ptd-EosFP-CD8, lyophilized DNA

Product Information Sheet
VS-FLPC1012

SUMMARY

shipped at RT; store at 4 ° C

For research use only

Product

“ptd-EosFP-CD8” is a mammalian expression vector encoding for a fluorescent protein, the green-to-red photoconvertible EosFP tandem dimer (functional monomer), fused to the N-terminus of human CD8 α-chain.

CD8 is a cell surface glycoprotein of cytotoxic T (T_C) cells that kill antigen-bearing target cells. The fusion protein td-EosFP-CD8 can be used as a localization marker for detecting CD8 proteins on living T_C cells by fluorescence microscopy. Thus it is well suitable for counting and cluster analysis of Tc cells. Based on its ability to be photoconverted, td-EosFP-CD8 is applicable to high resolution fluorescence microscopy e.g. PALM (Betzig et al., 2006). The photoconversion also enables regional optical marking of CD8 proteins.

Introduction

EosFP was isolated from the stony coral *Lobophyllia hemprichii* (Wiedenmann et al., 2004). The td-EosFP is a pseudomonomeric variant of EosFP in which two copies of an engineered EosFP variant are fused to form a tandem dimer that is expressed functionally in a wide range of pro- and eukaryotic cells at temperatures of 37 °C or below (Nienhaus et al., 2006).
EosFP 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., 2005). The wavelengths required for photoconversion and detection of the green and red fluorescent states can be easily separated. The fusion protein td-EosFP-CD8 is an excellent choice for localization studies of CD8 hence counting and cluster analysis of T_C cells in live cell imaging. Its photoconversion also allows for regional cellular marking of CD8 proteins.

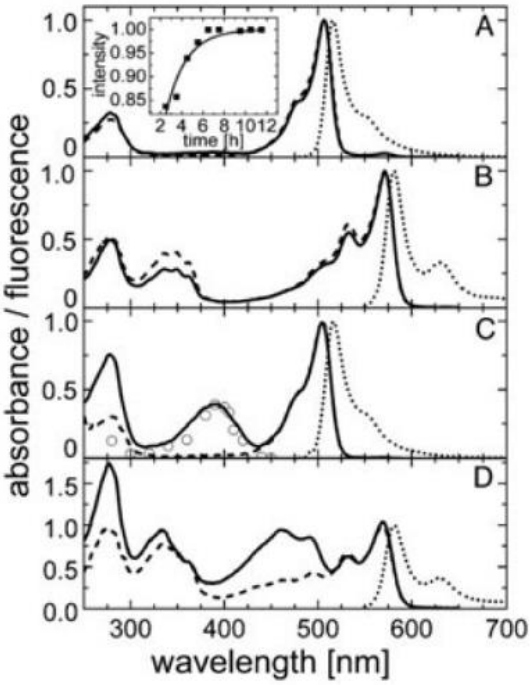
Detection

The green and the red fluorescent state of td-EosFP-CD8 can be detected with standard filter sets (FITC / GFP filters for the green state or TRITC / DsRed for the red state). This fusion protein is suitable for high resolution microscopy like PALM. 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.

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.



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 spectra were measured with emission set to 520 nm. Emission spectra were measured with excitation set to 490 nm. **open circles**: conversion yields scaled to the absorbance.

Inset in A: *in vitro* chromophore maturation at 27 °C indicated by the absorbance change at 506 nm (solid line: exponential fit).

B and D: Red species at pH 7 (**B**) and pH 5.5 (**D**). Excitation spectra were measured with emission set to 590 nm, emission spectra were measured with excitation set to 560 nm. (Wiedenmann et al., 2004)

Fluorescence Properties

	before photoconversion	after photoconversion
Excitation	506 nm	569 nm
Emission	516 nm	582 nm
Extinction coefficient	84'000 $M^{-1}cm^{-1}$	33'000 $M^{-1}cm^{-1}$
Fluorescence Quantum Yield	0.66	0.60

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 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 be flow cytometry for up to 9 days (Leutenegger et al., 2007).

Expression in Mammalian Cells

The vector "ptd-EosFP-CD8" can be transfected into mammalian cells by any known transfection method. We recommend chemical transfection using *Trans*IT®-2020 transfection reagent (Mirus, transfection protocol see below) or transfection by electroporation with INGENIO™ Electroporation Kits (Mirus). All products of Mirus are distributed by MoBiTec in Germany, Austria and Hungary (for other countries please inquire). The td-EosFP-CD8 protein will be expressed constitutively under the strong CMV promoter. The neomycin resistance gene enables selecting for stably transfected eukaryotic cells using G418.

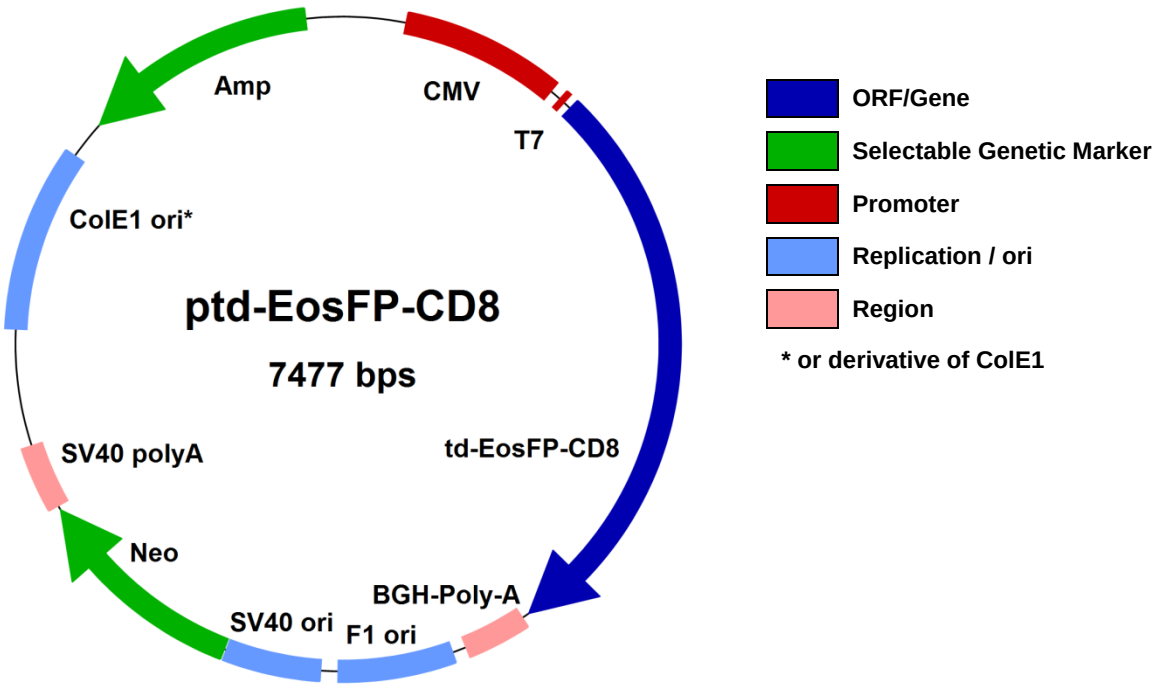
Chemical Transfection Protocol

- About 5 x 10^5 NIH 3T3 fibroblast cells are plated the day before transfection in a 60 mm dish in 5 ml complete growth medium (DMEM low glucose + 10 % Fetal Bovine Serum, growth conditions: 37 °C, 5 % CO_2). Cells should be 40 – 80 % confluent prior to transfection.
- 100 µl serum free medium (DMEM) is mixed briefly with 6 µl *Trans*IT®-2020 Reagent (Mirus) and 1.5 µg vector DNA, and incubated for 20 min at room temperature.
- The culture medium in the dish is exchanged for about 3.5 ml serum free medium and transfection mixture is dropwise added to the cells.
- 6 - 12 h later, the medium is exchanged for complete growth medium and cells are analyzed 24 - 48 h after transfection.

Long-Term Preparation

- Prior to adding transfection mixture, 3 - 4 coverslips are placed within the 60 mm dish. Transfection is done as outlined above.
- Prior to fixation, the medium is sucked off and cells are washed 3-times with PBS buffer (Phosphate Buffered Saline, pH 7.4)
- Cells are fixed with paraformaldehyde (4 %)/PBS solution for 10 min followed by washing 4-times with PBS.
- For embedding, microscope slides are prepared by adding 3 drops of mounting reagent to their surface (e.g., MobiGlow from MoBiTec or ProLong Gold® antifade from Life Technologies; for "Total Internal Reflection Fluorescence Microscopy" use Fluoro-Gel from Electron Microscopy Sciences).
- After the last washing step the coverslips are carefully taken out of the PBS buffer (with forceps) and remaining buffer is carefully removed with a clean laboratory wipe.
- Each coverslip is placed face down onto the prepared microscope slide and the sample is cured for 24 h in the dark.
- Following the curing time, the edges of the coverslips are completely sealed with optic adhesive (e.g., from Norland) or with Cytoseal 60 (Electron Microscopy Sciences). Sealing the edges retards oxidation and the sample can be stored at 4 °C.

Vector Map

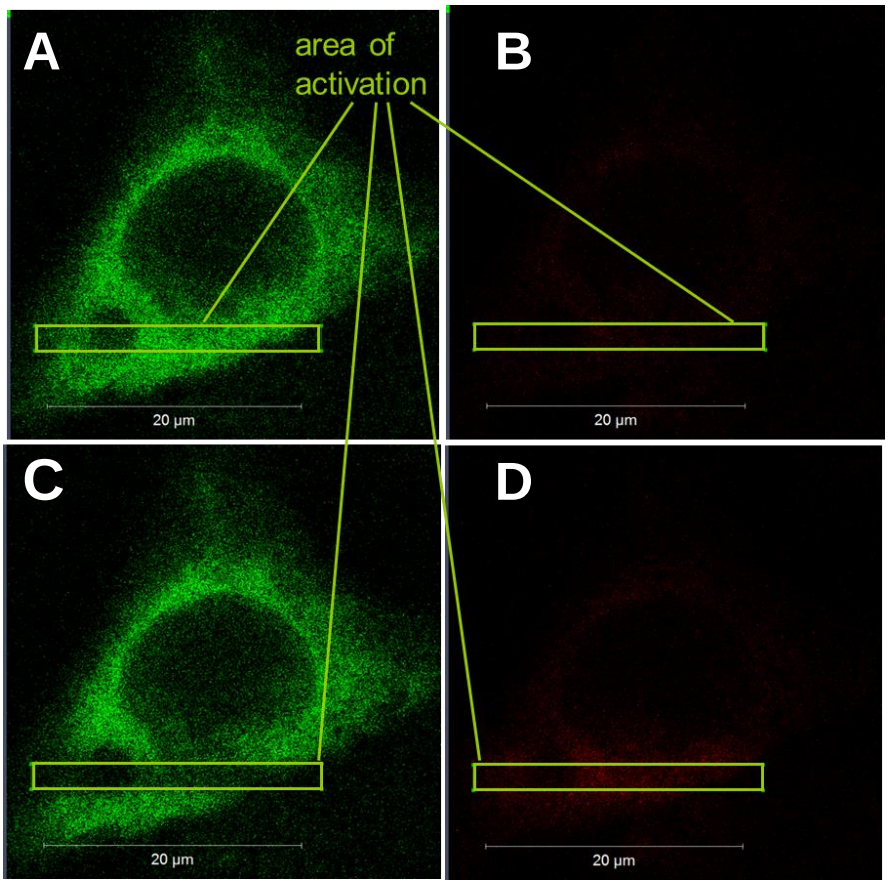

Amp
CMV
T7
ColE1 ori*
ptd-EosFP-CD8
7477 bps
SV40 polyA
td-EosFP-CD8
Neo
BGH-Poly-A
SV40 ori
F1 ori
ORF/Gene
Selectable Genetic Marker
Promoter
Replication / ori
Region
* or derivative of ColE1

Application Note

For localization analysis of CD8, we transfected human epithelial Hep2 cells with the plasmid ptd-EosFP-CD8 and analyzed the cells by fluorescence microscopy.

Results

By fluorescence microscopy (Zeiss, LSM710), we could detect EosFP-CD8 proteins localized on the cell surface. The green emitting form of the EosFP-CD8 was detected using a green filter (493-550 nm; Fig A and C). Upon irradiation with violet-blue light (405 nm) of a chosen area (framed, Fig. D), we activated photoconversion, leading to emission at 583 nm. Without irradiation no emission in the red light range is visible (Fig. B). EosFP-CD8 proteins that were photoconverted did not show emission in the green light range anymore (Fig. C, framed).



Before photoconversion

After photoconversion

Microscopy images of a Hep2 cell expressing the fusion protein td-EosFP-CD8, which is a cell surface glycoprotein of cytotoxic T cells.
EoFP-CD8 is localized on the cell surface of Hep2 cells. (A, B) Before photoconversion the green emitting form of EosFP-CD8 can be detected at 516 nm. (C, D) Upon irradiation with violet-blue light (405 nm) EosFP-CD8 is photoconverted, leading to a change in emission from green to red (516 to 583 nm). (A, C) Microscope image using a green filter, 493-550 nm or (B, D) using a red filter, 583-695 nm.

Methods

Transfection and Sample Preparation
Epithelial Hep2 cells were cultivated, transfected with ptd-EosFP-CD8, and analyzed as described in the chemical transfection protocol (p. 3).

Imaging Conditions

Microscope:	Zeiss LSM710,
Image:	Size 512 x 512 µm, 3 channels, 8 bit, zoom 4-times
Objective:	Plan Apochromat 63 x 1.4, oil, pixel dwell 1.58 µsec, average 1, pinhole 280 µm
Green filter:	493-550 nm, excitation laser: 488 nm, laser intensity 1%, gain 1122
Red Filter:	583-695 nm, excitation laser: 561 nm, laser intensity 1%, gain 1024
Photoconversion:	Excitation laser: 405 nm, 7% laser intensity, 13 iterations

References

Betzig E et al. (2006) Imaging intracellular fluorescent proteins at nanometer resolution; Science 313, 1642-1645

Leutenegger A et al. (2007) It's cheap to be colorful: Anthozoans show a slow turnover of GFP-like proteins; FEBS Journal 274, 2496–2505

Nienhaus GU et al. (2006) Photoconvertible Fluorescent Protein EosFP: Biophysical Properties and Cell Biology Applications; Photochem. Photobiol. 82, 351-358

Nienhaus K et al. (2005) Structural Basis for Photo-Induced Protein Cleavage and Green-to-Red Conversion of Fluorescent Protein EosFP; Proc. Natl. Acad. Sci. USA 102, 9156-9159

Shaner NC et al. (2007) Advances in fluorescent protein technology; J. Cell Sci. 120, 4247-4260.

Wacker S et al. (2006) A green to red photoconvertible protein as analyzing tool for early vertebrate development; Dev. Dyn. 236, 473-480

Wiedenmann J et al. (2004) EosFP, a fluorescent marker protein with UV-inducible green-to-red fluorescence conversion; Proc. Natl. Acad. Sci. USA 101, 15905-15910

Wiedenmann J & Nienhaus GU (2006) Live-cell imaging with EosFP and other Photoactivatable Marker Proteins of the GFP Family; Expert Rev. Proteomics 3, 361-374

Wiedenmann J et al. (2007) Fluoreszente Proteine aus den Ozeanen - Neue Werkzeuge für die zelluläre Bildgebung; BIOforum 30/5, 20-22

Order Information, Shipping and Storage

Order#	Product	Amount
VS-FLPC1012	ptd-EosFP-CD8, lyophilized DNA	20 µg
shipped at room temperature (RT); store at 4°C. Once the DNA has been dissolved in sterile water or buffer we recommend storage at -20 °C.		

Related Products

Order#	Product	Amount
MIR 5404	*Trans*IT®-2020 Transfection Reagent	0.4 ml
MIR 5400	*Trans*IT®-2020 Transfection Reagent	1 ml
MIR 5405	*Trans*IT®-2020 Transfection Reagent	5 x 1 ml
MIR 5406	*Trans*IT®-2020 Transfection Reagent	10 x 1 ml
MIR 50113	Ingenio™ Electroporation Kit with 0.4 cuvettes	25 RXN
MIR 50116	Ingenio™ Electroporation Kit with 0.4 cuvettes	50 RXN
MIR 50119	Ingenio™ Electroporation Kit with 0.4 cuvettes	100 RXN
MIR 50112	Ingenio™ Electroporation Kit with 0.2 cuvettes	25 RXN
MIR 50115	Ingenio™ Electroporation Kit with 0.2 cuvettes	50 RXN
MIR 50118	Ingenio™ Electroporation Kit with 0.2 cuvettes	100 RXN
MIR 50111	Ingenio™ Electroporation Solution	25 RXN
MIR 50114	Ingenio™ Electroporation Solution	50 RXN
MIR 50117	Ingenio™ Electroporation Solution	100 RXN
MIR 50120	Ingenio™ Cuvettes, 0.2 cm	25 PK
MIR 50121	Ingenio™ Cuvettes, 0.2 cm	50 PK
MIR 50122	Ingenio™ Cuvettes, 0.4 cm	25 PK
MIR 50123	Ingenio™ Cuvettes, 0.4 cm	50 PK
MIR 50124	Ingenio™ Cell Droppers	25 PK
MIR 50125	Ingenio™ Cell Droppers	50 PK
5160-30	Imaging Dish CG 1.0, 35 mm, 145 µm, TC-surface	30 PK
6160-30	Imaging Dish CG 1.5, 35 mm, 175 µm, TC-surface	30 PK
MGM01	MobiGLOW Mounting Medium	10 ml

Mirus products (MIR) are distributed by MoBiTec in Germany, Austria and Hungary. For other countries please inquire.

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