GORYO CHEMICAL

For research use only

Created: July 24, 2017

CaTM-3 AM

Table 1. Product information

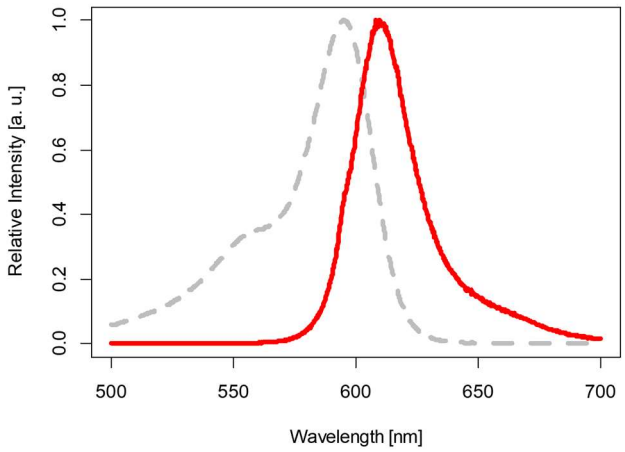
Catalog no.	Product name	Amount	Storage upon receipt	Stability
GC507	CaTM-3 AM	40 nmol × 5	≤−20°C, keep desiccated and protected from light.	1 year (when unopened and stored as described.)

1. About CaTM-3

CaTM-3 AM is a fluorescent probe to detect intracellular calcium ion by red fluorescence at an emission maximum of 609 nm corresponding to 590nm excitation. Its fluorescence intensity increases proportionally to the calcium concentration. The reagent, acetoxymethyl ester (AM) of CaTM-3 is easily incorporated into cytoplasm and hydrolyzed by intracellular esterases. The product is suitable for quantitative analysis of calcium ions because CaTM-3 usually distributes equally in the cytoplasm.

Specifications

Abs_{max}	595 nm
Em_{max}	609 nm
K_d (Ca^{2+})	0.19 μM
Φ	0.37



■ Storage

The reagent is shipped as a dried powder in a nitrogen gas filled tube. Upon receipt, store at ≤−20°C, keep desiccated and protected from light. Dissolve the reagents to DMSO just before the use. We provide no warranty for the reagents which was stored as a solution.

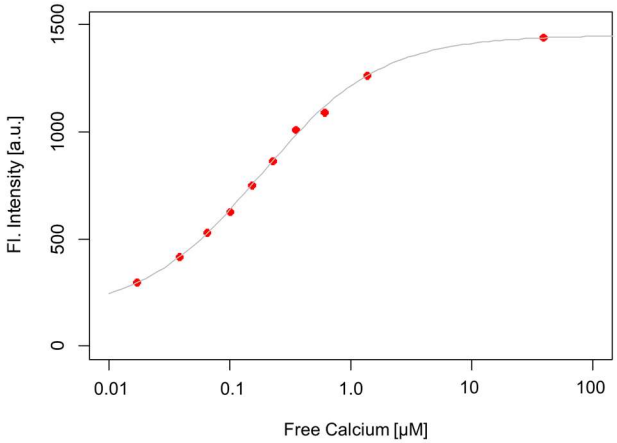


Fig. 2. Fluorescence intensity against calcium ion concentrations

2. Imaging protocols

■ Materials required but not provided

・Dimethyl sulfoxide (DMSO)
・Observation buffer (ex. Hank's Balanced Salt Solution, HBSS).

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■ Preparation of reagent

1. CaTM-3 AM is pale yellowish brown solid. Before opening the vial, spin down the solid to the bottom by a microcentrifuge or by a desktop centrifuge.
2. Warm the vial to the room temperature and add 40 µL of DMSO to one vial to make 1 mM stock solution, which will be a pale blue solution. Dissolve the solid completely.

■ An example observation of intracellular calcium signals

1. Seed HeLa cells to a poly-D-lysine coated glass-bottomed dish in DMEM + 10% FBS (with 1% penicillin and 1% streptomycin), then culture at 37°C, 5% CO_2 for >12 hours. Before loading the dye, remove the culture medium and rinse the cells three times with HBSS.
2. Dilute the stock solution of CaTM-3 AM with HBSS to make 3 µM staining solution. Add the solution to the dish to load the dye. Incubate at 37°C for 30 min.
3. Remove the staining solution and rinse the cells with HBSS three times.
4. Observe the cells in HBSS by fluorescence microscopy. See the 'Fluorescence observation' section for choosing the excitation/emission conditions.
5. Stimulate the cells by adding histamine (final 1 µM) to the solution during the observation. You may observe fluctuation of intracellular calcium concentration.
6. Add ionomysin (final 5 µM) to the solution. You may detect an increase in the intracellular calcium concentration.

※ In case of cultured cells, fluorescence decrease has been observed by the addition of 0.01% Pluronic F-127.

■ An example observation of calcium signals in a brain slice

1. Dissect a brain from a mouse at postnatal day 7 (P7) and prepare slices of hippocampus (thickness 300 µm).
2. Mount the slice on a membrane filter in culture media and incubate in at 37°C, 5% CO_2 for 7 days.
3. Replace the culture medium with 8 µM of CaTM-3 AM diluted in artificial cerebrospinal fluid (ACSF) containing 0.01% Pluronic F-127, 0.005% Cremophor EL, and 0.08% DMSO as a cosolvent. Incubate at 37°C for 40 minutes to load CaTM-3 AM to the slice.
4. Rinse the slice with ACSF for three times, and allowed to recover in 2 mL ACSF at 37°C for 45 minutes.
5. Transfer the slice into a recording chamber heated at 35°C and continuously perfused with ACSF. Acquire images using confocal microscope, etc.

※ For brain slices, addition of 0.01% Pluronic F-127 and 0.005% Cremophor EL is preferable, whereas addition of these reagents is not preferable for cultured cells.

■ Fluorescent observation

For confocal fluorescence microscopy, excite the dye with either 568 nm or 590 nm laser and observe the fluorescence ranging 610-680 nm. Alternatively, filter cube sets such as mCherry or TexasRed (for NIKON microscopes), U-FYW or U-FMCHE (for OLYMPUS microscopes) are recommended for observation by fluorescence microscopy.

■ Reference

Hirabayashi K, Hanaoka K, Egawa T, Kobayashi C, Takahashi S, Komatsu T, Ueno T, Terai T, Ikegaya Y, Nagano T, Urano Y. (2016) *Cell Calcium* **60** 256-265

Table 2. Related Products

Catalog no.	Product name	Major applications
GC401 GC402	CaSiR-1™	Detection of ferrous ions (Fe^{2+}) in Golgi.
GC403	CaSiR-1™ AM	Detection of Zn^{2+} ions.
GC404	CaSiR-1™ Assay Kit	For live cell imaging of Zn^{2+} ions.
GC501 GC502	CaTM-2™	Detection and live cell imaging of hypochlorous acid (HOCl).
GC503 GC504	CaTM-2™ AM	Detection of hydroxyl radical ($^{\cdot}OH$) and peroxynitrite ($ONOO^-$).
GC505	CaTM-2™ Assay Kit	Detection of hydroxyl radical ($^{\cdot}OH$), peroxynitrite ($ONOO^-$) and hypochlorous acid (HOCl).
SK1003-01	NiSPY-3	Detection of peroxynitrite ($ONOO^-$).
SK1004-01	DAF-FM DA	Detection of intracellular NO by green fluorescence.
SK1005-01	DAR-4M	Detection of NO by orange fluorescence.
SK1006-01	DAR-4M AM	Detection of intracellular NO by orange fluorescence.

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