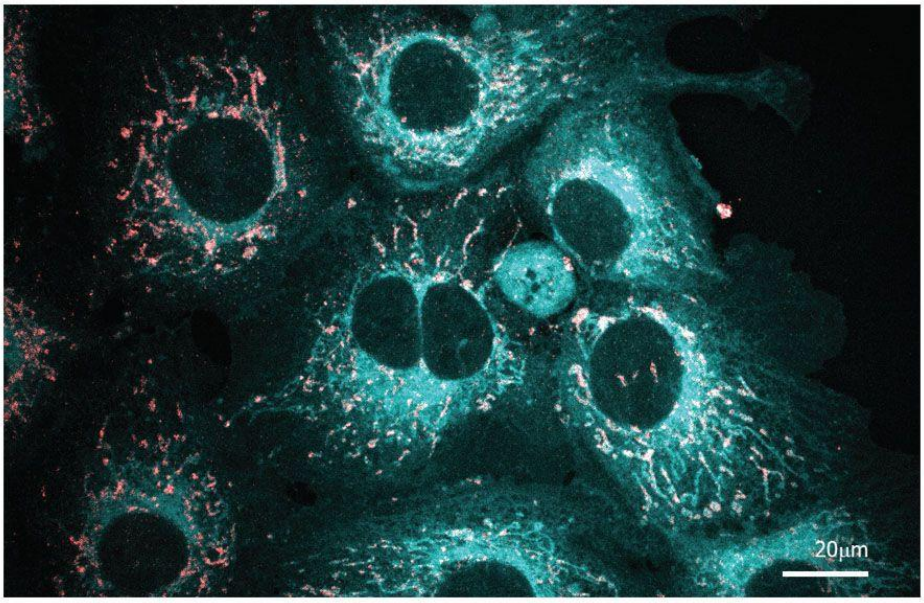
Biosensis® LipoFluor-ER2™ Ready-to-Dilute™, Endoplasmic Reticulum Blue Tracing Reagent

Catalog Number: Lipofluor™ TR-605-ER2



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.

Lipofluor™ TR-605-P2 localizes to the endoplasmic reticulum in live and fixed cells. It is highly cell-permeable, producing quick and reliable cell staining. TR-605-ER2 can detect the endoplasmic reticulum following antibody staining protocols and is compatible with various fluorescence applications. The dye, however, cannot be fixed within the cells with post fixation methods.

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

Advantages:

- Compatible with antibody staining
- Compatible with other dyes
- Simple and quick application
- Suitable for live or fixed cell imaging
- Low cytotoxicity
- Highly resistant to photobleaching
- Ideal for epi-fluorescence and confocal microscopy
- Stable at room temperature

2. Materials Provided

Four 0.5 mL microfuge vials containing
5.5 uL/vial of 50 mM stock solution of Lipofluor™ TR-605-ER2 in DMSO.
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™ TR-605-ER2 are 10µM-50µM.

3. Specifications

CAS Number: 376367-93-0; FW: 694.6

MF: $C_{28}H_{16}F_4IrN_3O_2$

Ex/Em: 405 nm/ 500 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-605-ER2 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-605-ER2 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:

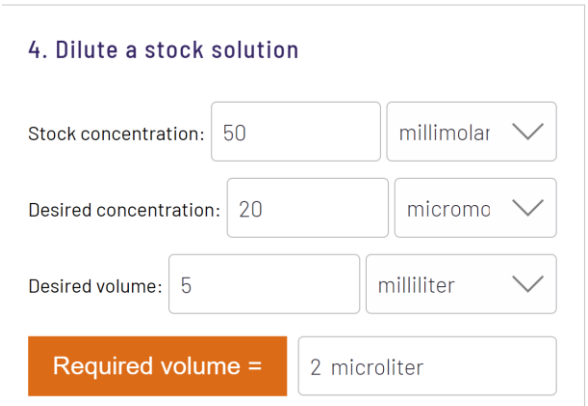
a) Take a 0.5 mL vial containing 5.5 µL of LipoFlour™ TR-605-ER2 in DMSO liquid.

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-605-ER2 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 4.98 µL of solvent to make ~5 mL of a 50 µM solution.

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



6. Storage Conditions

Lipofluor™ TR-605-ER2 stock 10 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

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

Staining Protocol for Live Cells

For Adherent Cells

1. Grow cells in live cell imaging chambers, with appropriate cell growth medium, and under appropriate growth conditions, to desired confluency (70 – 80%)
2. Remove the culture medium and add 10 – 50 µM of Lipofluor-ER2™ in pre-warmed PBS or growth medium (1:1000 – 1:2000 dilution of 50mM stock solution).
3. Incubate cells for 30 minutes under appropriate growth conditions.
4. Wash cells 2 times in PBS for 5 minutes per wash.
5. Replace with fresh growth media for imaging. For best results use phenol red free growth media.

For Suspended Cells

1. Take cells grown in suspension and centrifuge to obtain cell pellet. Remove the supernatant.
2. Resuspend cells 10 – 50 µM of TR-605-ER2 in pre-warmed PBS or growth medium (1:1000 – 1:2000 dilution of 50 mM stock solution).
3. Incubate cells for 30 minutes under appropriate growth conditions.
4. Re-pellet the cells by centrifugation and resuspend in PBS and allow to wash for 5 minutes.
5. Re-pellet the cells by centrifugation and resuspend in growth media for imaging.
6. Cells can be prepared as a wet mount or adhere cells to poly-L-lysine coated coverslips for imaging. For best results use phenol red free growth media.

For Co-Staining Experiment

1. Prior to co-staining, make sure that the spectral profiles of counter-staining agent and TR-605-ER2 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 replace PBS with phenol red free growth media for imaging

Staining Protocols for Fixed Cells

For Adherent and Non-Adherent Cells

1. For adherent cells: grow cells on glass coverslips in appropriate growth media to desired confluence (70 - 80%). For non-adherent cells: grow cells in suspension and adhere to poly-L-lysine treated coverslips following fixation and washing (steps 2 and 3).
2. Fix cells in pre-warmed 4% paraformaldehyde at room temperature for 10 to 20 minutes.
3. Wash cells 3 times in PBS for 5 minutes per wash.
4. Incubated fixed cells with 10 – 50 µM TR-605-ER2, prepared in PBS, for 30 minutes at room temperature.
5. Wash coverslips twice for 5 minutes in PBS.
6. Mount coverslips in an aqueous mounting media and image immediately for best results.

For Antibody Staining Experiment

1. Grow and fix cells as above (steps 1-3).
2. Following fixation, perform antibody staining according to standard protocols. Ensure the secondary antibody chosen is compatible with TR-605-ER2™ (e.g. does not excite with 400 nm excitation).
3. Upon completion of antibody staining incubate cells with 10 – 50 µM prepared in PBS, for 30 minutes at room temperature. Protect coverslips from light to reduce damage to secondary antibody.
4. Wash coverslips twice for 5 minutes in PBS. Protect coverslips from light to reduce damage to secondary antibody.
5. Mount coverslips in an aqueous mounting media and image immediately for best results.

8. Staining Protocol For Tissue Sections

Fresh or Frozen sections:
Prepare and mount tissue sections on slides using standard frozen/fresh tissue protocols.
Frozen samples to be stained should be kept in the dark at room temperature for approximately 20-30 minutes until thawed, then washed 3x 5 minutes with PBS to help remove blood and debris that could cause background.

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-605-ER2 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 OCT sectioned by microtone to your desired thickness.

Staining Sections

Incubate samples with 10 - 50 µM Lipofluor™ TR-605-ER2 in PBS, pH 7.2-7.6, or appropriate media for 1.5 hours 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. Optimization of concentration and incubation times will need to be determined for best results.

9. Fluorescent Imaging Settings

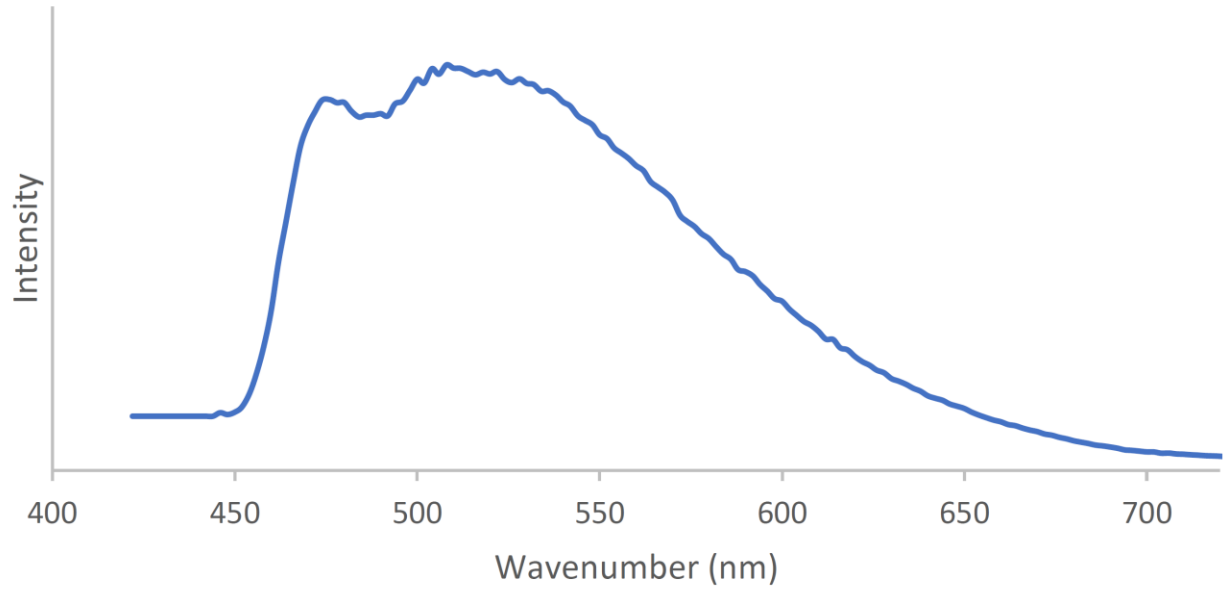
Epi-Fluorescence Microscopy

TR-605-ER2 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 450-570 nm.

Confocal or Two-Photon Microscopy

TR-605-ER2 can be excited by a 400 nm steady state laser, and emission should be collected using a detector suited to blue fluorophores such as DAPI. Alternatively, a spectral detector set for

the emission of TR-605-ER2 at 450 - 570 nm (Emax = 500nm) can be used.



Emissoin profile of Lipofluor-ER2™ with 403nm excitation