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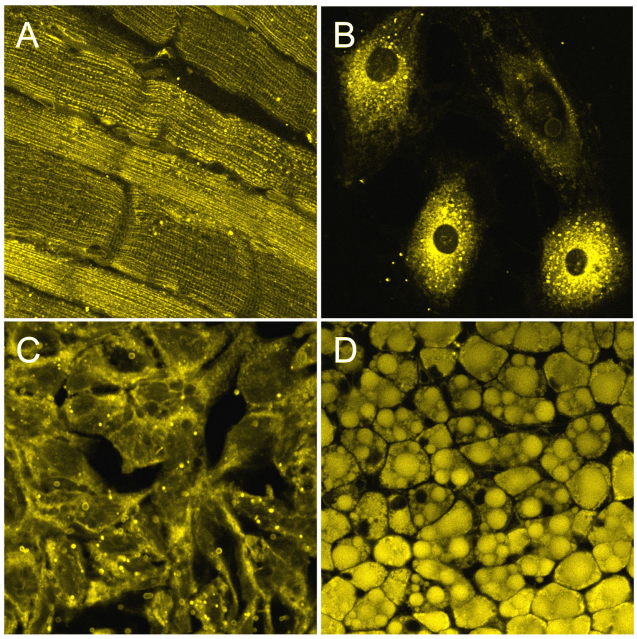
Biosensis P1™ Polar Lipid Tracing Reagent

Catalogue Number: TR-600-P1



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

1. Intended Use

TR-600-P1 localises with polar lipids allowing the labelling of lipid droplets and other high lipid content compartments in a range of cell and tissue types. TR-600-P1 is compatible with live and fixed samples and can be imaged by single photon or multi photon microscopy. TR-600-P1 is also compatible with vibrational spectroscopy as this complex is infrared and Raman active.

Cell penetration and localisation of TR-600-P1 has been confirmed in a range of cell lines, including adipocytes (3T3-L1), prostate cells (PNT2, PNT1a, LNCaP, 22RV1 and DU145), cardiomyocytes (H9c2) and neuronal cells (PC-12), and tissues, including adipose tissue (sheep and Drosophila), muscle tissue (sheep cardiac and skeletal) and brain (murine).

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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 405/550 nm)
- Compatible with other fluorescent dyes
- Ideal for epi-fluorescence, confocal and multiphoton imaging
- Infrared and raman-active
- 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-600-P1 will perform as specified if stored at room temperature and protected from light once reconstituted in DMSO. Use within 6 months of reconstitution in DMSO. Refer to the datasheet for further details.

5. Reagent Preparation

Reconstitute the product with 300 µL of DMSO to obtain a 10 mM stock solution. Mix thoroughly before use. This stock solution can be stored at room temperature, protected from light.
For use, stock solution should be diluted in an appropriate buffer or cell culture media to a concentration of 10-20 µM immediately before use (this solution should not be stored for later use).

Note: It is not recommended that detergents such as Tween20 or supplements with high lipid content such as foetal calf serum are used in preparation of TR-600-P1. Low solubility in aqueous solutions may cause precipitate of dye if used at concentrations higher than those recommended.

6. Staining Protocol For Live Cells

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

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 or serum-free medium containing 10 – 20 µM of TR-600-P1 (1:1,000 – 1:500 dilution of 10 mM stock solution)
4. Incubate cells for 30 minutes under appropriate growth conditions

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-600-P1 (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 or serum-free culture medium
5. Cells can be prepared as wet mounted or adhered 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-600-P1 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 the manufactuer's instructions
4. Following washes, mount in an aqueous mounting media for immediate imaging

7. Staining Protocol For Fixed Cells

Cell Fixation

We recommend to fix samples in 4% paraformaldehyde for 20 minutes at room temperature. Wash samples 3 x 10 minutes in PBS, pH 7.2-7.6.

Staining Fixed Cells

1. Incubate fixed cells in PBS, pH 7.2-7.6, containing 10-20 µM TR-600-P1 for 30 minutes at room temperature.
2. For best results, provide gentle agitation by a platform rocker (or similar) at low rpm.
3. Wash coverslips twice for one minute in PBS, pH 7.2-7.6 with agitation.
4. Mount coverslips in an aqueous mounting media and image immediately for best results.

8. Staining Protocol For Frozen Tissue Sections

Sample preparation

Prepare and mount tissue sections on slides using standard protocols for frozen tissue. Once sectioned, samples should be kept in the dark at room temperature until thawed (~20-30 minutes). Wash samples 3 times for five minutes in PBS, pH 7.2-7.6.

Note: 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.

Staining sections

1. Incubate samples with 10-20 µM TR-600-P1 in PBS, pH 7.2-7.6 (1:1,000 - 1:500 dilution of 10 mM stock solution) for 2 hours at room temperature with gentle agitation provided by a platform rocker (or similar) at low rpm.
2. Wash samples three times for five minutes in PBS, pH 7.2-7.6 at room temperature with agitation.
3. Mount coverslips in an aqueous mounting media and image immediately for best results.

9. Staining Protocol For *Drosophila* Tissue

Sample preparation

1. Dissect Drosophila tissues in PBS, pH 7.2-7.6, with clean tools.
2. Gently adhere tissues to Poly-L-Lysine or Poly-L-ornithine covered coverslips.
3. For fixation of fat body tissues, 2% paraformaldehyde in PBS for 30 minutes on ice followed by 3 x 10 minute washes in PBS, pH 7.2-7.6 at room temperature is recommended.

Tissue staining

Live and fixed tissue can be stained using this protocol.

1. Incubate tissue with 10-20 µM TR-600-P1 in PBS, pH 7.2-7.6 (1:1,000 - 1:500 dilution of 10 mM stock solution) for 30 minutes at room temperature.
2. Wash tissue twice for one minute in PBS, pH 7.2-7.6.
3. Mount coverslips in an aqueous mounting media and image immediately for best results.

Note: Image coupling gels such as cabomer-940 are ideal for tissue mounting as they support tissue integrate (see Rothstein EC *et al.* (2006) J Microsc 222: 58-64. for protocol).

10. Fluorescent Imaging Settings

Epi-Fluorescence Microscopy

TR-600-P1 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-600-P1 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-600-P1, 500-600 nm (E_{max} = 550 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.

Vibrational Spectroscopy

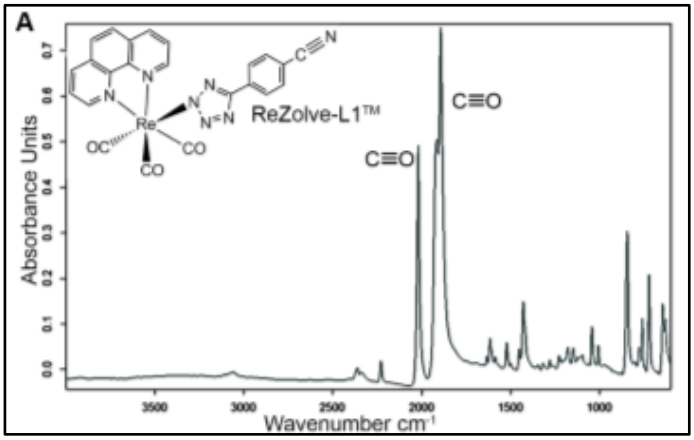
TR-600-P1 can be detected by infrared and Raman spectroscopy methods. Sample preparation will need to be adapted appropriately for these techniques.

Sample preparation

Adherent cell culture can be grown on silicon nitride substrate which are compatible with infrared, or calcium fluoride slides which is compatible with Raman spectroscopy. Fixation with cold methanol is recommended for best preservation of lipids. Following staining, dehydration maybe required as these techniques can be hindered by water content. (Bader CA, *et al.* Mol Biosyst 2016; 12:2064-8).

Infrared Spectroscopy

The FTIR spectrum of solid TR-600-P1 is dominated by the strong Re carbonyl stretching bands centred at ~2027 cm^{-1} and the doublet 1915/1893 cm^{-1}, characteristic of facial tricarbonyl complexes. This is a spectral region where vibrational modes due to biochemical components are conveniently negligible.



Raman spectroscopy

TR-600-P1 excitation can be achieved at 785 nm with a spectra as shown below.