funakoshi
FRONTIERS IN LIFE SCIENCE

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Cellular Thermoprobe™ for Fluorescence Ratio

Catalog NO. FDV-0005

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

Product Background

"Cellular Thermoprobe™ for Fluorescence Ratio" is a fluorescent polymeric thermometer for living cells. It diffuses throughout the cells and gives the information about intracellular temperature distribution by fluorescence ratio. **"Cellular Thermoprobe™ for Fluorescence Ratio"** can be delivered into cell without microinjection. In addition, it does not require Fluorescence Lifetime for measuring. That means it is easy-to-use. With its cell permeability, **"Cellular Thermoprobe™ for Fluorescence Ratio"** is applicable for both adherent and suspension cells. It enables researchers to distinguish intracellular regional temperature at organelles in cultured mammalian cells. "**Cellular Thermoprobe™ for Fluorescence Ratio"** is an innovative new tool that can provide unprecedented scientific insight.

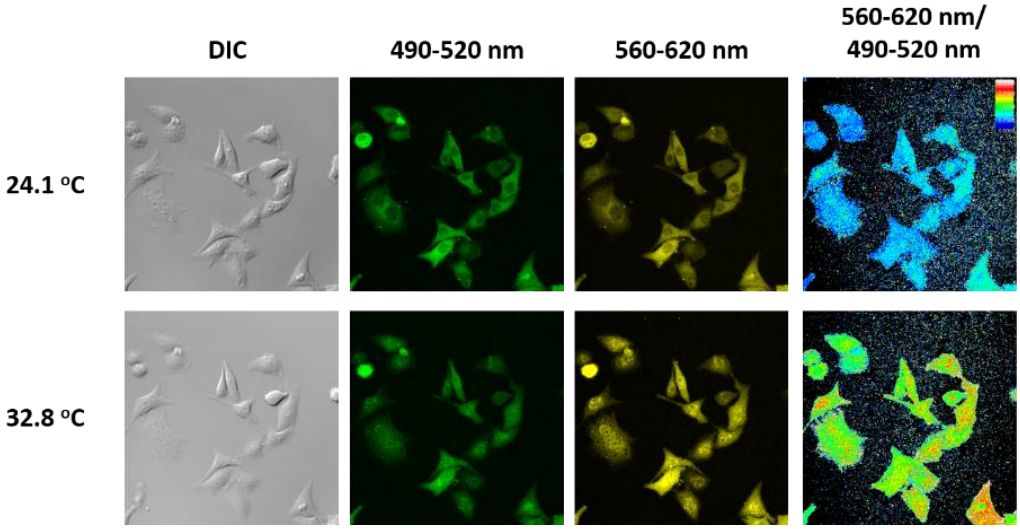
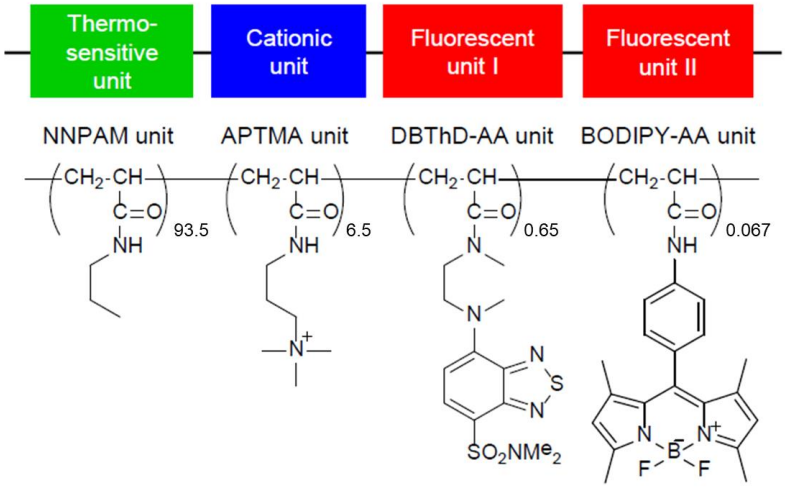


Figure 1. "Cellular Thermoprobe™ Fluorescence Ratio" in living HeLa cells.
Confocal fluorescent images at excitation 473 nm and the emission 490-520 nm and 570-610 nm of the **"Cellular Thermoprobe™ for Fluorescence Ratio"** in HeLa cells and its ratiometric (560-620 nm / 490-520 nm) image.

Description

Catalog Number: FDV-0005
Size: 200 µg or 3 x 200 µg
Lot No.: 23C0306
Polymer structure: See right figure
Average Molecular Weight: 20,800
Appearance: Yellow powder
Solubility: Soluble in water
Spatial Resolution: 240 nm
Temperature Resolution: 0.01-0.25°C

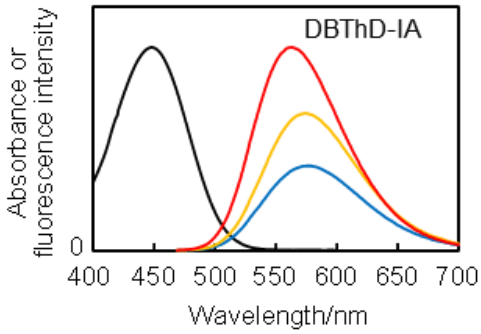


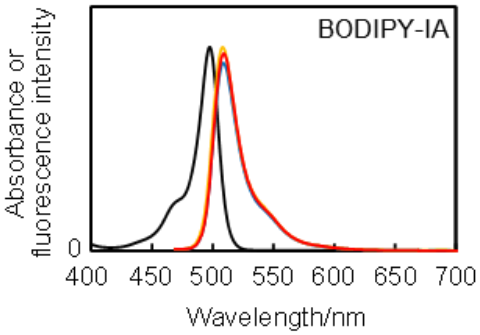
Storage

Storage (powder): Store powder at ambient temperature
Storage (solution): After reconstitution in water, aliquot and store at -20 °C. Avoid repeated freeze-thaw cycles and protect from light.

Representative absorption and fluorescence spectra of DBThD-IA and BODIPY-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 458 or 473 nm and emission1 at 490-530 nm for BODIPY-IA (temperature independent dye), and emission2 at 570-610 nm for DBThD-IA (temperature dependent dye) 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 Ratio"** 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 Ratio"**. 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 x 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 $MgCl_2$, 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 Ratio"** 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 ratio ($F_{DBThD\text{-}AI}/F_{BODIPY\text{-}IA}$) after the medium temperature becomes steady.
5. Adjust the medium temperature at your choice (e.g. 26 °C).
6. Measure the fluorescence ratio after the medium temperature becomes steady.
7. Repeat step 5-6 until reaching the maximum temperature of the stage heater.
8. Plot the fluorescence ratio 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 temperature control.

Introduction of **"Cellular Thermoprobe™ for Fluorescence Ratio"** 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 x 10^6 cells/ml.
4. Add **"Cellular Thermoprobe™ for Fluorescence Ratio"** 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 Ratio"** 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 Ratio"** 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 Ratio"** 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 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 $CaCl_2$ may improve it.

[*6] Optimal dilution rate of **"Cellular Thermoprobe™ for Fluorescence Ratio"** depends on cell types.

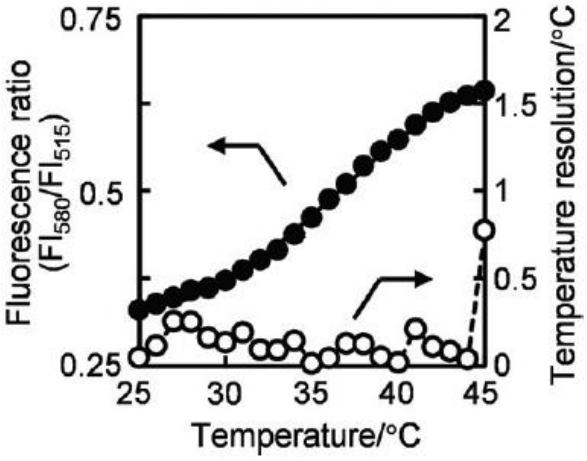
[*7] The volume of the solution depends on the culture dish type.

[*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 MOLT-4 cells

Fluorescence response (closed, left axis) and temperature resolution (open, right axis) in MOLT-4 cells. The temperature resolution of **"Cellular Thermoprobe™ for Fluorescence Ratio"** was 0.01 - 0.25°C in the temperature range between 25 and 44°C.



Reference data

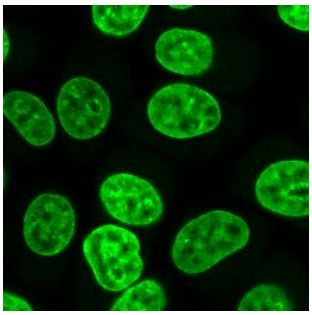
1. Uchiyama, *et al, Analyst*, **140**, 4498-4506 (2015) A cationic fluorescent polymeric thermometer for the ratiometric sensing of intracellular temperature
2. Uchiyama and Gota, *Rev. Anal. Chem.*, **36**, 1 (2017) Luminescent molecular thermometers for the ratiometric sensing of intracellular temperature
3. Tsuji, *et. al., Sci. Rep.*, **7**, 12889 (2017) Difference in intracellular temperature rise between matured and precursor brown adipocytes in response to uncoupler and β-adrenergic agonist stimuli
4. Uchiyama, *et al., Chem. Commun.*, **53**, 10976-10992 (2017) Intracellular temperature measurements with fluorescent polymeric thermometers

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.

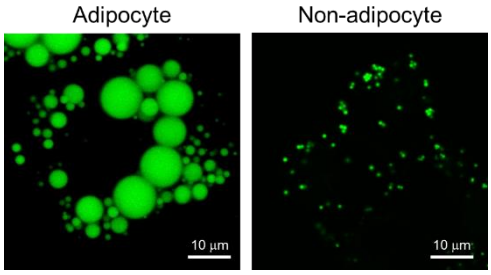
Catalog No. FDV-0029
Size 0.1 mg
Features
- Easy and quick procedure
- Compatible with 10% FBS
- Validated for both adherent cells and floating cells
- Little influence on cellular functions
- Ex/Em: 488 nm/520 nm (commercial FITC filters are available)



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.

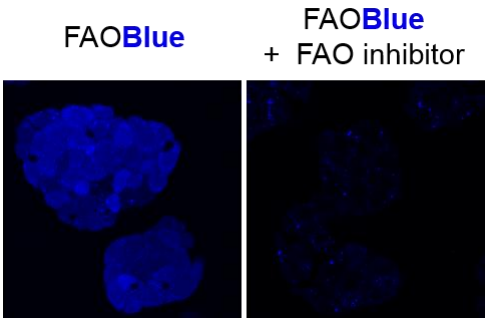
Catalog No. FDV-0027
Size 0.1 mg
Features
- Recommended Ex/Em:400-500 nm / 490-550 nm
- Enable to detect <1 μm lipid droplets
- Suitable for long-term live cell imaging
- Extremely photostable compared with conventional dyes
- Compatible with both live and fixed cells



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.



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