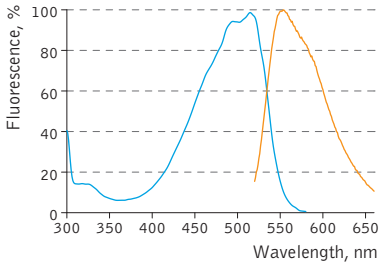
Genetically-encoded photosensitizer KillerOrange

- Blue and green light-induced production of reactive oxygen species
- Direct expression in cells, easy targeting to various subcellular compartments
- No exogenous chemical compounds required for chromophore maturation
- Not toxic before activation by blue or green light irradiation
- Recommended for selective light-induced protein inactivation and cell killing

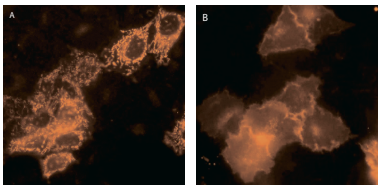
KillerOrange represents a mutant of KillerRed with a bright orange fluorescence (excitation maximum at 512 nm and emission maximum at 555 nm) [Sarkisyan et al. 2015, **id-ref1228**]. In contrast to KillerRed, which becomes phototoxic after illumination with green or orange (540-590 nm) light, KillerOrange was shown to be phototoxic to *E. coli* after illumination with blue or green (450-540 nm) light.

The blue-shifted spectrum of KillerOrange makes it potentially more suitable for two-photon microscopy than the parental KillerRed. Also, the large Stokes shift of over 40 nm should make it possible to spectrally separate signals of KillerOrange from cyan and green fluorescent proteins when the proteins are excited simultaneously by blue light. One can thus use green and cyan indicators to observe the effects of phototoxicity in real time without the need to change the excitation light.

KillerOrange-KillerRed pair can potentially be used to independently ablate two cell populations. This pair also promises the orthogonal optical control of the propagation of signaling cascades either by chromophore-assisted light inactivation of the participating proteins or by triggering cascades with hydrogen peroxide produced by KillerRed and likely by KillerOrange. KillerOrange-KillerRed tandem fusions or combination of various photosensitizers in one cassette may enhance phototoxicity under white light irradiation and may be useful as a research tool in biology.



KillerOrange normalized exitation – blue line (em@600 nm) and emission – orange line (ex@510 nm) spectra.
Complete KillerOrange spectra in Excel format can be downloaded from the Evrogen Web site at http://evrogen.com

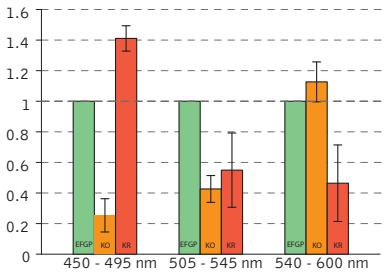


KillerOrange expression in mammalian cells.
(A) Transiently transfected HeLa cells expressing mitochondria-targeted KillerOrange.
(B) Transiently transfected HeLa cells expressing membrane-targeted KillerOrange.

Main properties of KillerOrange

Characteristic	
Molecular weight, kDa	27
Polypeptide length, aa	239
Structure	dimer
Aggregation	no
Maturation rate at 37°C	fast
Activating light	blue or green (e.g. 450-540 nm)
Fluorescence color	orange
Excitation maximum, nm	512
Emission maximum, nm	555
Quantum yield	0.42
Extinction coefficient, $M^{-1}cm^{-1}$	41200
Brightness*	17.3

* Brightness is a product of extinction coefficient and quantum yield, divided by 1 000.



KillerOrange (KO) and KillerRed (KR) toxicity under light illumination at different wavelengths.
Illumination with 540-600 nm light resulted in the selective removal of KillerRed-expressing cells, while the illumination with 450-495 nm light killed the majority of KillerOrange-expressing cells. Interestingly, 505-545 nm light illumination was almost equally efficient in killing both KillerOrange and KillerRed cells. Thus, by combining different light sources one can achieve precise control over cell populations expressing KillerOrange and KillerRed.
Green column – EGFP, orange column – KillerOrange, red column – KillerRed. Error bars represent SD, N=4.

Performance and use

KillerOrange can be used for *in vivo* killing of selected cells. It can be expressed and induced in various experimental systems, including bacteria and mammalian cells.

KillerOrange's suitability for light-induced killing of prokaryotic cells: has been demonstrated using *E. coli* XL1-Blue strain.

Recommended antibodies, filter sets, and activating parameters

KillerOrange can be recognized using Anti-KillerRed antibody (Cat.# AB961) available from Evrogen.

KillerOrange phototoxicity can be induced by blue or green light irradiation (450-540 nm) and depends on light intensity, irradiation time and KillerOrange concentration and localization. LEDs light is strongly recommended; laser-light irradiation in confocal mode is much less efficient. KillerOrange-mediated ROS production is accompanied by profound KillerOrange photobleaching. The resulting cell events (cell fate after irradiation, effect on protein localization) can be monitored using another fluorescent reporter, for example a green fluorescent protein. We recommend that you use TurboGFP for cell and organelle, or TagGFP2 for protein labeling.

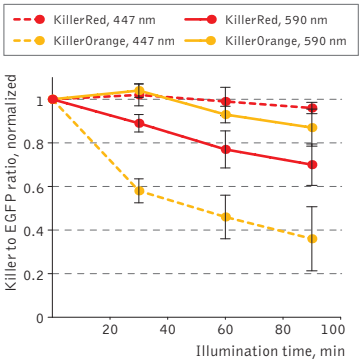
Available variants and fusions

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

The available vectors encoding KillerOrange are listed below in the section KillerOrange-related products. For most updated product information, please visit Evrogen website www.evrogen.com. If you need KillerOrange 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 KillerOrange 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.



Phototoxicity of mitochondria-targeted versions of KillerOrange and KillerRed in mammalian cells. KillerOrange, KillerRed and EGFP-expressing cells were mixed and illuminated with 477 nm or 590 nm light. Y-axis depicts changes in fractions of KillerOrange (yellow lines) or KillerRed-expressing (red lines) cells in the population. Cell fractions were normalized to the fractions in non-illuminated sample.
Error bars represent SD, N=3.

References

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

Sarkisyan, KS. et al. (2015). PLoS One, 10 (12): e0145287 / pmid: 26679300

KillerOrange-related products

Product	Cat.#	Description	Size
KillerOrange expression/source vectors			
pKillerOrange-C	FP221	Mammalian expression vector encoding humanized KillerOrange, allowing generation of fusions to the KillerOrange C-terminus	20 μg
pKillerOrange-N	FP222	Mammalian expression vector encoding humanized KillerOrange, allowing generation of fusions to the KillerOrange N-terminus	20 μg
pKillerOrange-B	FP223	Bacterial expression vector encoding humanized KillerOrange	20 μg
pKillerOrange-dMito	FP224	Mammalian expression vector encoding mitochondria-targeted KillerOrange	20 μg
pKillerOrange-mem	FP226	Mammalian expression vector encoding membrane-targeted KillerOrange	20 μg
Antibodies against KillerOrange			
Anti-KillerRed antibody	AB961	Rabbit polyclonal antibody against KillerRed, KillerOrange, ArrestRed, and JRed	100 μg

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