funakoshi
FRONTIERS IN LIFE SCIENCE

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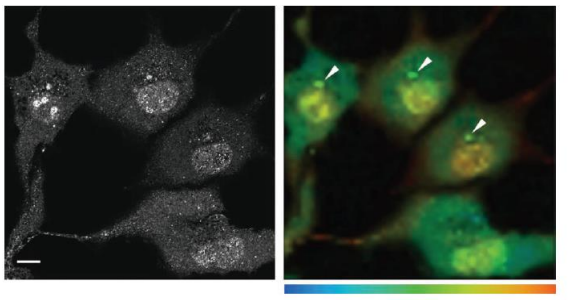
Diffusive Thermoprobe
(for Intracellular temperature mapping)

Product Background

Diffusive Thermoprobe 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. Diffusive Thermoprobe can distinguish intracellular temperature among organelle. For instance, the previous report demonstrated that the average temperature difference between the nucleus and the cytoplasm was 0.96℃ (reference 1). In addition, it was found temperature gap between the nucleus and cytoplasm was dependent on the cell cycle. Diffusive Thermoprobe is an innovative new reagent, and it provides unprecedented experimental approach.



Temperature mapping in living COS7 cells

Confocal fluorescence image (left) and fluorescence lifetime image (right) of Diffusive Thermoprobe.

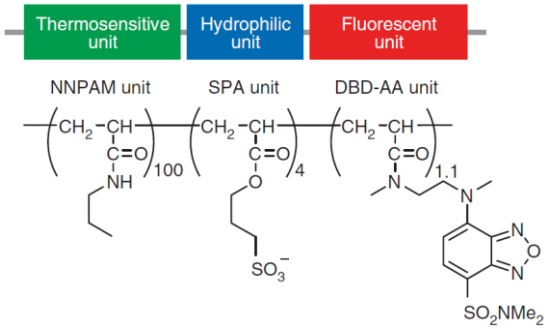
Description

Catalog Number: FDV-0002

Lot Number: see vial label

Size: 100μg

Chemical structure



Average Molecular weight: 19,300

Purity: >99%

Appearance: Yellow powder

Solubility: Soluble in water

Spatial resolution: 200 nm

Temperature resolution: 0.18-0.58℃

License: This product is licensed by Tokyo University

Reconstitution and Storage

Shipping: Shipped on ambient temperature

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

Reconstitution: Reconstitute at 10 mg/ml in 80 mM KCl, 10 mM K_2HPO_4, 4 mM NaCl.

Instruction

1. Before open the top, spin vial down briefly.
2. Reconstitute 100μg powder of Diffusive Thermoprobe at 10μl of 80 mM KCl, 10 mM K_2HPO_4, 4 mM NaCl.
3. Dissolve it completely by vortex or tapping etc.
4. Stand it at +4℃ for overnight (at least 8 hours).
5. Take 1μl solution from vial, and fill it up to glass capillary needle for microinjection.
6. Microinject it into cytoplasm with a glass capillary needle below 30℃*1.
7. Leave cells for 30min.
8. Analyze intracellular temperature by fluorescence lifetime imaging microscopy with an excitation at 460 nm and an emission at 560 nm.

*1 At higher temperature, it may cause clog.

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 $MgCl_2$, 0.1 mM EDTA, 20% glycerol, pH 7.8).
2. Lyse the cells using a 25-G needle with a syringe.
3. Centrifuge the dispersion (11,000 r.p.m., 15 min, 4℃) and collect the supernatant.
4. Dilute the supernatant by 40% with water to adjust its KCl concentration to 0.15M.

How to generate calibration curve

1. Dilute 1 μl of Diffusive Thermoprobe in water (1% w/v) by cell extract (100 μl).
2. Put the solution on a glass bottom dish.
3. Set the temperature at the lowest you want (e.g. 22℃).
4. Measure the fluorescence lifetime after the medium temperature got a steady.
5. Increase the temperature at your choice (e.g. 23℃).
6. Measure the fluorescence lifetime after the medium temperature got a steady.
7. Repeat step 5-6 until to obtain a calibration curve.
8. Estimate the temperature of your sample based on the calibration curve.

Note: Above methods (Reconstitution and microinjection, Preparation of cell extract for calibration curve, and How to generate calibration curve) is not only way to measure/calibrate cellular temperature. Optimize and establish the best condition at your own.

Reference

1) Okabe K, et. al, Nat Commun. 2012 Feb 28;3:705.

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