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FRONTIERS IN LIFE SCIENCE

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LipiDye-M <Lipid Metabolism Tracer>

Catalog NO. FDV-0028

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This product has been commercialized under the license from Nagoya University

Product Background

Lipids are the fundamental components of cells to make membrane structures and are used as signaling molecules. Fatty acids (FAs) are the smallest and the most important building block of lipids. FAs are not only biochemically synthesized in the cell but also taken up from extracellular space by FA-transporters. Intracellular FAs are metabolically converted into various lipids, including acyl-CoA, phospholipids, glycolipids, diacylglycerols (DAGs), and triacylglycerols (TAGs), and also degrade by mainly the mitochondrial fatty acid beta-oxidation (FAO) pathway to produce energy and by lipid droplet-selective autophagy, lipophagy. Lipid metabolism is strictly regulated by various enzymes in the cell, and its abnormal regulation induces diseases, including obesity, diabetes, etc. In understanding lipid metabolism, especially FA metabolism, fluorescent dye-labeled FAs have been employed with fluorescent imaging techniques. Although these FA-derivatives contribute to evaluating intracellular lipid metabolism, conventional fluorescent dyes cannot distinguish lipid metabolites and their localization.

LipiDye-M is a novel fluorescent dye-labeled C12 fatty acid (Figure 1 left) with a negative solvatochromic dye called 3a-azapyren-4-one (AP). LipiDye-M (original compound name AP-C12 in Ref.1) was originally developed by Dr. Shigehiro Yamaguchi and Dr. Masayasu Taki, Nagoya University. LipiDye-M is a mimic of FA, which is approximately the same length as C18-FA, such as stearic acid and oleic acid. The AP dye senses environmental polarity and changes its absorption and fluorescent spectrum (Figure 1, right). Although AP dye exhibits red fluorescence in lower polarity, such as hydrophobic oil, it emits green fluorescence in higher polarity, such as aqueous solution. Based on AP's solvatochromic property, LipiDye-M can change its fluorescent colors in various intracellular environments such as cytosol, organelle membranes, and lipid droplets. Likely to native fatty acids, LipiDye-M 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. According to these two features, LipiDye-M exhibits green-to-red fluorescence depending on its lipid structure and its localization (Figure 2). Combined with these fluorescent properties and conventional confocal microscopy imaging, LipiDye-M 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) (Figure 3). Under the indicated green and red channel conditions, the green channel detects cytosol, mitochondrial matrix, organelle membranes, and the red channel detects organelle membranes and lipid droplets. The merged image from the green and red channel shows that cytosol and mitochondrial matrix exhibits green, organelle membranes exhibit yellow, and lipid droplets exhibit red color. Ref.1 shows various application data applying LipiDye-M to cellular imaging of FA metabolism. For example, distribution of LipiDye-M metabolites in adipocytes in normal conditions and HepG2 in starved conditions were observed. Furthermore, the effects of lipid metabolism inhibitors on the distribution of LipiDye-M metabolites are also validated. Not only qualitative three-color imaging by the merged image but also quantitative analysis by the ratiometric image (Green/Red intensity) can be validated. Detailed information is described in Ref.1.

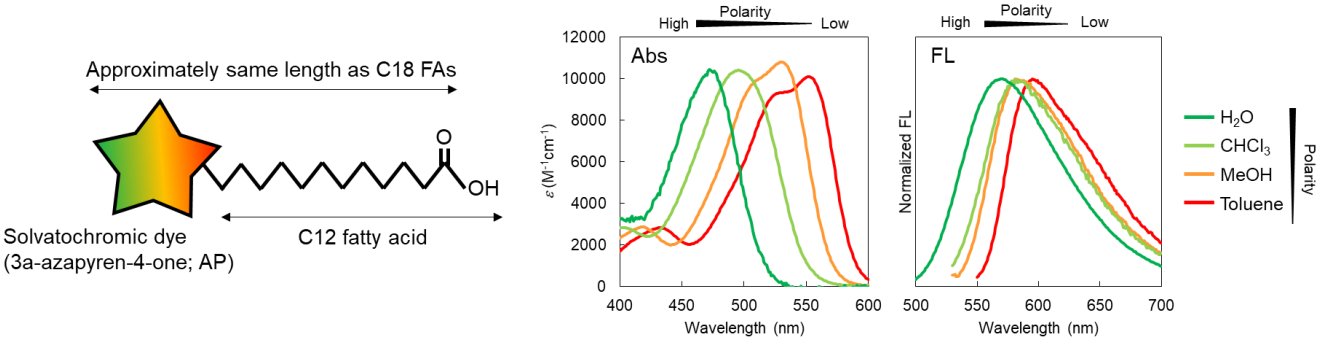


Figure 1. Structure of LipiDye-M and AP's fluorescent property

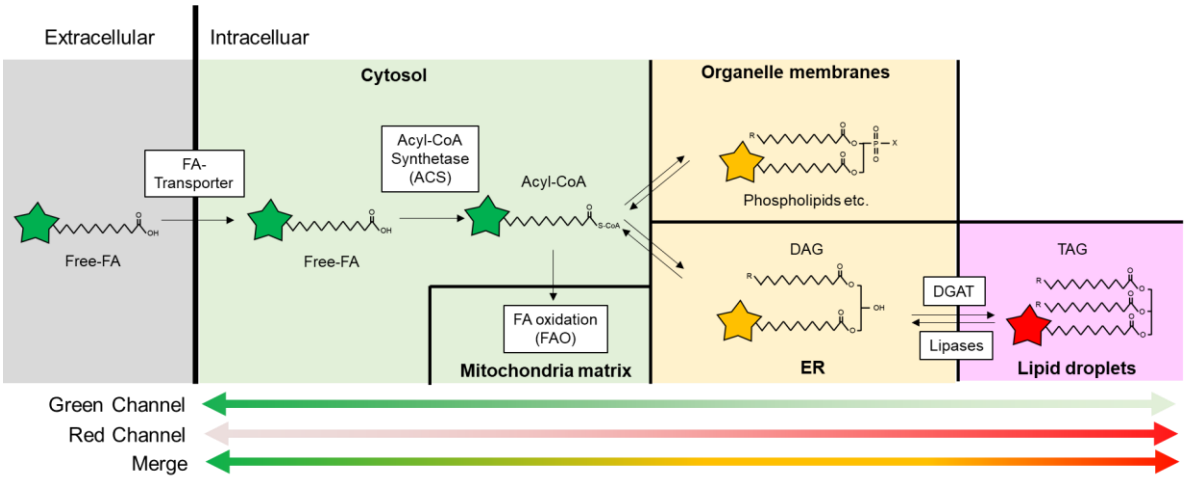


Figure 2. Scheme of LipiDye-M metabolites and fluorescent color

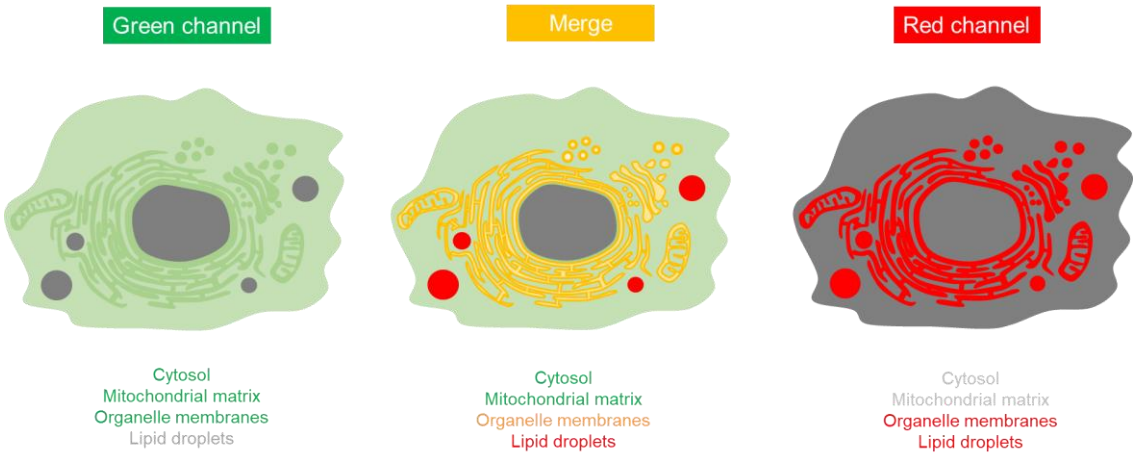


Figure 3. Three colors merged imaging for LipiDye-M metabolites

Description

Catalog Number: FDV-0028
Size: 0.1 mg
Formulation: $C_{28}H_{31}NO_5$
Molecular weight: 461.5 g/mol
Solubility: Soluble in DMSO
Fluorescent characteristics:
To distinguish each metabolite, the confocal laser microscopy system is highly recommended.
Excitation for green channel: 450-490 nm, recommended laser 457, 473 nm
for red channel: 540-600 nm, recommended laser 559 nm
Emission for green channel: 490-540 nm
for red channel: 570-620 nm
Analysis of merged image (Green + Red) is only for qualitative analysis.
If you would like to perform quantitative analysis by ratiometric imaging, please refer Ref.1.

Reconstitution and Storage

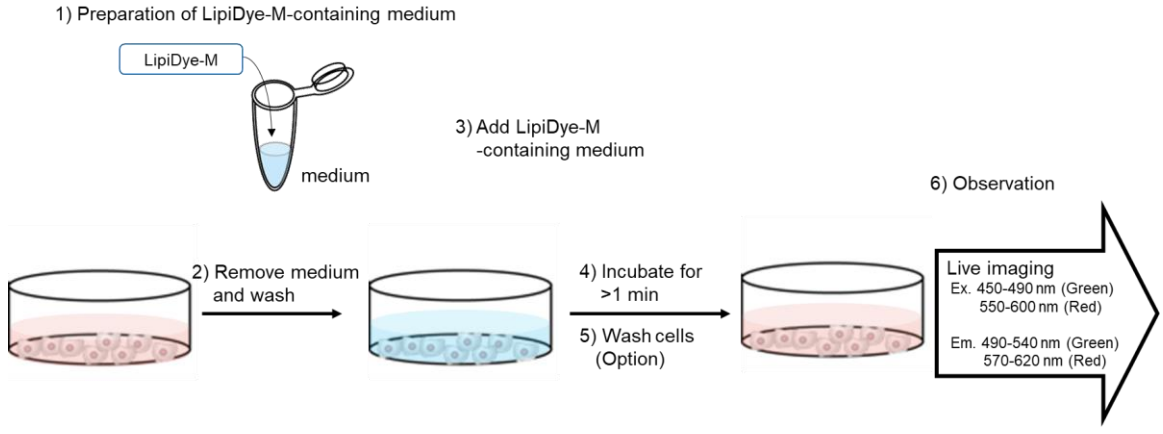
Reconstitution: Stock solution recommended concentration 1 mM in 100% DMSO.
Storage (powder): Store powder at -20°C
Storage (solution): After reconstitution in DMSO, aliquot and store at -20°C.
Avoid repeated freeze-thaw cycles.

How to use

General procedure for live cell imaging

*This procedure is an example of live cell imaging

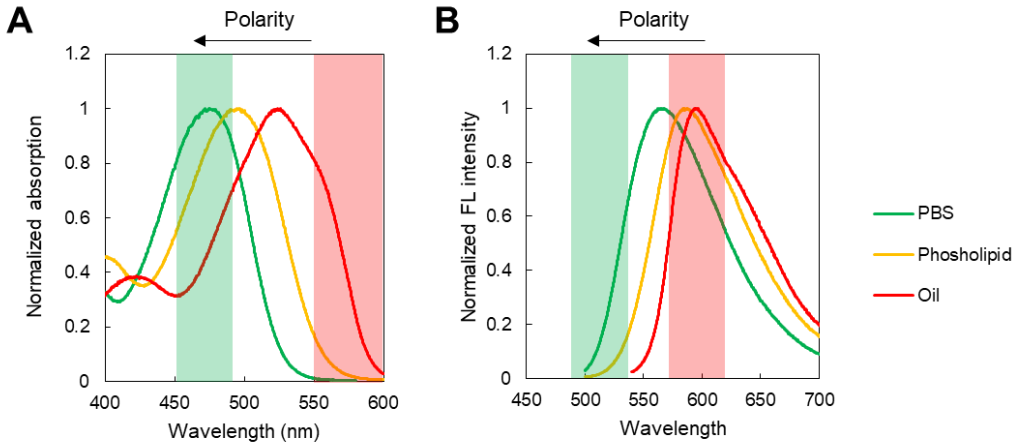
1. Prepare 5-10 μM LipiDye-M in serum-free and phenol red-free medium such as HBSS (Hank's balanced salt solution with Ca^{2+} and Mg^{2+})
   NOTE: Empirically optimize and determine the concentration of LipiDye-M and types of culture medium for your experiments. Culture medium affects various FA metabolic activities, including FA transportation and FAO activity.
2. Remove culture medium and wash cells HBSS several times
3. Add LipiDye-M-containing medium prepared in 1) to cells
4. Incubate cells at 37 °C for the appropriate time
   NOTE: Empirically optimize incubation time for your experiments and purposes.
5. Wash cells with PBS, HBSS, or medium and add fresh medium (Optional)
6. Observe cells by confocal microscopy
   NOTE: Cell fixation is not compatible with LipiDye-M. Observe cells under live cell conditions.



Reference data

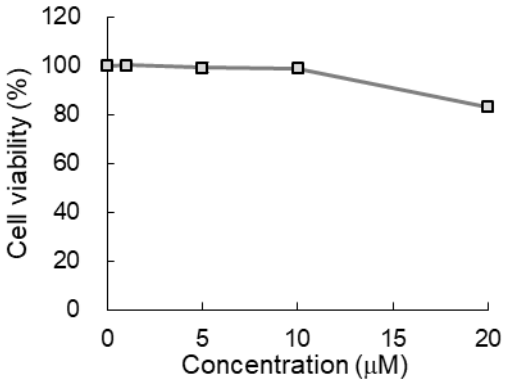
Spectrum of LipiDye-M

Absorption spectrum (A) and emission spectrum (B) in PBS, phospholipid bilayers of large unilamellar vesicles (LUVs), and soybean oil. PBS, phospholipid LUV, and soybean oil are mimicked for cytosol, organelle membranes, and lipid droplets, respectively. Based on the spectral features, the following two channels: a green channel (Ex. 450-490 nm, recommended laser 473 nm / Em.490-540 nm) and a red channel (Ex. 550-600 nm, recommended laser 559 nm / Em. 570-620 nm) are recommended to distinguish lipid metabolites.



Cytotoxicity of LipiDye-M

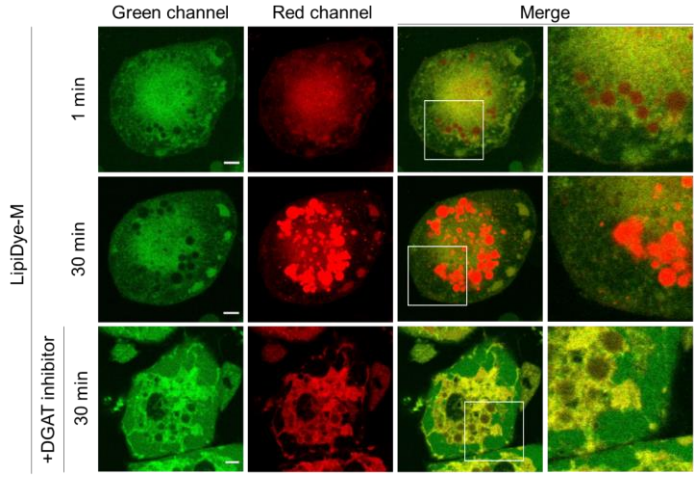
To observe the cytotoxicity of LipiDye-M, HepG2 cells were treated with various concentrations of LipiDye-M for 24 hours. Afterward, cell viability was assessed by MTT assay. Under 10 μM LipiDye-M shows little effect on HepG2 viability. However, 20 μM LipiDye-M exhibits weak cytotoxicity (~20%) on HepG2 cells.



Application data

Distribution of LipiDye-M metabolites in live adipocytes

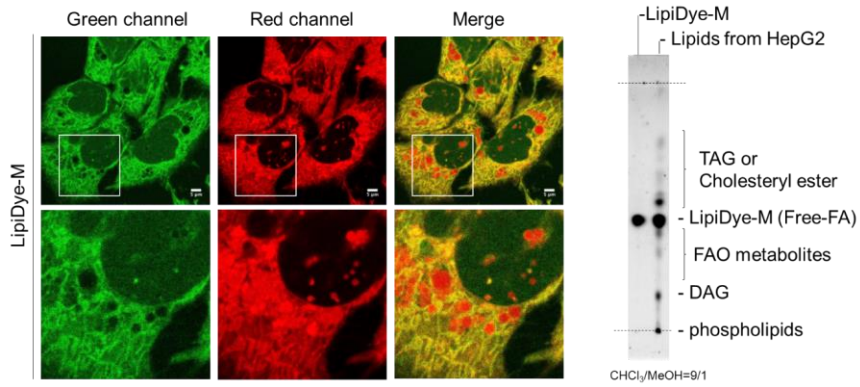
Adipocytes derived from 3T3-L1 cells were treated with LipiDye-M (5 μM) and observed after 1 min or 30 min with confocal fluorescent microscopy without a wash step. Confocal images were recorded in the green channel (Ex. 473 nm/Em 490-540 nm) and red channel (Ex. 559 nm/Em 570-620 nm). The images captured at 1 min shows strong green fluorescence intensity from cytosol and organelle membrane and weak red intensity from lipid droplets. This indicates major metabolites of LipiDye-M are free-FA, and acyl-CoA and LipiDye-M are insufficient to incorporate into TAGs. After 30 min, the intensity of the green channel decreased, and the red fluorescent signal from lipid droplets clearly increased. These images suggest LipiDye-M was sufficiently converted into TAG lipids and accumulated into lipid droplets. Under treatment with a DGAT inhibitor to block TAG synthesis, a slight red signal is detected from lipid droplets, and the yellow signal of organelle membranes on the merged image dramatically increased. This result indicates DGAT inhibitor blocks TAG biosynthesis on ER membrane, and metabolites of LipiDye-M were highly accumulated in organelle membranes as DAG species.



Distribution of LipiDye-M metabolites in live HepG2

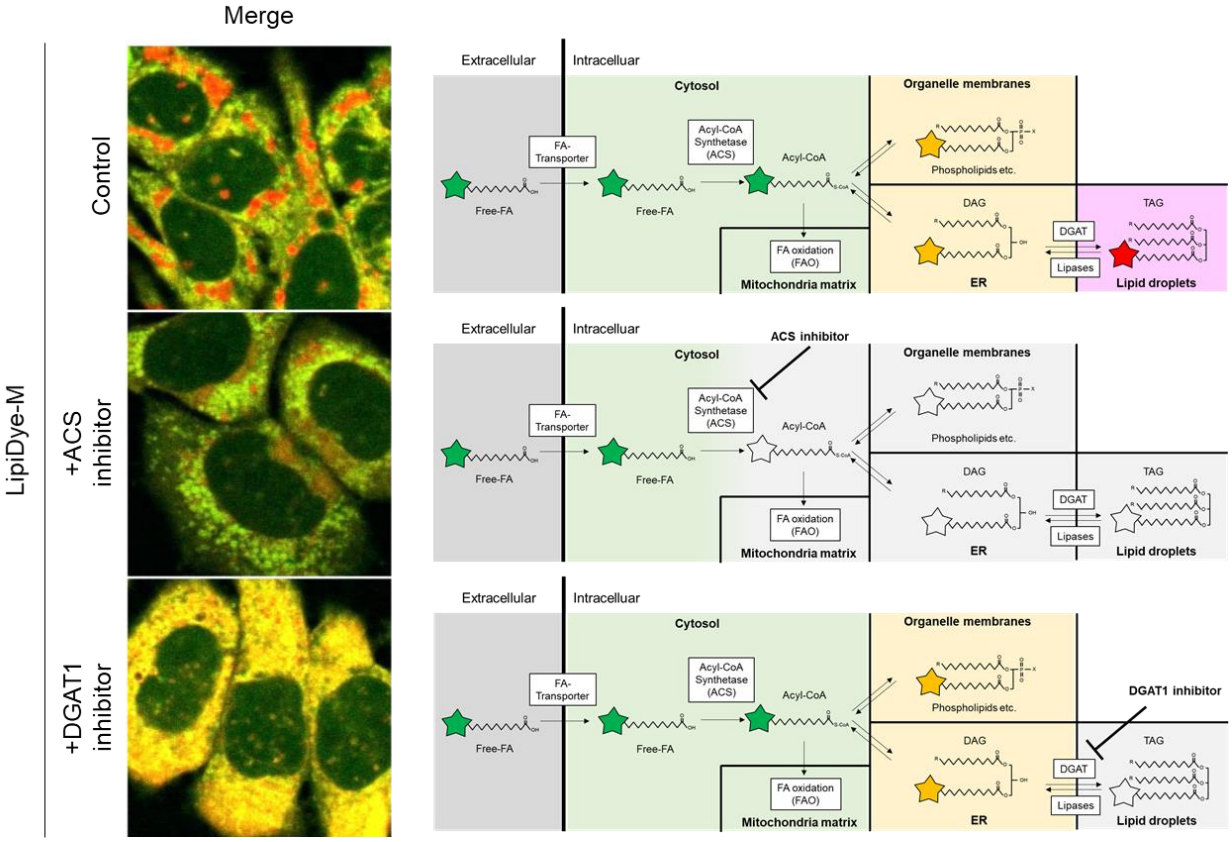
(Left) HepG2 cells were treated with oleic acid/palmitic acid in a complete medium containing 10% FBS to mature lipid droplets. Afterward, the culture media was exchanged with HBSS containing LipiDye-M (5 μM), the cells were incubated for 1 hour and observed by confocal fluorescent microscopy without a wash step. Confocal images were recorded in the green channel (Ex. 473 nm/Em 490-540 nm) and red channel (Ex. 559 nm/Em 570-620 nm). Under serum-starved conditions, the merged image showed LipiDye-M effectively incorporated into the cells, and its metabolites were distributed in not only the cytosol (green), ER (yellow), and lipid droplets (red) but also mitochondria as yellow.

(Right) To investigate metabolites of LipiDye-M in the cells, lipid fractions were biochemically extracted from the cells and separated by thin-layer chromatography (TLC) with fluorescent detection. Many metabolites of LipiDye-M were detected in the lipid extracts from HepG2 and were rationalized as esterified products (TAG or cholesteryl ester), phospholipids and FAO metabolites.



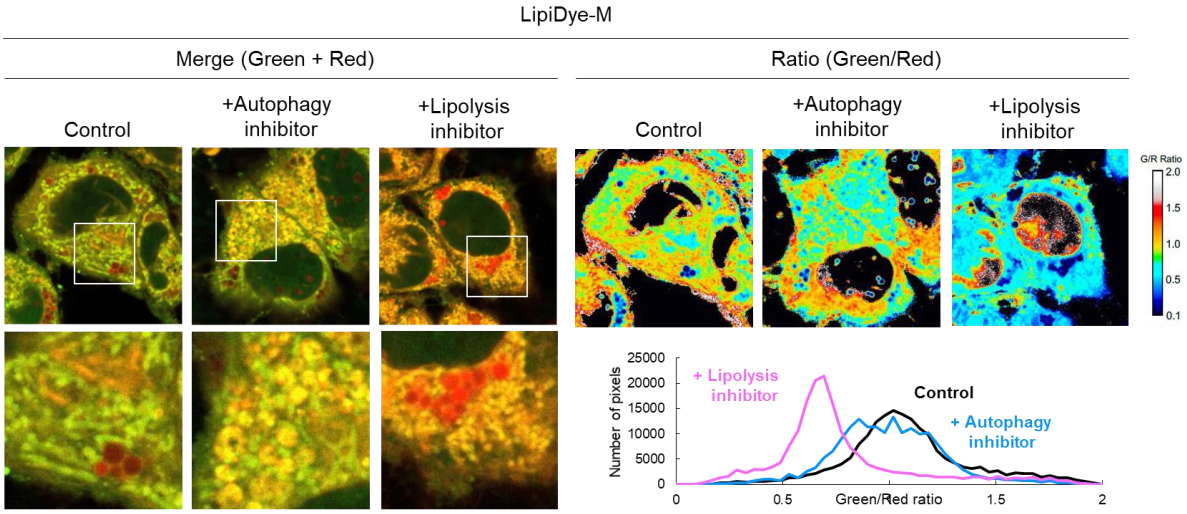
Effect of FA metabolism inhibitors on the distribution of LipiDye-M metabolites

HepG2 cells were treated with FA metabolism inhibitors, acyl-CoA synthase (ACS) inhibitor (Triacsin C, 5 μM) or DGAT1 inhibitor (T863, 20 μM), in complete medium for 18 hours. Afterward, the media was replaced with HBSS containing 5 μM LipiDye-M, 0.5 mM oleic acid, and the indicated inhibitor. The cells were incubated for 6 hours and observed. Confocal images were recorded in the green channel (Ex. 473 nm/Em 490-540 nm) and the red channel (Ex. 559 nm/Em 570-620 nm). Merged images are shown below on the left, and proposed metabolic pathways under treatment of each inhibitor are shown on the right. In the control cell, strong red signals from lipid droplets were observed. Under treatment with ACS inhibitor, both red and yellow signals dramatically decreased, and green signals increased. This result indicates ACS inhibitor suppresses the conversion of free-FA to acyl-CoA, and the free-FA form of LipiDye-M is accumulated in the cytosol and organelles. Under treatment of DGAT inhibitor, yellow signals clearly increased, while a little red signal from lipid droplet was observed. This data shows DGAT1 inhibitor blocked the conversion of DAG to TAG, and excess DAG was accumulated in the ER.



Effect of inhibitors for lipid degradation pathway on the distribution of LipiDye-M metabolites

HepG2 cells were cultured in HBSS containing LipiDye-M (5 μM) and autophagy inhibitor (50 nM Bafilomycin A1) or lipolysis (100 μM DEUP) for 6 hours. Afterward, confocal images were recorded in the green channel (Ex. 473 nm/Em 490-540 nm) and red channel (Ex. 559 nm/Em 570-620 nm). Both images merged (Green + Red; left), and ratio images (Green/Red; right) were calculated. In the control condition, mitochondria (green) and lipid droplets (red) were mainly observed in the merged image. Under the treatment of autophagy inhibitor, vesicle-structure indicating autophagosomes were detected by yellow color in the merged image. This data indicates lipid degradation by lipophagy was suppressed by the autophagy inhibitor. Under the treatment of lipolysis inhibitor, overall red signals in the merged image significantly increased, and the Green/Red ratio was clearly decreased in the ratio image and the pixel plot. This data indicate lipids were abnormally accumulated in lipid droplets and organelle membranes by suppression of the lipolysis pathway. Detailed information about the ratiometric analysis is described in Ref.1.



Reference

1. Kajiwara *et al.*,*Nat. Commun.*, 13, 2533 (2022) A negative-solvatochromic fluorescent probe for visualizing intracellular distributions of fatty acid metabolites.

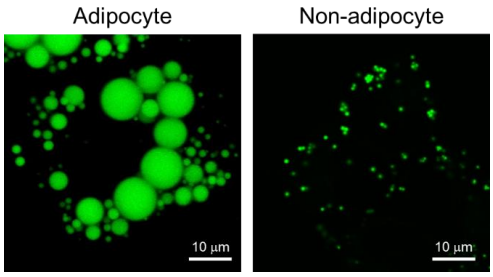
Related products

LipiDye II <Live Imaging>

LipiDye II is a highly sensitive lipid droplet staining dye with extremely photostable properties. This dye is the second generation of our previous reagent, LipiDye. This dye allows the detection of small lipid droplets (<1 μm) in non-adipocytes and is suitable for long-term live cell imaging for dynamic lipid droplet movements.

Catalog No. FDV-0027
Size 0.1 mg
Features

- Recommended Ex/Em:400-500 nm / 490-550 nm
- Enable to detect <1 μm lipid droplets
- Suitable for long-term live cell imaging
- Extremely photostable compared with conventional dyes
- Compatible with both live and fixed cells

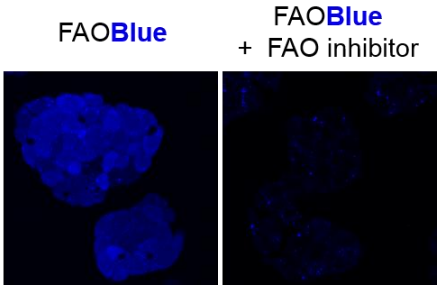


FAOBlue <Fatty Acid Oxidation Detection Reagent>

FAOBlue is a cell-based fatty acid beta-oxidation (FAO) detection dye that 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
- Monitor the drug-induced change of FAO activity

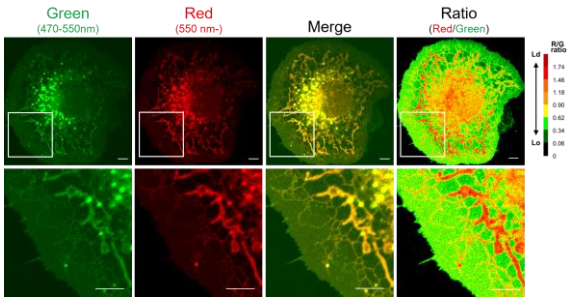


LipiORDER <Membrane Lipid Order Imaging Dye>

LipiORDER is a solvatochromic dye for membrane lipid order imaging. LipiORDER exhibits green fluorescence with Lo phase and exhibits red fluorescence with Ld phase. The ratiometric analysis (F_{red}/F_{green}) enables the quantitative visualization of membrane lipid order.

Catalog No. FDV-0041
Size 0.1 mg
Features

- Recommended Ex/Em: ~405 nm / 500-550 nm (Green channel) and 550-650 nm (Red channel)
- Quantitatively monitor lipid order on plasma and inner membranes in live cells
- Highly photostable and cellularly stable compared with similar conventional dyes.



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