funakoshi
FRONTIERS IN LIFE SCIENCE

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

# LipiRADICAL™ Green <Lipid Radical Detection Reagent>

Catalog NO. FDV-0042

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

日本語版はこちらから
ダウンロードできます。
①弊社ウェブサイトより
Webページ番号検索にて
【70873】で検索
②QRコードより

## Product Background

Lipid peroxidation (LPO) is one of the several degradation processes of lipids under oxidative stress (Figure 1). Primary products in LPO are lipid radicals and there are two major initiators to induce LPO process, pro-oxidants and lipid oxidative enzymes, including lipoxygenase (LOX) and cytochrome P450 (CYP). 1) For pro-oxidant-induced LPO, lipids containing unsaturated fatty acid, especially polyunsaturated fatty acids (PUFAs), are attacked by pro-oxidants, including reactive oxygen species (ROS) and form lipid-derived radicals. Lipid radical (L・) can be easily oxidized to lipid peroxyl radical (LOO・). Unstable LOO・ immediately extracts a hydrogen from another lipid molecule generating a lipid hydroperoxide (LOOH) and a new lipid radical (L・). 2) Another pathway enzyme-induced LPO, lipids containing PUFAs are oxidized to lipid hydroperoxides (LOOH), which decomposes to lipid peroxyl radicals LOO・ or alkoxyl radicals LO・ by metal ions ($Fe^{2+}$ etc.). Once lipid radical is produced by the above two processes, lipid radicals expand the radical chain reaction (radical propagation step). In the termination reaction, antioxidants donate a hydrogen atom to the lipid peroxy radical (LOO・) species resulting in the formation of many different aldehydes, including malondialdehyde (MDA), acrolein, propanal, hexanal, and 4-hydroxynonenal (4-HNE). These aldehydes are cytotoxic because reactive aldehydes attack biomolecules (proteins, DNA/RNA, etc.) to form secondary products. These reactive aldehydes are considered causative factors of organ injury, ferroptosis and ER-stress. To understand the molecular mechanism and physiological relevance of LPO, detection and quantification methods for lipid radicals are required. However, the conventional detection methods are highly limited. For example, electron spin resonance (ESR) is a major strategy to detect radical products but not applied to cell-based applications.

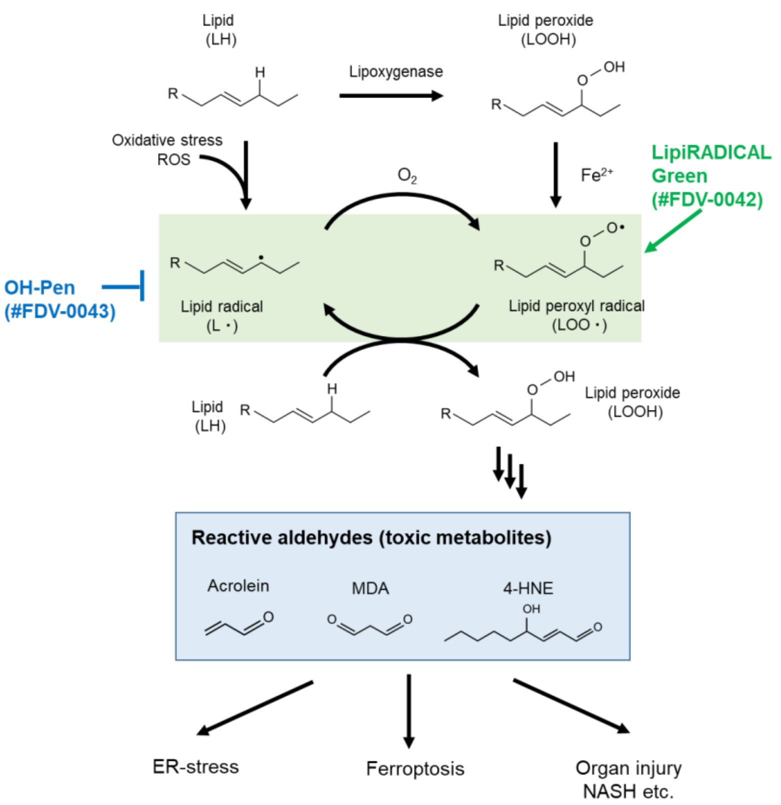



**Figure 1. Overview of lipid radicals in LPO pathway**

"**LipiRADICAL™ Green**" is the world's first lipid radical-responsive fluorescent dye (original compound name **NBD-Pen** in Ref. 1). **"LipiRADICAL™ Green"** is a green fluorescent dye NBD-conjugated nitroxyl radical-derivative (Figure 2). Although this compound contains NBD, the probe's fluorescence is highly quenched by intramolecular radical moiety. When the probe reacts with lipid radicals via radical-radical coupling forming a covalent bond to lipids, the fluorescent intensity is drastically recovered. **"LipiRADICAL™ Green"** is well validated to selectively detect lipid radicals, not reactive oxygen radicals. **"LipiRADICAL™ Green"** enable us to semi-quantification of lipid radicals in biological samples, to image cellular lipid radicals and to identify and analyze the molecular structure of lipid radicals with LC/MS system. **"LipiRADICAL™ Green"** is an innovative and powerful tool for LPO research. Funakoshi also has lipid radical-specific inhibitor, OH-Pen (#FDV-0043).

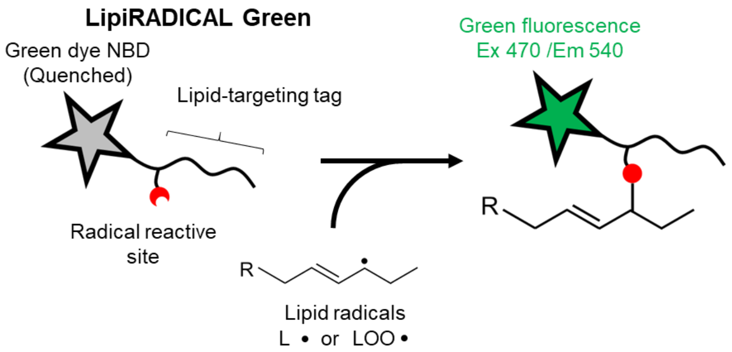
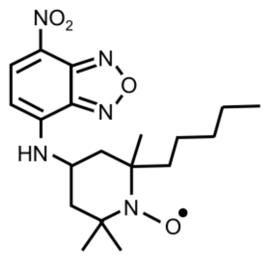



**Figure 2. Principle of LipiRADICAL™ Green**

## Description




Catalog Number: FDV-0042
Size: 0.1 mg
Formulation: $C_{19}H_{28}N_5O_4$ •
Molecular weight: 390.21g/mol
Solubility: Soluble in DMSO
Ex/Em:
Ex. 420-500 nm (maximum ~470 nm)
Em. 520-600 nm (maximum ~540 nm)
*Conventional FITC filter sets are compatible.

## Application

- *In vitro* detection of lipid radicals by fluorescent detection
- Live cell imaging of lipid radicals by fluorescent microscopy
- Screening of LPO suppressor or antioxidant both *in vitro* or *in cellulo*
- Structural analysis of lipid radicals by fluorescent-LC/MS-MS

## Reconstitution and Storage

Reconstitution: stock solution recommended concentration 1 mM in 100% DMSO.
Storage :
Store powder at -20°C.
After reconstitution in DMSO, aliquot and store at -20 °C, avoid repeated freeze-thaw cycles.
Protect from light.

## How to use

### General procedure of lipid radical detection *in vitro*

In the case of using pure lipids with any radical inducers

1. Prepare reaction mixture containing lipids such as PUFA and any radical inducers
2. Add "**LipiRADICAL™ Green"** to the reaction mixture
3. Measure fluorescence (Ex 470 nm/ Em 540 nm) after the appropriate incubation time

In the case of using any biological samples such as cell lysate, blood, biofluids etc.

1. Prepare biological samples with or without any radical inducers
2. Add **"LipiRADICAL™ Green"** to the sample
3. Measure fluorescence (Ex 470 nm/Em 540 nm) after the appropriate incubation time

### General procedure of lipid radical imaging in live cells

1. Prepare 1 μM **"LipiRADICAL™ Green"** in serum-free and phenol red-free medium or appropriate buffer such as HEPES-buffered saline (HBS) etc..
2. Remove culture medium and wash cells PBS several times
3. Add **"LipiRADICAL™ Green"**-containing medium to cells.
4. Incubate cells at 37°C for over 10 min.
5. (Optional) Wash cells with PBS or medium to remove excess reagent
6. (Optional) Add any pro-oxidants to cells to promote the production of lipid radical
7. Observe cells under live condition

### General procedure of lipid radical detection *in vivo*

*In vivo* animal experiments of **"LipiRADICAL™ Green"** requires experimental optimization.
Please refer to Ref.1-5 and optimize proper usage.

### General procedure of structural analysis of lipid radicals

Please see the **Appendix**.

## Appendix: Structural analysis of lipid radicals

Not only detecting lipid radicals *in vitro* and cells but also **"LipiRADICAL$^{TM}$ Green"** applies to the comprehensive identification and structural analysis of lipid radicals. This appendix describes basic instruction for structural analysis of lipid radicals with **"LipiRADICAL$^{TM}$ Green"**. Figure A1 shows a diagram of the structural analysis of lipid radicals.

**Step-1** Fluorescent labeling of lipid radicals by **"LipiRADICAL$^{TM}$ Green"**

Using any biological samples containing lipid radicals, **"LipiRADICAL$^{TM}$ Green"** can label lipid radicals with the NBD fluorescent dye. Lipid fraction is extracted by conventional lipid purification methods such as Bligh/Dyer method.

**Step-2** Fluorescent LC /MS-MS analysis

The labeled and extracted lipid mixture is applied to liquid chromatography (LC) with fluorescent detection (LC-FL)-high resolution tandem mass spectrometry (HRMS-MS). Labeled lipid-adducts are detected by LC-FL and its mass is detected by HRMS-MS

**Step-3** Structural estimation

Lipid radical structures are estimated from MS signals detected by LC-FL/HRMS-MS. The theoretical molecular weight of LipiRADICAL$^{TM}$ adduct is 389.2068 (calculated) and the original molecular weight is estimated with the following equation.

[Lipid radical] = [Total MS (detected MS)] – [LipiRADICAL$^{TM}$ adduct (389.2068; calculated)]

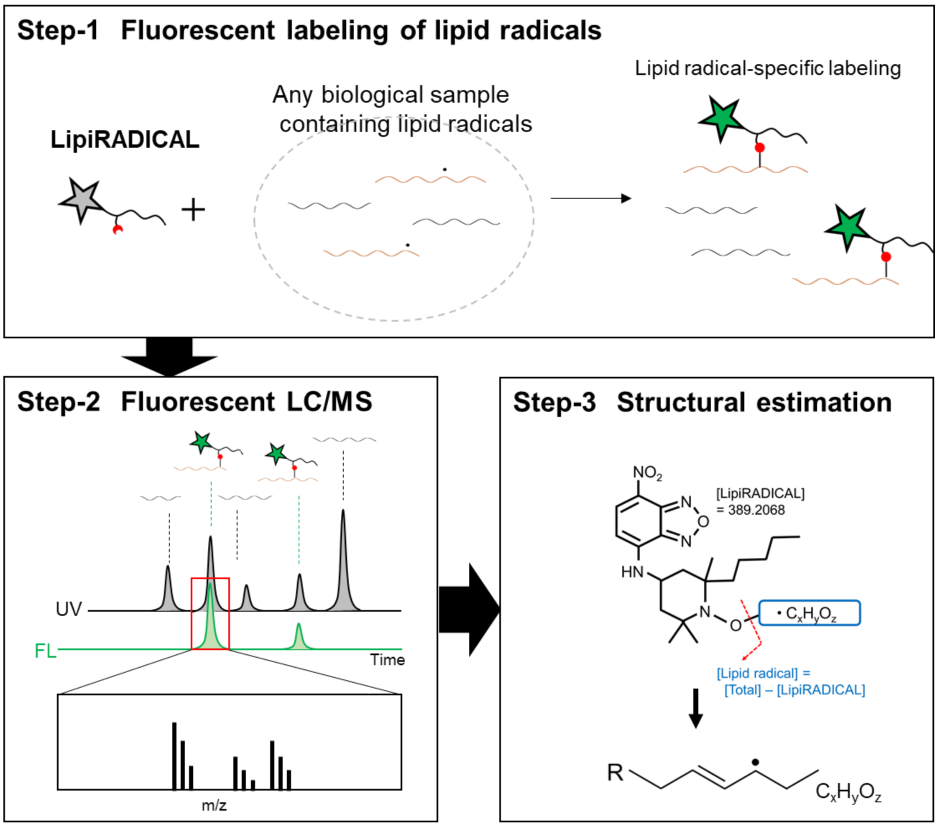


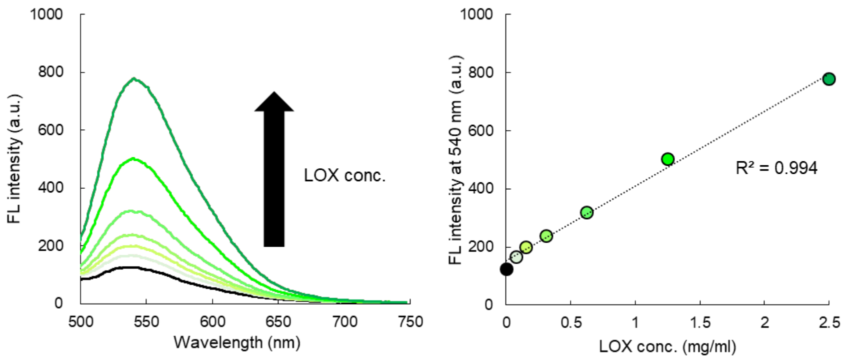

**Figure A1. Diagram of Structural Analysis of Lipid Radicals**

Detail protocol and analytical methods are shown in Ref 5. Ref 5 shows 132 lipid radicals derived from 5 PUFAs with LOX or pro-oxidants *in vitro*.

# Reference data

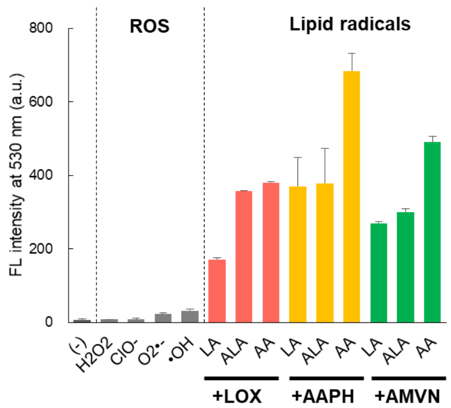
## Fluorescent spectrum

**" LipiRADICAL™ Green"** was added to arachidonic acid-lipoxygenase (LOX) mixtures and observed fluorescence excited by 470 nm light. In the absence of LOX enzyme, the fluorescent signal was highly quenched (Black line). In the presence of LOX enzyme, green fluorescence (500-650 nm, maximum ~540 nm) was detected in LOX dose-dependent manner.




## Specificity

**" LipiRADICAL™ Green"** was treated with the following reagents and fluorescent intensity (Ex 470 nm/Em 530 nm) was observed. All reactive oxygen species had little effects on the fluorescent intensity of "**LipiRADICAL™ Green"**. Green fluorescence was only observed under the polyunsaturated lipids (laulic acid (LA), α-laulic acid (ALA) or arachidonic acid (AA)) with LOX enzyme or pro-oxidants including AAPH and MeO-AMVN.




Reagents and conditions

LipiRADICAL™ Green (5 μM)

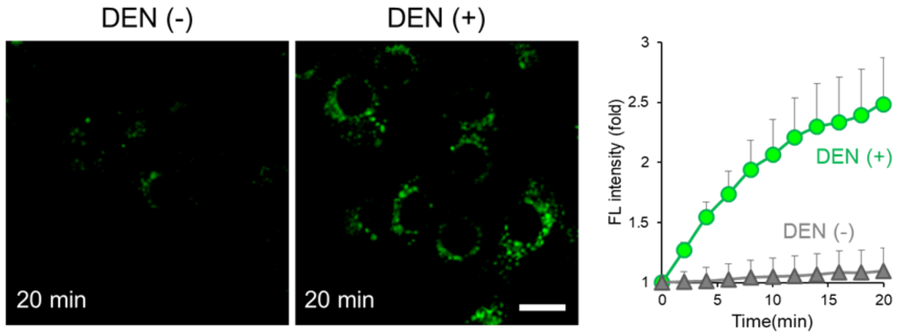
$H_2O_2$, $ClO^-$, $KO_2$ for $O^{2-}$・and ・OH : 0.5 mM

Lipids (0.5 mM) with LOX (2.5 μg/ml) , AAPH (10 mM) or MeO-AMVN 50 μM

# Application data

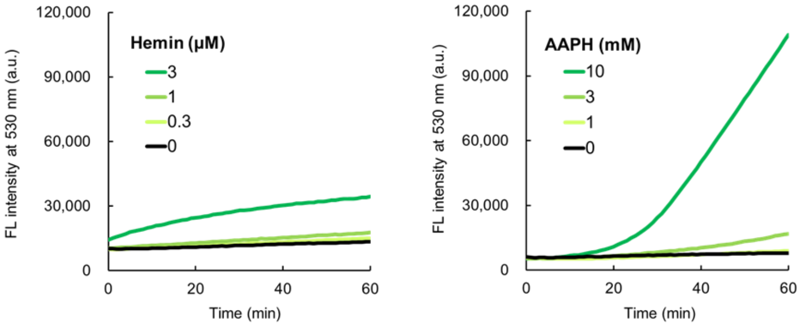
## Cell-based detection of lipid radicals induced by diethylnitrosamine (DEN)

Hepa1-6 cells were treated with 1 μM of **"LipiRADICAL™ Green"** for 20 min. For inducing an LPO signal, the cells were co-treated with diethylnitrosamine (DEN, 30 mM), an LPO initiator, and **"LipiRADICAL™ Green"**. Immediately after DEN addition, the cells were observed by confocal microscopy (Ex.458 nm/ Em. 490-674 nm) for 20 min with 2 min interval. The fluorescent signal of **"LipiRADICAL™ Green"** from the DEN-treated cells clearly increased.



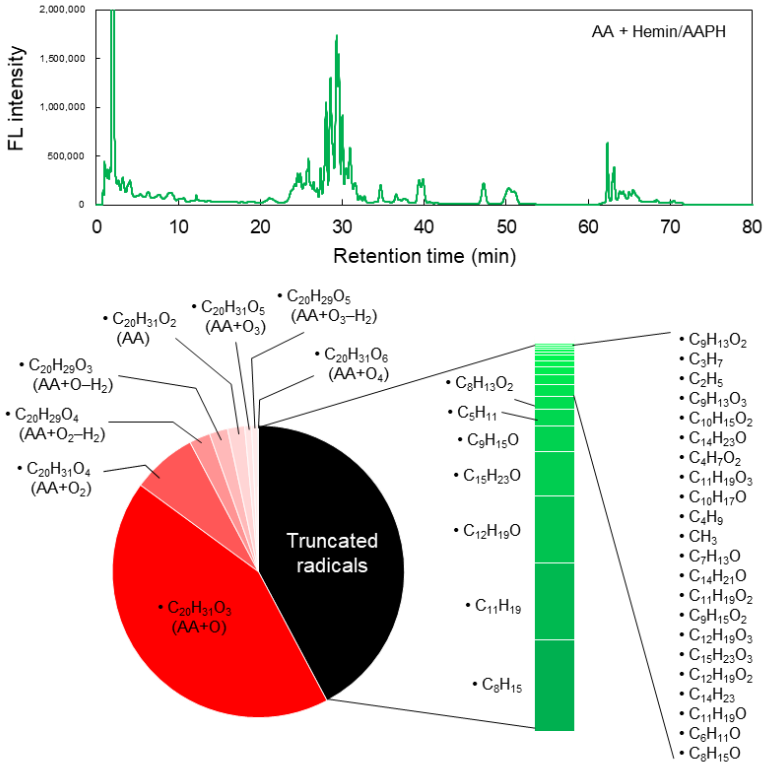
## *in vitro* detection of lipid radicals derived from LDL

Purified low-density lipoprotein (LDL, 20 µg protein/mL) was mixed with pro-oxidants hemin or AAPH and "**LipiRADICAL$^{TM}$ Green**" (10 µM)and the green fluorescence (Ex. 470 nm/ Em 530 nm) was measured for 60 min at 37$^{o}$C. Both hemin and AAPH increased green fluorescence indicating the production of lipid radicals from LDL particles in a time-dependent manner.



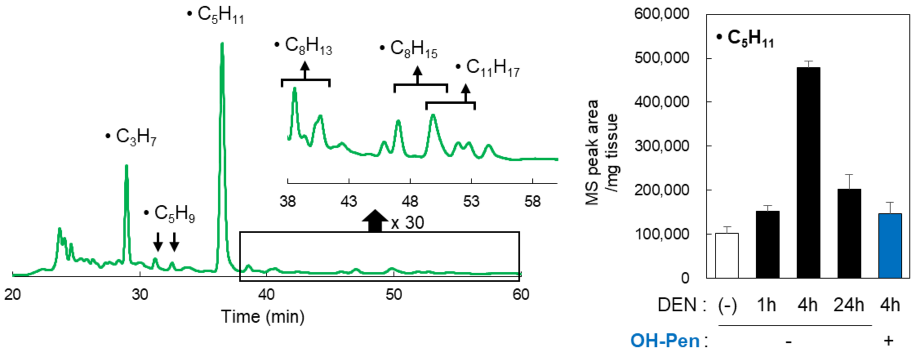

## Structural analysis of lipid radicals derived from arachidonic acid *in vitro*

Arachidonic acid (AA; 500 µM) was incubated with pro-oxidants hemin (10 µM) and AAPH (50 mM) mixture for 60 min. After incubation, 5 µM of **"LipiRADICAL$^{TM}$ Green"** was added to the reaction mixture and incubated for 15 min at R.T. Lipid components were extracted by the Bligh and Dyer method and analyzed by the LC-FL/MS-MS technique. (Upper panel) The fluorescent chromatogram is shown (Ex. 470/E. 530 nm). Several fluorescent peaks were observed and each peak was further analyzed by MS-MS. (Lower panel) Product profiles of AA-derived radicals are shown. MS-MS analysis identified a total of 8 full-length AA radicals and 29 truncated radicals. The relative abundances of each radical were calculated from each peak area. Detailed experimental protocol and analytical procedure are described in Ref.5.



### Structural analysis of lipid radicals *in vivo*

A well-known carcinogen, diethylnitrosamine (DEN, 100 mg/kg body weight), was injected intraperitoneally into mice and after 1, 4 and 24 hours, mice were anesthetized. Anesthetized mice then received intraperitoneal injections of “**LipiRADICAL™ Green”** (2.5 µmol/kg body weight). To check the specificity of “**LipiRADICAL™ Green”**, OH-Pen, a specific inhibitor of lipid radical (Catalog no. #FDV-0042; 10 µmol/kg body weight) was also injected into the mice before “**LipiRADICAL™ Green”** injection. The liver was removed from the mice and homogenized with methanol. Lipid solution was extracted from the liver homogenate according to the Bligh and Dyer method. Lipid samples were applied to LC-FL/MS-MS for analysis (Left). After 4 hours of treatment of DEN, there was a high production of lipid radicals. A total of 11 lipid radicals were identified. (Right) An example, a ・ $C_5H_{11}$ radical. OH-Pen-preinjection clearly inhibited the production of lipid radicals derived from DEN treatment.




## Reference

1. Yamada *et al., Nat. Chem. Biol.*, **12**, 608-613 (2016) Fluorescence probes to detect lipid-derived radicals.
2. Enoki *et al., Chem. Commun.*, **53**, 10922-10925 (2017) Lipid radicals cause light-induced retinal degeneration.
3. Ishida *et al., Free Radical Biol. Med.*, **113**, 487-493 (2017) Detection and inhibition of lipid-derived radicals in low-density lipoprotein.
4. Mishima *et al., J. Am. Soc. Nephrol.*, **31**, 280-296 (2020) Drug Repurposed as antiferroptosis agents suppress organ damage, including AKI, by functioning as lipid peroxyl radical scavengers.
5. Matsuoka *et al., Anal. Chem.*, **92**, 6993-7002, (2020) Method for structural determination of lipid-derived radicals

**Disclaimer/免責事項**

This product has been commercialized by Funakoshi Co., Ltd. based on the results of academic research, and the advertisement text, figures and manuals (hereinafter “Product information”) have been prepared based on published research reports on November, 2020. The academic interpretation at the time of creation of the Product Information may change in accordance with future developments in the relevant research field and expansion of various scientific findings, and the latest version and certainty of the Product Information are not guaranteed. The specifications of this product and the Product Information are subject to change without notice. Please contact us for the latest information.

本商品は学術研究成果を基にフナコシ株式会社が商品化したもので、2020 年 11 月時点における公開研究報告を基に広告文章およびマニュアル（以下、商品資料）を作成しています。今後の当該研究分野の発展および各種学術知見の拡大にともない、商品資料作成時の学術的解釈が変更になる可能性があり、最新性・確実性を保証するものではありません。また、本商品の仕様および商品資料を予告なく変更する場合がございます。最新の情報に関しましては、弊社までご確認いただきますようお願い申し上げます。

E-mail Newsletter Sign Up

Japanese

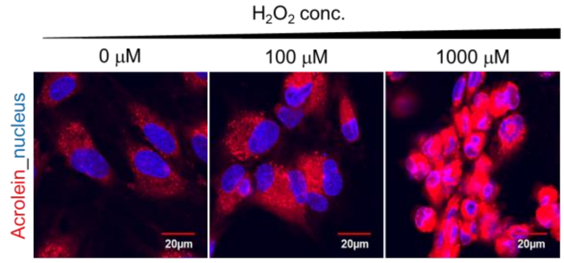
English

## Related products

### AcroleinRED™ <Cell-based Acrolein Detection Reagent>

Acrolein is a LPO downstream aldehyde and one of the most toxic oxidative stress marker. AcroleinRED™ is the world first cell-based acrolein detection reagent.

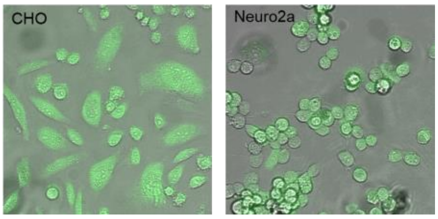
Catalog No. FDV-0022
Size 0.5 mg
Features
- Easy and quick protocol
- Enable to monitor acrolein production under live cells with various stimulations




### CellFluor™ GST <Cell-based GST Activity Assay Reagent >

Glutathione *S*-Transferases (GSTs) are major detoxication enzyme family which neutralize LPO-derived toxic aldehydes. CellFluor™ GST is a novel fluorescent probe for monitoring wide GST members' activity both in cell and *in vitro*. CellFluor™ GST releases green fluorophore rhodamine 110 upon GST activities. This probe has cell-permeability and can detect intracellular GST activity.

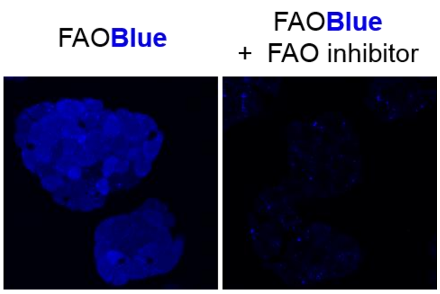
Catalog No. FDV-0031
Size 0.1 µmol
Features
- Easy and quick protocol
- Broad specificity for various GST family members
- Ex/Em: 496 nm/520 nm (commercial FITC filters are available)




### 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.





funakoshi
FRONTIERS IN LIFE SCIENCE
URL: http://funakoshi.co.jp
9-7 Hongo 2-Chome, Bunkyo-ku, Tokyo 113-0033