Calcium ion sensor Case12

- High dynamic range calcium indicator
- Direct expression in cells
- High selectivity and sensitivity
- High brightness of fluorescent response
- Fast maturation at 37°C
- Relatively high pH stability
- Recommended for monitoring change of calcium concentration inside living cells

Description

Case12 is a high dynamic range genetically encoded fluorescent sensor for detection of intracellular Ca^{2+} changes (Souslova et al., 2007). The sensor allows direct measurement of changes of calcium concentration in a physiological range from hundred nanomoles to micromoles with a high signal-to-noise ratio. Binding of Ca^{2+} is fast and reversible, allowing monitoring high-frequency-Ca^{2+} oscillations. In response to Ca^{2+} concentration rise, Case12 shows up to 12-fold increase of fluorescence brightness. Fluorescence of Case12 is characterized by single excitation/emission maxima peaked at 491/516 nm.

Case12 is recommended for monitoring change of calcium concentration inside living cells during various physiological and pathological conditions.

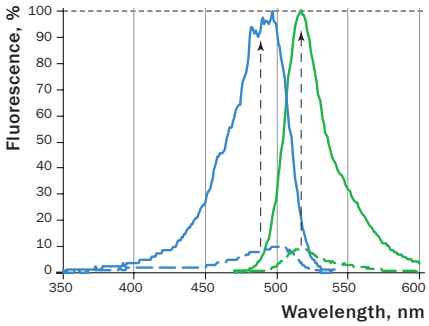
Main properties of Case12

Characteristic	
Molecular weight	46.4 kDa
Polypeptide length	415 aa
Fluorescence color	green
Excitation max	491 nm
Emission max	516 nm
Specificity	Ca^{2+}
Kd for Ca^{2+}	1 μM
pKa	7.2
Structure	monomer
Aggregation	no
Maturation at 37°C	fast

*Brightness is a product of extinction coefficient and quantum yield, divided by 1000.

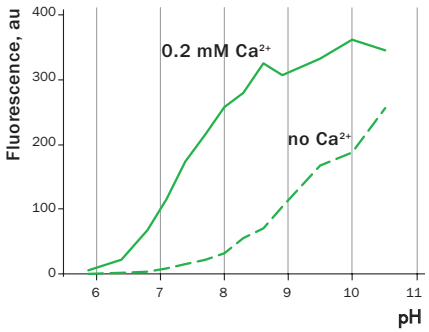
Performance and use

Case12 is characterized by fast maturation at 37°C and bright fluorescent response to Ca^{2+}. It can be directly expressed by target cells, both individually and in fusion with a specific localization signal. No aggregation is observed upon long-term (5 days) expression of Case12 in transiently transfected cells, indicating that Case12 is suitable for the generation of stable cell lines and transgenic animals. Case12 can be used for monitoring intracellular Ca^{2+} changes in various physiological and pathological conditions.



Case12 normalized excitation and emission spectra without Ca^{2+} (dotted line) and in the presence of 1 mM of Ca^{2+} (solid line).

Case12 shows multi-fold brightness increase of fluorescence in the response to 1 mM Ca^{2+}. Image from (Souslova *et al.*, 2007).



Dependence of Case12 fluorescence on pH in the presence (solid lines) and in the absence (dashed lines) of Ca^{2+} .

The common weak point of conventional calcium sensors is their low pH stability. For example, pKa (meaning of pH at which fluorescence brightness is 50% of maximum) for Pericams reaches as high as 8.0. Therefore, at physiological pH (7.2-7.5) such sensors exhibit low brightness and dynamic range (Nagai *et al.*, 2001a). In contrast, the pKa of Case12 is 7.2 (in the presence of 10 μM Ca^{2+}) close to that reported for G-CaMP (Nagai *et al.*, 2001b). This relatively high pH stability makes Case12 well suitable for *in vivo* use. Image from (Souslova *et al.*, 2007).

Monitoring changes in green emission of Case12 in response to intracellular changes of Ca^{2+} concentration should be carried out by excitation by blue light (488 nm laser line or standard GFP filter set). Emission can be collected at approximately 500-540 nm. Intensity of excitation light should be individually determined for particular biological system and instrumentation. In general, we recommend that you minimize excitation light intensity and duration.

Note: Yellow fluorescent core of Case12 undergoes partial photoconversion to a dark state upon irradiation with blue light. It means that an apparent "bleaching" effect occurs at the beginning of time series imaging of cells expressing Case12 protein. Unlike the real bleaching, in the case of Case12, signal drops to the level of dynamic equilibrium between fluorescent and dark state of the chromophore, and then remains stable.

Maximum dynamic range in HeLa cells

HeLa cells transfected with Case12 showed relatively weak green fluorescence, which was detected with a Leica microscope DM IRE2, confocal TCS-SP2, objective HCX-PL-APO-63x/1.40-0.60/OIL. Addition of 20 μM calcium ionophore A23187, allowing calcium to enter cells (2 mM Ca^{2+} in the medium), resulted in 5-6-fold increase in green fluorescence brightness. Subsequent addition of 20 mM EGTA removed Ca^{2+} and decreased the fluorescence signal close to baseline level, with the final contrast of 11-12 fold.

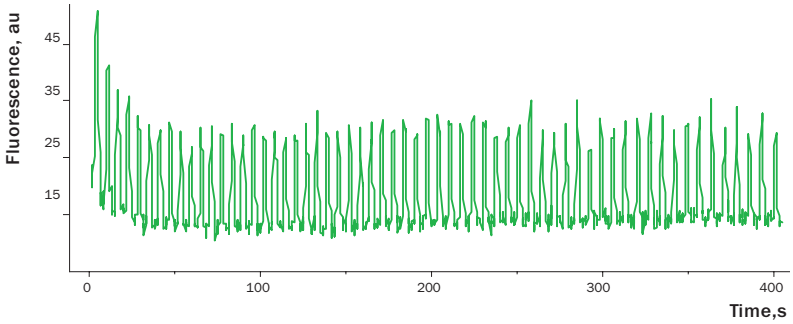
Monitoring of Ca^{2+} changes under physiological conditions

Mammalian cells expressing Case12 displayed a nice high dynamic range response upon addition of ATP at a final concentration of 100 μM. This experiment clearly showed that Case12 fluorescence response to Ca^{2+} oscillations is fast and reversible. It also demonstrated that the sensor responds to changes in Ca^{2+} concentration in living cells in the nanomolar range.

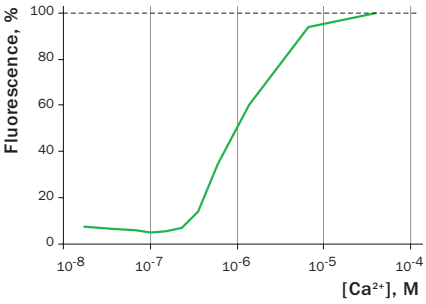
Recommended filter sets and antibodies

Case12 can be recognized using Anti-Tag(CGY)FP antibody (Cat.# AB121-AB122) available from Evrogen.

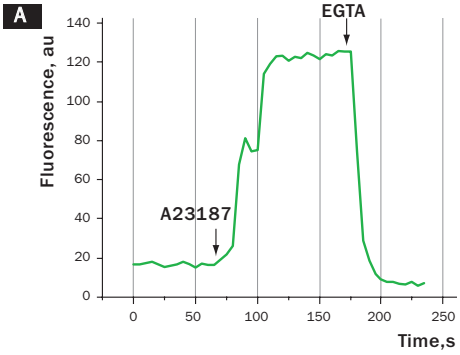
We recommend standard GFP filter sets. Appropriate Omega Optical filter sets for Case12 are QMAX-Green, XF100-2 and XF100-3. It can also be detected using Chroma Technology Corp. filter sets 41001, 41017, 41020, 41025 or similar.

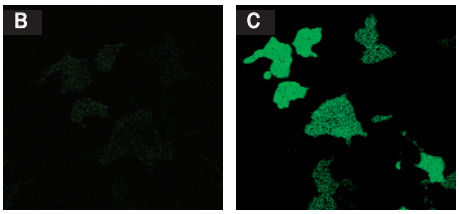


Fluorescence changes of human melanoma-derived M21 cells expressing Case12 in response to 100 μM ATP.
Images were captured every 0.294 sec on the confocal microscope.



Ca^{2+} titration curves.
The apparent Kd for Ca^{2+} binding was found to be 1 μM, which lies within the physiological range of Ca^{2+} concentrations. Image from (Souslova et al., 2007).





Testing Case12 in living cells.
A — Typical response of HeLa cells expressing Case12 to calcium ionophore A23187.
B, C — HeLa cells expressing Case12 shown before (B) and after (C) ionophore addition.
Image from (Souslova et al., 2007).

References

Haas *et al*. (1996) Curr. Biol. 6: 315–324.

Nagai et al. (2001a) Proc Natl Acad Sci U S A 98: 3197-3202.

Nakai et al. (2001b) Nat Biotechnol 19: 137-141.

Souslova et al. (2007) BMC Biotechnology, 7: 37.

Case12-related products

Product	Cat.#	Description
Case12 expression/source vectors		
pCase12-cyto	FP991	Mammalian expression vector comprising Case12 gene and allowing Case12 expression in cytosol
pCase12-mito	FP992	Mammalian expression vector encoding mitochondria-targeted Case12
pCase12-mem	FP993	Mammalian expression vector encoding membrane-targeted Case12
Gateway® Case12-cyto	FP994	Gateway® entry clone for transfer of Case12-cyto into Gateway® destination vectors
Gateway® Case12-mito	FP995	Gateway® entry clone for transfer of Case12-mito into Gateway® destination vectors
Antibody against Case12		
Anti-Tag(CGY)FP antibody	AB121 AB122	Rabbit polyclonal antibody against TagCFP, TagGFP, TagGFP2, TagYFP, PS-CFP2, Case12, HyPer, and EGFP

Please contact your local distributor for exact prices and delivery information.