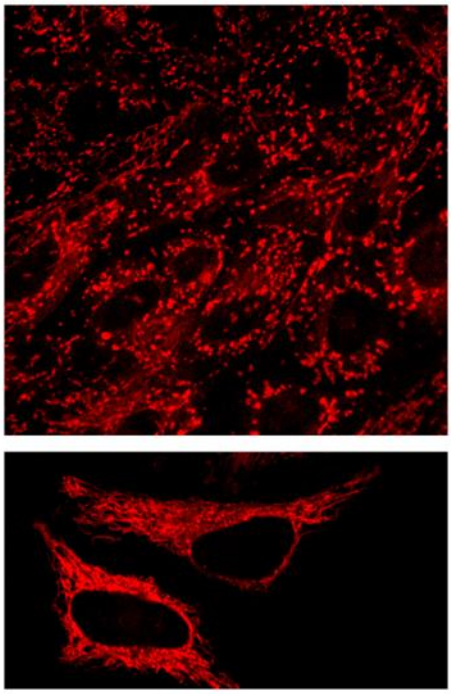
Biosensis® LipoFluor-MR™ Ready-to-Dilute™, Mitochondria Tracing Reagent

Catalog Number: Lipofluor™ TR-602-Mito



For research use only, not for use in clinical and diagnostic procedures.

1. Intended Use

Lipofluor-MR™ TR-602-Mito localizes to mitochondria in live cells and tissue. Lipofluor-MR™ TR-602-Mito is also suitable for detecting mitochondria in fixed and frozen tissue samples. This agent is ideal for a range of fluorescence applications, including imaging by confocal microscopy and multi-photon microscopy.

Tested Applications: Live cell culture, 4% PFA fixed, frozen, for fresh tissues. Dye does not stain mitochondria in fixed cells in culture for unknown reasons.

Validated systems:

Cell penetration and localization of Lipofluor-MR™ TR-602-Mito have been confirmed in live prostate cells (PNT2 and 22RV1), cardiomyocytes (H9c2), and cancer cell lines (HeLa). Mitochondrial localization has been shown in live tissues, including live adipose tissue (sheep), muscle tissue (sheep cardiac and skeletal), and frozen or PFA fixed muscle tissue (sheep skeletal).

2. Materials Provided

Four 0.5 mL microfuge vials containing
5.5 uL/vial of 20 mM stock solution of Lipofluor™ TR-602-Mito in DMSO. 100.8 µg per vial.
Each vial can be diluted 100X to 2000X depending upon the final working concentration of the dye in the experiment. Typical working concentrations for Lipofluor™ Lipofluor™ TR-602-Mito are 10µM-50µM.

3. Specifications

CAS Number: 2172800-69-8; FW: 907.84
MF: $C_{36}H_{26}IrN_{8*}F_6P$
Ex/Em: 405/600 nm: Form: Liquid:
Working concentrations: 10-50 µM

4. Precautions for Use

Before performing the staining procedure for fixed or live cell imaging, please read the entire procedure and consider the safety data sheet. For laboratory use only. Not fully tested. Not for drug, household, human, or veterinary uses.

5. Reagent Preparation

Lipofluor-MR™ TR-602-Mito undiluted stock solution vials should be stored at 2-8ºC protected from light for up to 12 months from receipt. Diluted working solutions are short-lived. Dilute to working solution just before use. Excess solutions do not store well. See “Storage conditions” below for more information.

To create a 20 µM, 5.445 mL working solution, follow these steps for each 0.5 mL vial:

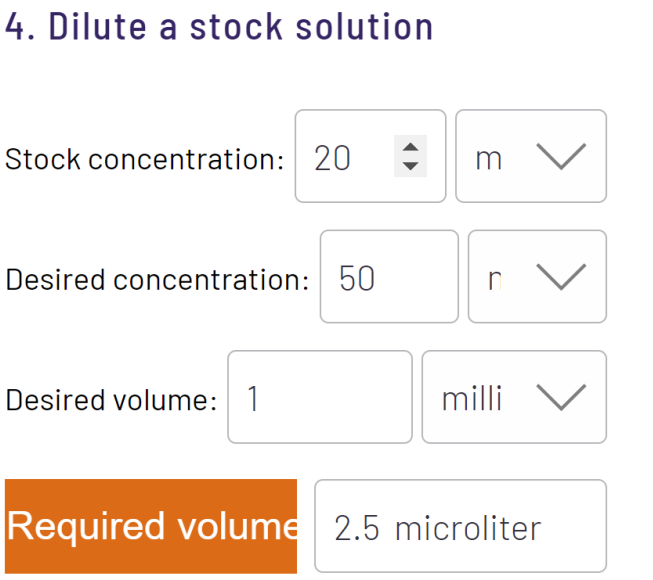
a) Take a 0.5 mL vial containing 5.5 µL of LipoFlour™ Lipofluor™ TR-602-Mito in DMSO liquid.

b) Dilute the DMSO liquid with 49.5 µL of an aqueous solution (such as PBS or cell media) to create a 10x dilution.

c) Transfer this mixture to a larger vial and dilute it further with 5.445 mL of the aqueous solution (100x dilution) to make a total of 5.5 mL of Lipofluor-MR™ TR-602-Mito solution at 20 µM. This operating solution is enough to generate solutions for more than 24 wells per vial with 200 µL per well.

d) If you need to make a specific solution concentration, you can use a basic chemistry formula to calculate how much of the original stock solution to use. The formula is C1V1=C2V2, where C1 is the concentration of the stock solution, V1 is the volume of the stock solution needed, C2 is the desired concentration of the final solution, and V2 is the final volume of the solution. Thus, one will solve for V1's volume. For example, if you need to make 1 milliliter of a 50 µM solution using a 20 mM stock solution, you can use an online calculator or solve the formula manually. In this case, C1 is 20 mM, C2 is 50 µM, and V2 is 1 mL. Solving for V1, you get V1=C2V2/C1, which means adding 2.5 µL of the stock solution to 998 µL of solvent to make ~1 mL of a 50 µM solution.

Useful on-link calculators: https://bit.ly/3IeWFbR



6. Storage Conditions

Lipofluor™ TR-602-Mito stock 20 mM DMSO solution vials should be stored, tightly sealed, and well protected from light at 2-8°C for up to 12 months from receipt. Opened stock tubes are stable if stored tightly sealed and protected from light for six months from purchase. Working or diluted solutions are unstable and should be made just before use. We have no data on frozen solutions; thus, we cannot recommend freezing the DMSO stocks or diluted solutions.

7. Staining Protocol For Live Cells

For Adherent Cells

1. Grow cells in 6 well-plate on coverslips with appropriate culture medium and under appropriate growth conditions
2. Grow cells to the desired confluence (70 – 80%)
3. Remove culture medium and add pre-warmed PBS, pH 7.2-7.6, or cell culture media containing 10 – 50 µM of Lipofluor=-MR™ TR-602-Mito (1:2000 – 1:400 dilution of 20 mM stock solution)
4. Incubate cells for 30 minutes under appropriate growth conditions
5. Wash coverslips twice for one minute in PBS, pH 7.2-7.6
6. Mount coverslips in aqueous mounting media for imaging

Note: Glycerol-based mounting media may reduce the fluorescence intensity of Lipofluor-MR™ TR-602-Mito.

For Suspended Living Cells

1. Centrifuge cell suspension to obtain cell pellet and remove the supernatant
2. Resuspend cells in pre-warmed PBS, pH 7.2-7.6 (37°C) or serum-free medium containing 10 – 50 µM of Lipofluor-MR™ TR-602-Mito (1:2000 – 1:400 dilution of 20 mM stock solution)
3. Incubate cells for 30 minutes under appropriate growth conditions
4. Re-pellet the cells by centrifugation and resuspend in PBS, pH 7.2-7.6, or cell culture medium
5. Cells can be prepared as a wet mounted or adhere to poly-L-lysine coated coverslips and mounted in an aqueous mounting media for immediate imaging

For Co-Staining Live cell Experiment

1. Before co-staining, ensure the spectral profiles of the counter-staining agent and Lipofluor-MR™ TR-602-Mito can be appropriately resolved. Generally, dyes that do not excite with 405 nm excitation can be imaged alongside Lipofluor-MR™ TR-602-Mito. Blue dyes such as DAPI are also compatible as they emit at a lower wavelength than Lipofluor-MR™ TR-602-Mito
2. Stain cells as described above with a reduced washing step to 30 seconds following incubation
3. Stain cells with a counter-staining agent according to the manufacturer's instructions
4. Following washes, mount in an aqueous mounting media for imaging

Note: Lipofluor-MR™ TR-602-Mito is not suitable for fixed cell staining of mitochondria

8. Staining Protocol For Tissue Sections

Unlike the conventional mitochondrial stains, paraformaldehyde-fixed, fresh, and fresh-frozen tissue sections have been successfully stained with Lipofluor-MR™ TR-602-Mito. Other fixation methods have not been attempted to date.

If endogenous fluorescence is an issue in your tissue sample, quenching can assist in imaging.

We recommend incubating samples in 100 mM glycine in PBS (pH to 7.4 with 1 M tris base, if required) for quenching endogenous fluorescence for 20 minutes at room temperature. Other treatments, such as UV irradiation, may also help quench endogenous fluorescence; however, avoid harsh treatments that may leach lipids from samples or interfere with lipid binding.

Sample Preparation

Tissues can be stained immediately upon collection or stored for later staining. We recommend 4% paraformaldehyde fixation or flash freezing for tissue storage. Sample preparation will depend on the tissue type and imaging platform. In general, Lipofluor-MR™ TR-602-Mito can stain tissue sections of up to 5 mm thick. Live samples can be sectioned using a sharp scalpel or knife. Fixed and frozen can also be prepared in OCT sectioned by microtone to your desired thickness.

Staining Sections

Incubate samples with 10 - 50 µM Lipofluor-MR™ TR-602-Mito in PBS, pH 7.2-7.6, or appropriate media (1:2,000 - 1:400 dilution of 20 mM stock solution) for 30 minutes at room temperature with gentle agitation provided by a platform rocker (or similar) at low rpm. Wash samples three times for five minutes in PBS, pH 7.2-7.6, at room temperature with agitation. Mount tissue in aqueous mounting media and image immediately for best results.

9. Fluorescent Imaging Settings

Epi-Fluorescence Microscopy

Lipofluor-MR™ TR-602-Mito can be excited by UV (~365 nm) or blue light (405 nm) sources with emissions collected using a wideband pass filter or narrowband pass filter within an emission range of 550-650 nm (green to red light).

Confocal or Two-Photon Microscopy

Lipofluor-MR™ TR-602-Mito can be excited by a 400 nm steady-state laser or at 800-830 nm using a two-photon pulse laser. Ideally, image the specimen with a spectral detector set for the emission of Lipofluor-MR™ TR-602-Mito, 500-650 nm (E_{max} = 600 nm). Alternatively, detect by using an emission filter suited for detecting FITC-based fluorophores.

Note: Time-gated imaging can be performed with this product and is ideal for samples with high levels of endogenous fluorescence. Probe emission lifetime is ~30 microseconds.