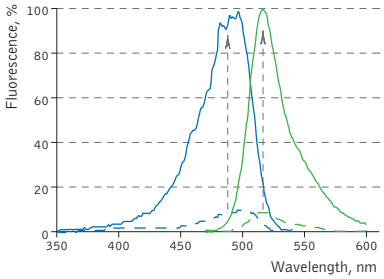
Calcium ion sensor Case12

- High dynamic range detection of intracellular Ca^{2+} level changes
- High selectivity and sensitivity, relatively high pH stability
- Fast maturation, high brightness of fluorescent response
- Direct expression in cells, easy targeting to various subcellular compartments
- No exogenous chemical compounds required
- Recommended for monitoring changes in Ca^{2+} concentration inside living cells

Case12 is a high dynamic range genetically encoded fluorescent sensor for direct measurement of changes of intracellular Ca^{2+} under various physiological and pathological conditions [Souslova et al. 2007]. The sensor is sensitive to changes of calcium concentration in a physiological range from a hundred nanomoles to micromoles with a high signal-to-noise ratio. Binding of Ca^{2+} is fast and reversible, allowing monitoring of 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.

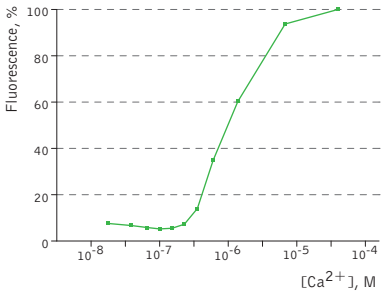
Case12 is recommended for monitoring change of calcium concentration inside living cells during various physiological and pathological conditions.



Case12 normalized excitation (blue line) and emission (green line) spectra without Ca^{2+} (dashed lines) and in the presence of 1 mM of Ca^{2+} (solid lines)[Souslova et al. 2007].
Case12 shows multi-fold brightness increase of fluorescence in the response to 1 mM Ca^{2+}. Complete Case12 spectra in Excel format can be downloaded from the Evrogen Web site at http://www.evrogen.com

Main properties of Case12

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



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

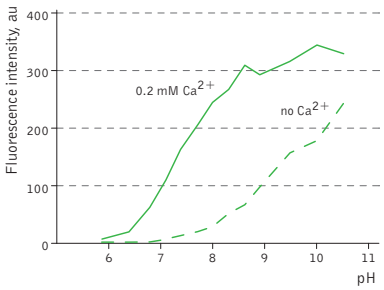
Performance and use

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. 2001]. In contrast, the pKa of Case12 is 7.2 (in the presence of 10 µM Ca^{2+}) close to that reported for G-CaMP [Nakai, Ohkura, and Imoto 2001]. This relatively high pH stability makes Case12 well suitable for *in vivo* 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.

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



Dependence of Case12 fluorescence on pH in the presence (solid line) and in the absence (dashed line) of Ca^{2+}. Image from Souslova et al. 2007.

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), resultes in 5-6-fold increase in green fluorescence brightness. Subsequent addition of 20 mM EGTA removes Ca^{2+} and decreases 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 display a nice high dynamic range response upon addition of ATP at a final concentration of 100 μM. This experiment clearly showes that Case12 fluorescence response to Ca^{2+} oscillations is fast and reversible. It also demonstrates that the sensor responds to changes in Ca^{2+} concentration in living cells in the nanomolar range.

Case12 suitability to generate stably transfected cells has been proven by Marinpharm company. Cell lines expressing Case12 are commercially available.

Recommended filter sets and antibodies

Case12 can be recognized using Anti-GFP antibody and Anti-Tag(CGY)FP antibody (Cat.# AB121) 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.

Available variants and fusions

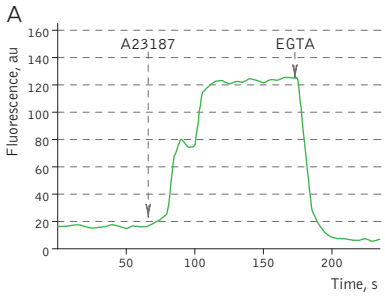
Case12 mammalian expression vectors contain Case12 coding sequence with codon usage optimized for high expression in mammalian cells, i.e. humanized [Haas, Park, and Seed 1996]. Humanized Case12 can also be expressed in *E. coli* and some other heterological systems upon subcloning into appropriate vector.

The available vectors encoding Case12 variants and fusions are listed below in the section Case12-related products. For most updated product information, please visit Evrogen website www.evrogen.com.

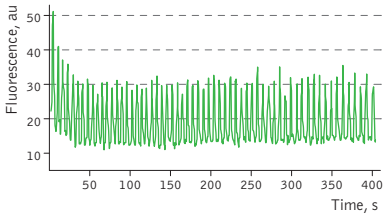
If you need Case12 codon variant or fusion construct that is not listed on our website, please contact us at product@evrogen.com.

Licensing opportunities

Evrogen technology embodied in Case12 is available for expanded and commercial use with an adaptable licensing program. Benefits from flexible and market driven license options are offered for upgrade and novel development of products and applications. For licensing information, please contact Evrogen at license@evrogen.com.



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.



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.

References

Haas, J., E. C. Park, and B. Seed (1996). Curr Biol, 6 (3): 315–324 / pmid: 8805248

Nagai, T et al. (2001). Proc Natl Acad Sci U S A, 98 (6): 3197–3202 / pmid: 11248055

Nakai, J, M. Ohkura, and K. Imoto (2001). Nat Biotechnol, 19 (2): 137–141 / pmid: 11175727

Souslova, EA et al. (2007). BMC Biotechnol, 7 (1): 7366–7375 / pmid: 17603870

Case12-related products

Product	Cat.#	Description	Size
Case12 expression/source vectors			
pCase12-cyto	FP991	Mammalian expression vector allowing Case12 expression in cytosol under the control of CMV promoter	20 µg
pCase12-mito	FP992	Mammalian expression vector encoding mitochondria-targeted Case12	20 µg
Antibodies against Case12			
Anti-Tag(CGY)FP	AB121	Rabbit polyclonal antibody against TagCFP, TagGFP, TagGFP2, TagYFP, PS-CFP2, Case12, HyPer, and EGFP	100 µg

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