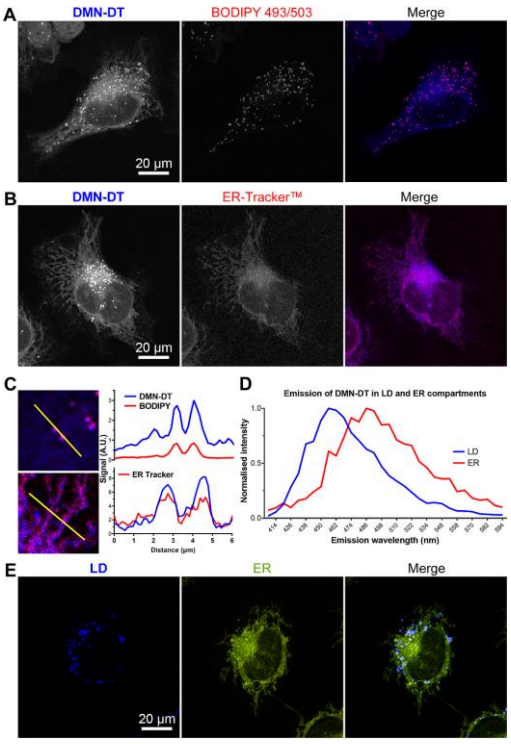
Biosensis® LipoFluor- DMN-DT™ Ready-to-Dilute™, Lipid Droplets & ER positive vesicle Tracing Reagent

Catalog Number: TR-607-DT



Tested Applications: **Tested Applications**: Live cell culture and fixed brain tissue.

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

1. Intended Use

LipoFluor- DMN-DT ™ is a new fluorescent imaging agent that can discern both LDs (lipid droplets) and ER (endoplasmic reticulum) compartments using a single excitation wavelength. LipoFluor- DMN-DT ™ is useful for imaging and studying the microenvironments within biological systems, as its emission wavelength directly relates to the polarity of its environment.

DMN-DT can track the biogenesis of LDs (which originates at the ER) and their subsequent dynamic movements, both within cells for a range of cell types and between adjacent cells (as demonstrated in 22Rv1 cells). These actions can be visualised in real time given both regions of the cell emit at different wavelengths after treatment with LipoFluor- DMN-DT ™.

LipoFluor- DMN-DT ™ is non-toxic (up to 100 µM) with only a low concentration (10 µM) required for confocal imaging experiments. LipoFluor- DMN-DT ™ does not induce LD formation and has been used in a range of cell and tissue types.

Highlights*

Simultaneous imaging of lipid droplets and endoplasmic reticulum in live cells.

LipoFluor- DMN-DT ™ facilitates organelle-specific emission maxima

- Captures lipid droplet biogenesis and intercellular trafficking.
- Demonstrated myelin lipid staining in brain tissue.

* Shane M. Hickey, Ian R.D. Johnson, Elena Dallerba, Mark J. Hackett, Massimiliano Massi, Joanna Lazniewska, Lauren A. Thurgood, Frederick M. Pfeffer, Douglas A. Brooks, Trent D. Ashton, A fluorescent and solvatochromic 1,8-naphthalimide probe for detection of lipid droplet trafficking and biogenesis, *Dyes and Pigments*, Volume 217, 2023, 111382, ISSN 0143-7208, https://doi.org/10.1016/j.dyepig.2023.111382.

Tested Applications: Live cell culture and fixed brain tissue. Validated Cell lines: LipoFluor-DMN-DT ™ to stain multiple cell lines, a range of malignant (22Rv1, LNCaP, PC3, and HCT-8) and non-malignant (PNT1a and PNT2) cell types. Other cell lines are expected to stain as well when alive.

Excitation/Emission: *λ*ex 403 nm. For regions of LD staining, the emission maximum was centered at 456 nm (blue light), while this was red-shifted for the ER (*λ*em 486 nm), a more (blue-green) light emission.

2. Materials Provided

Four 0.5 mL microfuge vials containing
5.5 uL/vial of 10 mM stock solution of Lipofluor™ TR-607-DT in DMSO.

Each vial can be diluted 1000X depending upon the final working concentration of the dye in the experiment. Typical working concentrations for Lipofluor™ TR-607-DT are 10 µM to a maximum of 100 µM. This concentration was suitable for live cell imaging. It yielded high-quality images of lipid droplets with no evidence of toxicity, demonstrating the efficacy of DMN-DT at this concentration for visualizing lipid droplets in cellular contexts.

3. Specifications

CAS Number: N/A FW: 454.12

MF: $C_{23}H_{22}N_2O_6S$

Ex: 403nm: Form: Liquid: Em: 456 nm (blue light), while this is red-shifted for the ER (*λ*em 486 nm), a blue-green light.

Working concentrations: 10-100 µM, preferred 10 µM for most applications.

4. Precautions for Use

Before performing the staining procedure for fixed or live cell imaging, please read the entire procedure and consider the safety data sheet. This is for laboratory use only. It has not been fully tested. It is not for drug, household, human, or veterinary use. The LipoFluor™ Dyes are NOT compatible with glycerol-based mounting media. PBS, water, or a phenol-red free media base are recommended for living OR fixed cell preparations when imaging.

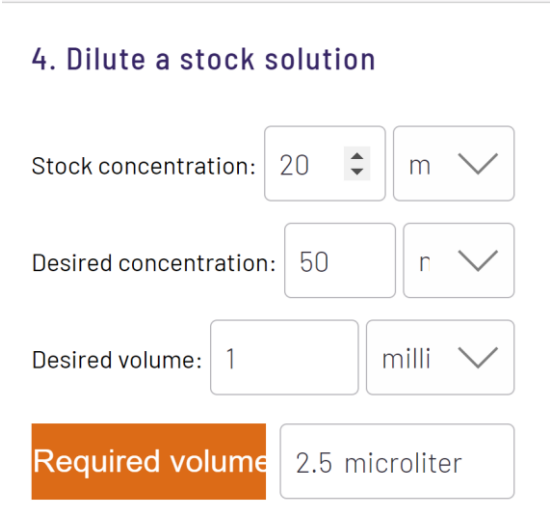
5. Reagent Preparation

LipoFluor-DMN-DT™ TR-607-DT 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, 5.5 mL working solution, follow these steps for each 0.5 mL vial :

Perform the first of two dilutions:
First, perform a 1:10 (add 49.5 ul of PBS to the 5 5 µL vial of dye = 55 µL total volume).
Second: Complete a further 1:100 dilution (5.445 mL of PBS to the 55xµL) to give a final working concentration of 0.01mM (10 µM)

a) 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, if you need to make 1 milliliter of a 50 µM solution using a 20 mM stock solution, you can use an online calculator or solve the formula manually. In this case, C1 is 20 mM, C2 is 50 µM, and V2 is 1 mL. Solving for V1, you get V1=C2V2/C1, which means adding 2.5 µL of the stock solution to 998 µL of solvent to make ~1 mL of a 50 µM solution.



b) Useful on-link calculators: https://bit.ly/3IeWFbR

6. Storage Conditions

Lipofluor™ TR-607-DT 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

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 cell culture media containing 10 µM of TR-607-DT (1:1000 dilution of 10 mM stock solution)
4. Incubate cells for 10 minutes under appropriate growth conditions
5. Wash coverslips twice for one minute in PBS, pH 7.2-7.6
6. Mount coverslips in PBS or phenol-red-free media base for imaging

For Suspended Living 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 µM of LipoFluor- DMN-DT™ TR-607-DT (1000X dilution of 10 mM stock solution)
3. Incubate cells for 10 minutes under appropriate growth conditions
4. Re-pellet the cells by centrifugation and resuspend in PBS, pH 7.2-7.6, or cell culture medium
5. Cells can be prepared as a wet mounted or adhere to poly-L-lysine coated coverslips and mounted in a PBS or phenol-red-free media base for immediate imaging

For Co-Staining Live cell Experiment

1. Before co-staining, ensure the spectral profiles of the counter-staining agent and LipoFluor- DMN-DT™ TR-607-DT can be appropriately resolved. Generally, dyes that do not excite with 403-05 nm excitation can be imaged alongside LipoFluor- DMN-DT. Blue dyes such as DAPI are compatible as they emit at about 461nm, which is reasonably close to 456 of DMN-DT, but in a different cellular location; however, the actual color will resemble that of DMN-DT too.
2. Stain cells as described above with a reduced washing step to 30 seconds following incubation
3. Stain cells with a counter-staining agent according to the manufacturer's instructions
4. Following washes, mount in a PBS or phenol-red-free media base for imaging

Fixed tissues: Lipofluor-DMN-DT™ has been demonstrated to effectively stain myelin in fixed mouse brain tissue (Hickey, SM et al., 2023). The provided protocol serves as an example of DMN-DT's application in fixed tissues. While other tissues or fixation methods remain untested, Biosensis believes that with end-user optimization, successful use in fixed cells or tissues should be achievable.

Protocol for Imaging Fixed Mouse Brain Sections Using Nikon A1+ Confocal Microscope

Adapted from: Shane M. Hickey, Ian R.D. Johnson, Elena Dallerba, Mark J. Hackett, Massimiliano Massi, Joanna Lazniewska, Lauren A. Thurgood, Frederick M. Pfeffer, Douglas A. Brooks, Trent D. Ashton

"A fluorescent and solvatochromic 1,8-naphthalimide probe for detection of lipid droplet trafficking and biogenesis,"

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(https://www.sciencedirect.com/science/article/pii/S014372082300308X)

Materials Needed:

- Mouse brain tissue
- Phosphate-buffered saline (PBS)
- 4% Paraformaldehyde (PFA) in PBS
- 15% and 30% sucrose solutions in PBS
- Supercharge+ slides
- PAP pen
- Slide rack
- 10 µM DMN-DT solution in PBS
- #1.5 coverslip
- Double-sided tape
- Nikon A1+ confocal microscope

Procedure:

1. **Dissection and Fixation:**
    - Dissect the mouse brain and wash it in PBS.
    - Fix the tissue in 4% paraformaldehyde (PFA) in PBS for 48 hours at 4°C with gentle agitation.
    - Wash the tissue three times for 5 minutes each in ice-cold PBS.
2. Sucrose Cryoprotection:
    - Place the tissue in 15% sucrose in PBS for 6 hours at 4°C with gentle agitation.
    - Transfer the tissue to 30% sucrose in PBS overnight at 4°C with gentle agitation.
3. Freezing and Storage**:**
    - Freeze the tissue at −65°C on a cold plate.
    - Store the tissue at −80°C until ready for sectioning.
4. Sectioning:
    - Cut tissue sections at 4 µm thickness.
    - Place sections on Supercharge+ slides.
5. Preparation for Imaging:
    - Thaw the tissue slide at room temperature for 5 minutes.
    - Draw a boundary around the tissue section using a PAP pen.
    - Place the slides in a slide rack.
    - Gently wash the slides three times for 3 minutes each to remove OCT and remaining sucrose.
6. Staining:
    - Dilute 100 µL of 10 µM DMN-DT in PBS.
    - Add the diluted DMN-DT solution to the tissue section.
    - Attach a #1.5 coverslip using double-sided tape on each side of the sample.
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7. Imaging on Nikon A1+ Confocal Microscope:

    Mouse brain sections were imaged using a Nikon A1+ confocal microscope at 2048 px resolution with a Galvano scanner. The lenses used included a Plan Apo λ 10× (0.45 aperture), a Plan Apo VC 20× DIC N2 (0.75 aperture), and a Plan Apo λ 60× Oil (1.4 aperture). Pinhole sizes ranged from 14.05 µm to

29.37 µm, with scanner zoom levels between 1.45× and 2×, and 2× line averaging. DMN-DT excitation was consistently at 403.4 nm, with laser power settings of 3.0%, 4.0%, and 6.0%, and PMT hV settings of 90, 100, and 110, respectively.

Fluorescent properties of DMT-DT.

Adapted from S.M. Hickey et al. Figure 1

When live HeLa cells are incubated with DMN-DT (10 µM) and excited at 403 nm, confocal microscopy reveals rapid dye uptake within 30 seconds. The dye exhibits bright punctate fluorescence in the 425-475 nm detection window and less intense reticular staining in the 500-550 nm window. Co-localization experiments confirm that DMN-DT accumulates in LDs, as evidenced by overlapping fluorescence in punctate regions with proven lipid droplet dyes and high Manders' and Pearson coefficient values (0.65 and 0.61, respectively).

Additionally, DMN-DT illuminates the endoplasmic reticulum (ER) of HeLa cells, shown by strong co-localization with an ER-specific marker, with Manders' and Pearson coefficient values of 0.78 and 0.81, respectively.

The emission profiles recorded from both compartments show an emission maximum at 456 nm for LDs and a red-shifted maximum at 486 nm for the ER, reflecting the relative lipophilic nature of these organelles.

Spectral unmixing demonstrates that DMN-DT can simultaneously stain both LD and ER regions in live cells using a single excitation light source. This dual fluorescent property makes DMN-DT a valuable tool for identifying and studying lipid droplets and the endoplasmic reticulum in live cells.

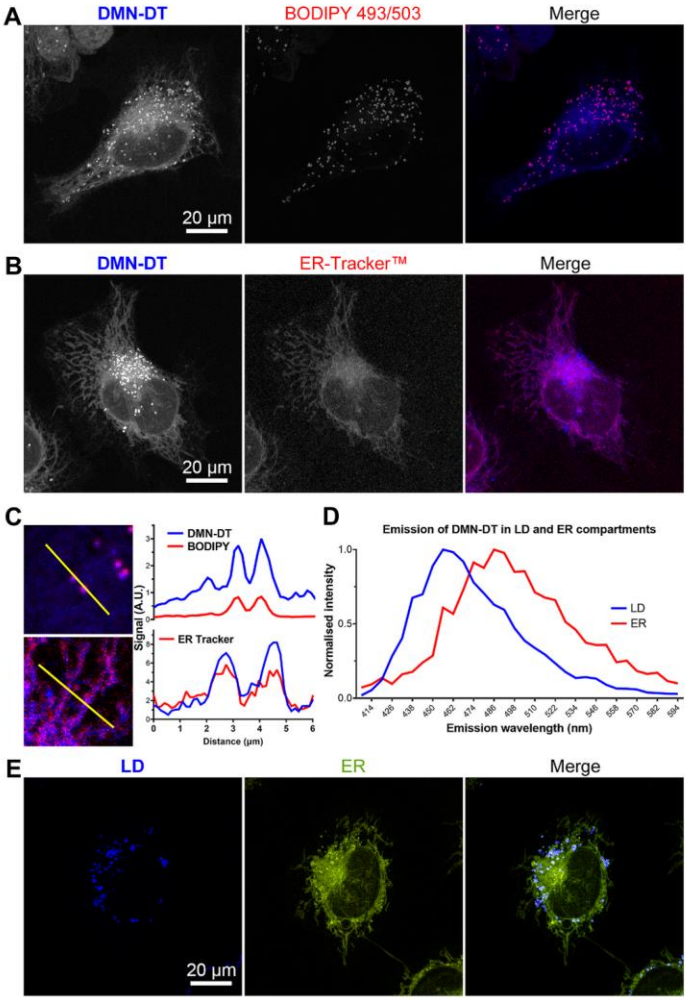

Fig. 1. Representative micrographs of BODIPY 493/503 and ER-Tracker™ co-stain experiment with **DMN-DT** on live HeLa cells. A) From left to right; 2 µM **DMN-DT** was excited at 403 nm with 2.0% laser power and a detector PMT HV gain of 110, BODIPY 493/503 (5 µM) was excited at 488 nm with 0.3% laser power and a detector PMT HV gain of 25. Overlapping signal is indicated in the merged micrograph by magenta colour. Scale bar = 20 µm. B) From left to right; 2 µM **DMN-DT** was excited at 403 nm with 2.0% laser power and a detector PMT HV gain of 110, ER-Tracker™ (1 µM) was excited at 561 nm with 3.0% laser power and a detector PMT HV gain of 70. Overlapping signal is indicated in the merged micrograph by magenta colour. Scale bar = 20 µm. C) Line profiles (indicated by yellow line) of signal intensity taken from a single frame of a z-stack from a subsection of images A (upper panel) and B (lower panel). D) Normalised emission intensity spectra of **DMN-DT** localised in LDs (blue line) and ER (red line). E) Maximum-intensity confocal micrograph from spectral-unmixing of **DMN-DT** fluorescence, displaying LD (blue), ER (green) and merged signal. Scale bar = 20 µm.