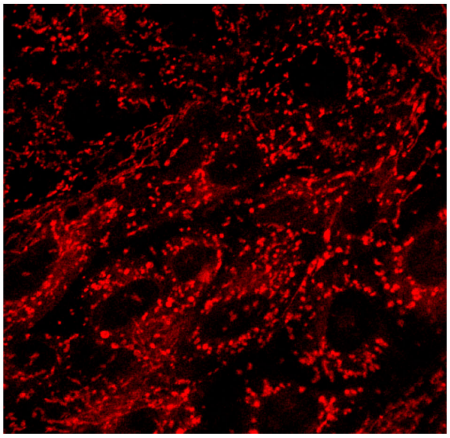
Biosensis Mito-R™ Mitochondria Tracing Reagent

Catalogue Number: TR-602-MR



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

1. Intended Use

TR-602-MR localises to mitochondria in live cells and tissue. TR-602-MR is also suitable for detection of mitochondria in fixed and frozen tissue samples. This agent is suitable for a range of fluorescence applications including imaging by confocal microscopy and multi-photon microscopy.

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

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2. Specifications

- Simple and quick application
- Suitable for live cell imaging
- Suitable for live and fixed tissue
- Low cytotoxicity
- Highly resistant to photobleaching
- Large Stokes shift (Ex/Em 405/600 nm)
- Compatible with other fluorescent dyes
- Ideal for epi-fluorescence, confocal and multiphoton imaging

3. Precautions for Use

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

4. Storage Conditions

As a solid, TR-602-MR should be stored at 2-8^{0}C. Product is stable for up to 6 months if stored as specified. Once reconstituted in DMSO, TR-602-MR should be stored at 2-8^{0}C. Reconstituted stock solution should be used within 2 months of reconstituting for best staining results. Refer to the datasheet for further details.

5. Reagent Preparation

Reconstitute the product with 55 µL of DMSO to obtain a 10 mM stock solution. Mix thoroughly before use. This stock solution should be stored at 2-8^{0}C, protected from light.

Note: TR-602-MR should not be reconstituted in aqueous solutions such as phosphate-buffered saline (PBS) or cell culture media. For use, TR-602-MR should be diluted in a buffer or cell culture media to a concentration of 10 µM - 50 µM immediately before use (this solution should not be stored for later use).

6. 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 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 TR-602-MR (1:1,000 – 1:200 dilution of 10 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 TR-602-MR.

For Suspended 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 TR-602-MR (1:1,000 – 1:200 dilution of 10 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 Experiment

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

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

7. Staining Protocol For Tissue Sections

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

If endogenous fluorescence is an issue in your tissue sample, quenching can assist in imaging. For quenching endogenous fluorescence, we recommend incubating samples in 100 mM glycine in PBS (pH to 7.4 with 1 M tris base, if required) for 20 minutes at room temperature.

Other treatments such as UV irradiation may also be useful for quenching endogenous fluorescence, however avoid harsh treatments which 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, TR-602-MR 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 this manner or in OCT sectioned by microtone to your desired thickness.

Staining Sections

Incubate samples with 10 - 50 µM TR-602-MR in PBS, pH 7.2-7.6, or appropriate media (1:1,000 - 1:200 dilution of 10 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.

8. Fluorescent Imaging Settings

Epi-Fluorescence Microscopy

TR-602-MR 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 a emission range of 550-650 nm.

Confocal or Two-Photon Microscopy

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

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