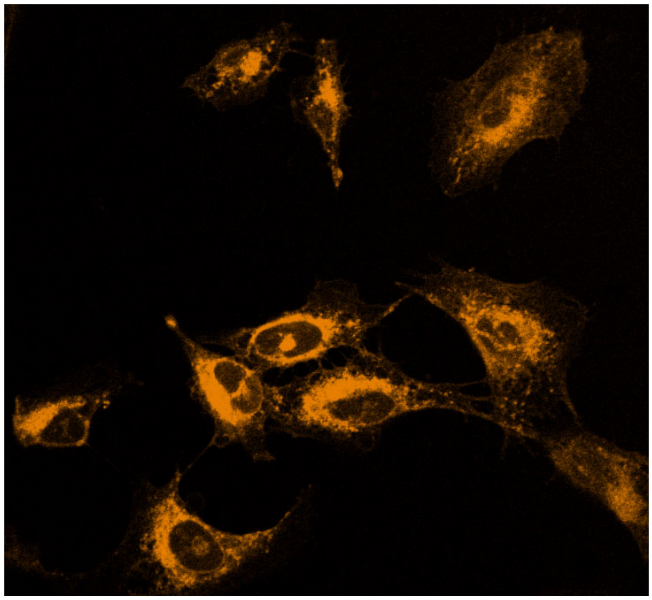
Biosensis ER-O™ Endoplasmic Reticulum Tracing Reagent

Catalogue Number: TR-601-ER1



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

1. Intended Use

TR-601-ER1 labels the endoplasmic reticulum in live and fixed cells. TR-601-ER1 passively diffuses across the plasma membrane into the cell, stains at low concentrations and has minimal cytotoxic effects. It can be used as a real-time imaging reagent which can be imaged within minutes of addition and has minimal photobleaching. TR-601-ER1 is easily washed from cells, and therefore is ideal for protocols which require intermittent monitoring of endoplasmic reticulum structures.

TR-601-ER1 has been successfully imaged using epifluorescent microscopy, confocal microscopy and two-photon microscopy. Cell penetration and localisation of TR-601-ER1 has been confirmed in a range of cell lines, including prostate cells (PNT2, PNT1a, LNCaP, 22RV1 and DU145), cardiomyocytes (H9c2) and neuronal cells (PC-12).

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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 405/570 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-601-ER1 will perform as specified if stored at room temperature and protected from light. Once reconstituted in DMSO use within 6 months. 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.

Note: TR-601-ER1 should not be reconstituted in aqueous solutions such as phosphate-buffered saline (PBS) or cell culture media. For use, TR-601-ER1 should be diluted in a buffer or cell culture media to a concentration of 50 µM -100 µM immediately before use (this solution should not be stored for later use).

6. Staining Protocol For Live Cells

Staining

For adherent cells, remove the medium from the culture dish and replace it with media containing 50-100 µM of TR-601-ER1. The optimal staining concentrations of TR-601-ER1 may vary between cell lines.

Imaging

TR-601-ER1 can be observed in cells within minutes following addition. For the brightest staining allow cells to incubate with TR-601-ER1 for 15 minutes prior to imaging. Do not wash cells. Maintain TR-601-ER1 in media for the duration of the imaging protocol.

Removal of TR-601-ER1

To remove TR-601-ER1 from cells, aspirate the TR-601-ER1 containing media, briefly wash cells with PBS, pH 7.2-7.6. Replace this with cell culture media which does not contain TR-601-ER1. Some cells may require several wash steps.

Co-Staining Experiments

Prior to co-staining experiments, make sure that the spectral profiles of counterstaining agent and TR-601-ER1 can be appropriately resolved. Stain cells with counter-staining agent according to manufacturer's instructions. Following washes, add TR-601-ER1 and stain cells as described above for image.

7. Staining Protocol For Fixed Cells

Unlike the conventional endoplasmic reticulum stains, cells fixed with 4% paraformaldehyde have been successfully stained with TR-601-ER1. Other fixation methods have not been attempted to date.

Cell Fixation

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

Incubate fixed cells with 50-100 µM TR-601-ER1 prepared in PBS, pH 7.2-7.6 for 15 minutes at room temperature.

Imaging

Mount coverslips on cells in TR-601-ER1 solution for imaging, provide gentle agitation by a platform rocker (or similar) at low rpm.

8. Fluorescent Imaging Settings

Epi-Fluorescence Microscopy

TR-601-ER1 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-601-ER1 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-601-ER1, 500-600 nm (E_{max} = 570 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.