funakoshi
FRONTIERS IN LIFE SCIENCE

9-7 Hongo 2-Chome, Bunkyo-Ku
Tokyo 113-0033, Japan

LipiORDER <Membrane Lipid Order Imaging Dye>

Catalog NO. FDV-0041

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

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Product Background

Membrane lipid order is a biophysical parameter that defines a membrane organization and is often described by the degree of lipid packing. For example, phospholipids only containing saturated lipids create high packing and thick lipid bilayer, called liquid-order (Lo) phase. On the other hand, phospholipids containing unsaturated lipids, which have bent structure, form low packing and thin membrane structure, called liquid-disorder (Ld) phase. In the model membrane mixing saturated lipids and unsaturated lipids, Lo and Ld are clearly separated and create individual domains. While the model membrane composition can be discussed membrane lipid order (Lo/Ld) easily, actual cells have numerous types of lipids and form very complicated membrane lipid orders. Furthermore, the lipid order is also influenced by various factors in cellular membranes, including sterol lipids such as cholesterol and membrane proteins, etc. Lipid raft, a continuous interest topic in biology, which serves as functional microdomains on cellular membranes, is one of the specialized Lo domains, with highly accumulated saturated lipids such as sphingomyelin, cholesterol, functional membrane proteins and lipidated proteins. Membrane lipid order has been considered as a fundamental factor in providing physical properties of cellular membranes, such as membrane fluidity, membrane tension and membrane curvature. Observation of cellular lipid order may lead to an understanding of the various function of cellular membranes.

To measure membrane lipid order, some solvatochromic dyes which change fluorescence intensity and color in response to their solvent polarity are applied. These solvatochromic dye fluorescent properties change depending on membrane lipid order. Among them, Laurdan is the most well-known dye for membrane lipid order imaging. However, conventional dyes have some limitations. For example, Laurdan requires UV light excitation and exhibits low photostability. So Laurdan is not suitable for live-cell imaging. Dyes which can be excited by longer wavelength with more photostability and chemically stable in cells are desirable traits for cellular imaging of membrane lipid order. **LipiORDER** is a novel solvatochromic dye for membrane lipid order imaging originally developed by Dr. Yosuke Niko, Kochi University, and Dr. Andrey S. Klymchenko, University of Strasbourg (original compound name PK in Ref.1). LipiORDER is excited at around 400 nm wavelength, which is compatible with live-cell imaging and changes its emission fluorescent color from green to red depending on membrane lipid order. **LipiORDER** also has high photostability and chemical stability on the cell membranes. LipiORDER is a convincing tool to monitor cellular membrane lipid order imaging on live-cells.

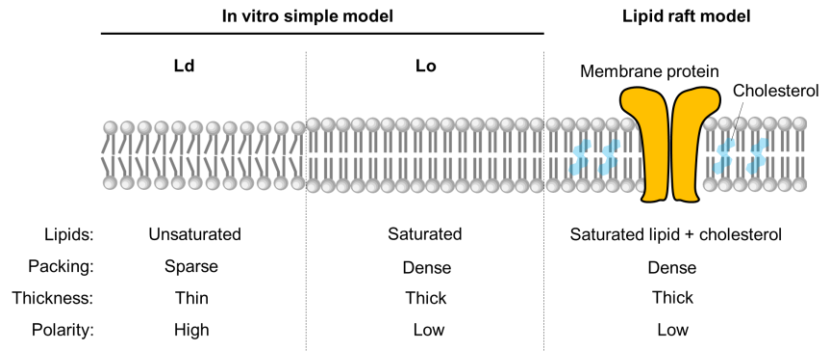


Figure 1. Overview of membrane lipid order

Principle and Reference data

Sensing of lipid order by using LipiORDER is based on the following two unique properties. 1) LipiORDER is a pyren-based solvatochromic fluorescent dye which changes fluorescent property in response to their solvent environment (Figure P1). In low polaric solvents such as toluene, LipiORDER shows green fluorescence. On the other hand, in highly polaric solvents such as DMSO and methanol, this dye changes color to orange or red. 2) LipiORDER is a highly hydrophobic compound and quickly accumulates in the various biological membranes. Combining the two features above, LipiORDER can sense the local environment in a lipid bilayer. Generally, Lo is a high packing lipid bilayer and shows lower polarity, whereas Ld is a sparse packing lipid bilayer and shows high polarity. Based on polarity of lipid bilayer derived from lipid order, LipiORDER will change fluorescent color, from green on Lo membrane to red on Ld membrane (Figure P2). Ratiometric fluorescent value (F_{Red}/F_{Green}) is correlated to lipid order (Lo and Ld).

Actually, in sphingomyeline/cholesterol (SM/Chol) liposome, one of the model Lo, LipiORDER emits green fluorescence and in 1,2-dioleoyl-sn-glucero-3-phosphocholine (DOPC) liposome, a model Ld, shows red fluorescence. In DOPC/Chol, an intermediate model, the reagent show yellow to orange. The ratiometric values (F_{575}/F_{510}) clearly depend on lipid order, SM/Chol (Lo) is low and DOPC (Ld) is high (Figure P3).

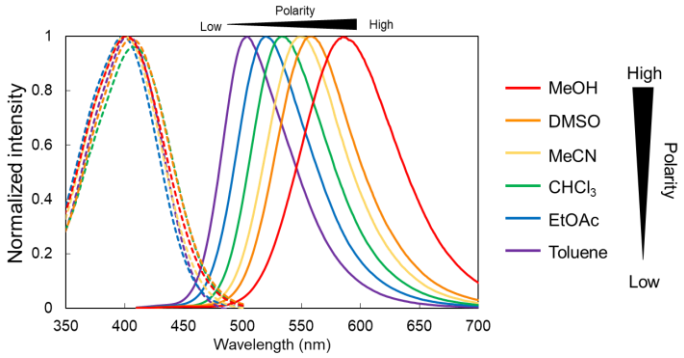


Figure P1　Absorption and fluorescent spectrum of LipiORDER in various solvent

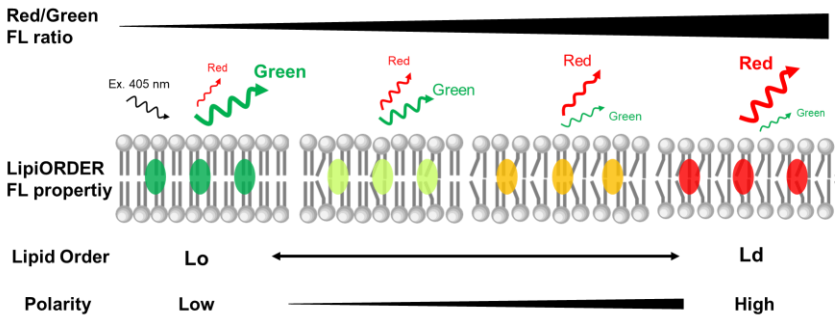


Figure P2　Graphical overview of lipid order-dependent fluorescent change of LipiORDER

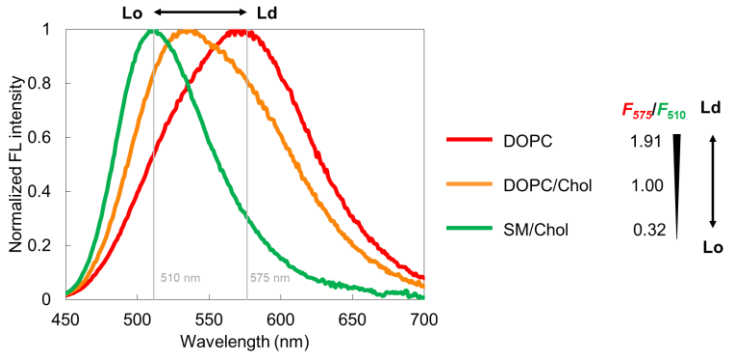


Figure P3　Fluorescent spectrum of LipiORDER in model liposomes

Description

Catalog Number: FDV-0041
Size: 0.1 mg
Formulation: $C_{23}H_{21}NO$
Molecular weight: 327.4 g/mol
Solubility: Soluble in DMSO
Fluorescent characteristics: Ex. 405 nm/Em. 450-650 nm (dependent on solvents)

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.

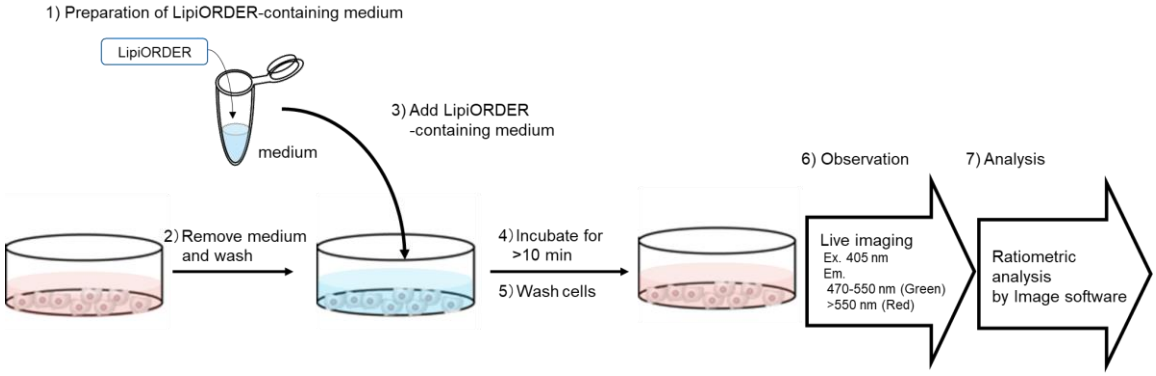
How to use and experimental setting

General procedure of live cell imaging

*This procedure is an example of cultured cell staining. For zebrafish staining, please find Ref.1.

1. Prepare 0.1-1 μM LipiORDER in serum-free and phenol red-free medium such as HBSS
2. Remove culture medium and wash cells PBS several times
3. Add LipiOREDR-containing medium to cells
4. Incubate cells at 37 °C for over 10 min
5. Wash cells with PBS or medium (Optional)
6. Observe cells under live condition with confocal laser microscopy and obtain green and red fluorescent images
7. Perform ratiometric analysis with image software using green and red fluorescent images

NOTE: The staining concentration of LipiORDER is dependent on cell type and experiments. Please empirically optimize to determine the suitable concentration for each experiment.



Fluorescent microscopy and analysis

For LipiORDER ratiometric imaging, LipiORDER is exited at 405 nm and its fluorescence is detected with two ranges, green channel and red channel. The recommended wavelength range of green channel and red channel is 500-550 nm and 550-650 nm, respectively. Ratiometric image analysis (F_{Red}/F_{Green}) is calculated by any image processing software such as ImageJ.

Option: For calibration control of each model lipid order, we recommend obtaining the following images.

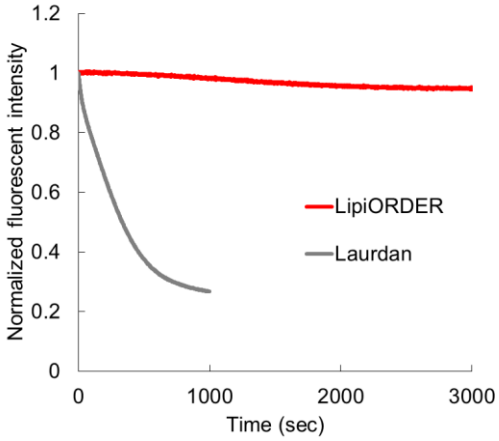
- Labrafac oil for lipid droplet,
- SM/Chol liposome for Lo model
- DOPC liposome for Ld model

NOTE: As you can see in Figure P1, the absorbance of LipiORDER at around 480 nm is negligible. LipiORDER is compatible with common green dyes (excited by ~480 nm laser) and red dyes (excited by ~560 nm) for multicolor staining.

Application data

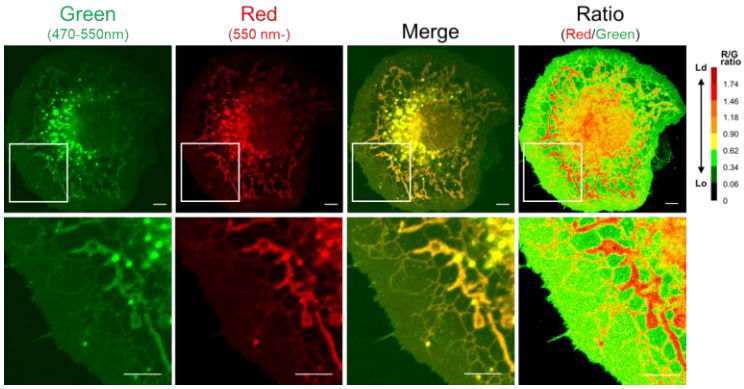
Photostability of LipiORDER

LipiORDER and Laurdan, a conventional membrane lipid order imaging dye in lipid vesicles composed of 0.2 mM DOPC in 20 mM HEPES (pH 7.4) were irradiated with Xe lamp. LipiORDER and Laurdan were excited at 405 nm and 360 nm, respectively and fluorescent intensity was measured. Laurdan was quickly photodegraded, whereas LipiORDER maintains fluorescent intensity for at least 1 hour. LipiORDER is highly stable compared to Laurdan.



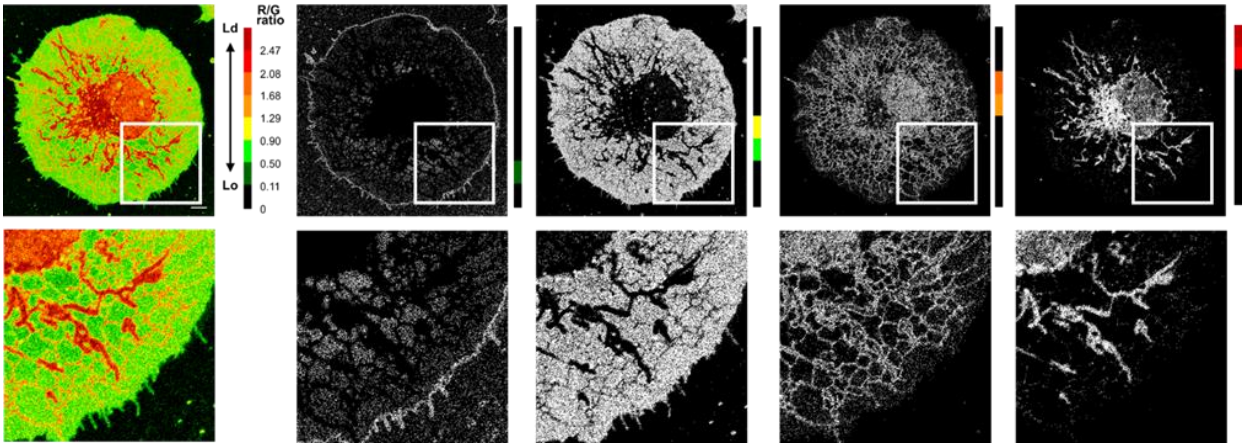
Ratiometric imaging of COS7 cells

COS7 cells were treated with 300 nM LipiORDER in HBSS for 10 min and observed by confocal laser microscopy (Ex. 405 nm, Em 470-550 nm for Green channel and >550 nm for Red channel). Ratiometric analysis was performed with ImageJ using green and red channel data and lipid order was shown by green-to-red pseudocolor (**Lo** ■■■■■■■■ **Ld**). Plasma membrane and intramembranes are shown Lo and Ld, respectively.



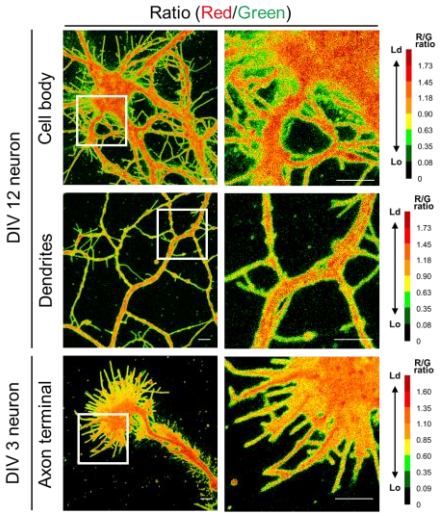
*An example of membrane lipid order analysis

Each layer of ratiometric pseudocolor was extracted. Low ratio value ■ shows plasma membrane structure mainly and high ratio value ■■ and ■■ mainly shows how ER-like and mitochondria-like structure, respectively.

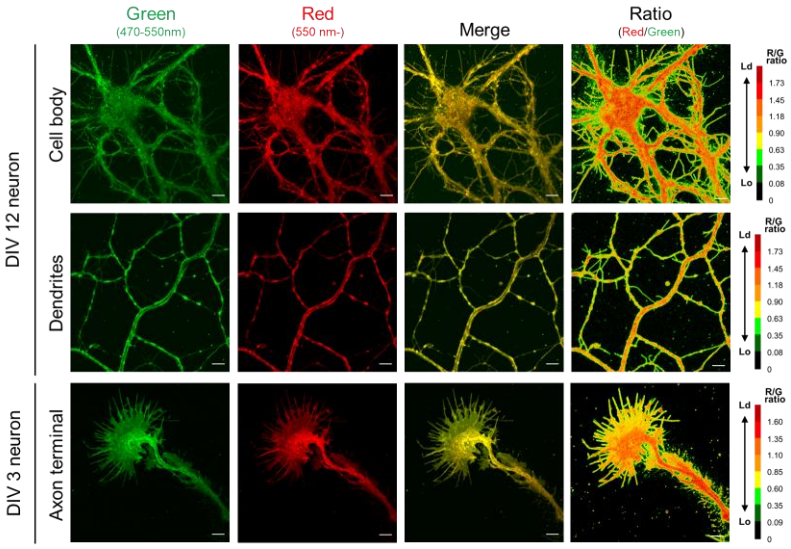


Ratiometric imaging of neuronal cells

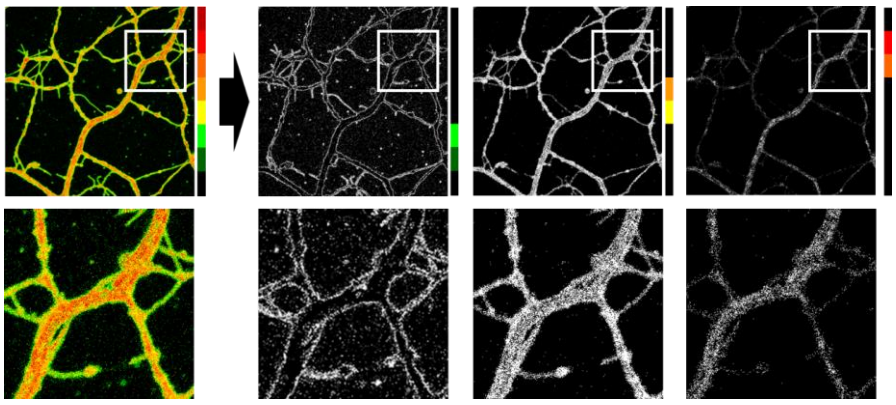
Primary cultured hippocampal neurons (DIV 3 or DIV 12) from E17.5 mice were stained with 300 nM LipiORDER in HBSS for 10 min and observed by confocal laser microscopy (Ex. 405 nm, Em. 470-550 nm for Green channel and >550 nm for Red channel). Ratiometric analysis was performed with ImageJ using green and red channel data and lipid order was shown by green-to-red pseudocolor (**Lo** ■■■■■■■ **Ld**).



* Each fluorescent imaging data is shown below. The ratiometric data was calculated using the following pictures.

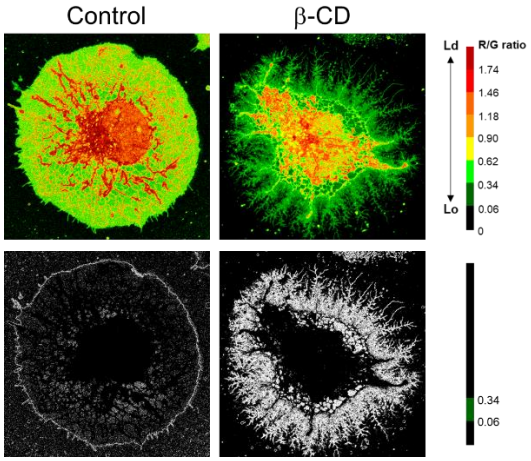


*An example of membrane lipid order analysis (Dendrites in DIV12 neurons). Near Lo phases (■■) clearly shows plasma membrane structures. On the other hand, intermediate (■■) and Ld phases (■■) were observed from intracellular compartments.



Drug-induced cellular lipid order changes

COS7 cells were treated with 15 mM β-cyclodextrin (β-CD), a membrane-disrupting chemical via removing endogenous cholesterol, for 4 hours. After β-CD treatment, cells were washed and stained with 300 nM LipiORDER in HBSS for 10 min. The cells were observed by confocal laser microscopy (Ex. 405 nm, Em. 470-550 nm for Green channel and >550 nm for Red channel). Ratiometric analysis was performed with ImageJ using green and red channel data and lipid order is shown by green-to-red pseudocolor (**Lo** ■ ■ ■ ■ ■ ■ ■ **Ld**). The cell structure was dramatically changed by β-CD and at the same time, the distribution of Lo phase (■) clearly changed.



Notes

All spectrum data and a photostability data were obtained by Dr. Yosuke Niko, Kochi University.

All cell imaging data were obtained by Dr. Mitsuharu Hattori, Nagoya City University.

Reference

1. Valanciunaite *et al., Anal. Chem.*, **92**, 6512-6520 (2020) Polarity Mapping of Cells and Embryos by Improved Fluorescent Solvatochromic Pyrene Probe.

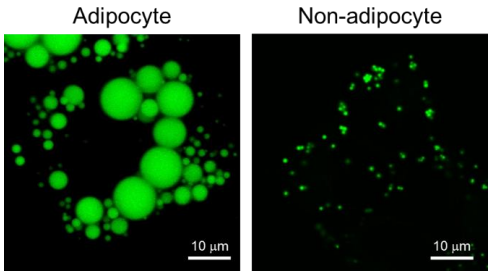
Related products

LipiDye II <Live Imaging>

LipiDye II is a highly sensitive lipid droplet staining dye with extremely photostable property. This dye is the second generation of our previous reagent, LipiDye. 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

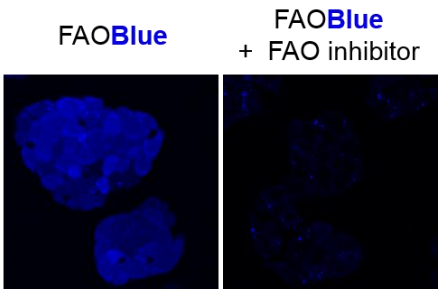


FAOBlue <Fatty Acid Oxidation Detection Reagent>

FAOBlue is a cell-based fatty acid beta-oxidation (FAO) detection dye which emits blue fluorescence upon FAO activity. FAOBlue enables to quantitatively monitor cellular FAO activities under various conditions.

Catalog No. FDV-0033
Size 0.2 mg
Features

- Recommended Ex/Em:~405 nm / 460 nm
- Enable to detect cellular FAO activity directly without any specific equipment, only need microscopy.
- Monitor drug-induced change of FAO activity quantitatively.

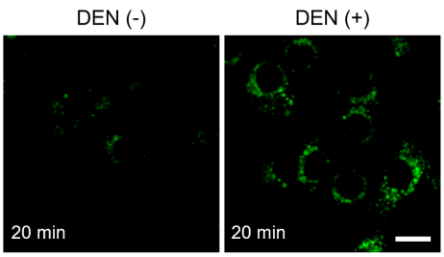


LipiRADICAL Green <Lipid Radical Detection Reagent>

LipiRADICAL Green is a specific fluorescent dye for lipid-derived radicals which are the most upstream factor of lipid peroxidation (LPO). LipiRADICAL Green can be applied into both *in vitro* assay and cell-based assay to monitor lipid radical productions.

Catalog No. FDV-0042
Size 0.1 mg
Features

- Recommended Ex/Em:~480 nm / 520 nm
- Enable to detect very unstable lipid-derived radicals
- Compatible with *in vitro* assay and in cell-based assay
- An innovative reagent for comprehensive identification of lipid-derived radicals by lipidomics



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