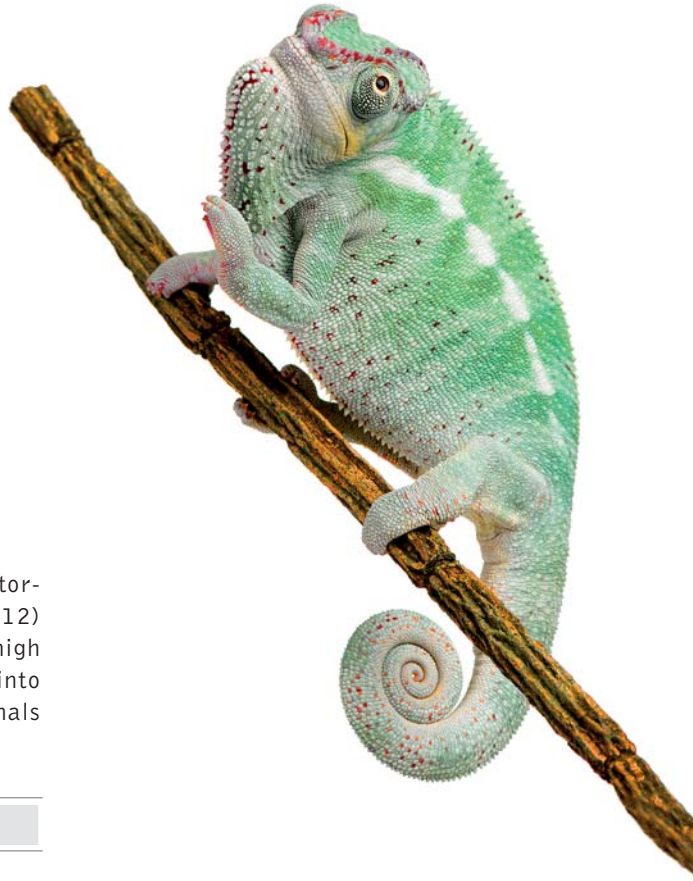
evrogen



Fluorescent sensors for detection of intracellular Ca^{2+} and H_2O_2

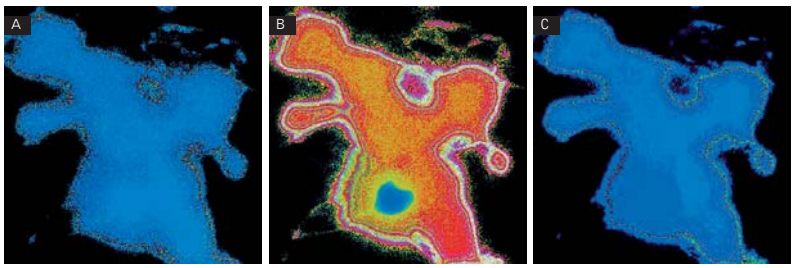
Evrogen offers genetically-encoded fluorescent sensors for monitoring changes in intracellular concentration of calcium ions (Case12) and hydrogen peroxide (HyPer). Both indicators demonstrate high selectivity and sensitivity of detection, allow precise targeting into various subcellular compartments and real time measuring of signals in natural intracellular surroundings.

Protein	HyPer	Case12
Fluorescence color	green	green
Excitation max, nm	420 and 500	491
Emission max, nm	516	516
Specificity	H_2O_2	Ca^{2+}
Aggregation	not observed	not observed

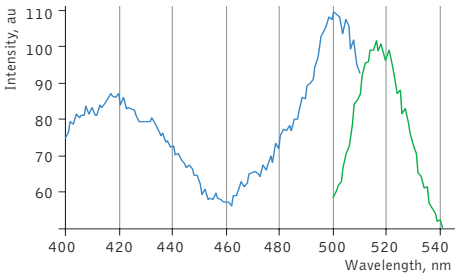
HyPer

Ratiometric sensor for hydrogen peroxide, HyPer, demonstrates submicromolar affinity to H_2O_2 and is insensitive to other oxidants tested, like superoxide, oxidized glutathione, nitric oxide, and peroxinitrite. Unlike many chemical sensors, HyPer does not cause artifactual ROS generation and can be used for detection of fast changes of H_2O_2 concentration in different cell compartments.

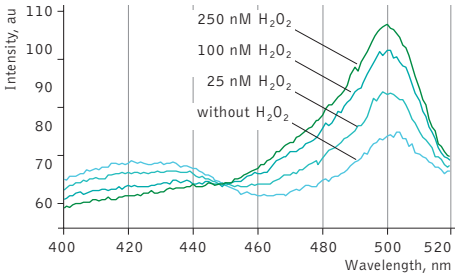
Without H_2O_2, HyPer has two excitation peaks with maxima at 420 nm and 500 nm, and one emission peak with maximum at 516 nm. Upon exposure to H_2O_2, the excitation peak at 420 nm decreases proportionally to the increase in the peak at 500 nm, allowing ratiometric measurement of H_2O_2. Oxidized HyPer can be reduced inside cells.



Ratiometric images of the group of HeLa cells before (A), 20 sec after (B), and 600 sec after (C) addition of 180 ul of H_2O_2. Images were pseudocolored using "ratio" lookup table of NIH ImageJ software: blue-green-red-white colors represent lowest-intermediate-high-highest level of H_2O_2.



HyPer excitation (blue line) and emission (green line) spectra.

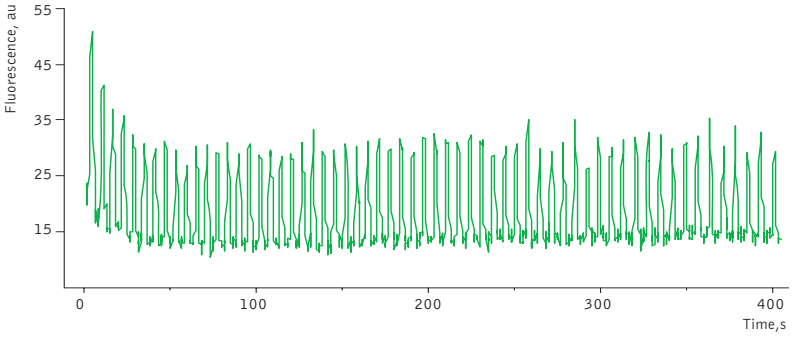


Changes in the excitation spectrum of isolated HyPer in response to H_2O_2 addition. Emission was measured at 530 nm.

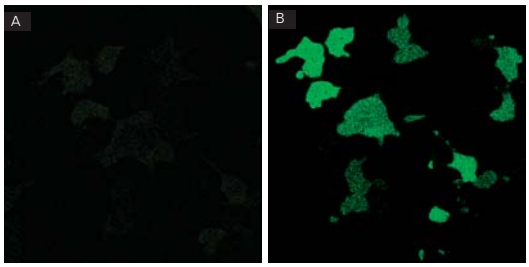
Case12

High dynamic range sensor for calcium ions, Case12, allows 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, easily detectable using standard GFP filter sets.

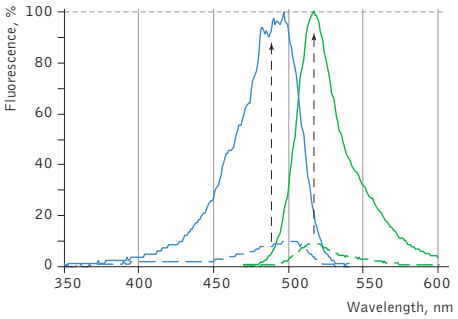
In contrast to conventional calcium sensors, Case12 is stable under physiological pH (pKa 7.2 in the presence of 10 μM Ca^{2+}). This makes it well suitable for *in vivo* use.



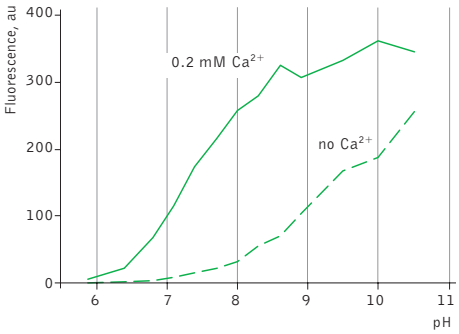
Fluorescence changes of human melanoma-derived M21 cells expressing Case12 in response to 100 μM ATP.



Testing Case12 in living cells. HeLa cells expressing Case12 shown before (A) and after (B) ionophore addition.



Case12 normalized excitation (blue) and emission (green) 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+}.



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

Available vectors

Vector	Cat#
pCase12-cyto	FP991
pCase12-mem	FP993
pCase12-mito	FP992
pHyPer-cyto	FP941
pHyPer-dMito	FP942
pHyPer-nuc	FP944

For licensing information, please contact Evrogen at license@evrogen.com

For more information, please visit our web-site: **www.evrogen.com**