Kindling red fluorescent protein KFP-Red

- Reversible or irreversible photoactivation
- Activated by green light that does not damage cells and tissues
- Quenching by blue light
- Recommended for tracking cells and cellular organelle movements

KFP-Red (also referred to as KFP1) is a photoactivatable GFP-like protein generated on the basis of *Anemonia sulcata* chromoprotein, asFP595 [Lukyanov et al. 2000; Chudakov, Belousov, et al. 2003; Chudakov, Feofanov, et al. 2003]. KFP-Red switches from a non-fluorescent to a red fluorescent form (with excitation/emission maxima at 580 nm and 600 nm, respectively) under the exposure to intense green light irradiation. A green light laser does not damage cells and tissues. Activated KFP-Red can be easily detected because its emission spectrum is beyond the region of cell autofluorescence.

KFP-Red can be used for *in vivo* monitoring cell and cellular organelle movement.

Main properties of KFP-Red

Characteristic	before / after photoactivation
Fluorescence color	NO / red
Excitation maximum, nm	580
Emission maximum, nm	600
Quantum yield	<0.001 / 0.07
Extinction coefficient, $M^{-1}cm^{-1}$	123 000 / 59 000
Brightness*	0 / 4.1
Activating light	green (530-560 nm)
Calculated contrast, fold	35-70
Structure	tetramer
Cell toxicity	not observed
Aggregation	no
Maturation rate at 37°C	medium
Molecular weight, kDa	26
Polypeptide length, aa	238

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

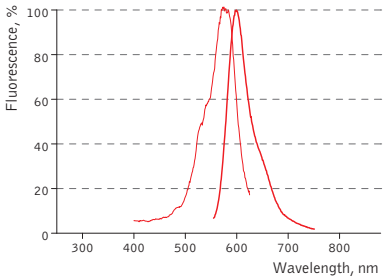
Performance and use

KFP-Red was successfully expressed and tested in various experimental models, including bacteria, *Xenopus* embryo, and cultured mammalian cells. Like other Anthozoa GFP-like proteins, KFP-Red is a tetramer. This restricts the wide use of KFP-Red as a fusion partner for cellular proteins.

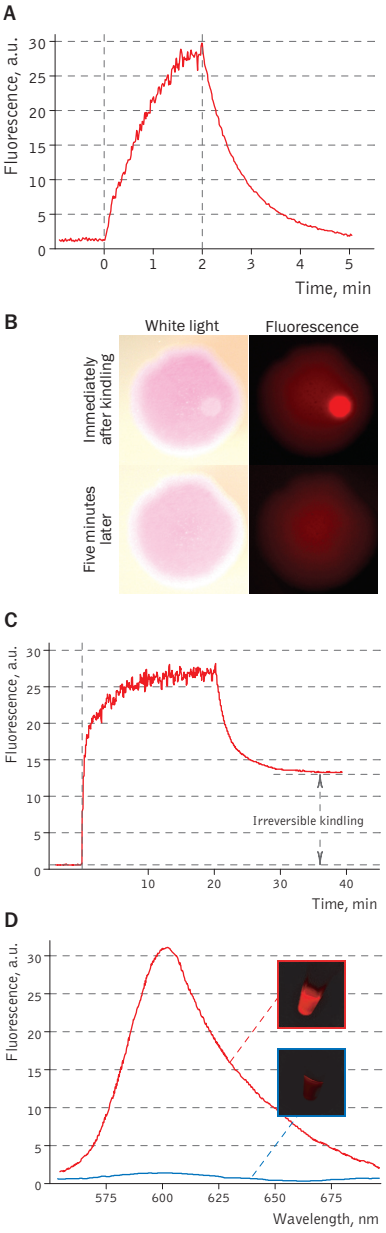
Reversible or irreversible kindling: Depending on the kindling light intensity KFP-Red can be photoactivated reversibly or irreversibly allowing the monitoring of both short- and long-term cell processes.

A reversibly kindled KFP-Red relaxes to the initial non-fluorescent form (half-life 50 seconds), or can be quenched instantly by blue light (430-490 nm). Reversible kindling results in about 70 times increase of the red fluorescence intensity comparing to unkindled protein. Reversible kindling and quenching can be repeated many times.

An irreversibly kindled KFP-Red gives stable red fluorescence which is at least 35 times brighter than that of the protein before kindling. An irreversibly kindled KFP-Red remains stable and brightly fluorescent for more than 72 hrs in living cells and for at least a year in protein samples. An irreversibly kindled KFP-Red can be partially quenched by blue light, but then it restores its brightness within several minutes. Therefore, in some applications, blue light can be used to quench a reversibly kindled KFP-Red, whereas an irreversibly kindled KFP-Red remains fluorescent.



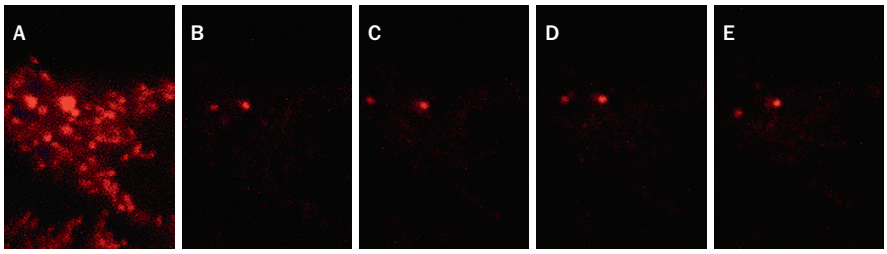
KFP-Red normalized excitation (thin line) and emission (thick line) spectra.
Complete KFP-Red spectra in Excel format can be downloaded from the Evrogen Web site at http://www.evrogen.com



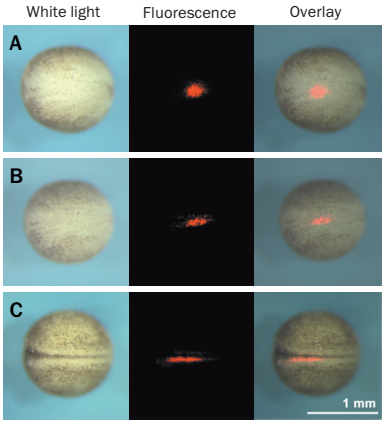
KFP-Red reversible and irreversible kindling.
(A) Kindling and relaxation kinetics. Zero time is set at the commencement of irradiation with kindling light (532 nm laser light, 1% power). Kindling irradiation was stopped after 2 min. (B) Reversible photoactivation of KFP-Red in *E. coli*. The round-shaped part of the *E. coli*. colony exp-ressing KFP-Red was irreversibly kindled with intense green light. This region fluoresces brightly, while its absorption is low. After several minutes, the kindled protein relaxed to the non-fluorescent state, while its absorption recovered. (C) Irreversible kindling. Zero time is set at the commencement of irradiation with kindling light (532 nm laser light, 20% power). Kindling irradiation was stopped after 20 min. (D) Irreversibly kindled (red line) and "unkindled" (blue line) KFP-Red fluorescence spectra and brightness ratio.

Application of KFP-Red to track cell migration was demonstrated using embryonic fate mapping as an example. *Xenopus* embryos were taken at the stage of two blastomeres and KFP-Red mRNA was microinjected into the animal poles of both blastomeres. At the early neurula stage, a round-shaped group of cells within the neural plate was kindled irreversibly. Irradiated cells became brightly fluorescent and their migration in the developing embryo was monitored. Longitudinal extension accompanied by transversal convergence of the labeled group of cells was visible after the first two hours after kindling. At the end of neurulation, the labeled spot appeared as a thin stripe on the surface of the left neural fold.

KFP-Red suitability for tracking movement of cell organelles was demonstrated on PC12 cells transfected with a mitochondria-targeted KFP-Red expressing vector. After 25 hours of incubation, mitochondria remained non-fluorescent (no kindling observed) upon irradiation using a 1% power scanning green laser (HeNe laser line 543 nm, 1 mW, once per 10 seconds; the number of scans is not limited). After several scans with a 5-10% power laser, mitochondria became brightly fluorescent and were observed using a 1% power laser for several minutes. Brief irradiation (about 20 seconds in fast mode) with a 30% power green laser light induced irreversible kindling of KFP-Red in mitochondria within the irradiated field. Irreversibly kindled mitochondria were monitored.



Monitoring of mitochondrial movement using KFP-Red in PC12 cells.
(A) Reversibly and irreversibly kindled mitochondria. Irradiation with weak blue laser light caused instantaneous quenching of reversibly kindled mitochondria, while the irreversibly kindled mitochondria (compare A and B) remained fluorescent; (B-E) Irreversibly kindled mitochondria tracking using a 1% power green laser.



Monitoring of cell migration during *Xenopus* neural plate development using KFP-Red.
(A) At the early neurula stage, a round-shaped group of cells within the neural plate was irreversibly "kindled"; (B) longitudinal extension of the labeled group of cells after two hours after kindling; (C) thin stripe of the labeled cells at the end of neurulation.
Experimental data were presented by Dr. A. Zaraisky (Institute of Bioorganic Chemistry RAS, Moscow, Russia).

See additional examples of KFP-Red use at www.olympusconfocal.com

Recommended filter sets and laser lines

KFP-Red is non-fluorescent before light activation. Upon green-light irradiation, the protein kindles to its red fluorescent form. Green light of low intensity (e.g. 1% power scanning green laser, HeNe laser line 543 nm, 1 mW, scan per 10 seconds; the number of scans is not limited) does not cause kindling and may be used as excitation light for KFP-Red visualization.

Scanning with about 5-10% power laser results in reversible kindling of KFP-Red. More intensive-light irradiation is required for irreversible KFP-Red kindling (e.g. irradiation for 20 seconds in fast mode with a 30% power green laser light induces irreversible kindling of KFP-Red in mitochondria within the irradiated field). Irradiation with weak blue laser light causes instantaneous quenching of reversibly kindled KFP-Red, whereas for the irreversibly kindled KFP-Red, quenching is not so pronounced.

TRITC filter set or similar can be used for visualization of activated KFP-Red. Omega Optical filter sets QMAX-Red and XF174 are recommended.

Kindling effect depends on temperature. Light intensity required for kindling goes down when the temperature decreases and goes up when the temperature rises. This property can be used to achieve kindling at lower light intensities by sample cooling.

Available variants and fusions

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

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

Chudakov, D.M., V.V. Belousov, et al. (2003). Nat Biotechnol, 21 (2): 191–194 / pmid: 12524551

Chudakov, D.M., A.V. Feofanov, et al. (2003). J Biol Chem, 278 (9): 7215–7219 / pmid: 12496281

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

Lukyanov, K.A. et al. (2000). J Biol Chem, 275 (34): 25879–25882 / pmid: 10852900

KFP-Red-related products

Product	Cat.#	Description	Size
KFP-Red expression/source vectors			
pKindling-Red-N	FP301	Mammalian expression vector encoding humanized KFP-Red and allowing its expression and generation of fusions to the KFP-Red N-terminus	20 µg
pKindling-Red-B	FP302	Bacterial expression vector; source of the KFP-Red coding sequence	20 µg
Recombinant protein			
rKFP-Red	FP351	Purified recombinant kindling red fluorescent protein	100 µg

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