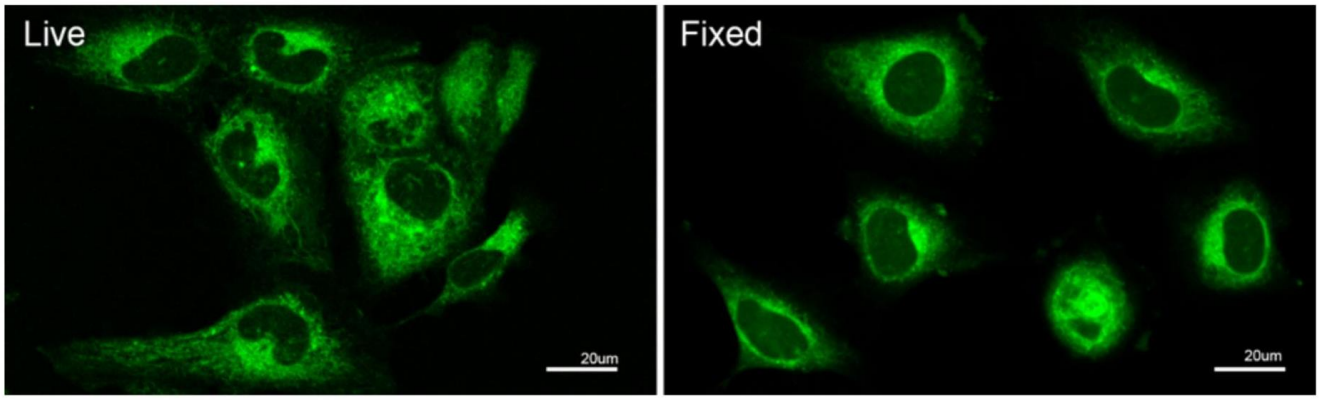
Biosensis® LipoFluor-ER1™ Ready-to-Dilute™, Endoplasmic Reticulum Staining Reagent

Catalog Number: Lipofluor™ TR-601-ER1



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

1. Intended Use

The Endoplasmic Reticulum (ER) is an essential organelle that maintains lipid metabolism and homeostasis. It performs various functions like synthesizing membranes and secreting proteins, protein folding, calcium storage, and lipid biogenesis. The ER is a crucial point for synthesizing proteins and lipids, making its function vital for maintaining protein and lipid balance. Changes in lipid metabolism can affect the cell's response to protein-induced stress, highlighting the interaction between lipid metabolism and protein quality. The ER also forms physical contact sites with other organelles like mitochondria, peroxisomes, and lipid droplets, which is crucial for lipid homeostasis. Changes in lipid homeostasis can lead to ER dysfunction and the development of metabolic pathologies like insulin resistance and fatty liver disease. Understanding the dynamic relationship between lipid homeostasis and proteostasis in the ER may provide insights into treating diseases associated with altered lipid biosynthesis.

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 that require intermittent monitoring of endoplasmic reticulum structures.

Tested Applications: Live or fixed cell cultures and fixed tissues or unfixed tissues.

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

2. Materials Provided

Four 0.5 mL microfuge vials containing
5.5 uL/vial of 50 mM stock solution of Lipofluor™ TR-601-ER1 in DMSO. 164.1 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-601-ER1 are 10µM-20µM.

3. Specifications

CAS Number: 1404104-40-0; FW: 596.57
MF: $C_{21}H_{12}N_7O_3Re$
Ex/Em: 405 nm/ 570 nm: Form: Liquid:
Working concentrations: 10µM-50µM

4. Precautions for Use

It is not recommended that detergents such as Tween20 or supplements with high lipid content, such as fetal calf serum, be used to prepare these reagents as the lipid in the solution will bind the dye, reducing the cell signal. Low solubility in aqueous solutions may cause dye precipitate if used at concentrations higher than recommended. Before performing the staining procedure for fixed or live cell imaging, please read the entire procedure and consider the safety data sheet. TR-601-ER1 should be diluted in an appropriate buffer or cell culture media to a concentration of 10µM-50µM immediately before

use. Diluted solutions are unstable, and the diluted solution should not be stored for later use. For laboratory use only. Not fully tested. Not for drug, household, human, or veterinary uses.

5. Reagent Preparation

Note: Lipofluor™ TR-600-P1 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 50 µM, 5.445 mL working solution, follow these steps for each 0.5 mL vial:

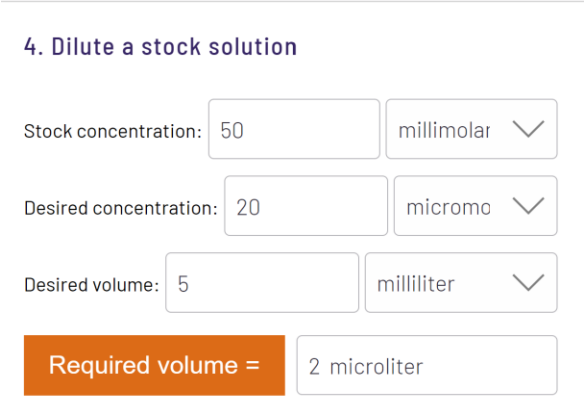
a) Take a 0.5 mL vial containing 5.5 µL of LipoFlour™ TR-601-ER1 in DMSO liquid.

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™ TR-601-ER1 solution at 50 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, suppose you need to make 5 milliliters of a 20 µM solution using a 50 mM stock solution. In that case, you can use an online calculator (see figure below) or solve the Cformula manually. In this case, C1 is 50 mM, C2 is 20 µM, and V2 is 5 mL. Solving for V1, you get V1=C2V2/C1, which means adding 2.0 µL of the stock solution to 4.998 µL of solvent to make ~5 mL of a 20 µM solution.

Calculating Molar Dilutions:
For example, this online source: https://bit.ly/3IeWFbR



6. Storage Conditions

Lipofluor-ER1™ TR-601-ER1 stock 50 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

Remove the medium from the culture dish or growing well and replace it with media containing 50-100 µM of TR-601-ER1 for adherent cells. The optimal staining concentrations of TR-601-ER1 may vary between cell lines. The stain can be observed in cells within minutes following addition. For the brightest staining, allow cells to incubate with Lipofluor-ER1™ for 15 minutes before imaging. Do not wash cells and maintain Lipofluor-ER1™ in media for the duration of the imaging protocol.

Imaging

Excite with laser or light at wavelengths of ~405 nm. 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 before 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 LIVE cells, aspirate the TR-601-ER1 containing media and 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

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

8. Staining Protocol For Fixed Cells Cell Fixation

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 and provide gentle agitation by a platform rocker (or similar) at low rpm.

9. Staining Protocol For Tissue Sections

Unlike the conventional endoplasmic reticulum stains, paraformaldehyde-fixed, fresh, and fresh-frozen tissue sections have been successfully stained with Lipofluor™ TR-601-ER1. 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™ TR-600-ER1 can stain tissue sections of up to 2 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 50 - 100 µM Lipofluor™ TR-601-ER1 in PBS, pH 7.2-7.6, or appropriate media (1:500-1:000 dilution of 50 mM stock solution) for 30 minutes at room temperature with gentle agitation provided by a platform rocker (or similar) at low rpm. Wash samples briefly 2 times for 1-2 minutes in PBS, pH 7.2-7.6, at room temperature with agitation. Note the timing and number of washing steps must be optimized. If overdone, the Lipofluor-ER1 will be washed out. Mount tissue in aqueous mounting media and image immediately for best results. Optimization of concentration and incubation times will need to be determined for best results.

10. 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 an 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, an image with a spectral detector should be set for the emission of TR-601-ER1, 500-600 nm (E_{max} = 570 nm). Alternatively, detect by using an emission filter suited to 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.

Lipofluor-ER1™ matches leading competitor material BUT also works with both live and fixed cells unlike its leading competitor.