evrogen

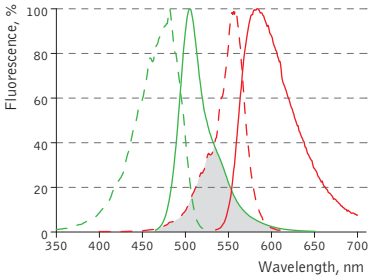
Caspase-3 apoptosis sensor Casper3-GR

- Early detection of apoptosis in the living cells
- High sensitivity
- Direct expression
- No exogenous chemical compounds or cofactors required
- Proven suitability for FLIM-based screenings

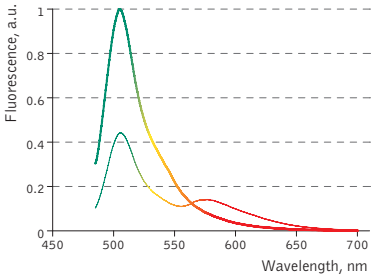
Casper3-GR is a FRET based sensor that can be used for detection of caspase-3 mediated apoptosis in living cells. The sensor consists of green and red fluorescent proteins TagGFP and TagRFP connected by the linker containing caspase-3 cleavage sequence, DEVD. The high fluorescence quantum yield of TagGFP along with the high molar extinction coefficient of TagRFP and excellent overlap of donor emission and acceptor excitation spectra result in highly effective FRET between these fluorescent proteins. The activation of caspase-3 during apoptosis leads to cleavage of DEVD sequence and elimination of FRET that can be detected as decrease in the red emission of TagRFP and a simultaneous increase in green emission of TagGFP.
The calculated Forster distance R_0= 5.7 nm for TagGFP - TagRFP pair is one of the largest among the values reported. At the same time, since TagGFP and TagRFP emission peaks are spaced by as much as 79 nm, the emission signal for these two proteins can be easily separated in any imaging system. As an additional advantage, shifting the wavelengths toward the red part of the spectrum (comparing to traditional cyan and yellow FRET partners) reduces input of cellular autofluorescence.

Main properties of Casper3-GR

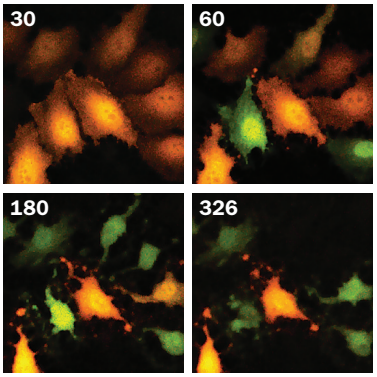
Characteristic	
Calculated Forster distance (R_0)	5.70
FRET efficiency (E)	0.50
Specificity	caspase-3 activity
Response	elimination of FRET
Polypeptide length, aa	484
Molecular weight, kDa	54
FRET donor	TagGFP
Fluorescence color	green
Excitation maximum, nm	482
Emission maximum, nm	505
Brightness, % of EGFP	104
pKa	4,7
FRET acceptor	TagRFP
Fluorescence color	red
Excitation maximum, nm	555
Emission maximum, nm	584
Brightness, % of EGFP	148
pKa	3,8



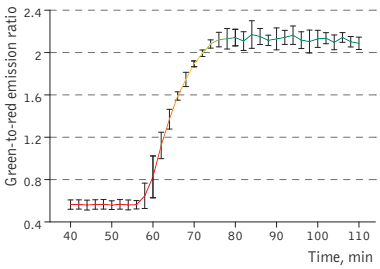
Excitation (dashed lines) and emission (solid lines) spectra of TagGFP (green) and TagRFP (red) are shown individually. Spectral overlap is filled with gray.



Emission spectra of Casper3-GR before (thin line) and after digestion by Caspase-3 (thick line).



Two channel fluorescence imaging of Casper3-GR upon staurosporine-induced apoptosis in HeLa cells. Time (in min.) is shown after staurosporine infusion.

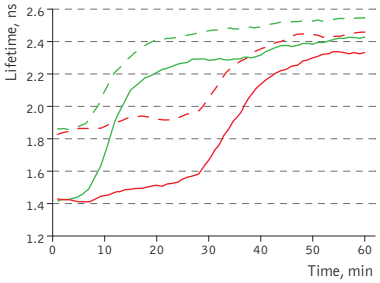


Green-to-red emission ratio change of Casper3-GR upon staurosporine-induced apoptosis.
Approximately 40-50 min after staurosporine infusion, cells demonstrated pronounced changes fluorescence signal ratio. Emission ratio shown for 5 cells, time point aligned to the median of ratio changes, individual for each cell. Excitation at 488 nm, emission was detected at 500-530 nm and 560-600 nm.

Performance and use

The excellent performance of Casper3-GR sensor has been demonstrated *in vivo* on the example of HeLa cells staurosporine-induced apoptosis [Subach et al. 2008]. Living cells were monitored at 37°C with Leica SP2 confocal microscope (excitation using 488 nm laser line, emission collected at 500-530 nm and 560-650 nm). The fluorescence was evenly distributed in the cytosol and nucleus with no aggregation or non-specific localization observed. Importantly, both green and red signals were reliably stable upon various irradiation conditions for hours. No reversible or irreversible fluorescence bleaching or photoconversion was observed.

Approximately 30-40 min after 2 μM staurosporine infusion, cells demonstrated rapid (within 10 min) and pronounced changes in green-to-red fluorescence signal ratio, indicating activation of caspase-3. Later these cells demonstrated characteristic membrane blebbing. The average contrast in living cells (calculated as donor/acceptor emission ratio change for 5 cells, time point aligned to the median of ratio changes, individual for each cell) reached 3.8-fold.
Measurement of Casper3-GR apoptosis induced FRET changes by FLIM revealed the dramatic increase of TagGFP fluorescence lifetime from 1.5 ns to 2.5 ns. The FRET efficiency of the uncleaved Casper3-GR (38% based on the phase lifetime) is among the highest measured by FLIM. Since the FRET efficiency of the cleaved substrate is zero, the dynamic range of the sensor is rather high, indicating that Casper3-GR can be successfully used for the high content FLIM based screenings on living cells.



TagGFP fluorescence phase lifetime (solid lines) and average modulation lifetime (dashed lines) changes for Casper3 during staurosporine-induced apoptosis. Excitation was at 488 nm and donor fluorescence emission was passed through a 500-530 nm bandpass filter.

Recommended filter sets

The excitation wavelength required to visualize FRET changes of Casper3-GR by ratio-imaging is provided by an ordinary FITC/GFP excitation filter or ubiquitous 488 nm laser line, and the two emission signals are acquired using a 500-530 nm (FITC/GFP emission filter) bandpass filter and a 560-600 nm bandpass filter (Cy3/DsRed emission filter) or a 560LP longpass filter.

Licensing opportunities

Evrogen technology embodied in Casper3-GR 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.

References

Subach et al. (2008) “Conversion of Red Fluorescent Protein into a Bright Blue Probe.” Chemistry & Biology, 15 (10): 1116–1124 / pmid: 18940671

Casper3-GR-related products

Product	Cat.#	Description	Size
pCasper3-GR	FP971	Mammalian expression vector allowing Casper3-GR expression in cytoplasm under the control of CMV promoter	20 μg

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