pmIrisFP, FLAG®-tagged, lyophilized DNA

Product Information Sheet
 # VS-FLP10050

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SUMMARY

shipped at room temperature; store at -20 °C

For research use only

Product: pmIrisFP with monomeric IrisFP and FLAG®-tag.

Introduction

pmIrisFP is a photoactivatable fluorescent protein that combines irreversible photo-conversion from a green- to a red-emitting form with reversible photoswitching between a fluorescent and a non-fluorescent state in both forms. Suitable laser lines for photoswitching are indicated in Fig. 1. pmIrisFP is a monomeric variant of IrisFP with low tendency of dimer formation. Its multiple photoactivation modes can be used for pulse-chase experiments combined with subdiffraction-resolution imaging in living cells by using dual-color photo-activation localization microscopy (PALM)[1].

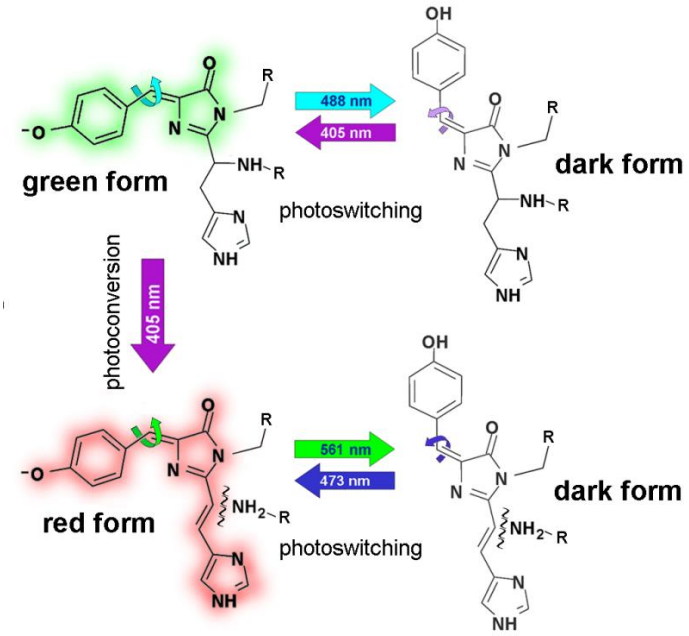

Fig. 1 Schematic view of the light-driven transformations of the mIrisFP chromophore. Suitable wavelengths for photoconversion/ photoswitching are given in the arrows.

Advantages

- Low tendency for dimer formation.
- Expected localization of fusion proteins shown for various proteins.
- Suitable for pulse-chase experiments, measurements of k_{on}- and k_{off} rates and high-resolution microscopy (PALM).

Source

pmIrisFP is an advanced variant of the green to red photoconvertible protein EosFP[2] that was isolated from the stony coral *Lobophyllia hemprichii*. pmIrisFP differs from monomeric pmEosFP by four additional mutations[3], A69V, F173S, K145I and Y189A.

Table 1: pmIrisFP is compared with mEos2

Fluorescent Protein	pmIrisFP		mEos2[4]	
Form	**green**	**red**	**green**	**red**
Excitation / Emission (nm)	486 / 516	546 /578	506 / 519	573 / 584
Extinction coefficient (M^{-1} cm^{-1})	47,000	33,000	56,000	46,000
Quantum yield	0.54	0.59	0.84	0.6
Brightness x 1000	25.5	19.3	47.0	30.4
Photoactivation mode	reversible / irreversible		irreversible	
Switching half-time off / on (s)	8.8 / 6.4[a]	16.0 / 4.9[b]	–	–
Relaxation half-time (min)	53	24	–	–
Photoswitching mode	negative	negative	–	–
Detected photons per burst	176	354	–	360

[a] off-switching: 473 nm light, 30 mW cm^{-2}, on-switching: 405 nm light: 4.5 mW cm^{-2}
[b] off-switching: 561 nm light, 6 mW cm^{-2}, on-switching: 473 nm light: 0.5 mW cm^{-2}

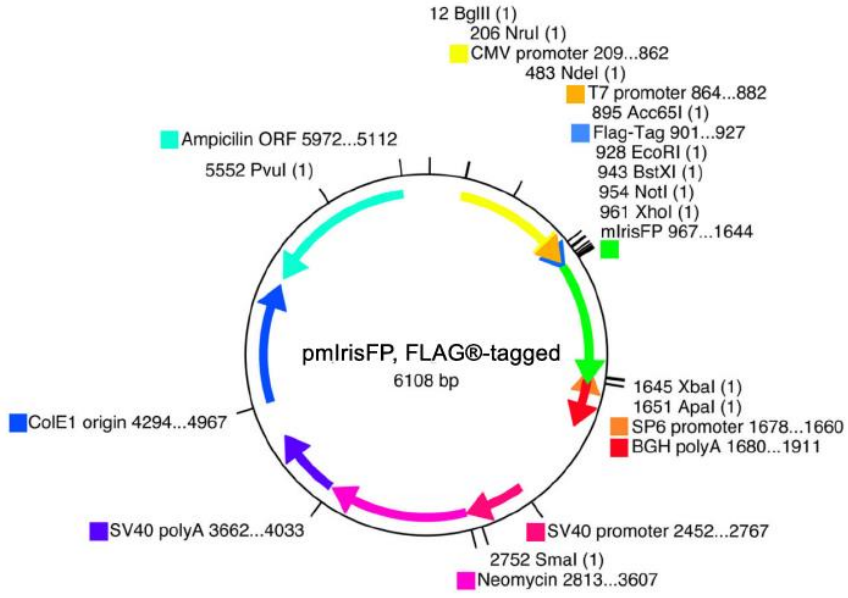

Fig. 2: Vector map of FLAG®-tagged pmIrisFP, FLAG®-tagged

Optical parameters

pmIrisFPs green fluorescent form has a peak extinction coefficient of 47,000 M^{-1} cm^{-1} at 486 nm and emits fluorescence with a high quantum yield of 0.54 peaking at 516 nm.
pmIrisFPs red fluorescent form has a peak extinction coefficient of 33,000 M^{-1} cm^{-1} at 546 nm and emits fluorescence with a high quantum yield of 0.59 peaking at 578 nm (Fig. 3).
The chromophores of both the green and red forms are in the cis conformation. Excitation of either fluorescent species induces chromophore isomerization to the non-fluorescent trans conformation. The green trans chromophore absorbs maximally at 386 nm with peak extinction coefficient of 12,000 M^{-1} cm^{-1}, the red trans chromophore absorbs maximally at 446 nm with peak extinction coefficient of 21,000 M^{-1} cm^{-1}, and isomerizes spontaneously to the fluorescent cis form.

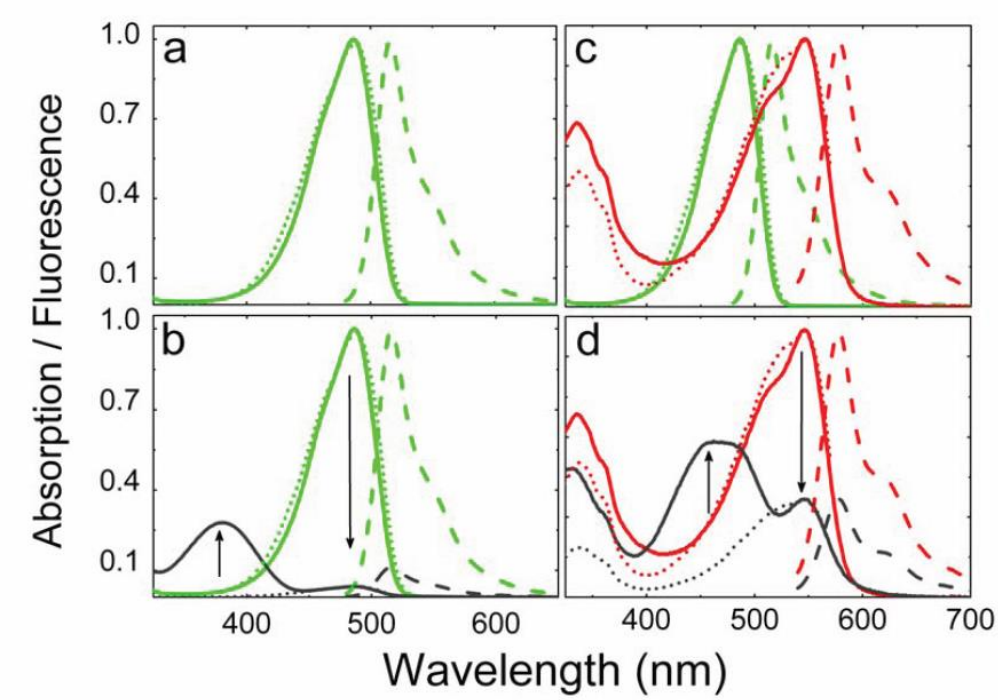

Fig. 3: Absorption, excitation and emission spectra, depicted by solid, dotted and dashed lines, respectively, were scaled to equal peak amplitudes. Emission spectra of green (red) pmIrisFP were taken with excitation at 473 (532) nm; excitation spectra of green (red) IrisFP were measured via the emission at 540 (580) nm. (**a**) Green pmIrisFP and (**b**) green pmIrisFP before (green lines) and after (black lines) illumination with 473-nm light. (**c**) Red pmIrisFP (red lines) after photoconversion of green pmIrisFP (green lines) with 405 nm light, and (**d**) red pmIrisFP before (red lines) and after (black lines) illumination with 532 nm light (corrected for background). All spectra were recorded in sodium phosphate buffer, pH 10.

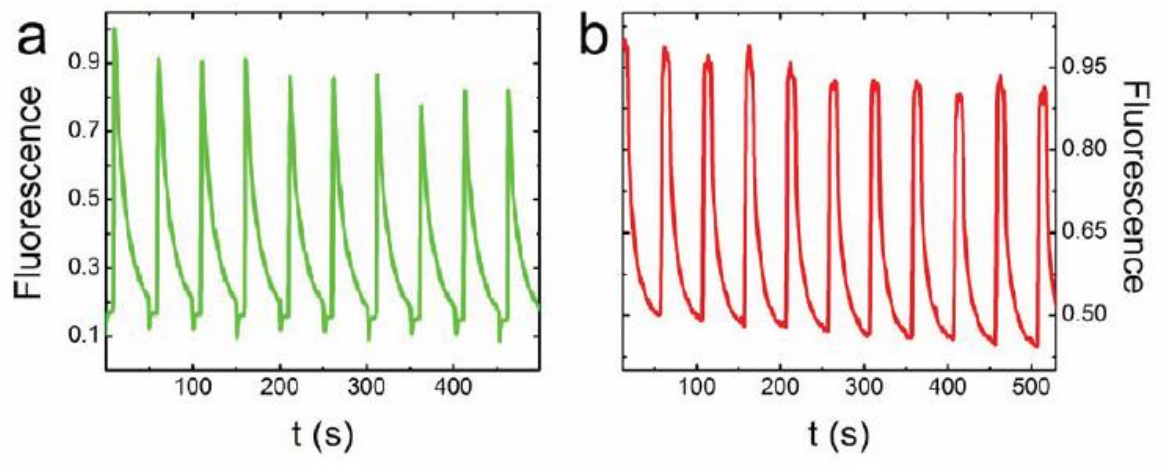

Fig. 4: Alternating off- and on-switching of (**a**) green pmIrisFP with 473 (30 mW cm^{-2}) and 405 nm (5 mW cm^{-2}) light, respectively, and (**b**) red pmIrisFP with 561 (60 mW cm^{-2}) and 473 nm (5 mW cm^{-2}) light, respectively.

Maturation

in vitro: pmIrisFP matures with a half-life of 14 min at 37 °C, faster than many engineered fluorescent proteins.

in vivo: in HEK293 cells, pmIrisFP green fluorescence appears 15 h after transfection, similar to EGFP (13 h).

References

Betzig *et al*. (2006) Imaging Intracellular Fluorescent Proteins at Nanometer Resolution. *Science* 313, 1642 – 1645.

Wiedenmann, J., Ivanchenko, S., Oswald, F., Schmitt, F., Röcker, C., Salih, A., Spindler, K.D., & Nienhaus, G.U. (2004). EosFP, a fluorescent marker protein with UV-inducible green-to-red fluorescence conversion. *Proc. Natl. Acad. Sci*. U.S.A. 101, 15905-15910.

Fuchs, J., Boehme, S., Oswald, F., Hedde, P.N., Krause, M, Wiedenmann, J., and Nienhaus, G.U. (2010). Imaging Protein Movements in Live Cells with Super-resolution Using mIrisFP. *Nature Methods* 7, 627 – 630.

McKinney, S.A., Murphy, C.S., Hazelwood, K.L., Davidson, M.W. & Looger, L.L. (2009). A bright and photostable photoconvertible fluorescent protein. *Nature Methods* 6, 131-133.

Order Information, Shipping and Storage

Order#	Product	Quantity
VS-FLP10050	pmIrisFP, FLAG®-tagged, lyophilized DNA	10 µg
shipped at room temperature; store at -20 °C		

Related Products

Order#	Product	Quantity
VS-FLP10010	pwt-EosFP, + mitochondrial targeting signal, lyophilized DNA	10 µg
VS-FLP10020	pwt-EosFP, FLAG®-tagged, lyophilized DNA	10 µg
VS-FLP10030	ptd-EosFP, FLAG®-tagged, lyophilized DNA	10 µg
VS-FLP10040	pmEosFP(Thermostab), FLAG®-tagged, lyophilized DNA	10 µg
shipped at room temperature; store at -20 °C		