

## SUMMARY

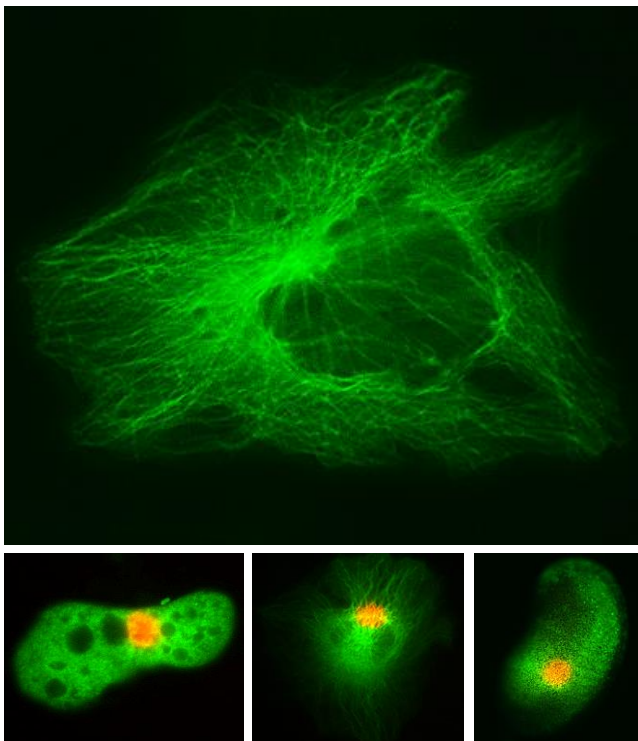
shipped at room temperature; store at -20 °C

For research use only

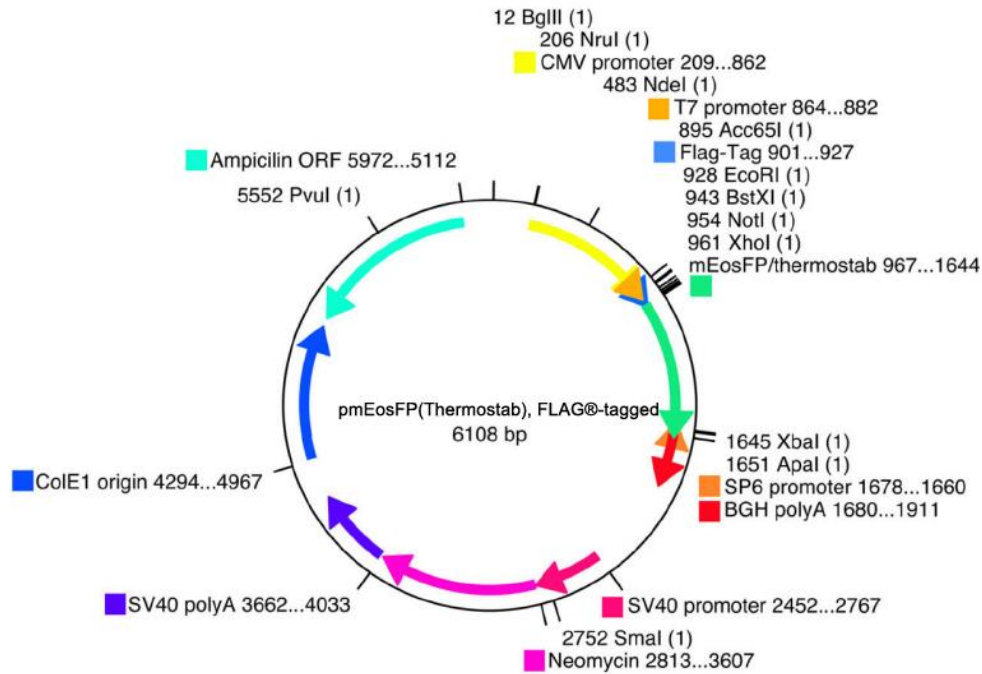
**Product:** pmEosFP(Thermostab) with monomeric EosFP(Thermostab) and FLAG<sup>®</sup>-tag.

## Introduction

pmEosFP(Thermostab) is an advanced variant of the green-to-red photoconvertible fluorescent protein EosFP<sup>1,2</sup>. The marker was optimized for functional expression at 37 °C, especially in fusion with other proteins. pmEosFP(Thermostab) retains the monomeric nature of its predecessor and shows an excellent performance in fusion even with demanding partners such as tubulin (Fig. 1). pmEosFP(Thermostab) can be efficiently converted from green to red by a light pulse at wavelengths between 360 and 430 nm. Nuclear localization of the recombinant signal-binding protein pmEosFP(Thermostab) and tubulin-pmEosFP(Thermostab) is shown in Figure 1 (bottom left and center). Subsequently, the protein of interest can be tracked by the red fluorescence of the photoconverted form of pmEosFP(Thermostab). The marker protein can be also used to study cell movement, e.g. during embryonic development<sup>3</sup> (Fig. 1, bottom right).



**Fig. 1:** pmEosFP(Thermostab) expressing cells. pmEosFP(Thermostab) fused to tubulin (upper image). Conversion from green to red with a light pulse between 360 and 430 nm is shown in bottom pictures. Nuclear localization of RBP-pmEosFP(Thermostab) (bottom left) and tubulin-pmEosFP(Thermostab) (bottom center). pmEosFP(Thermostab) used for cell tracking in a Xenopus embryo (bottom right).



**Fig. 2:** Vector map of pmEosFP(Thermostab), FLAG<sup>®</sup>-tagged

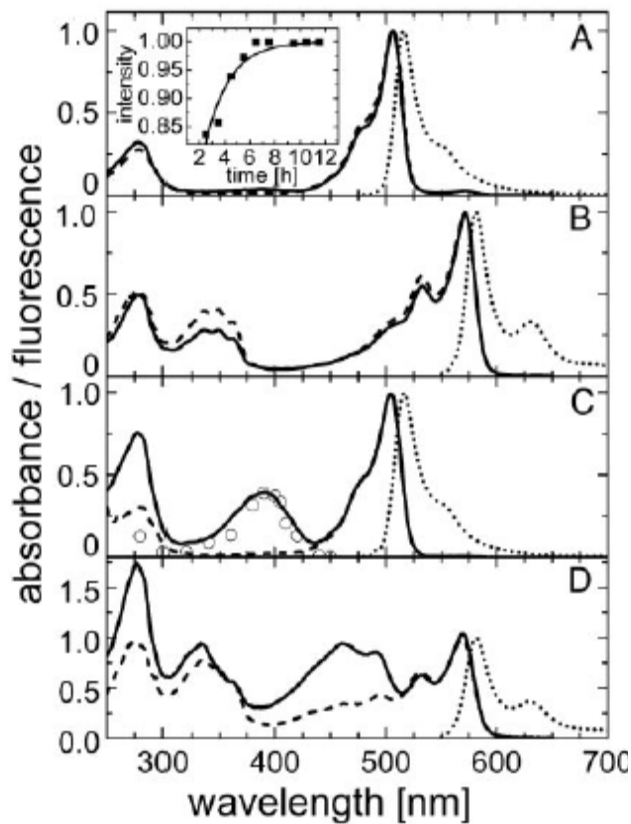
## Optical parameters

pmEosFP(Thermostab) can be efficiently converted from green to red by a light pulse at wavelengths between 360 and 430 nm.

	before photoconversion	after photoconversion
Excitation	506 nm	571 nm
Emission	516 nm	581 nm
Extinction coefficient	72'000 M <sup>-1</sup> cm <sup>-1</sup>	41'000 M <sup>-1</sup> cm <sup>-1</sup>
Fluorescence Quantum Yield	0.70	0.55

## Turnover of the red fluorescent state

Both the green and the red form of EosFP(Thermostab) are highly stable at cytosolic pH values. A half-life of ~3 weeks was determined for the red form of wild type EosFP in coral cells. In developing embryos of *Xenopus laevis*, the photoconverted stage could be tracked up to 14 days. In dividing cell cultures (HEK293), the red fluorescence could be traced by flow cytometry for up to 9 days.



**Fig. 3.:** Spectra of the green and red states of pmEosFP(Thermostab) at pH 7 and pH 5.5. Solid lines, absorbance; dashed lines, excitation; dotted lines, emission spectra. (A and C) Green species at pH 7 (A) and pH 5.5 (C). Excitation (emission) spectra were measured with emission (excitation) set to 520 (490) nm. ○, conversion yields scaled to the absorbance. (Inset) In vitro chromophore maturation at 27 °C determined from the absorbance at 506 nm (solid line, exponential fit). (B and D) Red species at pH 7 (B) and pH 5.5 (D). Excitation (emission) spectra were measured with emission (excitation) set to 590 (560) nm.

## Detection

The green and the red fluorescent state of pmEosFP(Thermostab) can be detected with standard filter sets (FITC/GFP filters for the green state or TRITC/DsRed for the red state). Fluorescence of the red state can be detected instantaneously after photoconversion. Green fluorescence can be monitored starting between 6.5 and 12 h after transfection/microinjection of vector/mRNA. Microinjection of purified pmEosFP(Thermostab) allows immediate cell labeling by photoconversion.

## Photoconversion

Photoconversion can be achieved by irradiation with light of wavelengths between 350 and 440 nm with a maximal efficiency at ~390 nm. Therefore, standard DAPI filter sets can be used for photoconversion as well as customized filters with maximal transmission at 400 - 440 nm and appropriate lasers, e.g. a 405 nm laser diode.

Photoconversion can usually be achieved within a few seconds, depending on the energy output of the light source. However, an increase of the energy beyond a limit set by the maximal conversion rate of pmEosFP(Thermostab) might result in an unwanted bleaching of the red fluorescent state. In such cases, prolonged irradiation with lower light levels should be applied. At present, no negative effects of the photoconversion on expressing cells were reported.

# pmEosFP(Thermostab), FLAG<sup>®</sup>-tagged, lyophilized DNA

Product Information Sheet  
# VS-FLP10040



## References

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 mlrisFP. *Nature Methods* 7, 627–630.

Wacker, S., Oswald, F., Wiedenmann, J., & Knöchel, W. (2006). A green to red photoconvertible protein as analyzing tool for early vertebrate development. *Dev. Dyn.*, 236, 473-480.

## Order Information, Shipping and Storage

Order#	Product	Quantity
VS-FLP10040	pmEosFP(Thermostab), FLAG <sup>®</sup> -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 <sup>®</sup> -tagged, lyophilized DNA	10 µg
VS-FLP10030	ptd-EosFP, FLAG <sup>®</sup> -tagged, lyophilized DNA	10 µg
VS-FLP10050	pmlrisFP, FLAG <sup>®</sup> -tagged, lyophilized DNA	10 µg
shipped at room temperature; store at -20 °C		

FLAG<sup>®</sup> is a registered trademark of Sigma-Aldrich Co

## Contact and Support

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