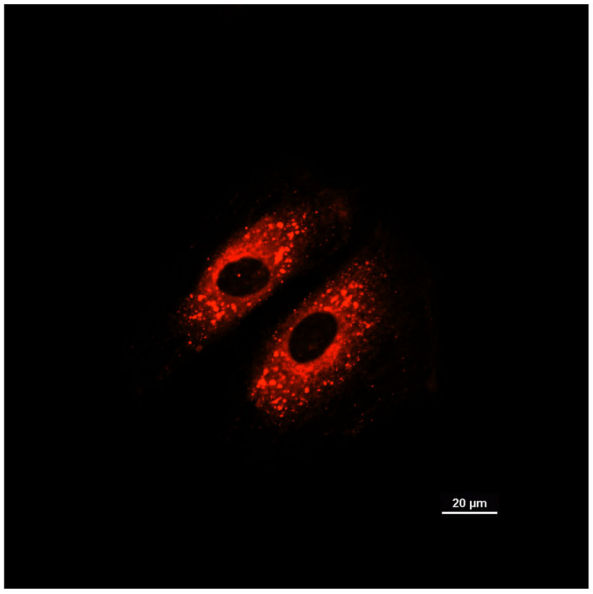
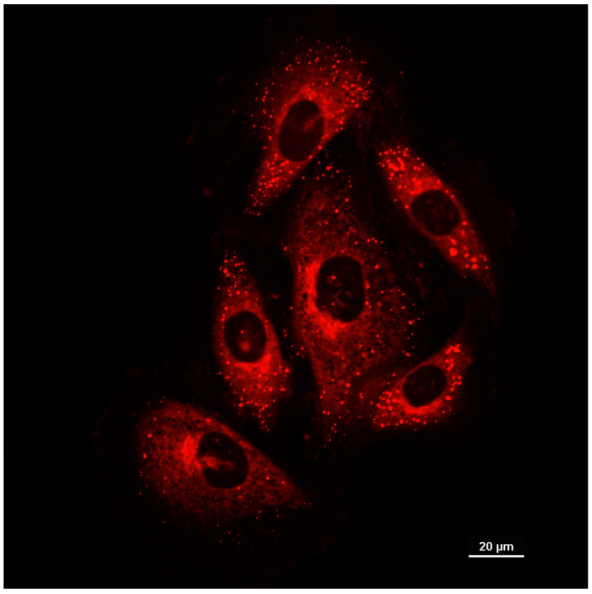
Biosensis P2™ Polar Lipid and Endoplasmic Reticulum Tracing Reagent

Catalogue Number: TR-603-P2





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

1. Intended Use

TR-603-P2 is a cell permeant stain for labelling intracellular lipid droplets and the endoplasmic reticulum. The large Stokes shift (Ex/Em 405/600 nm) provides users with greater flexibility in experimental design, ideal for dual and multi-colour labelling experiments. TR-603-P2 has superior photostability and low cytotoxicity making it suitable for time lapse imaging of live cell. Easy to use with minimal sample preparation and a short staining required.

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2. Specifications

- Simple and quick application
- Suitable for fixed or live cell imaging
- 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
- Stable at room temperature

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

TR-603-P2 will perform as specified if stored at room temperature. and protect from light. Once reconstituted in DMSO, use within 6 months. Refer to the datasheet for further details.

5. Reagent Preparation

Reconstitute the product with 149 µL of DMSO to obtain a 10 mM stock solution. Mix thoroughly before use. Do not reconstitute in aqueous solutions such as phosphate-buffered saline (PBS) or cell culture media. TR-603-P2 should be diluted in an appropriate buffer or cell culture media to a concentration of 10 µM – 20 µM immediately before use (this solution should not be stored for later use).

6. Staining Protocol For Live Cells

Serum-free culture medium is recommended for staining, as the lipids in the serum can reduce staining intensity.

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 serum-free cell culture media containing 10 – 20 µM of TR-603-P2 (1:1,000 – 1:500 dilution of 10 mM stock solution)
4. Incubate cells for 30 minutes under appropriate growth conditions
5. Wash coverslips 3 times for one minute in PBS, pH 7.2-7.6
6. Mount coverslips in aqueous mounting media for imaging

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 – 20 µM of TR-602-LER (1:1,000 – 1:500 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 serum-free 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-603-P2 can be appropriately resolved.
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

7. Fluorescent Imaging Settings

Epi-Fluorescence Microscopy

TR-603-P2 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-603-P2 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-603-P2, 490-670 nm (E_{max} = 600 nm). Alternatively, detect by using an emission filter suited to the detection of red 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.