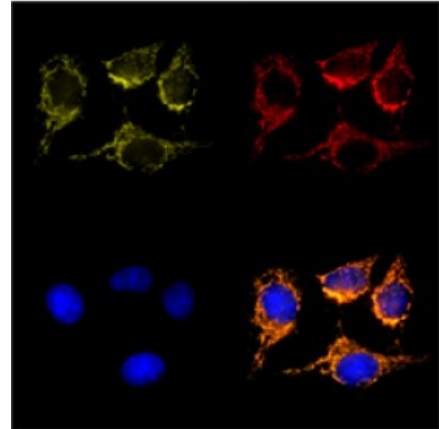


MitoSpy™ Orange CMTMRos

Catalog # / Size: 2724020 / 20 x 50 µg
2724015 / 5 x 50 µg

Preparation: The stock solution for MitoSpy™ Orange CMTMRos is prepared by dissolving the lyophilized probe in dimethyl sulfoxide (DMSO) to make a final concentration of 1 mM by adding 117 microL of DMSO to each vial.

Concentration: NULL



HeLa cells were stained with 250 nM of MitoSpy™ Orange CMTMRos (yellow) for 20 minutes, fixed with 4% paraformaldehyde (PFA) for ten minutes, and permeabilized with 0.1% Triton X-100 for ten minutes. Then the cells were stained with anti-Cytochrome

Applications:

Applications: Immunofluorescence

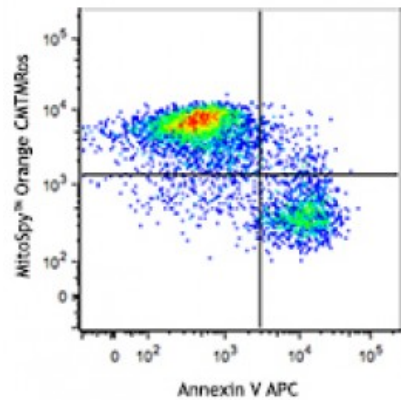
Recommended Usage: Each lot of this reagent is quality control tested by immunofluorescence staining. For immunofluorescence microscopy, a concentration range of 50nM to 500 nM is recommended. For flow cytometric staining, the suggested use of this reagent is 50nM. It is recommended that the reagent be titrated for optimal performance for each application.

Application Notes: MitoSpy™ Orange CMTMRos (chloromethyl-tetramethylrosamine) is excited at 551 nm and emits at 576 nm.

1. Prior to reconstitution, spin down the vial of lyophilized reagent in a microcentrifuge to ensure the reagent is at the bottom of the vial.

2. Reconstitute MitoSpy™ Orange CMTMRos to a 1 mM concentration with DMSO by adding 117 microL DMSO to an individual vial of lyophilized material. Protect the stock solution from light and keep frozen for storage

3. Prepare the working solutions for MitoSpy™ Orange CMTMRos in 37°C



Human T-cell leukemia cell line, Jurkat, was treated for five hours with LEAF™ purified anti-CD95 (clone EOS9.1), then stained with an impermeant nucleic acid stain, Annexin V APC, and MitoSpy™ Orange CMTMRos. Data shown was gated on live cell

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 - 250nM is recommended.

- If cells are labeled live and then subsequently fixed and permeabilized, the recommended concentration is between 250 nM to 500 nM.

4. Grow cells to a desired confluency and wash once with warm 1 x PBS.

5. Add the diluted MitoSpy™ Orange CMTMRos 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 and permeabilized:

A. Fix the cells with 2-4% PFA for ten minutes at room temperature.

B. Wash the cells twice with 1 X PBS.

C. (Optional) Permeabilize the cells with 0.25% triton X-100.

D. Regular IF staining protocol can be used for antibodies or other probe co-stains.

Description: MitoSpy™ mitochondrial localization probes are cell-permeant, fluorogenic chemical reagents that are used for labeling mitochondria in living cells. MitoSpy™ Orange CMTMRos localizes to the mitochondria based on its membrane potential and is useful to indicate cell health as well as for localization. It also can be fixed and permeabilized for further antibody-based detection.