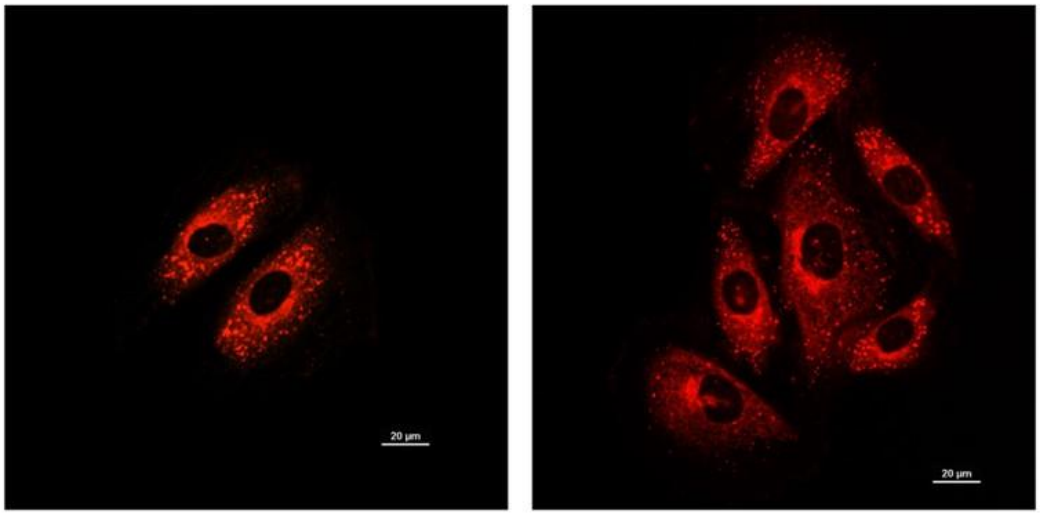
Biosensis® LipoFluor-P2™ Ready-to-Dilute™, Polar Lipid Droplets and Endoplasmic Reticulum Tracing` Reagent

Catalog Number: Lipofluor™ TR-603-P2



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

1. Intended Use

Lipid droplets are cell organelles that play a crucial role in maintaining lipid and membrane balance. They help to protect the cell's integrity and function during times of stress by performing various tasks such as sequestering potentially toxic lipids and proteins, maintaining energy and redox balance, preserving membrane and organelle homeostasis, and modulating autophagy. Abnormal lipid droplet dynamics have been linked to the pathophysiology of numerous metabolic disorders, including obesity, diabetes, atherosclerosis, fatty liver, and even cancer.

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

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

- 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

2. Materials Provided

Four 0.5 mL microfuge vials containing
5.5 uL/vial of 10 mM stock solution of Lipofluor™ TR-603-P2 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-603-P2 are 10µM-20µM.

3. Specifications

CAS Number: 2169684-98-2; FW: 671.74
MF: $C_{29}H_{19}IrN_8$
Ex/Em: 405 nm/ 600 nm: Form: Liquid:
Working concentrations: 10µM-20µM

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-603-P2 should be diluted in an appropriate buffer or cell culture media to a concentration of 10µM-20µM 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-603-P2 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 10 µM, 5.445 mL working solution, follow these steps for each 0.5 mL vial:

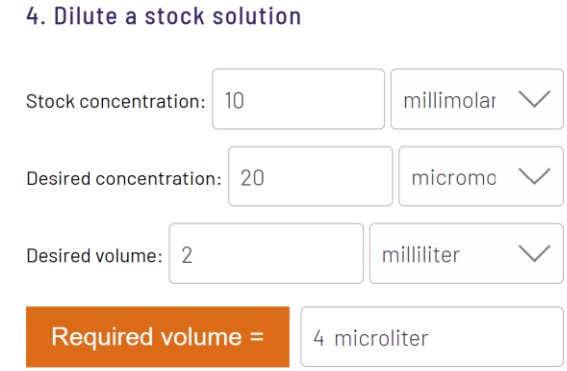
a) Take a 0.5 mL vial containing 5.5 µL of LipoFlour™ TR-603-P2 in DMSO liquid.

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-603-P2 solution at 10 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 2 milliliters of a 20 µM solution using a 10 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 10 mM, C2 is 20 µM, and V2 is 2 mL. Solving for V1, you get V1=C2V2/C1, which means adding 4.0 µL of the stock solution to 1.96 µL of solvent to make ~2 mL of a 20 µM solution.

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



6. Storage Conditions

Lipofluor™ TR-603-P2 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.

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 the 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.

8. 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-603-P2 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.

9. 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-603-P2 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 - 20 µM Lipofluor™ TR-603-P2 in PBS, pH 7.2-7.6, or appropriate media (1:500-1:000 dilution of 10 mM stock solution) 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.

10. 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 an 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 detecting 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 microsecond