

Kindling red fluorescent protein KFP-Red

- Photoactivatable red fluorescent protein
- Kindled by green laser that does not damage cells and tissues
- Can be kindled reversibly or irreversibly
- Recommended for tracking cell and cellular organelle movement

Description

KFP-Red (KFP1 in our scientific publications) is a photoactivatable GFP-like protein (Chudakov *et al.*, 2003a,b). It was generated by site-directed and random mutagenesis of *Anemonia sulcata* chromoprotein, asFP595 (Lukyanov *et al.*, 2000).

KFP-Red switches from a non-fluorescent to a red fluorescent form (with excitation maximum at 580 nm and emission maximum at 600 nm) 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		
Molecular weight	26 kDa	
Polypeptide length	232 aa	
Structure	tetramer	
Activating light	green (530-560 nm)	
Contrast, fold	35-70	
Aggregation	no	
Maturation rate at 37°C	medium	
	before photoconversion	after photoconversion
Excitation max	580 nm	580 nm
Emission max	600 nm	600 nm
Quantum yield	<0.001	0.07
Extinction coefficient, M ⁻¹ cm ⁻¹	123 000	59 000
Brightness	<0.1	4.1

*Brightness is a product of extinction coefficient and quantum yield, divided by 1000.

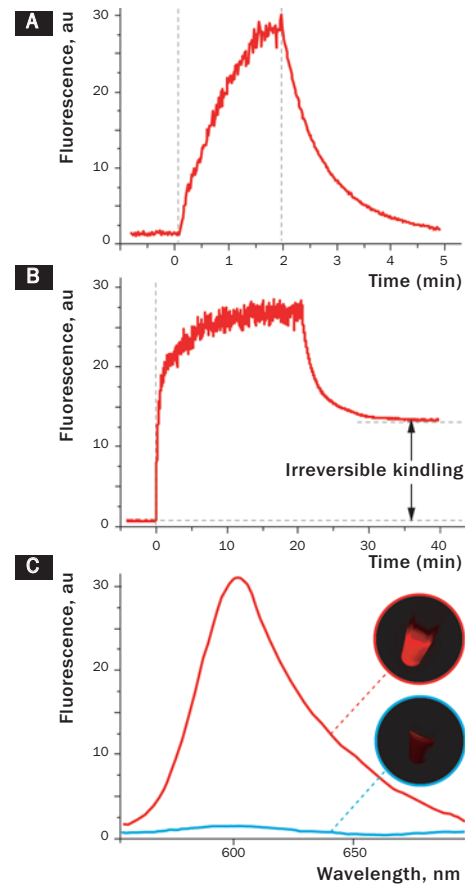
Performance and use

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

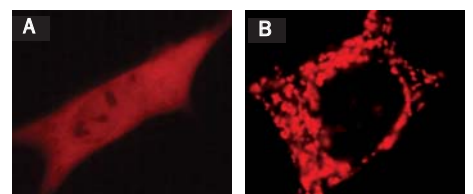
Depending on the kindling light intensity KFP-Red can be kindled reversibly or irreversibly allowing the monitoring of both short- and long-term cell processes.

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-



KFP-Red properties.

A — KFP-Red reversible 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 — KFP-Red 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. C — Irreversibly kindled (red line) and "unkindled" (blue line) KFP-Red fluorescence spectra and brightness ratio. The photo shows intact and irreversibly kindled KFP-Red samples after a year of incubation at room temperature. Spectra of irreversibly kindled KFP-Red in Excel format can be downloaded from the Evrogen Web site at www.evrogen.com/KFP-Red.shtml.



KFP-Red use for cell (A) and mitochondria labeling (B).

Microscopic images were obtained after reversible kindling of KFP-Red in HeLa cells.

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.

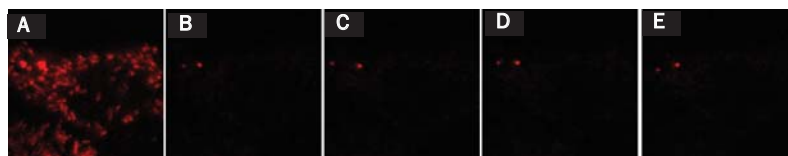
Application of KFP-Red to track cell migration was demonstrated using embryonic fate mapping as an example. *Xenopus* embryos were taken at the stage of two blastomeres and KFP-Red mRNA was microinjected into the animal poles of both blastomeres. At the early neurula stage, a round-shaped group of cells 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.

To show KFP-Red usability for tracking movement of cell organelles, PC12 cells were transfected with a mitochondria-targeted KFP-Red expressing vector. After 25 hrs 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 sec; 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 min. Brief irradiation (about 20 sec 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.

KFP-Red diffusion within an eukaryotic cell was also demonstrated. HeLa cells were transfected with pKindling-Red-N vector. After 24 hours of incubation they were observed in the Zeiss LSM 510 confocal microscope. Almost no fluorescence could be observed upon irradiation by 1% power of 1mW 543 nm laser. The upper part of the cell was shortly (about 5-10 seconds) irradiated by 100% power 543 nm laser, causing kindling of the KFP-Red. Starting immediately after kindling, diffusion of the activated protein was observed using 1% power laser as an excitation light source.

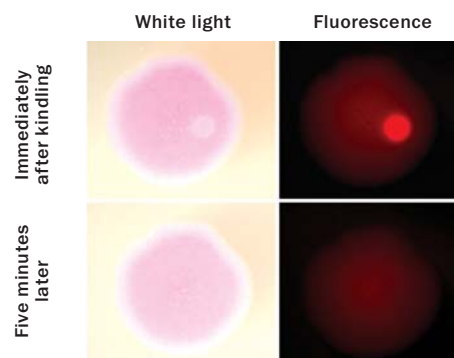
Available variants and fusions

KFP-Red codon usage is optimized for high expression in mammalian cells (Haas et al., 1996), but it can be successfully expressed in many other heterologous systems.



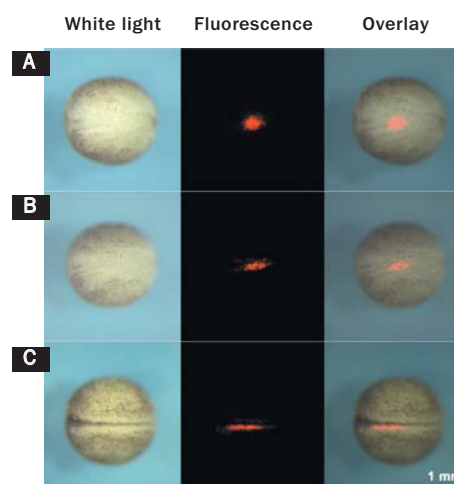
Monitoring of mitochondria movement using KFP-Red in PC12 cells.

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.



Reversible photoactivation of KFP-Red in *E. coli*.

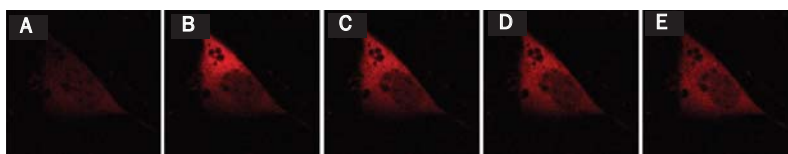
The round-shaped part of the *E. coli* colony expressing KFP-Red was irreversibly kindled with intense green light. This region fluoresces brightly, while its absorption is low. After several minutes, the kindled protein relaxed to the non-fluorescent state, while its absorption recovered.



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. Zairisky, Institute of Bioorganic Chemistry, RAS (Moscow, Russia).



KFP-Red diffusion within eukaryotic cell.

A — Non-activated KFP-Red in a mammalian cell; B — KFP-Red was irreversibly kindled in a cell part; C-E — tracking KFP-Red diffusion within the cell using a 1% power green laser.

KFP-Red-mito fusion: A mitochondrial targeting sequence (MTS) is linked to the KFP-Red N-terminus. MTS was derived from the subunit VIII of human cytochrome C oxidase (Rizzuto *et al.*, 1989; Rizzuto *et al.*, 1995). When expressed in mammalian cells, this variant provides red fluorescent labeling of mitochondria (after photoactivation).

Recommended filter sets, and visualization parameters

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.

References

- Chudakov *et al.* (2003 a) *Nature Biotechnology*, 2003, 21(2):191-194.
 Chudakov *et al.* (2003 b) *Journal of Biological Chemistry* 278(9): 7215-7219.
 Haas *et al.* (1996) *Curr. Biol.* 6: 315–324.
 Lukyanov *et al.* (2000) *Journal of Biological Chemistry* 275(34):25879-25882.
 Rizzuto *et al.* (1989) *J. Biol. Chem.* 264: 10595–10600.
 Rizzuto *et al.* (1995) *Curr. Biol.* 5: 635–642.

KFP-Red-related products

Product	Cat.#	Description	Size
KFP-Red expression/source vectors			
pKindling-Red-N	FP301	Mammalian expression vector encoding humanized KFP-Red for its expression in mammalian cells	20 µg
pKindling-Red-B	FP302	Bacterial expression vector; source of humanized KFP-Red coding sequence	20 µg
pKindling-Red-mito	FP401	Mammalian expression vector encoding humanized KFP-Red targeted to mitochondria	20 µg
Recombinant protein			
rKFP-Red	FP351	Purified recombinant KFP-Red	100 µg

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

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