the working solution for MitoSpy™ Green FM in 37°C culture medium (incomplete), this will vary by cell line and type of imaging required. • If labeling mitochondria for live cell imaging, a concentration between 50 - 250 nM is recommended. • If cells are labeled live and then subsequently fixed, a concentration between 250 - 500 nM is recommended. 4. Grow cells to a desired confluency and wash once with warm 1 x PBS. 5. Add the diluted MitoSpy™ Green FM solution to the live cells and place in the 37°C incubator for 20-30 minutes. 6. Wash the cells twice with warm 1 X PBS or culture media. 7. If the cells will be imaged live, they can now be imaged with a fluorescence microscope. If the cells need to be fixed: A. Fix the cells with 2-4% paraformaldehyde (PFA) for ten minutes at room temperature. B. Wash the cells twice with 1 x PBS. C. Regular IF staining protocol can be used for antibodies or other probe co-stains.
Product Citations	1. Galli A, *et al.* 2020. Biomedicines. :8. PubMed 2. Dent JR, *et al.* 2019. J Appl Physiol (1985). 127:1117. PubMed 3. Maffioli E, *et al.* 2020. Front Cell Dev Biol. 508:8. PubMed

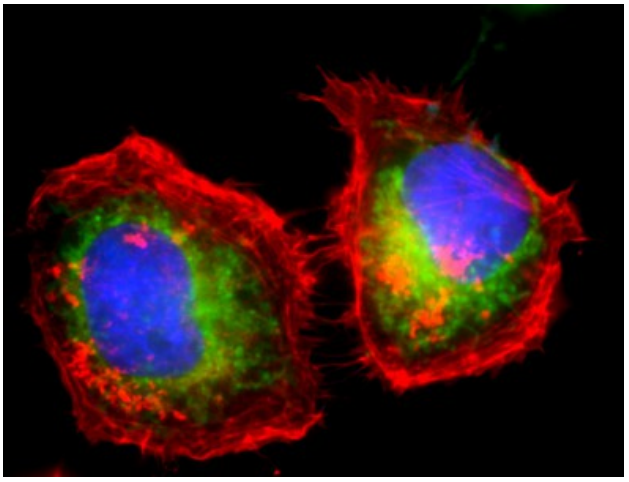
Antigen Details

Distribution	Mitochondria.
Biology Area	Apoptosis/Tumor Suppressors/Cell Death, Cell Biology, Mitochondrial Function, Neuroscience
Molecular Family	Mitochondrial Markers
Gene ID	NA

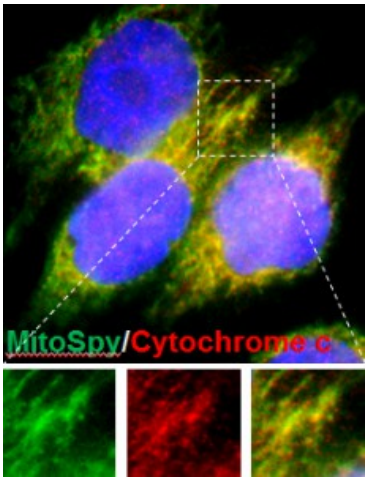
Related Protocols

Immunocytochemistry Staining Protocol

Product Data



HeLa cells were stained with 250 nM of MitoSpy™ Green FM (green) for 20 minutes and fixed with 4% paraformaldehyde (PFA) for ten minutes. Then the cells were stained with Alexa Fluor® 594 phalloidin for 20 minutes (red) and counterstained with DAPI (blue). The image was captured with a 60x objective.



HeLa cells were treated with 400 nM MitoSpy™ Green FM (Green) for 30 minutes, fixed with 4% paraformaldehyde (PFA) for fifteen minutes, permeabilized with 0.5% Triton X-100 for three minutes, and blocked with 5% FBS for 60 minutes. Then the cells were intracellularly stained with purified anti-Cytochrome c antibody (clone 7H8.2C12) overnight at 4°C followed by Alexa Fluor® 594 (Red) goat anti-mouse IgG for one hour at room temperature (Cat. No. 405326, 1:250 dilution, 2 µg/ml). Nuclei were counterstained with DAPI (Blue, Cat. No. 422801). The image was captured with a 60X objective using KEYENCE BZ-X700 fluorescence microscope. Exposure time (in seconds) is 1/20.

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