

Cellular Thermoprobe™ for Fluorescence Lifetime

Catalog NO. FDV-0004

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

Product Background

“Cellular Thermoprobe™ for Fluorescence Lifetime” is a fluorescent polymeric thermometer for living cells. It diffuses throughout the cells and gives the information about intracellular temperature distribution by fluorescence lifetime imaging microscopy. “Cellular Thermoprobe™ for Fluorescence Lifetime” can be delivered into cell without microinjection. It is highly photostable and easy-to-use. With its cell permeability, “Cellular Thermoprobe™ for Fluorescence Lifetime” is applicable for both adherent and suspension cells. It enables us to distinguish intracellular temperature in cultured mammalian cells at organelle level. For instance, the previous report demonstrated that the average temperature difference between the nucleus and the cytoplasm was 0.98°C (ref. 1). “Cellular Thermoprobe™ for Fluorescence Lifetime” is an innovative new reagent that provides unprecedented scientific insight.

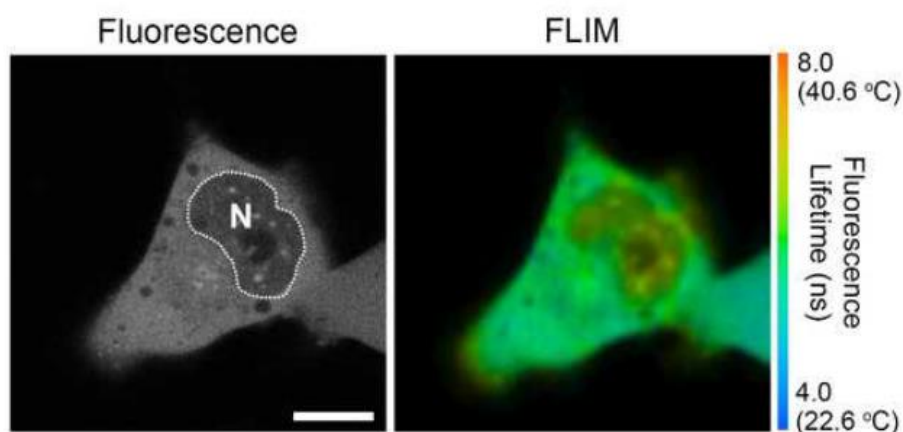


Figure 1. Temperature mapping in living HeLa cells.

Confocal fluorescence image (left) and fluorescence lifetime images (right) of the “Cellular Thermoprobe™ for Fluorescence Lifetime” in HeLa cells. N indicates the nucleus.

Description

Catalog Number: FDV-0004

Size: 200 μg or 3 x 200 μg

Lot No.: see vial label

Polymer structure: See right figure

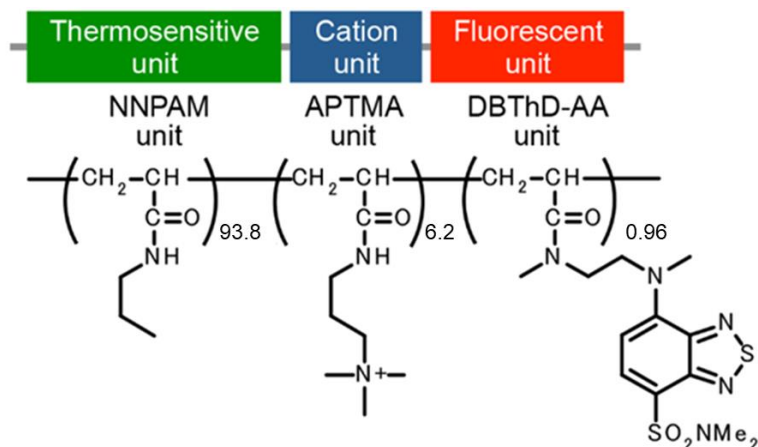
Average Molecular Weight: 12,300

Appearance: Yellow powder

Solubility: Soluble in water

Spatial Resolution: 200 nm

Temperature Resolution: 0.05-0.54 $^{\circ}\text{C}$



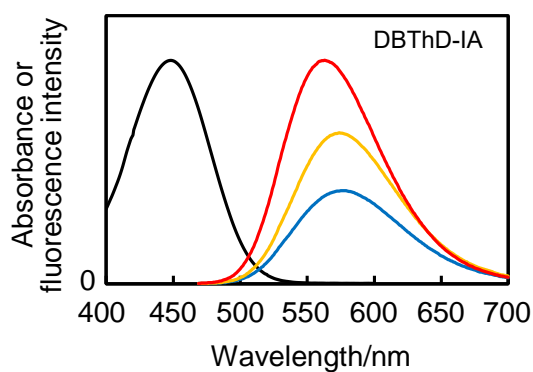
Storage

Storage (powder): Store powder at ambient temperature

Storage (solution): After reconstitution in water, aliquot and store at -20 $^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles and protect from light.

Representative absorption and fluorescence spectra of DBThD-IA

The absorption spectra were measured in acetonitrile (black). The fluorescence spectra were measured with excitation at 458 nm in ethyl acetate (red), acetonitrile (orange) and methanol (blue)



Optimal excitation and emission will be determined by your own. As an example, excitation at 405 nm and emission at 560-610 nm would work well.

How to use

Reconstitution

1. Before open the top, spin the vial down briefly.
2. Reconstitute 200 µg powder of “**Cellular Thermoprobe™ for Fluorescence Lifetime**” in 20 µl of ultrapure water to prepare 1% w/v stock solution *1.
3. Dissolve it completely by vortex or tapping.
4. The stock solution (1% w/v) can be stored at 4°C shortly with protecting from light but for long-term storage store at -20 °C is recommended. Note that the stock solution needs to be incubated at 4°C at least overnight before proceeding to experiments to obtain full extension of the polymer.

*1 Ionic solutions such as DMEM and PBS inhibit the incorporation of “**Cellular Thermoprobe™ for Fluorescence Lifetime**”. If you find poor solubility, put it on ice for a while until it dissolve.

Preparation of cell extract for calibration curve

1. Cell pellets (1×10^7) were collected from 100 mm dish and resuspended in hypertonic buffer (2.5 ml, containing 0.42 M KCl, 50 mM HEPES-KOH, 5 mM MgCl₂, 0.1 mM EDTA, 20% glycerol, pH 7.8).
2. Lyse cells using a 25-G needle with a syringe.
3. Centrifuge (11,000 rpm, 15 min, 4°C) and collect the supernatant.
4. Dilute the supernatant with water up to 40% to adjust its KCl concentration to 0.15M.

How to generate calibration curve*2

1. Dilute 1 µl of 1% w/v “**Cellular Thermoprobe™ for Fluorescence Lifetime**” stock solution with cell extract (20-100 µl) prepared above.
2. Put the solution on a glass bottom dish.
3. Set the temperature of the stage heater at the lowest you can (e.g. 25 °C).
4. Measure the fluorescence lifetime after the medium temperature becomes steady.
5. Adjust the medium temperature at your choice (e.g. 26 °C).
6. Measure the fluorescence lifetime after the medium temperature becomes steady.
7. Repeat step 5-6 until reaching the maximum temperature of the stage heater.
8. Plot the fluorescence lifetime against temperature to obtain a calibration curve. Estimate the temperature of your sample based on the calibration curve.

*2 Calibration curve can be also generated by Spectrofluorometer or Fluorescence Plate Reader equipped with fluorescence lifetime system and temperature control.

Introduction of “Cellular Thermoprobe for Fluorescence Lifetime” into suspension cells

1. Collect the suspension cells by centrifugation at 400 x g for 3 min and wash it with 1 ml of a 5 % glucose solution and centrifuge it.
2. Remove the supernatant.
3. Resuspend the cell pellets in a 5 % glucose solution at a density of 1×10^6 cells/ml.
4. Add “Cellular Thermoprobe™ for Fluorescence Lifetime” in water (1% w/v) to a 20-100 fold*³ volume of cell suspension.
5. Incubate the cells at 25 °C for 10 min.
6. Centrifuge it and remove supernatant, and add 1ml PBS.
7. Centrifuge it and remove supernatant, and resuspend in PBS.
8. For the fluorescence imaging, approximately 10 µl of the cell suspension is dropped onto a coverslip and observe it immediately*⁴.

*³ Optimal dilution rate of “Cellular Thermoprobe™ for Fluorescence Lifetime” depends on cell types.

*⁴ Set the appropriate temperature (e.g. 32-33°C) in a microscope cage incubation chamber based on your calibration curve and/or experimental condition.

Introduction of “Cellular Thermoprobe for Fluorescence Lifetime” into adherent cells

1. Prepare the cells at the 30 to 50 % confluency on glass bottom dish or equivalent.
2. Remove the medium and wash with a 5 % glucose solution*⁵.
3. Add 0.01-0.05 w/v%*⁶ of “Cellular Thermoprobe™ for Fluorescence Lifetime” in 5 % glucose solution*^{5,*7}.
4. Incubate the cells at 25 °C for 10 min.
5. Wash the cells with PBS three times.
6. Add phenol red-free culture medium and measure the fluorescence lifetime with appropriate temperature in a microscope cage incubation chamber*⁸.

*⁵ In the case that the dissociation of the adherent cells were observed in 5% glucose solution, 5% glucose solution with 0.1-0.3 mM CaCl₂ may improve it.

*⁶ Optimal dilution rate of “Cellular Thermoprobe™ for Fluorescence Lifetime” depends on cell types.

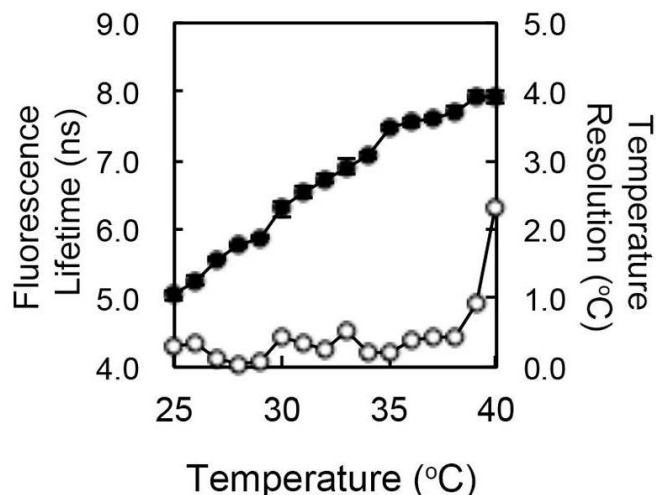
*⁷ The volume of the solution depends on the culture dish type.

*⁸ Set the appropriate temperature (e.g. 32-33°C) in a microscope cage incubation chamber based on your calibration curve and/or experimental condition.

Note: Above methods (Reconstitution, Preparation of cell extract for calibration curve, How to generate calibration curve and Introduction of the reagent into cells) should be optimized depending on the cell type and organisms you use.

Example of Calibration Curve in HeLa cell extracts

Fluorescence response (closed, left axis) and temperature resolution (open, right axis) in HeLa cell extracts. The temperature resolution of “Cellular Thermoprobe™ for Fluorescence Lifetime” was 0.05-0.54°C in the temperature range between 28 and 38°C



Reference data

1. Hayashi *et. al.*, *PLoS ONE*. **10**, e0117677 (2015) A Cell-Permeable Fluorescent Polymeric Thermometer for Intracellular Temperature Mapping in Mammalian Cell Lines

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 March, 2015. 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、2015年3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けますようお願い申し上げます。



E-mail Newsletter
Sign Up

Japanese



English



Related products

NucleoSeeing™ <Live Nucleus Green>

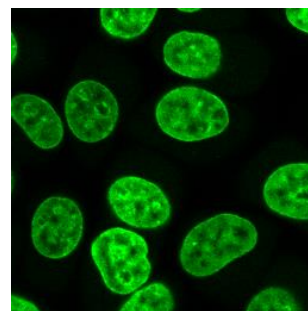
NucleoSeeing™ is DNA-responsive green dye for monitoring cell nucleus in live cells. As it shows low cytotoxicity and phototoxicity, it is very suitable for long-term live imaging of cell nucleus.

Catalog No. FDV-0029

Size 0.1 mg

Features

- Easy and quick procedure
- Compatible with 10% FBS
- Validated for both adherent cells and floating cells
- Little influence on cellular functions
- Ex/Em: 488 nm/520 nm (commercial FITC filters are available)



LipiDye™ II <Live Imaging>

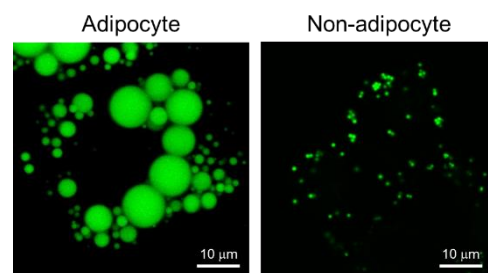
LipiDye™ II is a highly sensitive lipid droplet staining dye with extremely photostable property. This dye is the second generation of our previous reagent, LipiDye™. This dye allows us to detect small lipid droplets (<1 μm) in non-adipocytes and to apply into long-term live cell imaging for dynamic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

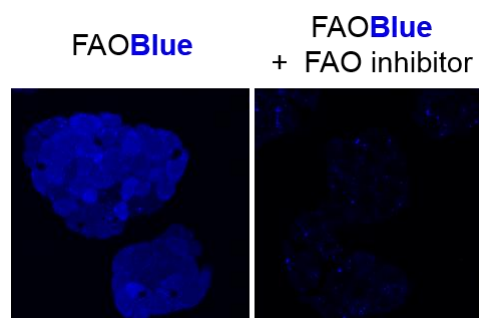
FAOBlue™ is a cell-based fatty acid beta-oxidation (FAO) detection dye which emits blue fluorescence upon FAO activity. FAOBlue™ enables to quantitatively monitor cellular FAO activities under various conditions.

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.



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