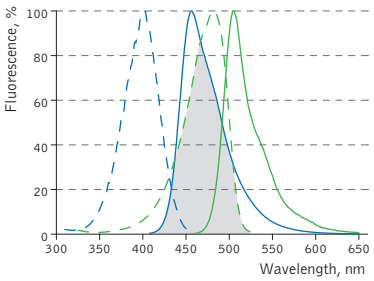
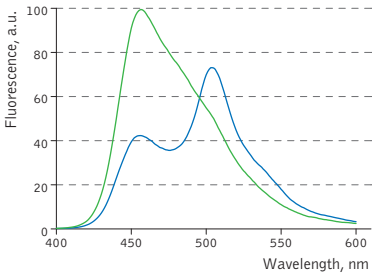
Caspase-3 apoptosis sensor Casper3-BG

- Early detection of apoptosis in the living cells
- High sensitivity
- Direct expression
- No exogenous chemical compounds or cofactors required

Casper3-BG is a FRET based sensor that can be used for detection of caspase-3 mediated apoptosis in living cells. The sensor consists of blue and green fluorescent proteins, TagBFP and TagGFP2, connected by the linker containing caspase-3 cleavage sequence, DEVD. Good overlap between the emission spectrum of TagBFP and the absorbance spectra of TagGFP2 ensures efficient FRET between these 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 green emission of TagGFP2 and a simultaneous increase in blue emission of TagBFP.
The calculated Forster distance and measured efficiency of FRET between TagBFP and TagGFP2 are larger than those reported for the standard ECFP-EYFP and mCyPet-mYPet pairs. Moreover, TagBFP and TagGFP2 proteins lack the ability to form heterodimers, which results in more than 6-fold lower background for FRET analysis than in case of weakly dimerizing FRET pairs, such as ECFP-EYFP [Subach et al. 2008].



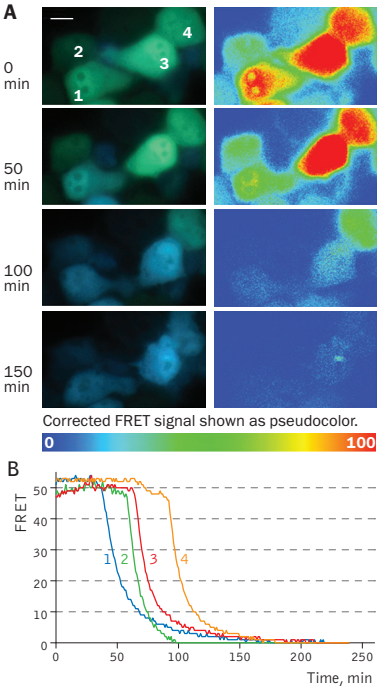
Excitation (dashed lines) and emission (solid lines) spectra of TagBFP (blue) and TagGFP2 (green) are shown individually. Spectral overlap is filled with gray.



Change in Casper3-BG excitation/emission spectra upon the cleavage of DEVD sequence *in vitro*. Before cleavage - blue line, after cleavage - green line.

Main properties of Casper3-BG

Characteristic	
Calculated Forster distance (R_0)	5.25
FRET efficiency (E)	0.57
Specificity	caspase-3 activity
Response	elimination of FRET
Polypeptide length, aa	490
Molecular weight, kDa	55
FRET donor	TagBFP
Fluorescence color	blue
Excitation maximum, nm	402
Emission maximum, nm	457
Brightness, % of EGFP	99
pKa	2,7
FRET acceptor	TagGFP2
Fluorescence color	green
Excitation maximum, nm	483
Emission maximum, nm	506
Brightness, % of EGFP	105
pKa	5.0



Imaging of FRET intensity in staurosporine-treated HeLa cells: (A) Fluorescent images of the cells after staurosporine treatment (left). The corrected FRET signals are shown as pseudocolor images (right). Scale bar, 10 mm. (B) time course of corrected FRET normalized per donor fluorescence observed in four cells indicated in (A).

Performance and use

The excellent performance of Casper3-BG sensor has been demonstrated *in vivo* on the example of HeLa cells staurosporine-induced apoptosis [Subach et al. 2008]. The two-filter method of sensitized FRET measurements [Gordon et al. 1998] on a pixel-by-pixel basis was applied, as described in [Galperin et al. 2004]. The initial mean FRET efficiency *in vivo* normalized to donor fluorescence was 51.5%.

Following 40-80 min exposure to 1 mM staurosporine, the FRET gradually dropped to zero before the shrinking of cells characteristic to apoptosis. The large FRET efficiency of the TagBFP-TagGFP2 pair enabled the detection of even weak proteolitic activity in each cell at the beginning of apoptosis, when only a fraction of the substrate was cleaved.

Recommended filter sets

The set of filters from Chroma (403/12 nm exciter, part #74673, 457/50 nm emitter, part #66974, and dichroic mirror, part #86100) or similar.

Licensing opportunities

Evrogen technology embodied in Casper3-BG 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

Galperin et al. (2004) "Three-chromophore FRET microscopy to analyze multiprotein interactions in living cells." Nat Methods, 1 (3): 209217 / pmid: 15782196

Gordon et al. (1998) "Quantitative fluorescence resonance energy transfer measurements using fluorescence microscopy." Biophys J, 74 (5): 2702–2013 / pmid: 9591694

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

Casper3-BG-related products

Product	Cat.#	Description	Size
pCasper3-BG	FP970	Mammalian expression vector allowing Casper3-BG expression in cytoplasm under the control of CMV promoter	20 μg

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