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FRONTIERS IN LIFE SCIENCE

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Tokyo 113-0033, Japan

LipiDye™ II, DMSO Solution

Catalog NO. FDV-0027S

Research use only, not for human or animal therapeutic or diagnostic use.

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Product Background

Lipid droplets (LDs) are organelles that have unique phospholipid monolayer and store neutral lipids such as triglycerides and sterol esters (Figure 1 left). LDs are historically found in adipose tissue and considered as sites for energy storage or lipid turnover. Recent studies discovered that LDs are not only in adipocytes, but also found ubiquitously in cells from yeast to mammalian cells. The numbers, size and composition of LDs largely differ depending on cell types or even within the same cell. For example, adipocytes usually have large LD structures (>10 μm), which can be observed by optical microscopy. On the other hand, non-adipocytes have a much smaller LD structure compared with adipocytes (Figure 1 right). LDs are produced from the endoplasmic reticulum (ER), exported to the cytoplasm and expand via fusion of LDs or incorporation of additionally synthesized neutral lipids. LDs contact with various organelles, including ER, mitochondria, lysosomes, nucleus and shows dynamic movement inside the cells. To observe the dynamic movement of LDs in live cells and investigate the physiological functions of LDs, a specific LD dye compatible with long-term live cell imaging is required.

Conventional fluorescent dyes for LDs such as Nile Red contribute to elucidate biological functions of LDs, but its sensitivity and selectivity are limited to detect relatively large LDs in adipocytes or cells treated with excess lipids. It is challenging for conventional dyes to detect small LDs often founds in non-adipocytes under live cell conditions. Funakoshi provides a green fluorescent dye LipiDye™ (catalog no. #FDV-0010), which shows high sensitivity and selectivity for LDs and can detect approximately 1 μm sizes of LDs. Although LipiDye™ is a powerful tool to monitor small LDs in non-adipocytes, LipiDye™ requires 405 nm excitation and has insufficient photostability, not suitable for long-term live cell imaging to observe dynamic LDs synthesis, movement or degradation. Here, **LipiDye™ II**, an upgrade version of LipiDye™, can be excited by less toxic 450-480 nm light and exhibits super-photostability. LipiDye™ II (original compound name LAQ1 in Ref.1) is very suitable for long-term live cell imaging, including Z-stack time-lapse imaging with multiple time excitations for short-term intervals. For example, LipiDye™ II was applied in long-term imaging for drug-induced LD-degradation processes for 12 hours with 3,000 image captures and visualized lipolysis of LDs and de novo synthesis of very small (<1 μm) LDs. Furthermore, LipiDye™ II is compatible with STED microscopy and enables detecting less than 500 nm LDs in HeLa cells.

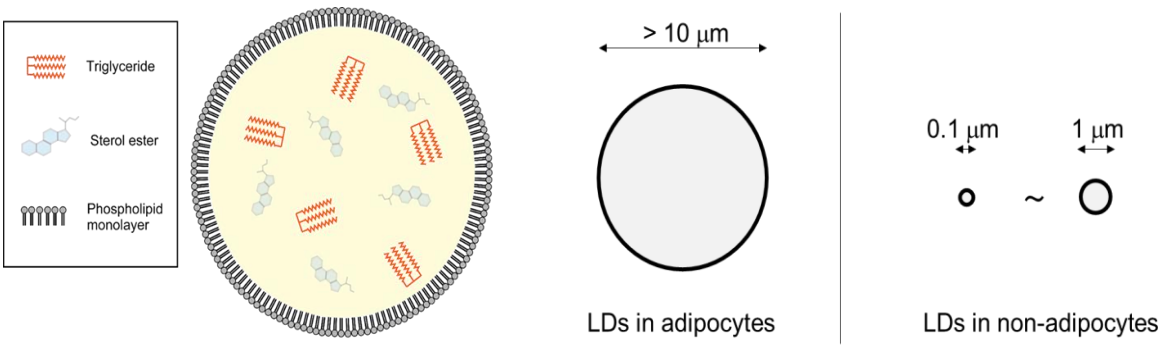


Figure 1. Overview of LDs

Left: Structural image and major composition of the LD, Right: Typical size of adipocytes and non-adipocytes

Description

Catalog Number: FDV-0027S
Size: 25 µL
Concentration: 1 mM
Formulation: $C_{26}H_{17}NO_2S_2$
Molecular weight: 439.5 g/mol
Fluorescent characteristics:

Ex. 400-500 nm (maximum ~420 nm)

Compatible with blue excitation lasers (ex. 405, 445, 458, 473 and 488 nm* lasers, etc.), Xenon lamp or LED with commercial FITC or GFP filters.

*Note 488 nm laser can excite LipiDye™ II but shows weak fluorescence compared with 473 nm excitation. If using 488 nm laser, please empirically optimize imaging conditions such as dye concentration etc. for your experiments.

Em. 450-650 nm (dependent on solvents)

Maximum ~510 nm in soybean oil similar to LDs. Around 490-550 nm range is recommended.

Storage

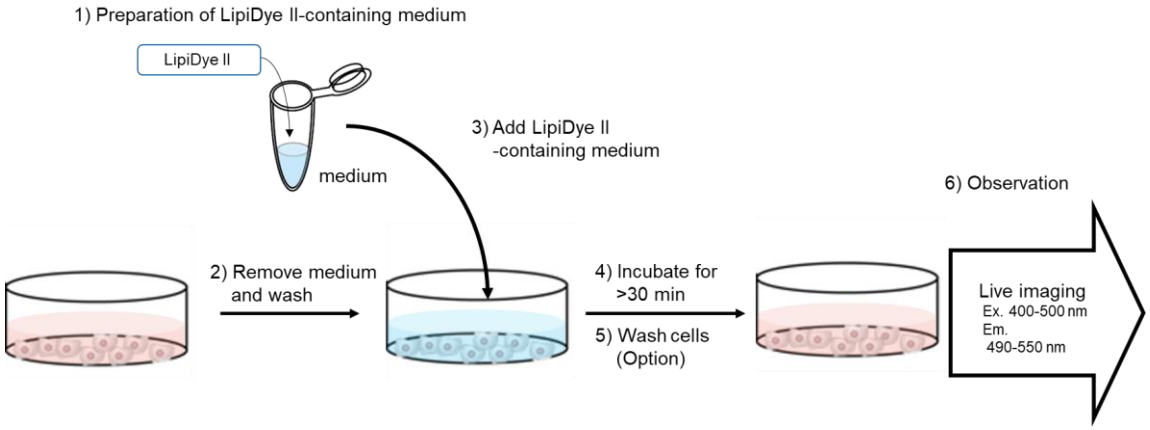
Spin down the solution before opening the cap and make aliquots.
Store the solution at -20°C and avoid repeated freeze-thaw cycles.

How to use

General procedure for live cell imaging

*This procedure is an example of cultured cell staining.

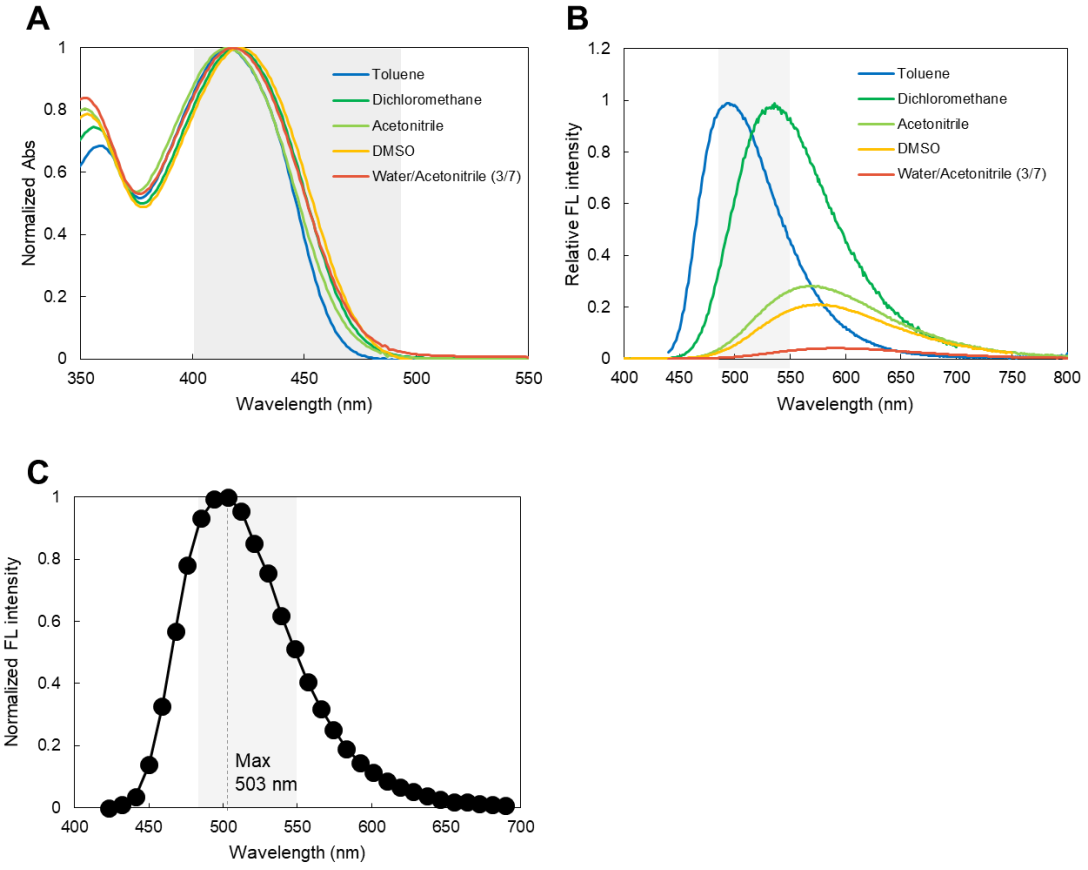
1. Prepare 1 µM LipiDye™ II in serum-free and phenol red-free medium such as DMEM
   NOTE-1: LipiDye™ II is compatible with FBS-containing medium but please optimize FBS and LipiDye™ II concentrations.
   NOTE-2: Recommended concentration is 0.1 µM for ~405 nm excitation and 1 µM for ~473 nm excitation. Empirically optimize and determine the concentration of LipiDye™ II for your experiments.
2. Remove culture medium and wash cells PBS several times
3. Add LipiDye™ II-containing medium to cells
4. Incubate cells at 37 °C for over 30 min
   NOTE-3: Empirically optimize incubation time for your experiments.
5. Wash cells with PBS or medium and add fresh medium (Optional)
6. Observe cells



Reference data

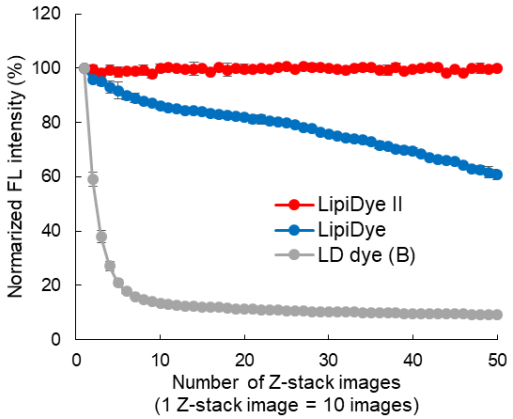
Spectrum of LipiDye™ II

(A) Absorption spectrum in various solvents.
(B) Emission spectrum in various solvents. LipiDye™ II is a solvatochromic dye and shows a different spectrum in each solvent. Under low polaric solvents, toluene and dichloromethane, emits from blue to green fluorescence with high quantum yield. On the other hand, under high polaric solvents, acetonitrile, DMSO and water, LipiDye™ II exhibits a weak fluorescence intensity with a red-shift fluorescence.
(C) Emission spectrum in cellular LDs measured by fluorescent microscopy.



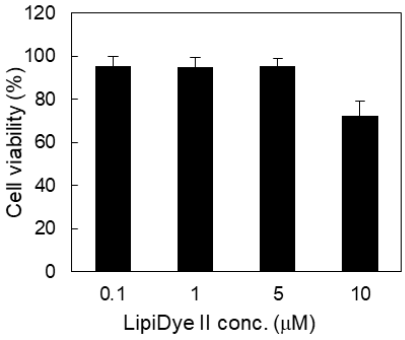
Photostability of LipiDye™ II in the cell

3T3-L1 adipocytes pre-fixed in 4% formaldehyde were stained with LipiDye™ II, prototype LipiDye™ and conventional LD dye (**B**). The free dyes were removed by washing and z-stack images (z=10 with a 2 µm step) of the adipocytes in the same area were repeatedly acquired by confocal microscopy (Ex 473 nm/Em 490-540 nm). The total fluorescence was measured and normalized intensity was calculated. The fluorescent intensities from conventional dye B dramatically reduced by repeated light irradiation, including LipiDye™ gradually decreased. LipiDye™ II maintained its fluorescence at least 50 z-stack images (total 500 images).



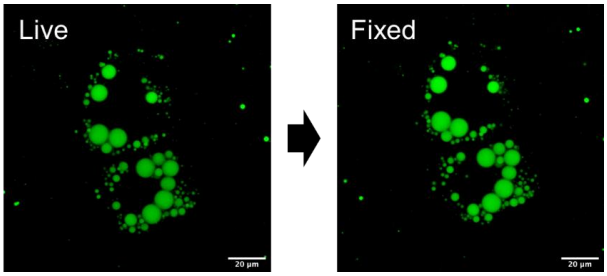
Cytotoxicity of LipiDye™ II

3T3-L1 adipocytes were treated with various concentrations of LipiDye™ II for 24 hours. After incubation, cell viability was evaluated by MTT assay. At least 5 µM showed little cytotoxicity on adipocytes. The recommended concentration of LipiDye™ II for LD-staining is 0.1-1 µM.



Comparison of the fluorescence intensity under live cell and after PFA fixation

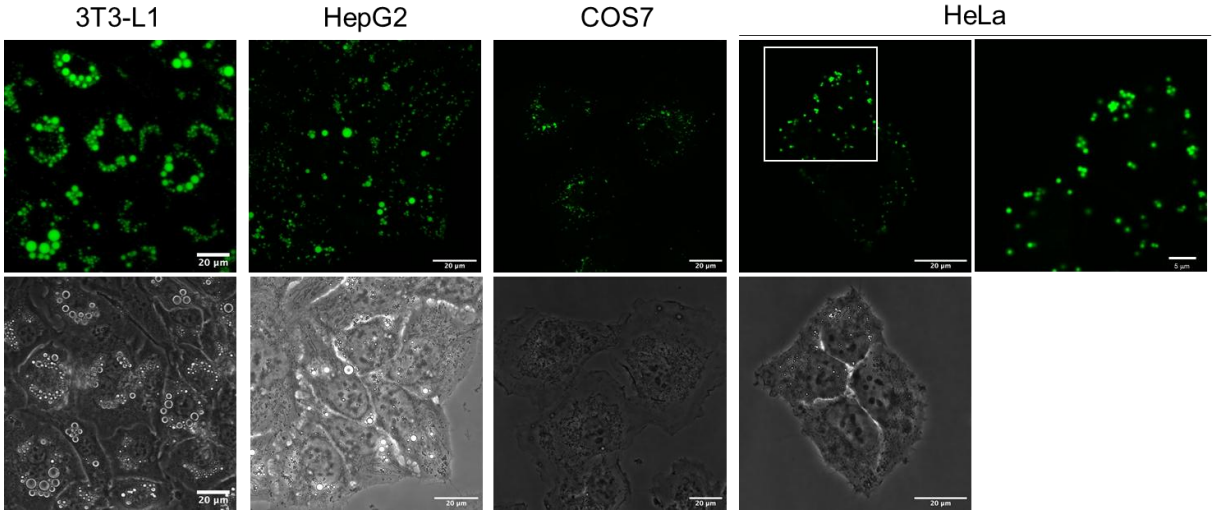
3T3-L1 adipocytes stained with LipiDye™ II and were observed under live cell conditions (left). After recording the live cell image, the cells were subsequently fixed with 4% PFA and observed by confocal microscopy (Ex. 473 nm/Em 490-540 nm). Fixation has little effect on the fluorescent intensity of LipiDye™ II. LipiDye™ II is compatible with any immunocytochemical experiments after the live cell imaging experiments.



Application data

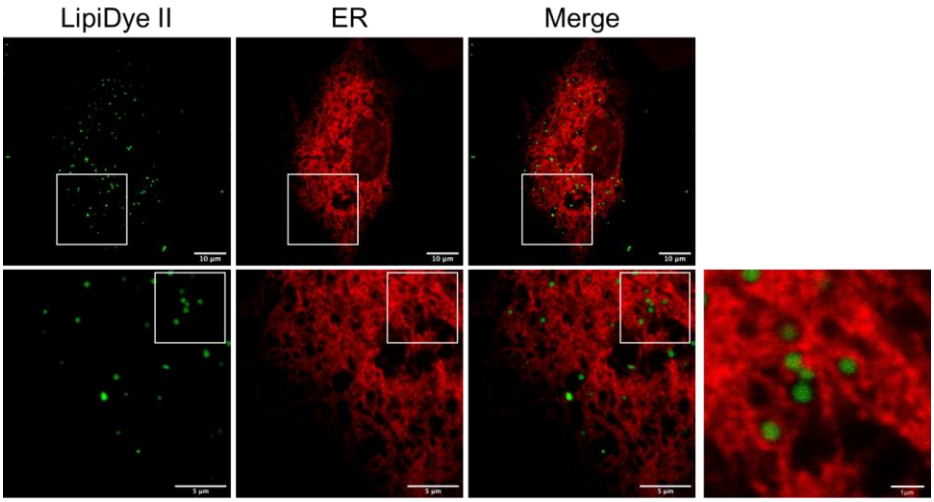
Staining of various cells

3T3-L1, HepG2, COS-7 and HeLa cells were stained with LipiDye™ II (1 µM) for 12 hours and observed by confocal microscopy (Ex. 473 nm/Em 490-540 nm). In the case of HepG2 cells were pretreated with palmitic acid (0.33 mM) /oleic acid (0.66 mM), one day before LipiDye™ II staining. In HeLa cells, small LDs of approximately 1 µm were clearly observed. (Scale bar: 20 µm, HeLa cell enlarged 5 µm)



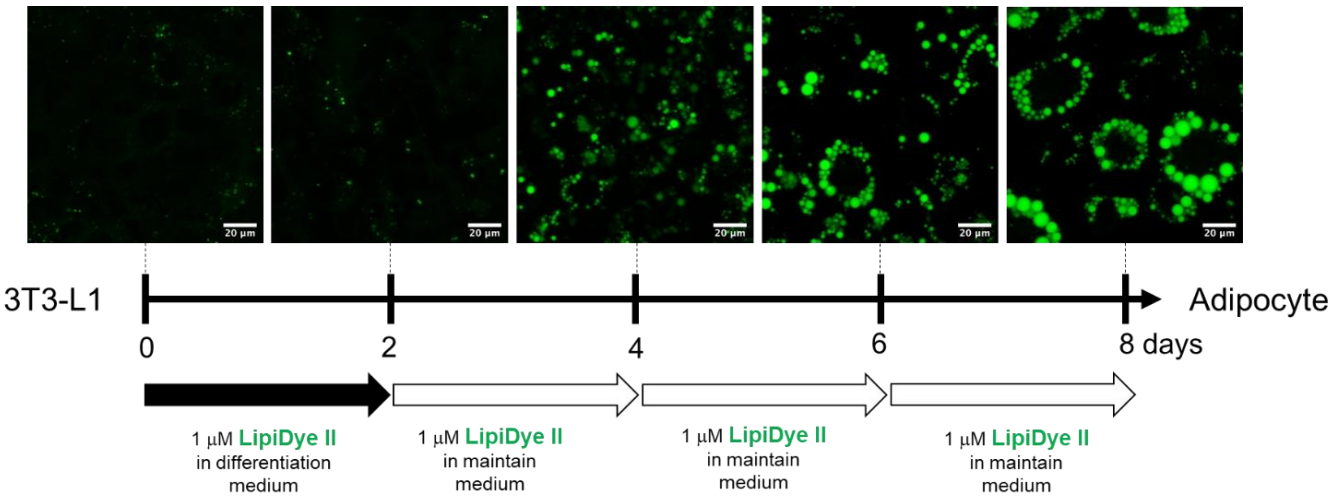
Multicolor imaging with ER marker

COS7 cells expressing ER-resident fluorescent protein (mKO1) were stained with LipiDye™ II (1 µM) for 12 hours. After washing, the cells were observed by confocal microscopy (LipiDye™ II; Ex. 473 nm/Em 490-540 nm, mKO1; Ex. 635 nm/Em 660-710 nm). Small LDs less than 1 µm were frequently observed in the network structure of ER. (Scale bar: 20 µm, 5 µm and 1 µm)



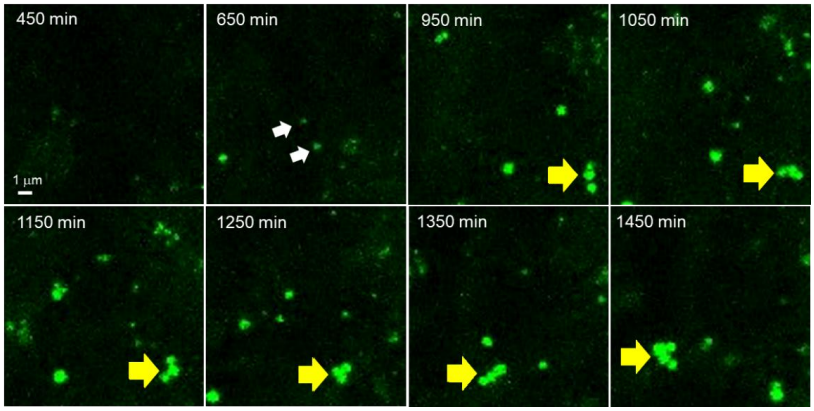
Long-term staining during adipocyte differentiation and maturation

Two days after confluence, 3T3-L1 preadipocytes were stained with LipiDye™ II for 12 hours. After washing with fresh medium, the cells were incubated with a differentiation medium containing 1 µM LipiDye™ II and the first image (0 days) was recorded by confocal microscopy (Ex. 473 nm/Em 490-540 nm). After two days of differentiation, the medium was replaced with a maintenance medium containing 1 µM of LipiDye™ II. During acquisition of the images, the medium containing LipiDye™ II was exchanged every 2 days. (Scale bar; 20 µm)



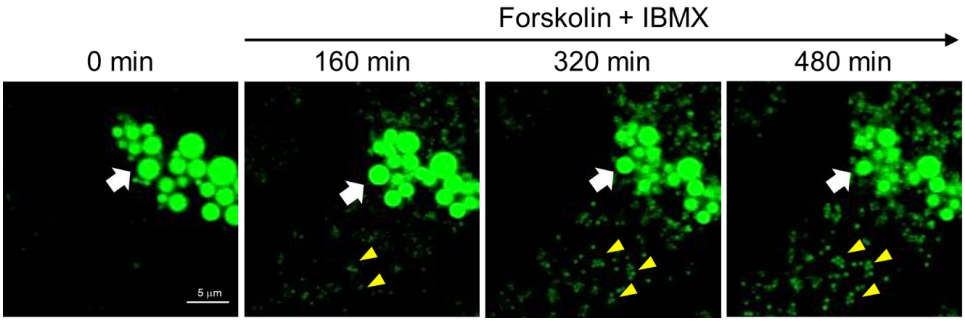
Time-lapse Z-stack imaging of adipogenesis

3T3-L1 preadipocytes cultured in differentiation medium containing 1 µM LipiDye™ II and time-lapse Z-stack imaging (20 z-images/10 min, for 24 hours) was performed by confocal microscopy (Ex. 473 nm/Em 490-540 nm). After ~10 hours differentiation, small LDs were observed (650 min, white arrows) and some LDs were docking with other LDs during adipogenesis (1050-1450 min, yellow allow). (Scale bar; 1 µm)



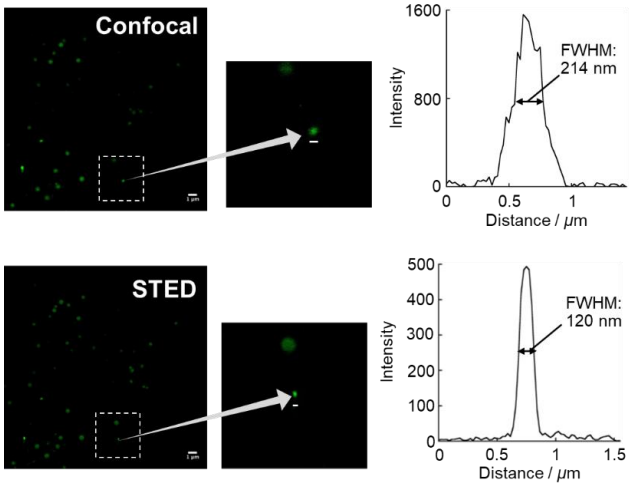
Time-lapse Z-stack imaging of lipolysis and lipogenesis

3T3-L1 adipocytes were incubated with 1 µM LipiDye™ II and washed with media to remove the free dye. After then the cells were treated with Forskolin (10 µM), an activator of adenylyl cyclases, and IBMX (100 nM), an inhibitor of phosphodiesterases. These drugs increased the intracellular concentration of cAMP and subsequently promoted the hydrolysis of triacylglycerols. Immediately after the addition of drugs, time-lapse Z-stack imaging (15 z-images/4 min, for 800 min, total 3000 images) were performed by confocal microscopy (Ex. 473 nm/Em 490-540 nm). Some large LDs clearly contracted or disappeared caused by the drugs. After two hours, numerous newly formed small LDs were observed. (Scale bar; 5 µm)



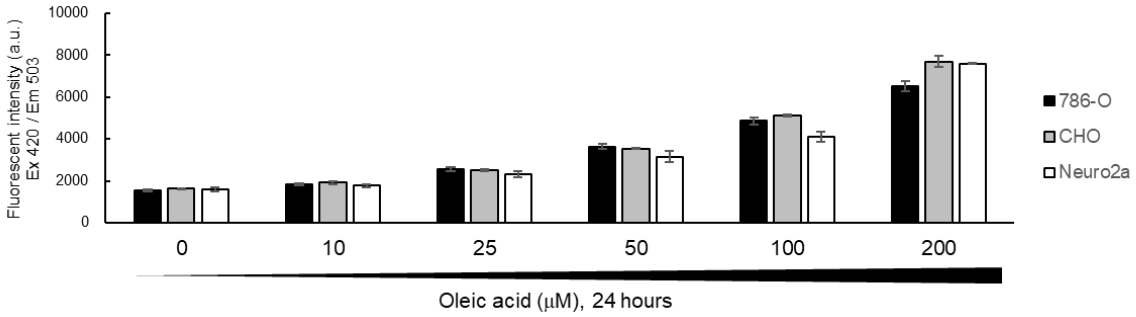
Live-cell STED super resolution microscopy imaging

HeLa cells were treated with 1 µM LipiDye™ II, washed and cultured in medium. The cells were imaged by confocal laser microscopy (Ex 473 nm/ Em 490-540 nm) and STED microscopy (Ex 473 nm/ Em 500-640 nm, depletion laser 660 nm). STED imaging detected ~120 nm (FWHM) small LD, which was not clearly detected by confocal microscopy. Detailed STED imaging condition and analysis methods were described in Ref.1. (Scale bar 1 µm)



Semi-quantitative analysis of cellular lipid droplets by fluorescent plate reader

Three cell lines (human renal cancer cell line 786-O, mouse neuroblastoma cell line Neuro2a, and Chinese hamster ovarian cell line CHO) were seeded in 96 well plate at 1 x 10^4 cell/well and cultured in DMEM containing 10% FBS (10% FBS/DMEM). After 24 hours, cells were treated with 10-200 µM oleic acid in 10% FBS/DMEM for further 24 hours to promote growth of lipid droplets. After washing cells, the cells were stained by 5 µM LipiDye™ II-containing 2% FBS/DMEM for 2 hours. Just before fluorescent plate leader measurement, cells were washed by PBS twice and fluorescent intensity (Ex 420 ±5 nm/Em 503 ±10 nm) of each well was measured. In all cell lines, oleic acid dose-dependency was observed.



Reference

1. Taki *et al.,ACS. Mater. Lett.*., **3**, 42-49 (2021), Fused Thiophene-*S,S*,-dioxide-Based Super-Photostable Fluorescent Marker for Lipid Droplets

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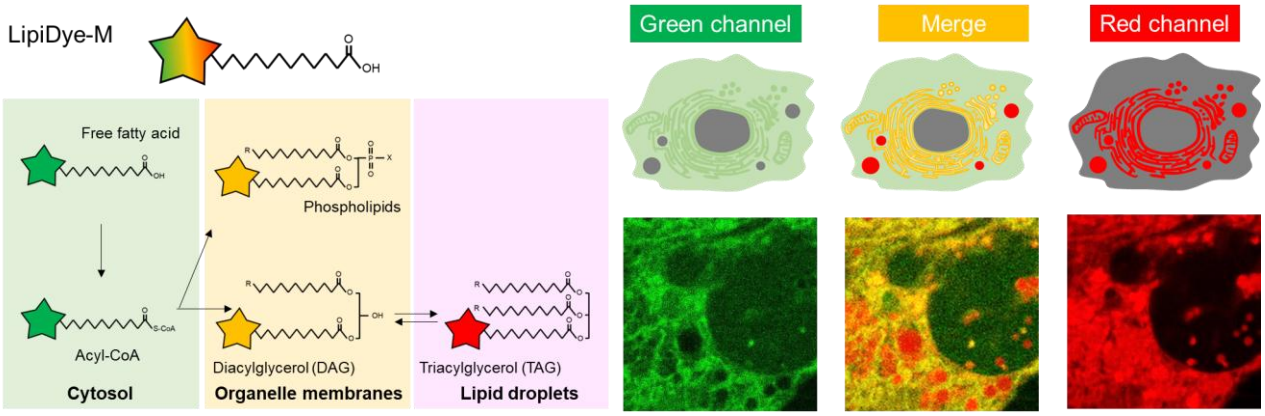
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LipiDye™-M <Lipid Metabolism Tracer>

LipiDye™-M is a C12 fatty acid mimic labeled with a novel solvatochromic dye. As LipiDye™-M exhibits green-to-red fluorescence depending on its lipid structure and its localization, LipiDye™-M can trace status of cellular fatty acid uptake and lipid metabolism in cells. LipiDye™-M is a powerful tool for both basic research and pharmaceutical research for lipid metabolism.



Catalog No. FDV-0028
Size 0.1 mg
Features

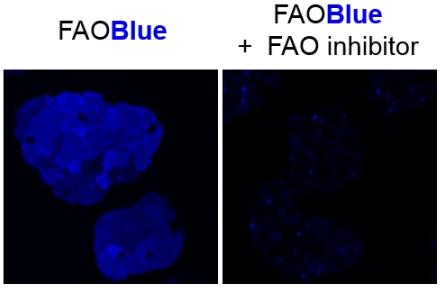
- allows to perform three-color imaging (green, yellow and red) by merging images from a green channel (Ex. 450-490 nm / Em. 490-540 nm) and red channel (Ex. 550-600 nm / Em. 570-620 nm)
- can also be taken up to cells by FA-transporters and converted into many types of lipids, including acyl-CoA, phospholipids, DAGs, TAGs, and degraded to small metabolites by the mitochondrial FAO pathway.
- Emits green fluorescence in high polarity (cytosol), yellow fluorescence in moderate polarity (organelle membranes) and red fluorescence in low polarity (lipid droplets)

FAOBlue™ <Fatty Acid Oxidation Detection Reagent>

FAOBlue™ is a cell-based fatty acid beta-oxidation (FAO) detection dye which emits blue fluorescence upon cellular FAO activity.

Catalog No. FDV-0033
Size 0.2 mg
Features

- Ex/Em:~405 nm / 460 nm
- Enable to directly detect cellular FAO activity in live cells
- Apply quantitative comparison of FAO activity between different cell types
- Can monitor the drug-induced change of FAO activity



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