

Lipidye™ RED <Lipid Droplet Live Imaging Red>

Catalog NO. FDV-0057

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

Product Background

Lipid droplets and Lipidye series

Lipid droplets (LDs) are organelles that have a unique phospholipid monolayer and store neutral lipids such as triacylglycerols (TAG) and sterol esters (Figure 1, left). LDs are historically found in adipose tissue and are considered sites for energy storage or lipid turnover. Recent studies discovered that LDs are not only found in adipocytes, but are also ubiquitously present in cells from yeast to mammalian cells. The numbers, sizes, and compositions of LDs vary widely across cell types and 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. LDs are produced from the endoplasmic reticulum (ER), exported to the cytoplasm, expand in size by either fusion of other LDs or by incorporating additional synthesized neutral lipids. LDs contact various organelles, including the ER, mitochondria, lysosomes, and the nucleus, and exhibit dynamic movement inside the cells. To observe the dynamic movement of LDs in live cells and investigate their physiological functions, a specific LD dye compatible with long-term live-cell imaging is required.

Funakoshi provides **Lipidye™ II** (#FDV-0027, green fluorescence) and **Lipidye™ RED** (#FDV-0057, red fluorescence), which are highly sensitive fluorescence detection reagents to detect LDs. They exhibit a high S/N ratio and selectivity for LDs, allowing for the detection of even small LDs in non-adipocytes. Additionally, they exhibit super-photostability, enabling long-term live cell imaging, including Z-stack and time-lapse imaging.

Analysis of lipid droplet compositional dynamics by Lipidye™ RED

As described above, the major components of LDs are triacylglycerol (TAG) and sterol esters. TAG in LDs is hydrolyzed into diacylglycerol (DAG) and fatty acid by lipase, and DAG is further hydrolyzed to monoacylglycerol (MAG), and MAG is finally hydrolyzed to glycerol. Among the stepwise hydrolysis processes known as lipolysis, the lipid composition of LDs dynamically changes from a TAG-rich composition to a DAG-rich composition. Another metabolic process, named lipophagy, in which LDs are degraded by autophagy, has also been reported. These LDs degradation and metabolic processes are regulated depending on the energy demands of cells. Fatty acids

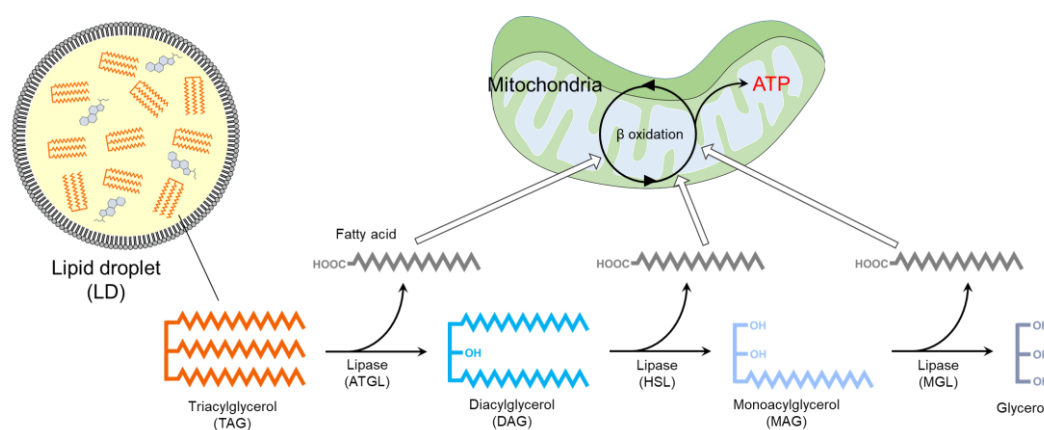


Figure 1 Degradation of lipid component in lipid droplet

released by LDs degradation, in which one molecule of TAG releases three fatty acid molecules, undergo β -oxidation in the mitochondria and are converted into chemical energy of ATP. It is known that failure of LDs degradation and metabolic processes causes various metabolic diseases. Therefore, it is important to analyze the progression of LDs degradation and the lipid composition of LDs in cells. Conventionally, LC/MS/MS analysis of lipid extracts from cells and tissues has been used to evaluate the lipid composition of LDs. However, this method is complicated and loses spatial information on the individual LDs composition. Although conventional LDs staining reagents can observe the spatial information and size of LDs, they cannot obtain information regarding the lipid composition or the degradation degree of LDs.

LipidDye™ RED can be used as a red fluorescence dye for LDs in typical fluorescence intensity imaging with fluorescence microscopy or confocal laser scanning microscopy (CLSM). Additionally, it is also possible to analyze the lipid composition of LDs by measuring their fluorescence lifetime with fluorescence lifetime imaging microscopy (FLIM). Generally, LDs exhibit a very low polarity environment. However, the polarity gradually increases as the lipid composition changes due to hydrolysis. LipidDye™ RED has a property in which fluorescence lifetime changes with the polarity of the surrounding environment, so the fluorescence lifetime changes depending on the degree of hydrolysis of LDs. Specifically, LipidDye™ RED exhibits a long fluorescence lifetime in LDs in a low-polar environment with a high TAG proportion. It exhibits a short fluorescence lifetime in LDs in a relatively high polar environment with a high proportion of DAG due to hydrolysis. By utilizing this property, it is possible to analyze the lipid composition of individual LDs based on differences in fluorescence lifetime. LipidDye™ RED has high photostability and intracellular retention, allowing long-term imaging and making it possible to analyze the hydrolysis process of LDs spatially and temporally.

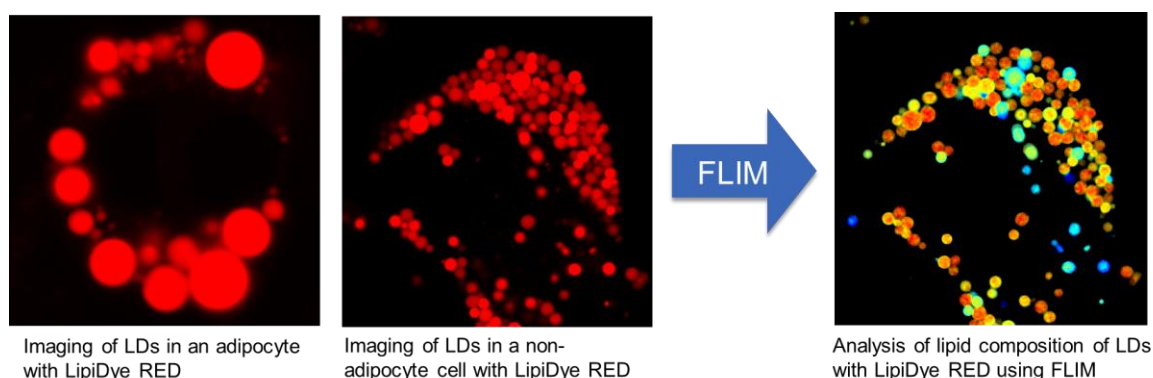


Figure 2 LDs imaging by LipidDye™ RED with fluorescence intensity and fluorescence lifetime

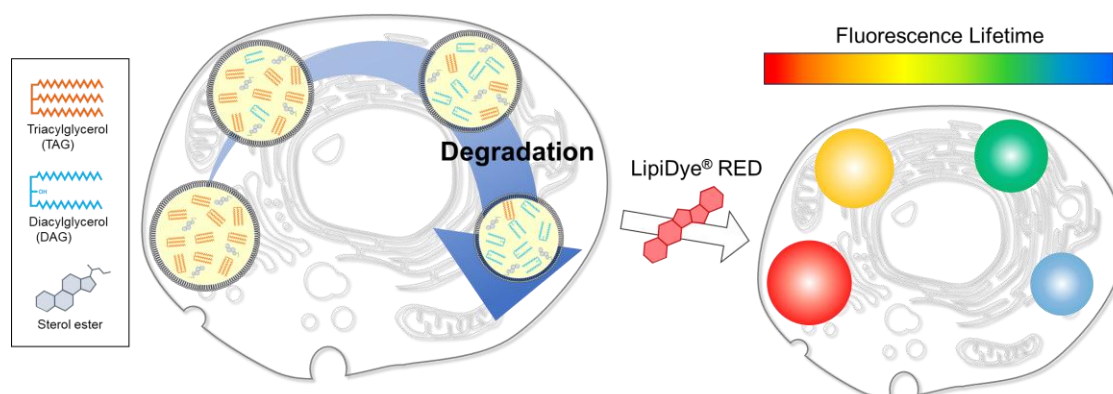


Figure 3 Degradation of LDs and analysis of lipid composition of LDs by LipidDye™ RED

Description

Catalog Number: FDV-0057

Size: 0.05 mg

Formulation: $C_{42}H_{29}NO_2P_2$

Molecular weight: 641.6 g/mol

Solubility: Soluble in DMSO

Fluorescent characteristics:

Ex. 470-560 nm

Compatible with excitation lasers (ex. 458, 473, 488, 514, 532, and 561 nm* lasers, etc.), Xenon lamp or LED with commercial Alexa555 or RFP filters.

***Note:** 561 nm laser can excite LipiDye™ RED but shows weak fluorescence compared with other excitations. When using a 561 nm laser, empirically optimize imaging conditions such as dye concentration, etc., for your experiments.

Em. 550-700 nm (Maximum ~600 nm in LDs)

Note: Multicolor staining with blue or green dyes is possible, but wavelength selection is essential. Especially when multiple staining with green dyes (FITC, GFP, etc.) using a 488 nm laser to excite the green dye, LipiDye™ RED is also excited. Therefore, to selectively detect green dye in multiple staining, use a bandpass filter that cuts fluorescence of 520 nm or longer. Conversely, to selectively detect LipiDye™ RED in multiple staining, use a 514 nm laser or longer to excite that and filter out fluorescence below 560 nm.

Reconstitution and Storage

Reconstitution: Stock solution at a recommended concentration of 0.5 to 1 mM in 100% DMSO.

Storage (powder): Store powder at RT

Storage (solution): After reconstitution in DMSO, make aliquots 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 cultured cell staining.

1. Prepare 0.5 μ M LipiDye™ RED in serum-free and phenol red-free medium such as DMEM.

NOTE-1: LipiDye™ RED is compatible with FBS-containing medium.

NOTE-2: Recommended concentration is 0.1-5 μ M. Empirically optimize and determine the concentration of LipiDye™ RED for your experiments.

2. Remove culture medium and wash cells with appropriate medium or PBS several times.

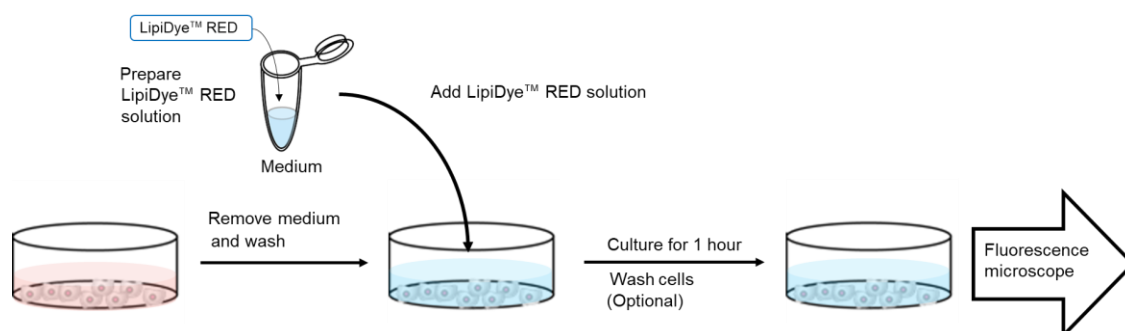
3. Add LipiDye™ RED -containing medium to cells.

4. Incubate cells at 37 °C for 1 hour.

NOTE-3: Empirically optimize incubation time for your experiments.

5. Wash cells with PBS or medium and add fresh medium (Optional)

6. Observe cells with a fluorescence microscope.



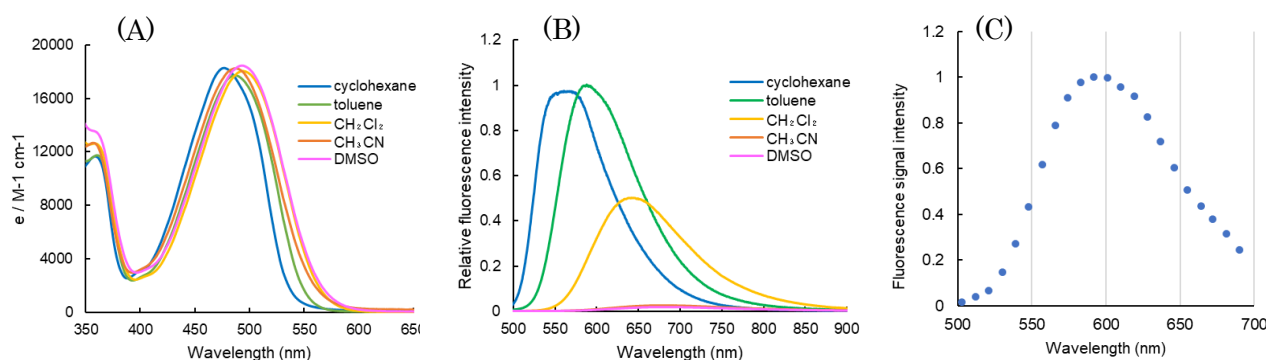
Reference data

Spectrum of LipiDye™ RED

(A) Absorption spectrum in various solvents.

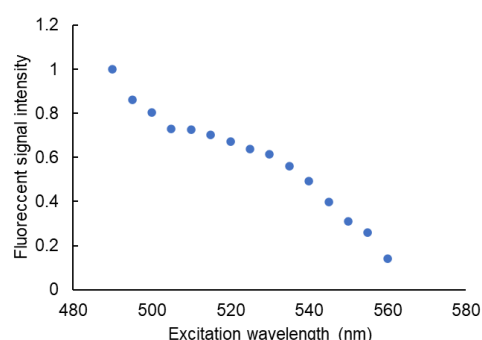
(B) Emission spectrum in various solvents. LipiDye™ RED is a solvatochromic dye and shows a different spectrum in each solvent. In low-polar solvents such as cyclohexane, toluene, and dichloromethane, LipiDye™ RED emits from orange to red fluorescence with a high quantum yield. On the other hand, under high-polar solvents such as acetonitrile and DMSO, LipiDye™ RED exhibits a weak fluorescence intensity with a red-shift fluorescence.

(C) Emission spectrum in cellular LDs measured by fluorescent microscopy.



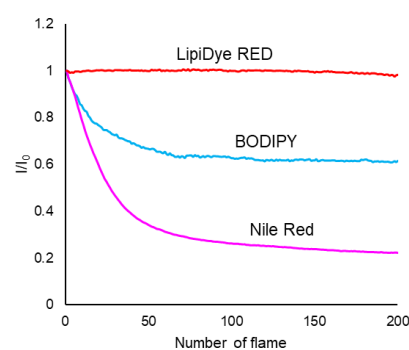
Applicability of excitation wavelength

LipiDye™ RED was used to stain LDs in HepG2 cells, and red fluorescence ($\lambda_{\text{em}} = 580\text{--}750 \text{ nm}$) intensity was measured with several different excitation lasers. It was found that excitation light up to about 560 nm can be applied.



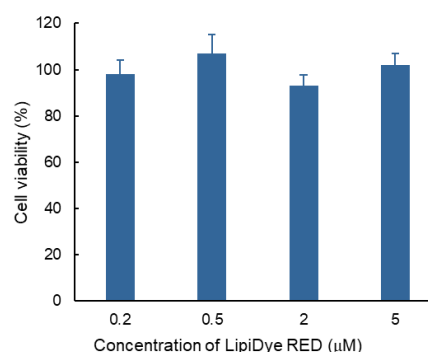
Photostability

After staining HeLa cells with LipiDye™ RED, Nile Red, and BODIPY493/503, images were repeatedly acquired using a high-power laser (500 nm) using a confocal laser scanning microscope, and changes in fluorescence intensity were observed. In contrast to Nile Red and BODIPY493/503, whose fluorescence attenuated significantly after multiple image acquisitions, LipiDye™ RED showed almost no change in fluorescence intensity even after a total of 200 image acquisitions.



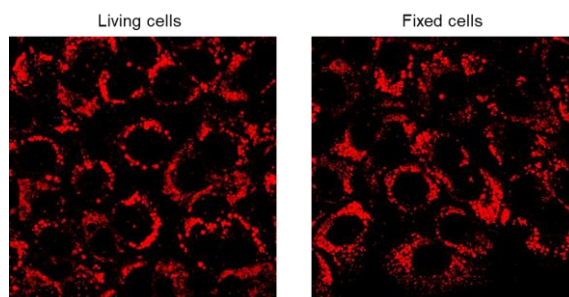
Cytotoxicity of LipiDye™ RED

HeLa cells were treated with various concentrations of LipiDye™ RED for 24 hours. After incubation, cell viability was evaluated by MTT assay. The concentration range (0.2–5 μM) showed little cytotoxicity in cells.



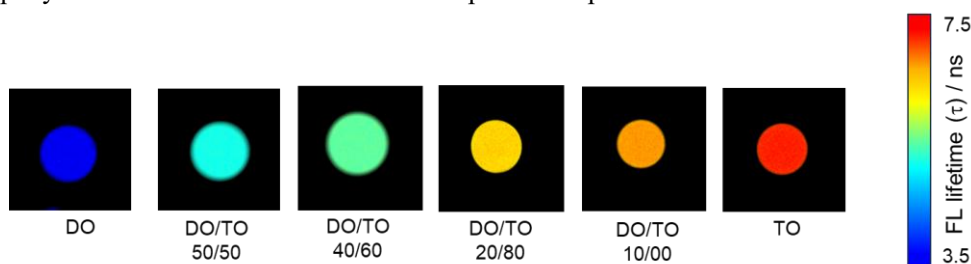
Changes in fluorescence lifetime depending on lipid composition

Huh-7 cells treated with oleic acid to form LDs were stained with LipiDye™ RED and fixed with 4% paraformaldehyde. Almost no change in the fluorescence signal was observed before and after the fixation treatment. It has been shown that LipiDye™ RED can be used for co-staining with immunostaining after fixing cells.



Changes in fluorescence lifetime depending on lipid composition

Artificial LDs with varying composition ratios of triolein (TO), a typical TAG, and diolein (DO), a typical DAG, were stained with LipiDye™ RED, and their fluorescence lifetime imaging was observed. The results showed that the higher the DO ratio, the shorter the fluorescence lifetime, and the higher the TO ratio, the longer the fluorescence lifetime ($\tau = 4.1$ ns to 7.5 ns). This indicates the DAG/TAG ratio of LDs can be evaluated using the fluorescence lifetime of LipiDye™ RED. Fluorescence lifetime is expressed in pseudo-color.

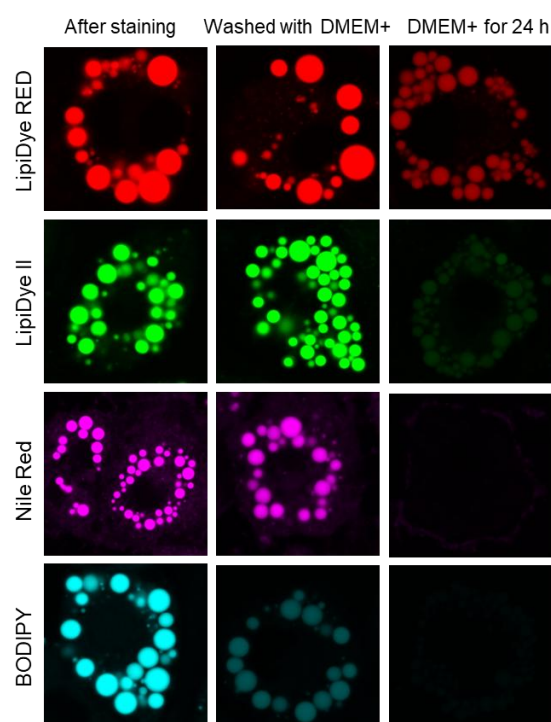


Application data

LDs imaging after medium exchange

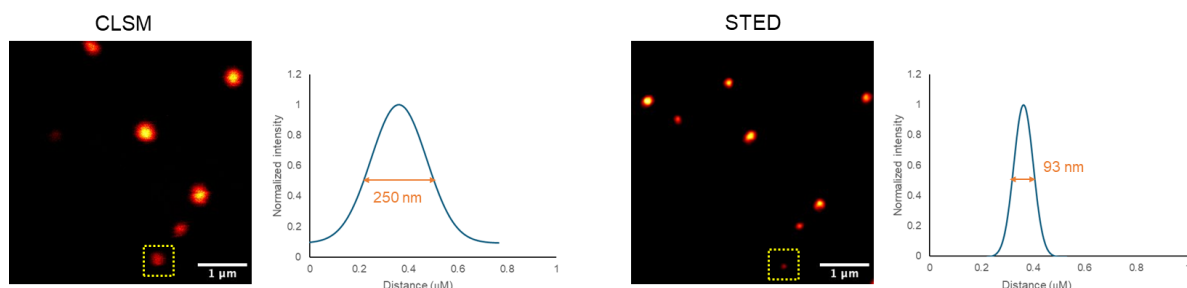
Adipocytes differentiated from 3T3-L1 cells (3T3-Adi) were stained with LipiDye™ RED, LipiDye™ II, Nile Red, and BODIPY493/503, and a fluorescence image of LDs was observed using a confocal laser scanning microscope. The fluorescence intensity of Nile Red and BODIPY493/503 decreased after cell washing, and almost no fluorescence signal was observed after culturing for 24 hours in an FBS-containing medium (DMEM+). LipiDye™ II maintained its fluorescent signal after washing, but the fluorescent signal significantly weakened after culturing in DMEM+ for 24 hours*. On the other hand, the fluorescence intensity of LipiDye™ RED did not decrease after washing, and remained even after culturing in DMEM+. This indicates that LipiDye™ RED exhibits greater intracellular retention compared to conventional reagents.

*Long-term fluorescence observation with LipiDye™ II is also possible by continuous culturing in a medium containing LipiDye™ II.



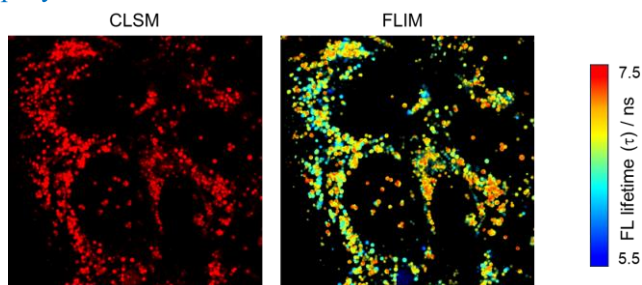
Live-cell STED super-resolution microscopy imaging

HeLa cells were treated with 0.5 μM LipiDye™ RED, washed, and cultured in medium. The cells were imaged by confocal laser microscopy (Ex 488 nm/ Em 560-750 nm) and STED microscopy (Ex 488 nm/ Em 560-750 nm, depletion laser 775 nm). STED imaging detected ~ 92 nm (FWHM) small LD, which was not clearly detected by confocal microscopy.



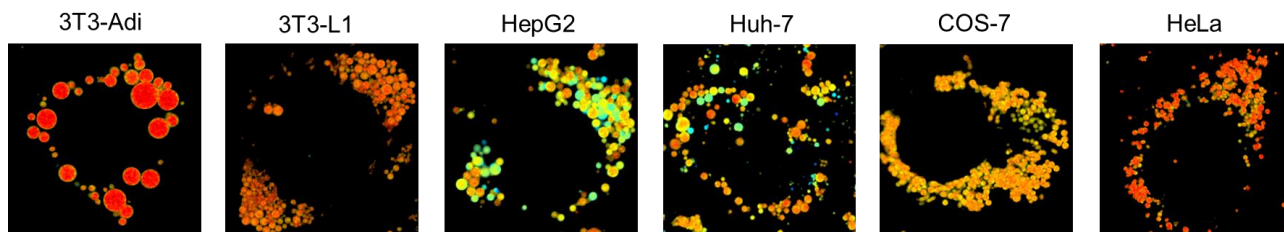
Fluorescence intensity and lifetime imaging of non-adipocyte cells

Huh-7 cells treated with oleic acid to form LDs were treated with 0.5 μM LipiDye™ RED, and fluorescence imaging and fluorescence lifetime imaging (Ex 488 nm/Em 560-750 nm) were performed using a confocal laser microscope. FLIM images are pseudo-colored with fluorescence lifetime (blue: $t = 5.5$ ns, red: $t = 7.5$ ns)



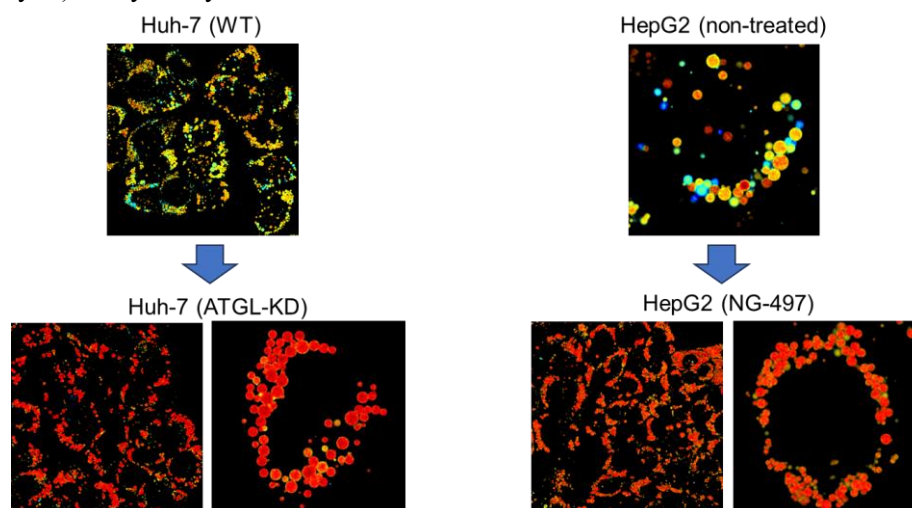
Fluorescence intensity and lifetime imaging of non-adipocyte cells

Adipocytes differentiated from 3T3-L1 cells (3T3-Adi), undifferentiated 3T3-L1 cells, HepG2 cells, Huh-7 cells, COS-7 cells, and HeLa cells were stained with LipiDye™ RED (0.5 μM), and fluorescence lifetime imaging was performed (excitation 488 nm/fluorescence 560-750 nm). Cells other than 3T3-Adi were treated with oleic acid for 1 day to form LDs and then observed. The fluorescence lifetimes of LipiDye™ RED-stained LDs in hepatoma-derived HepG2 cells and Huh-7 cells were not uniform, suggesting that the DAG/TAG composition of each LD differs largely. On the other hand, the fluorescence lifetimes of LDs in other cells were relatively uniform and longer than those of hepatoma cells, indicating that the overall lipid composition had a high proportion of TAG.



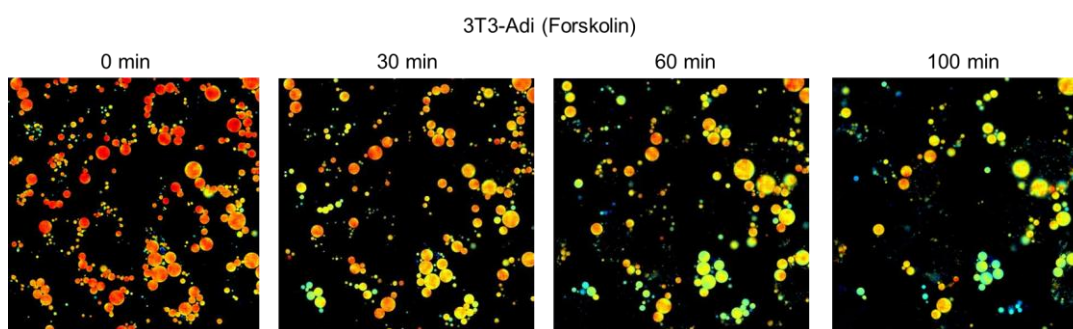
Evaluation of the influence of lipase activity on lipolysis

LipidDye™ RED fluorescence lifetime imaging was performed on Huh-7 cells in which adipose triglyceride lipase (ATGL) was knocked down using siRNA. The fluorescence lifetime of LDs in WT Huh-7 cells was heterogeneous, whereas the fluorescence lifetime in ATGL knockdown cells was uniform and long. The same phenomenon was also observed when HepG2 cells were treated with the ATGL inhibitor NG-497. These suggest the heterogeneity of the lipid composition of LDs in hepatoma cells is due to an increase in the proportion of DAG generated by LDs degradation (lipolysis) catalyzed by ATGL.



Time-lapse analysis of the change of lipid composition during lipolysis induction

Forskolin, an adenylate cyclase activator, was added to adipocytes differentiated from 3T3-L1 cells (3T3-Adi) to induce lipolysis, and time-lapse analysis of fluorescence lifetime imaging of LipidDye™ RED -stained LDs was performed for 100 minutes. The fluorescence lifetime of LDs became heterogeneous and shorter over time after drug treatment, suggesting that the proportion of DAG increased due to LDs degradation.



Reference

1. Wang *et al.*, *J. Am. Chem. Soc.*, **147**, 41514-41523 (2025), Single-Cell Fluorescence Analysis of Lipid Droplet Compositional Dynamics during Triacylglycerol Catabolism.

Related product

FAOBlue™ <Fatty Acid Oxidation Detection Reagent>

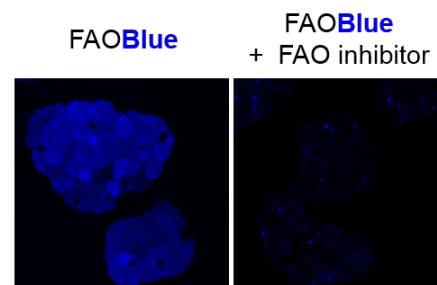
FAOBlue™ is a cell-based fatty acid beta-oxidation (FAO) detection dye which emits blue fluorescence upon cellular FAO activity.

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



LipidDye™ II <Lipid Droplet Live Imaging>

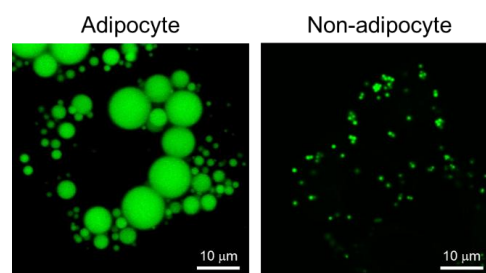
LipidDye™ II is a highly sensitive lipid droplet staining dye with extremely photostable property. This dye is the second generation of our previous reagent, LipidDye™. This dye allows us to detect small lipid droplets (<1 μm) in non-adipocytes and to apply into long-term live cell imaging for dynamic 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



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