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FRONTIERS IN LIFE SCIENCE

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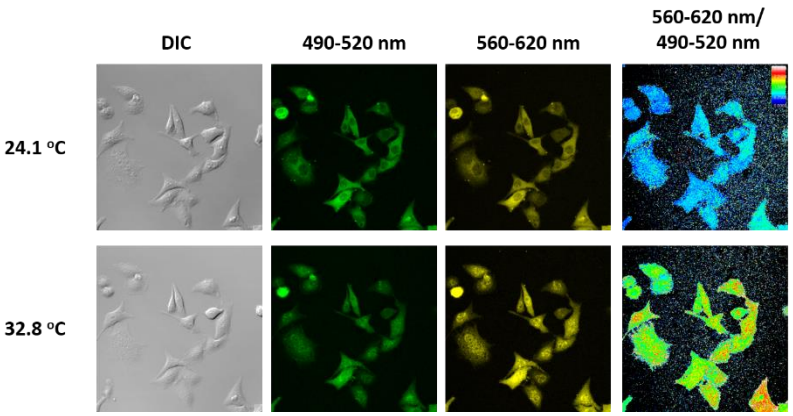
## Product Background

# Cellular Thermoprobe® for Fluorescence Ratio

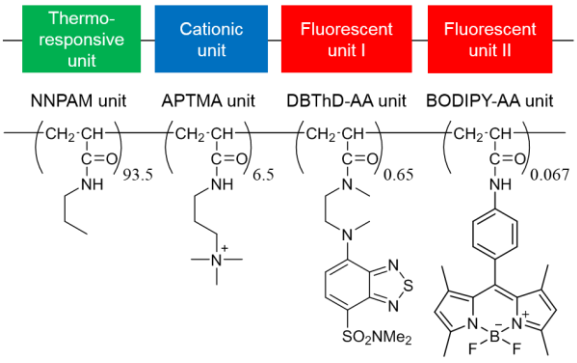
Cellular Thermoprobe® for Fluorescence Ratio is a fluorescent polymeric thermometer for living cells. It diffuses throughout the cells and gives the information about intracellular temperature distribution by fluorescence ratio. Cellular Thermoprobe® for Fluorescence Ratio can be delivered into cell 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.




**Fluorescence Ratio in living HeLa cells.**
Confocal fluorescence image and fluorescence image at the emission of 490-520 nm, 570-610 nm and 560-620 nm / 490-520 nm ratio of the "Cellular Thermoprobe® for Fluorescence Ratio" in HeLa cells.

## Description

**Catalog Number:** FDV-0005
**Lot Number:** 17C0921
**Size:** 200µg or 200µg x 3
**Chemical Structure**




**Average Molecular Weight:** 28,000
**Purity:** >99%
**Appearance:** Yellow powder
**Solubility:** Soluble in water
**Spatial Resolution:** 240 nm
**Temperature Resolution:** 0.01-0.25℃

**License:** This product is licensed by Tokyo University and KIRIN Co., Ltd

## Reconstitution and Storage

**Shipping:** Shipped at ambient temperature
**Storage:** Store at ambient temperature (powder). For reconstituted solution, store at +4℃. Protected from

light.

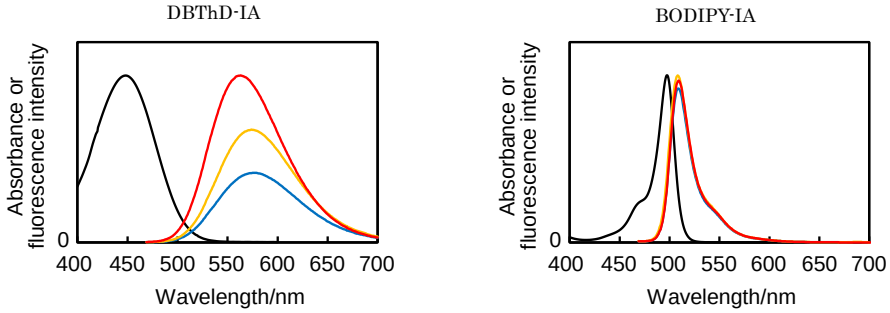
## Reconstitution

1. Before open the top, spin the vial down briefly.
2. Reconstitute 200μg powder of Cellular Thermoprobe® for Fluorescence Ratio in 20μl of ultrapure water*1.
3. Dissolve it completely by vortex or tapping.
4. Store the stock solution (1% w/v) at +4℃. Protect from light. Note that the stock solution needs to be incubated at +4℃ at least overnight before proceeding to experiment 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.

## 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 nm and emission1 at 490-530 nm, and emission2 at 570-610 nm would work well.

## 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 r.p.m., 15 min, 4℃) 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*1

1. Dilute 1μl of Cellular Thermoprobe® for Fluorescence Ratio in water (1% w/v) with cell extract (20-100 μl).
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℃).
4. Measure the fluorescence ratio after the medium temperature becomes steady.
5. Adjust the medium temperature at your choice (e.g. 26℃).
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.

*1 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*1 volume of cell suspension.
5. Incubate the cells at 25℃ 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 10µl of the cell suspension is dropped onto a coverslip and observe it immediately*2.

*1 Optimal dilution rate of Cellular Thermoprobe® for Fluorescence Ratio depends on cell types.

*2 Set the appropriate temperature (e.g. 32 ~ 33℃) 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*1.
3. Add 0.01-0.05 w/v%*2 of Cellular Thermoprobe® for Fluorescence Ratio in 5 % glucose solution*1*3.
4. Incubate the cells at 25℃ 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*4.

*1 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.

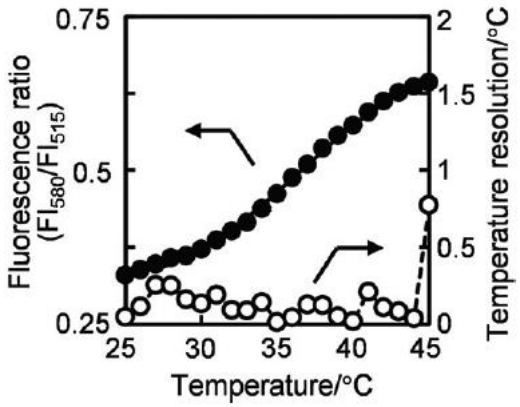
*2 Optimal dilution rate of Cellular Thermoprobe® for Fluorescence Ratio depends on cell types.

*3 The volume of the solution depends on the culture dish type. But we assume that minimum amount of volume (50-100 µl) is sufficient to measure the cellular temperature.

*4 Set the appropriate temperature (e.g. 32 ~ 33℃) 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) 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℃ in the temperature range between 25 and 44℃.

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## Reference


1) Uchiyama S, et. al, Analyst, 2015, 140, 4498-4506
2) Uchiyama, S. & Gota, C. Reviews in Analytical Chemistry, 36.1 (2016), from doi:10.1515/revac-2016-0021
3) Tsuji T., et. al., Sci Rep. 2017 Oct 10;7(1):12889. doi: 10.1038/s41598-017-12634-7
4) Uchiyama S., et. al., Chem Commun (Camb). 2017 Oct 5;53(80):10976-10992. doi: 10.1039/c7cc06203f



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