Recombinant kindling red fluorescent protein rKFP-Red

Cat.# FP351

Recombinant KFP-Red (rKFP-Red) is 26-kDA photoactivatable colored non-fluorescent protein. It has spectral properties identical to those of the expressed KFP-Red. rKFP-Red can be kindled by green light. Irreversibly kindled purified rKFP-Red retains red fluorescence for many months and can be used as a standard on protein gels and Western blots; control for fluorescence microscopy and for calibration of fluorometeres and FACS machines. Moreover, rKFP-Red may be microinjected into cells and tissues of interest, kindled, and used as a marker of these particular objects.

rKFP-Red is purified from transformed *E. coli* using acetone precipitation. Purity of the rKFP-Red determined on PAGE is about 95%.

Storage: Before photoactivation, rKFP-Red should be stored at +4°C in the dark place.

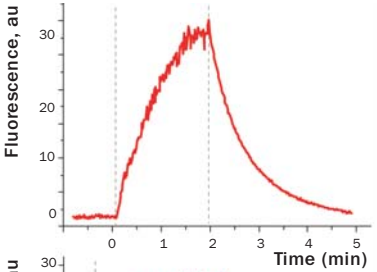
Recommendations for use

rKFP-Red becomes bright red fluorescent (kindles) in response to intense green light irradiation (530-570 nm). Kindled KFP-Red have excitation maximum at 575 nm and emission maximum at 600 nm. Depending on kindling light intensity KFP-Red can be kindled reversibly or irreversibly.

Kindling and excitation light intensities: Reversibility of kindling is controlled both by the light intensity level and total light dose. For example, irradiation of KFP-Red expressing *E. coli* colony in

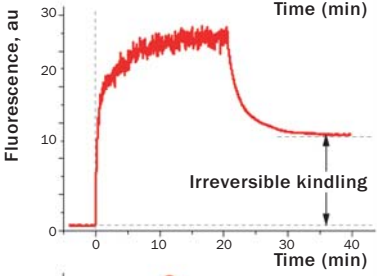
KFP-Red reversible kindling and relaxation kinetics.

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.



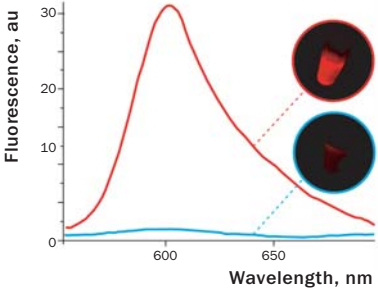
KFP-Red irreversible kindling.

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.



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.

fluorescent microscope through 5x objective (TRITC filter set, 100W lamp) causes very slow reversible kindling, which reaches its maximum in about 10 min. At this light intensity no irreversible kindling is observed independently of irradiation time. Under irradiation through 20x objective KFP-Red reaches its maximum brightness in about 4-5 sec, though this time is not enough to cause noticeable irreversible kindling. After 1 min of *E. coli* colony irradiation through 20x objective bright irreversible kindling is observed. Irradiation through 40x objective allows to achieve bright irreversible kindling in about 10 sec.

Low intensity green light causes no or very slow reversible kindling of KFP-Red and can therefore be used as excitation light to visualize both reversibly and irreversibly kindled KFP-Red without inducing background signal growth. This allows kindled objects to be tracked for a long time without loss of image contrast.

Importantly, kindling effect depends on temperature. Light intensity required for kindling comes down with the temperature decrease and grows when the temperature rises. This KFP property should be taken into account, when working at 37°C.

Kindling, quenching, and excitation light wavelengths: For reversible and irreversible kindling please use 530-570 nm light. Light wavelength between 500 nm and 520 nm will cause low kindling. Shorter wavelength (400-490 nm) will cause quenching. Excitation maximum for kindled KFP-Red is at 575 nm, emission at 600 nm. The brightest fluorescence is observed using these parameters. However, you can successfully use light between 540 nm and 600 nm as excitation light and observe emission between 580 nm and 630 nm.

rKFP-Red as standard in protein gel

When used as a standard on protein gel, irreversably kindled rKFP-Red can be used to correlate KFP-Red expression levels to

fluorescence intensity or to differentiate problems with detection of KFP-Red fluorescence from expression of KFP-Red protein.

When denatured by heating (2-3 min, 95-98°C), rKFP-Red is detected as a set of three bands on Coomassie stained SDS gel. The 28-kDa band corresponds to the full-length protein. The 8- and 20-kDa bands are the N- and C-terminal fragments of the protein, respectively (Martynov *et al.*, 2001). When loaded without pre-heating, besides three bands mentioned above, an additional major band at 90-100-kDa should be seen (native tetrameric form of rKFP-Red, strongly magenta colored under daylight before Coomassie staining).

If rKFP-Red is to be used as an internal standard in a Coomassie-stained minigel, we recommend loading 0.5-1.0 µg of rKFP-Red per lane. If rKFP-Red is added to a total cell/tissue lysate or other crude sample, the amount of total protein loaded per lane must be optimized for the particular application. Pre-denatured rKFP-Red will not fluoresce on an SDS gel, whereas non-denatured one will fluoresce on an SDS gel.

References: Martynov *et al.* (2001) J. Biol. Chem. 276(24): 21012-21016.