For research use only

CaSiR-1™ / CaSiR-1™ *AM*

Table 1. Product information

Catalog No.	Material	Amount	Storage	Stability
GC401 GC402	CaSiR-1™	1 mg×1 vials 50 µg×20vials	Store under -20°C, desiccate and protect from light Use up at once after probes are solved to DMSO	1 year (unopened)
GC403	CaSiR-1™ AM	50 µg×20vials		

1. Introduction

■ *About **CaSiR-1**™ / **CaSiR-1**™ AM*

The calcium ion is an critical intracellular second messenger and involved in lots of biological phenomena. Though fluorescence imaging is contributed to analysis of calcium behavior, the fluorescence wavelength area of general calcium probes are limited to around 500-580 nm.

CaSiR-1™ and CaSiR-1™ AM are near-infrared fluorescence calcium probes which have fluorescence maximum wavelength at 664 nm. Multicolor imaging is possible between CaSiR-1™ or CaSiR-1™ AM and fluorescent probes or fluorescent proteins which have fluorescent wavelength in visible area such as Hoechst, Fluorescein, Rhodamine, GFP, YFP and RFP etc. Near-infrared region has greater tissue penetration, less overlap with the spectrum of background autofluorescence and exhibits less phototoxicity to cells and tissue.

CaSiR-1™ changes fluorescent intensity greatly when it binds to calcium. For example, fluorescent intensity rises more than 1000-fold when calcium concentration is changed from 0 µM to 39 µM. Little fluorescence is detected when calcium concentration is 0 µM.

CaSiR-1™ is suited to cell introduce by microinjection, patchclamp and electroporation, etc. CaSiR-1™ AM, an acetoxymethyl ester of CaSiR-1™, can permeate cell membrane. After permeating cell membrane, CaSiR-1™ is hydrolyzed by esterase to give CaSiR-1™ and stay in the cell (Figure 1.). From these methods, it is possible that catching fluctuation of intracellular calcium concentration as the change of fluorescence intensity in CaSiR-1™ induced living cells. Action potential can be regarded as calcium concentration fluctuation in the cell when CaSiR-1™/ CaSiR-1™ AM is used to neuron.

2. Live cell staining protocol with CaSiR-1 AM

■ Materials Required but not Provided

- Anhydrous dimethylsulfoxide (DMSO)
- Appropreate medium or buffer such as Hank's Balanced Salt Solution (HBSS)
- 20 % Pluronic F-127 in DMSO

■ Preparation of Reagent and Cell Staining

1. To prepare a stock solution, dissolve the **CaSiR-1™ AM** 50 µg in 46 µL of DMSO() to 1 mM. To improve the induction efficiency and inhibit localization of the probe, the addition of Pluronic F-127 is recommended.
2. Dilute an aliquot of stock solution to a final concentration of 1-10 µM in appropreate loading medium or buffer such as HBSS(stain solution). Final concentration of Pluronic F-127 is around 0.01-0.05 %.
3. Remove the culture medium from cell culture dish and wash with loading medium.
   Caution : Glass bottom dish etc. are recommended as cell culture dish, because it has no intrinsic fluorescence.
4. Add stain solution to the dish and incubate 10 to 60 minutes under 37 °C, 5 % CO_2 conditions.
5. After staining, remove the stain solution from the dish and wash 2 or 3 times by medium or buffer which is not contained probe. Replace to HBSS buffer and observe the changes of intracellular fluorescence intensity using a fluorescence microscopy.

■ Fluorescence Imaging

650 nm are suited for excitation wavelength. Cy5 (Nikon Co. Ltd.) or U-DM-CY5.5-3 (Olympus Co. Ltd.) etc. are usable for filter. The maximum peak of fluorescent wavelength is detected at about 664 nm.

■ Storage

Probes are forwarded under conditions of N_2 atmosphere, dry and frozen state. After receipting, store under -20 °C, desiccate and protect from light. We recommend to use up at once after probes are solved to DMSO.

■ Reference

Egawa, T.; Hanaoka, K.; Koide, Y.; Ujita, S.; Takahashi, N.; Ikegaya, Y.; Matsuki, N.; Terai, T.; Ueno, T.; Komatsu, T.; Nagano, T. J. Am. Chem. Soc. 2011, 133, 14157-14159

CaSiR-1™ and **CaSiR-1™ AM** were commercialized by Goryo Chemical Company under the guidance of prof. Tetsuo Nagano (Professor of Graduate School of Pharmaceutical Sciences, Laboratory of Chemistry and Biology).

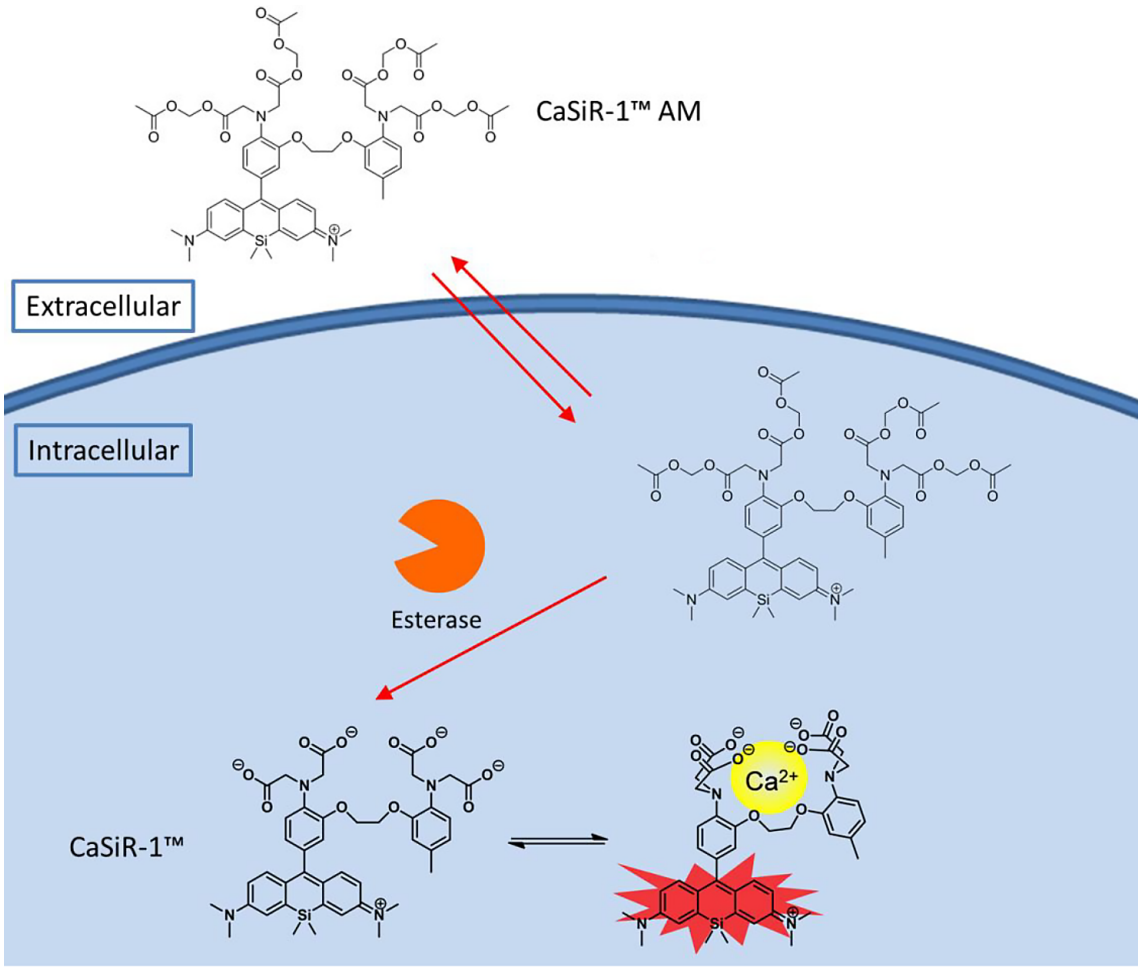

Figure 1. The mechanism which CaSiR-1™ AM is penetrated inside the cell and fluoresced by trapping calcium ion.

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