

FAOBlue <Fatty Acid Oxidation Detection Reagent>

Catalog NO. FDV-0033

Research use only. Not for use in human.

Product Background

Fatty acids (FAs) are basic building blocks for wide variety of lipids, essential components of cells, and are one of primary sources of energy. The major pathway for the degradation of FAs is mitochondrial FA beta-oxidation (FAO). FAO is a key metabolic pathway for energy homeostasis in organs such as the liver, heart and skeletal muscle. FAO is a complicated biochemical event containing many types of enzymes. First, FAs are converted to acyl-CoA form by acyl-CoA synthetase family. Second, acyl-CoA forms are incorporated into mitochondria via the carnitine shuttle pathway. Once acyl-CoA entering to mitochondrial matrix, acyl-CoA (C_n) is converted to acyl-CoA (C_{n-2}) and acetyl-CoA by four stepwise reactions. 1) Dehydrogenation: Acyl-CoA is oxidized to enoyl-CoA by acyl-CoA dehydrogenases, 2) Hydration: Enoyl-CoA is hydrated to 3-hydroxyacyl-CoA by crotonase, 3) Oxidation: 3-hydroxyacyl-CoA to 3-ketoacyl-CoA, 4) Thiolysis: 3-ketoacyl-CoA to acyl-CoA (C_{n-2}) and acetyl-CoA. Acetyl-CoA is further converted to ATP. The resulting acyl-CoA (C_{n-2}) enters another cycle of FAO to further produce acyl-CoA (C_{n-4}).

Abnormal FAO is involved in various diseases such as obesity and non-alcoholic fatty liver diseases (NAFLD). Although measurement of FAO activity in diseased cells is important, methods for measuring FAO activity are limited due to its complicated processes above. Only a few indirect methods such as using radio-isotope containing fatty acids or measuring oxygen consumption are commonly performed.

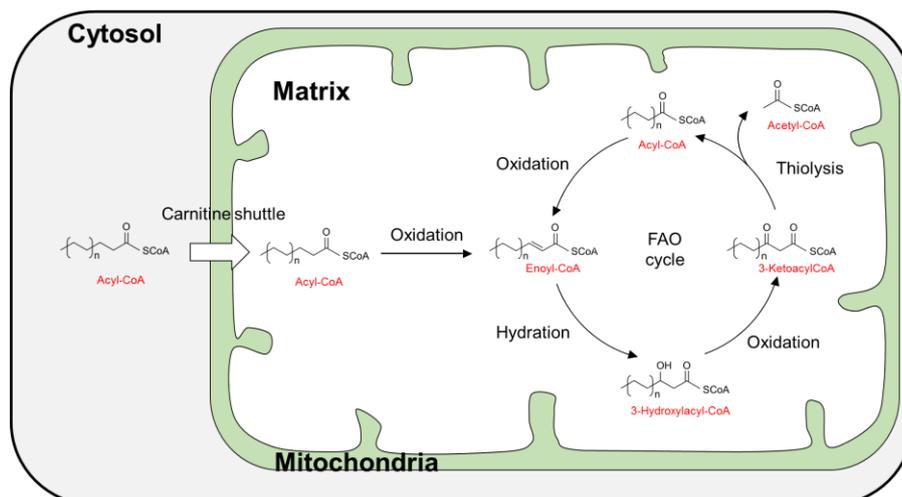


Figure 1. Overview of FAO cycle in mitochondria

FAOBlue is the world-first reagent for directly measuring FAO activity in living cells. FAOBlue is a coumarin dye possessing a nonanic acid (C9) protected by acetoxymethyl ester, and it shows no-fluorescence excited by 405 nm before metabolization by FAO. FAOBlue can enter into cells through direct penetration of cell membrane and its acetoxymethyl ester is hydrolyzed by intracellular esterases. Free FA type of FAOBlue is converted to acyl-CoA and further incorporated into FAO pathway. Acyl-CoA type of FAOBlue is degraded by three FAO cycles to non-fluorescent coumarin possessing a propionic acid (C3). After 4th FAO degradation, coumarin dye is released from propionic acid and diffused into whole cells. While FAOBlue shows no fluorescence excited by 405 nm before metabolizing by FAO, the released coumarin derived from FAO cycles shows strong blue fluorescence excited by 405 nm. FAOBlue-based FAO assay enables to measure FAO activity with an easy procedure. Inhibition of carnitine shuttle by etomoxir greatly diminishes the fluorescence enhancement in living cells. This data suggests that FAOBlue mainly detects mitochondrial FAO activity.

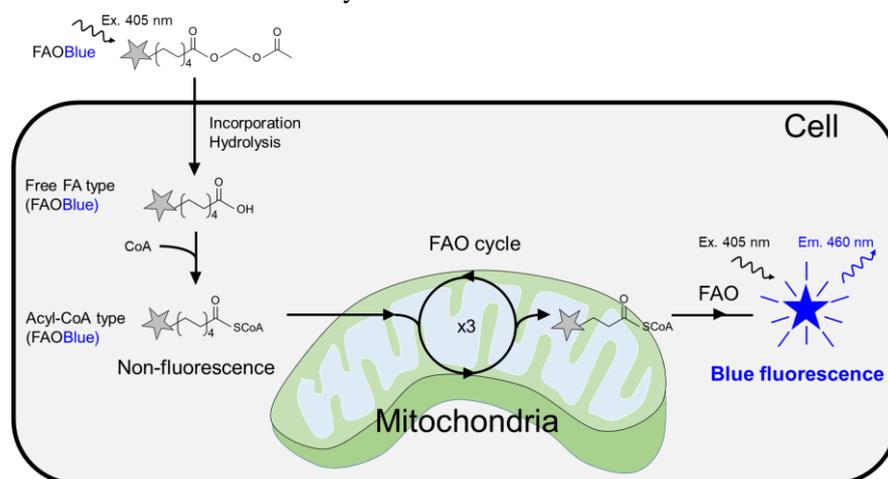


Figure 2. Sensing mechanism of FAOBlue in living cell.

Description

Catalog Number: FDV-0033
 Size : 0.2 mg
 Formulation : C₂₄H₃₁NO₉
 Molecular weight : 477.51 g/mol
 Solubility : Soluble in DMSO

Application

- Relative quantification of FAO activity
- Evaluation of drug effect on FAO activity

Reconstitution and Storage

Reconstitution : Add 100% DMSO into vial to prepare 1-10 mM stock solution.

Storage :

Powder : Store at -20°C.

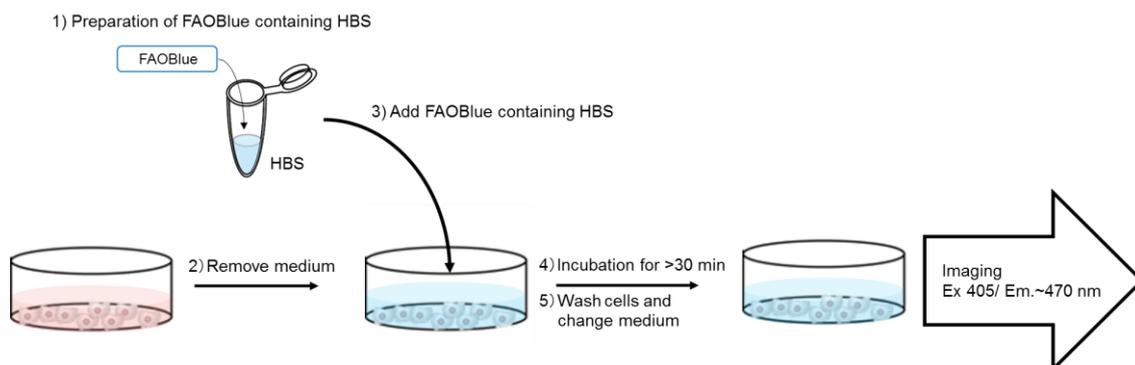
Stock solution :

- Make aliquots and store at -20 °C with protecting from light.
- Avoid repeated freeze-thaw cycles.

How to use

General procedure

1. Add FAOBlue (recommended final conc. 5-20 μM) in fresh HEPES-buffered saline (HBS)
NOTE: Instead of HBS, serum free culture media also suitable.
2. Remove the cultured medium and wash the cells with HBS twice
3. Add FAOBlue-containing HBS to cells
4. Incubate cells at 37°C for >30 min
5. Wash cells with HBS
6. Observe cells under live condition with blue fluorescence (Ex 405 nm/ Em. 430-480 nm)



Reference data and Experimental guide

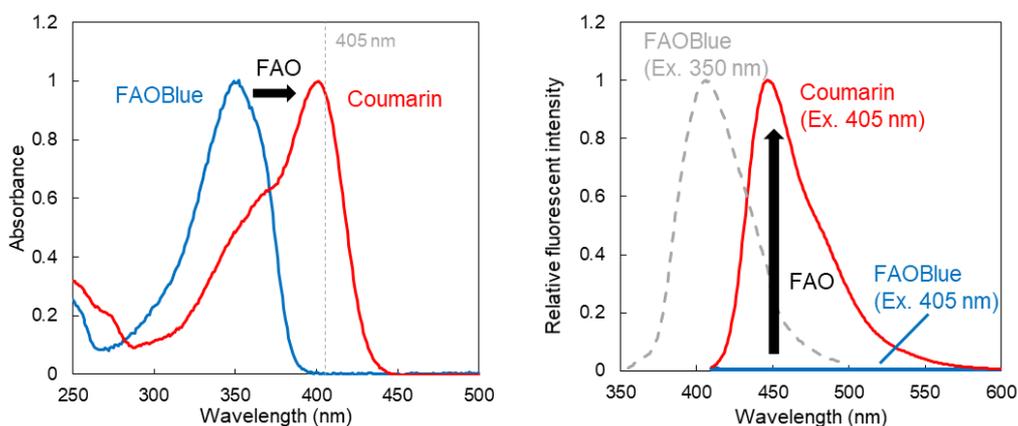
Absorbance and fluorescence spectrum of FAOBlue and coumarin-derivative

Absorbance (left) and fluorescence (right) spectra of FAOBlue (blue line) and the coumarin-derivative dye released after metabolization of FAO (red line) in PBS buffer (pH 7.4).

Absorbance spectra: an absorption peak of coumarin-derivative dye is clearly shifted by FAO from the peak of FAOBlue.

Fluorescence spectra: Coumarin-derivative dye shows strong blue fluorescence but FAOBlue emits little fluorescence, when both compounds are excited at 405 nm.

NOTE: FAOBlue shows blue fluorescence (gray line; 370-450 nm) when it is excited at 300-380 nm (max 350 nm).



Experimental guide for imaging

Confocal laser microscopy: Please use 405 nm laser equipped in the microscopy. Using 405 nm laser allows to detect only a fluorescent signal from coumarin-derivative dye.

Epifluorescence microscopy: Excitation filter is very important. Commercial DAPI filters are not compatible with this reagent, because DAPI filters excite both FAOBlue and coumarin-derivative dye. Excitation filters which pass 390-450 nm wavelength light are recommended.

Application data

Visualization of FAO activities in 4 cancer cell lines

Four cancer cell lines (HepG2, LNCaP, HeLa and A549) were treated with FAOBlue in HBS buffer with or without pre-treatment of etomoxir (40 μ M, 3 hours), a potent FAO inhibitor. After FAOBlue incubation, blue fluorescence (Ex. 405 nm/ Em. 430-480 nm) was observed. All cell lines showed blue fluorescence in cytosol, but pre-treatment of etomoxir clearly decreased fluorescent intensities. These results indicated the blue fluorescence was derived from FAO activity in the cells.

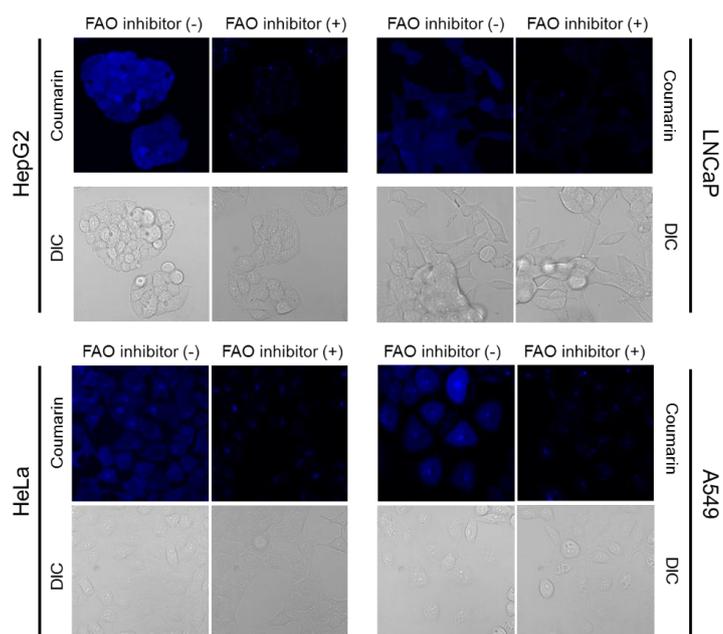
* Experimental condition:

HepG2, 5 μ M FAOBlue for 30 min.

LNCaP, 20 μ M FAOBlue for 120 min

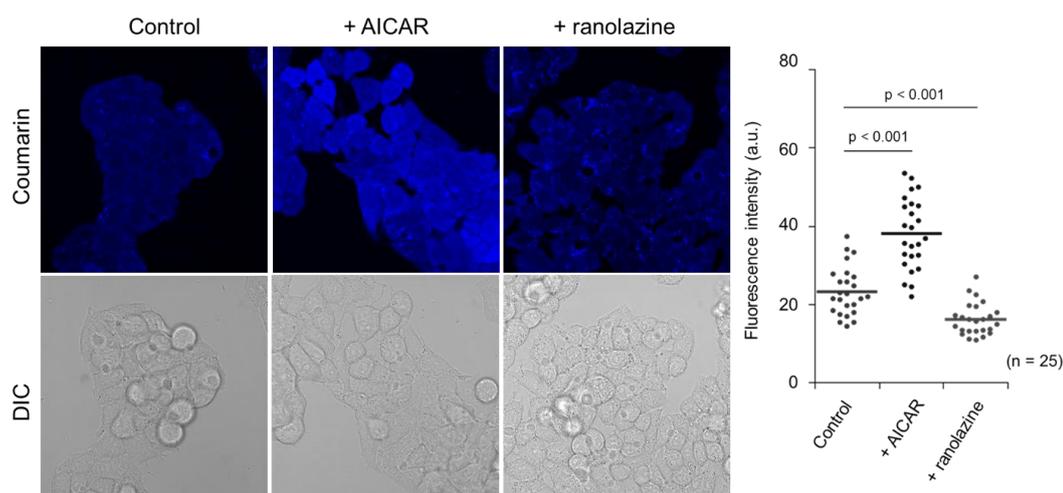
HeLa, 20 μ M FAOBlue for 120 min

A549, 5 μ M FAOBlue for 30 min



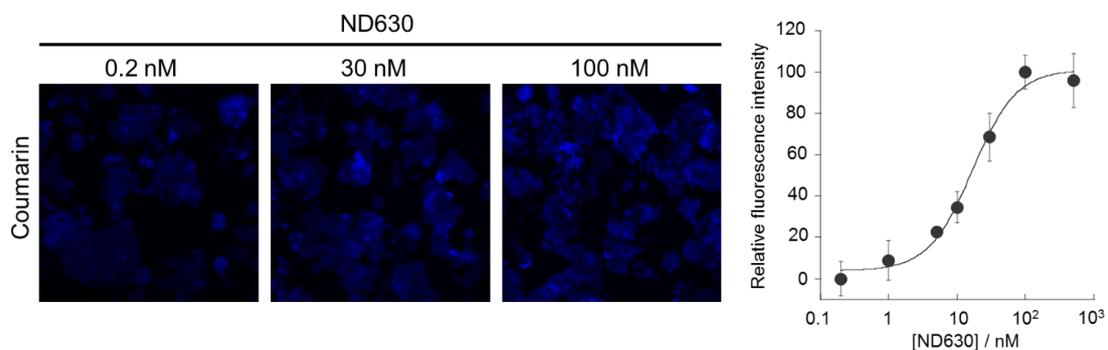
Perturbation of FAO activity by drugs

HepG2 cells were pre-incubated with 200 μ M AICAR, a FAO activator via AMPK activation, for 3 hours or 200 μ M of ranolazine, a partial FAO inhibitor, for 12 hours. After drug treatment, the cells were incubated with 5 μ M FAOBlue for 30 min. Compared with control cells, pretreatment with AICAR significantly increased blue fluorescent intensity. On the other hand, pretreatment with the partial FAO inhibitor ranolazine clearly decreased blue fluorescent intensity. Ex. 405 nm/ Em. 430-480 nm



Quantitative analysis of the drug effects on FAO activity

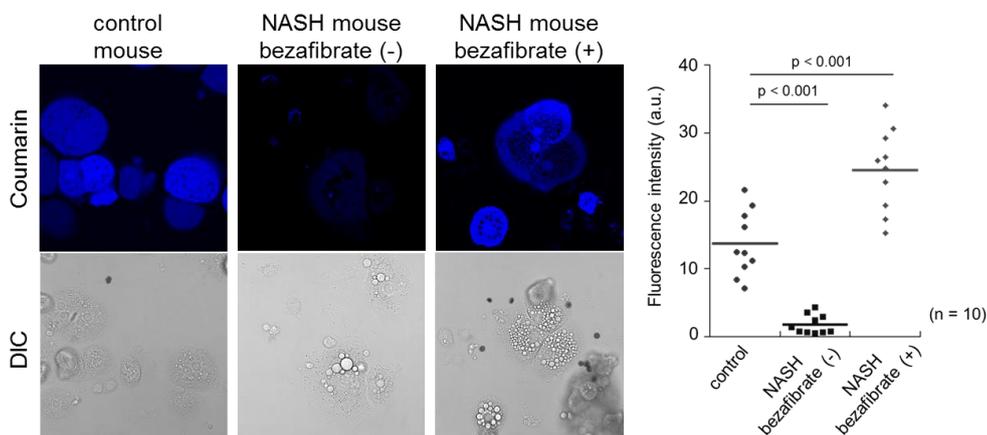
ND630 is an inhibitor of acetyl-CoA carboxylase and considered as a potential therapeutic drug for non-alcoholic fatty liver disease (NAFLD). HepG2 cells were pre-incubated with various concentration of ND630 for 4 hours. After ND630 treatment, cells were treated with 5 μM FAOBlue for 30 min. Blue fluorescent intensities of each concentration of ND630 were quantified. Ex. 405 nm/ Em. 430-480 nm



Analysis of FAO activity using NASH model mouse

Non-alcoholic steatohepatitis (NASH) is a typical disease which shows low metabolic activity of FAs. Control healthy mice and NASH model mice were orally administered with 400 mg/kg bezafibrate, a therapeutic agent of NASH. After 4 weeks administration, primary hepatocytes were isolated from control mice and NASH model mice and cultured in culture dishes. Primary hepatocytes were further treated with 5 μM FAOBlue for 30 min and fluorescence imaging was performed. Compared with control cells, NASH model mouse-derived hepatocytes showed low FAO activity. Bezafibrate dramatically recovered FAO activity of hepatocytes isolated from NASH model mouse. FAOBlue is a powerful tool to estimate drug effects and efficiency on FAO activity.

*Detail procedure of mice experiment is described in Ref.1. Ex. 405 nm/ Em. 430-480 nm



Reference

1. Uchinomiya *et al.*, *Chem. Commun.*, **56**, 3023-3026 (2020) Fluorescence Detection of Metabolic Activity of Fatty Acid Beta Oxidation Activity in Living Cells.

Related products

LipiDye <Lipid Droplet Green>

LipiDye is highly sensitive lipid droplet staining dye. This probe allows us to access small lipid droplets (<1 μm) in non-adipocytes.

Catalog No. FDV-0010

Size 0.1 mg

Features

- Enable to detect <1 μm lipid droplets
- Higher sensitive than Nile Red Dye.
- Compatible for both live and fixed cells
- Low cytotoxicity
- Ex/Em: 405 nm/521-530 nm

