ptd-EosFP, FLAG®-tagged, lyophilized DNA

Product Information Sheet
 # VS-FLP10020

SUMMARY

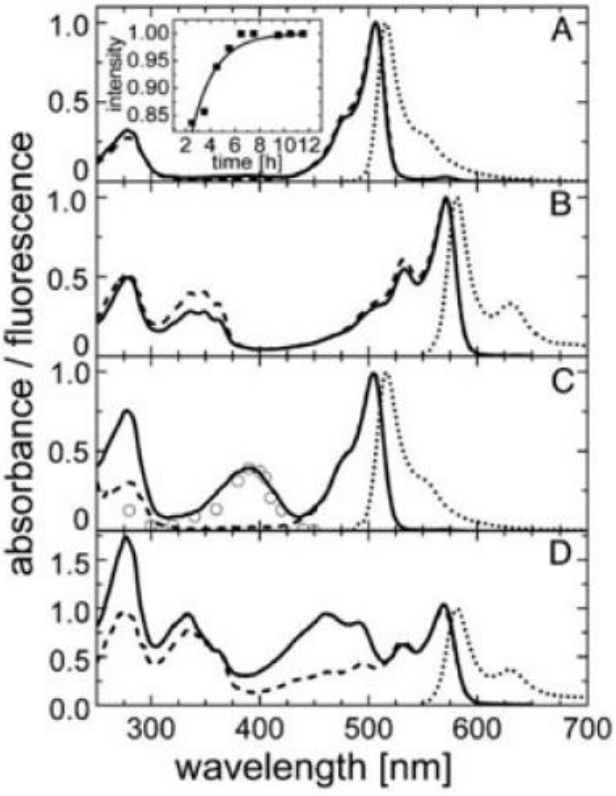
shipped at room temperature; store at -20 °C

For research use only

Product: ptd-EosFP with tandem dimer (functional monomer) EosFP and FLAG®-Tag.

Introduction

EosFP was isolated from the stony coral *Lobophyllia hemprichii* (Wiedenmann *et al*. 2004). Initially, the protein matures in a green fluorescent state with an emission maximum at 516 nm. Upon irradiation with violet-blue light the chromophore undergoes an irreversible photoconversion to a red state emitting at 581 nm (Nienhaus *et al*. 2005a). The wavelengths required for photoconversion and detection of the green and red fluorescent states can be easily separated, making EosFP an excellent choice for regional optical marking.



Spectra of the green and red states of EosFP 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.

o: 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.

MoBiTec GmbH, Germany Phone: +49 551 70722 0 Fax: +49 551 70722 22 E-Mail: info@mobitec.com www.mobitec.com

Fluorescence properties

Excitation

before photoconversion	506 nm
after photoconversion	569 nm

Emission

before photoconversion	516 nm
after photoconversion	582 nm

Extinction coefficient

before photoconversion	84'000 $M^{-1}cm^{-1}$
after photoconversion	33'000 $M^{-1}cm^{-1}$

Fluorescence Quantum Yield

before photoconversion	0.66
after photoconversion	0.60

Oligomerization tandem dimer, functional monomer

Detection

The green and the red fluorescent state of EosFP 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 EosFP 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 EosFP 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.

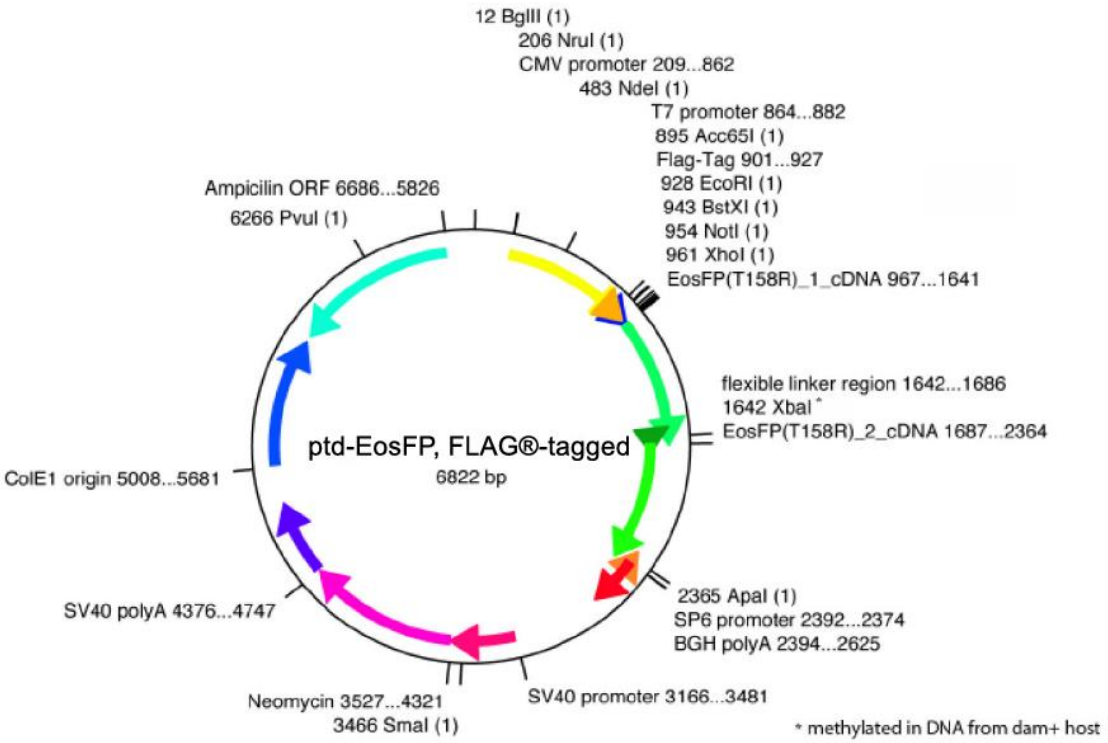
Turnover of the red fluorescent state

Both the green and the red form of EosFP are highly stable at cytosolic pH values. A half-life of ~3 weeks was determined for the red form of wildtype 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 be flow cytometry for up to 9 days.

Cell labeling vs. fusion proteins: choice of EosFP variants

Two variants of EosFP are available from MoBiTec: The tetrameric wildtype protein (EosFP) (Wiedenmann *et al*. 2004) and a pseudomonomeric variant in which two copies of an engineered EosFP variant are fused to form a tandem dimer (ptd-EosFP) (Nienhaus, G.U. *et al*. 2006). Both variants express functionally in a wide range of pro- and eukaryotic cells at a temperatures of 37 °C or below. For the labeling of cells or tissues, tetrameric EosFP is the construct of choice. For labeling of subcellular compartements using short oligopeptide signals attached to the marker, both EosFP and ptd-EosFP can be considered. Although some fusion proteins with tetrameric EosFP are possible, the pseudomonomeric variant ptd-EosFP is the recommended construct for protein labeling. Fusions to the N-terminus of ptd-EosFP usually work well. Fusions to the C-terminus are also possible, however, some fusion might fail with proteins requiring a strictly monomeric marker, for instance tubulin.

ptd-EosFP with tandem dimer (functional monomer) EosFP and FLAG®-Tag.



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Order Information, Shipping and Storage

Order#	Product	Quantity
VS-FLP10030	ptd-EosFP, FLAG®-tagged, lyophilized DNA	10 µg
shipped at room temperature; store at -20 °C		

Contact and Support

MoBiTec GmbH ● Lotzestrasse 22a ● D-37083 Goettingen ● Germany

Customer Service – General inquiries & orders
phone: +49 (0)551 707 22 0
fax: +49 (0)551 707 22 22
e-mail: order@mobitec.com

Technical Service – Product information
phone: +49 (0)551 707 22 70
fax: +49 (0)551 707 22 77
e-mail: info@mobitec.com

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