ptd-EosFP-Paxillin, lyophilized DNA

Product Information Sheet
VS-FLPC1011

SUMMARY

shipped at RT; store at 4 °C

For research use only

Product

"ptd-EosFP-Paxillin" 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 chicken Paxillin (*G. gallus*). The fusion protein contains a FLAG®-tag[1].

Paxillin is a signal transduction adaptor protein associated with focal adhesions. The fusion protein td-EosFP-Paxillin can be used as a localization marker for detecting Paxillin within living cells by fluorescence microscopy. Based on its ability to be photoconverted, td-EosFP-Paxillin is well suitable to high resolution fluorescence microscopy (Betzig et al., 2006). The photoconversion also enables regional optical marking of Paxillin proteins. In particular, ptd-EosFP-Paxillin transfected cells can be used as adjusting sample for PALM microscopy.

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 td-EosFP-Paxillin protein is an excellent choice for localization and co-localization studies of Paxillin in live cell imaging. Its photoconversion allows for regional cellular marking of Paxillin proteins.

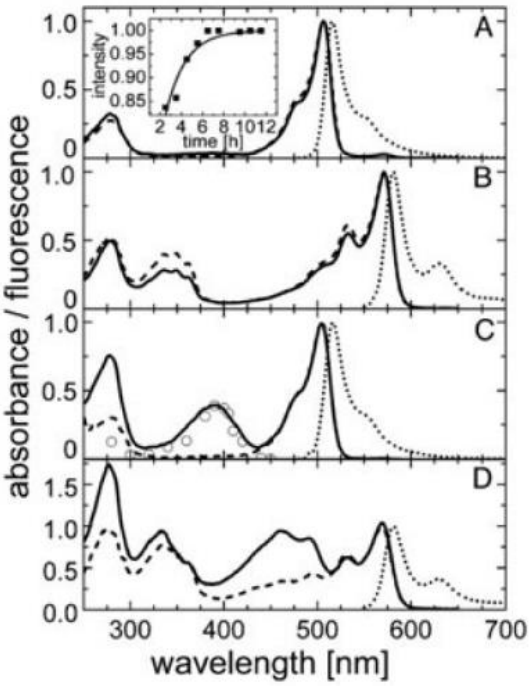
Detection

The green and the red fluorescent state of td-EosFP-Paxillin 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 by flow cytometry for up to 9 days (Leutenegger et al., 2007).

Expression in Mammalian Cells

The vector "ptd-EosFP-Paxillin" 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 protein td-EosFP-Paxillin will be expressed constitutively under the strong CMV promoter. The neomycin resistance gene enables selecting for stably transfected eukaryotic cells using G418.

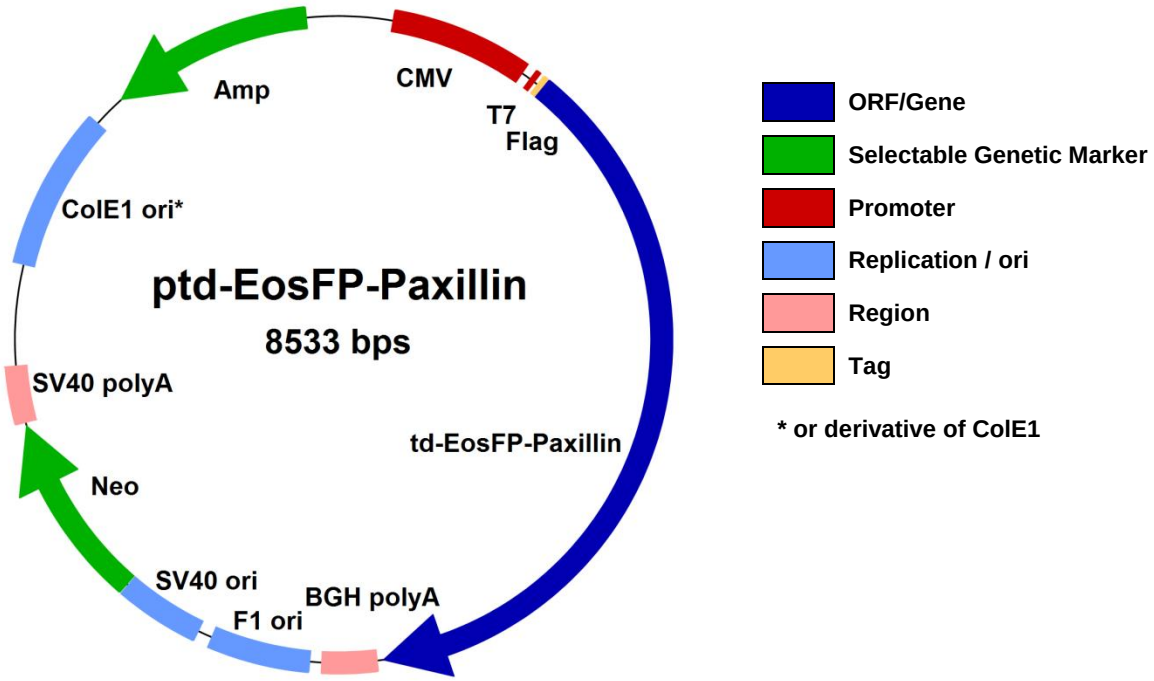
Chemical Transfection Protocol

- 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

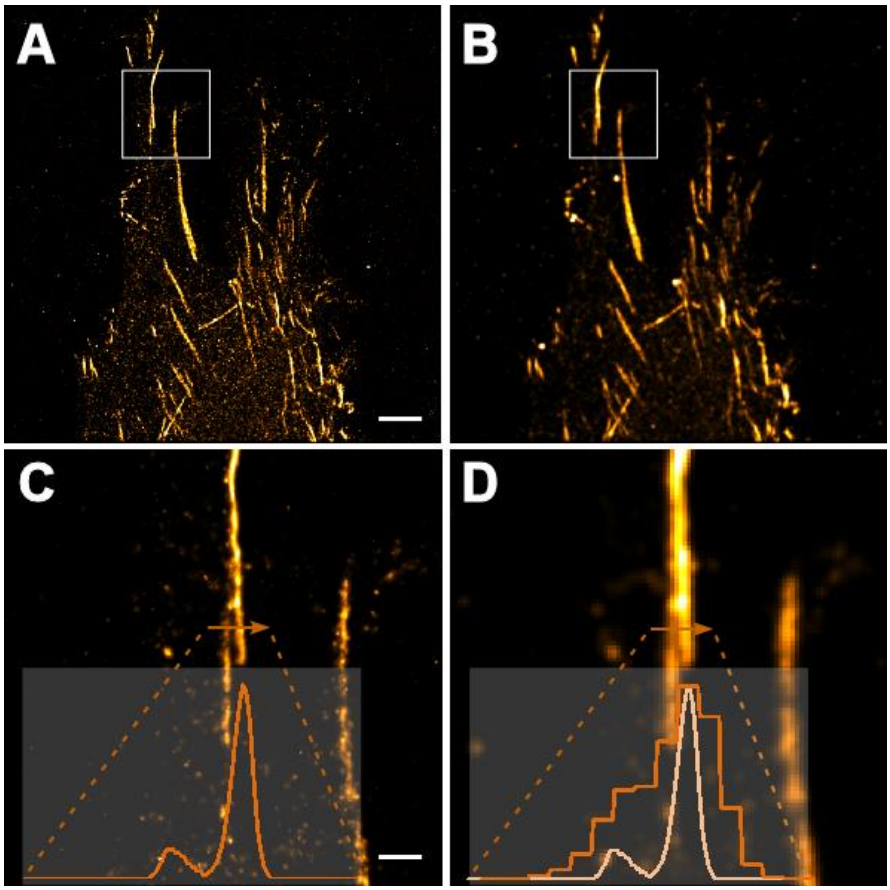

Amp
CMV
T7
Flag
ColE1 ori*
ptd-EosFP-Paxillin
8533 bps
SV40 polyA
td-EosFP-Paxillin
Neo
SV40 ori
F1 ori
BGH polyA
ORF/Gene
Selectable Genetic Marker
Promoter
Replication / ori
Region
Tag
* or derivative of ColE1

Application Note

For localization analysis of Paxillin, we transfected NIH 3T3 mammalian fibroblast cells with the plasmid ptd-EosFP-Paxillin and analyzed the cells by 2D-PALM and Sum Wide Field Microscopy.

Results

By 2D-PALM and Sum Wide Field Microscopy, we could detect focal adhesions as relative dense elongated structures. By PALM we measured 1 - 2 million localizations with 100 ± 15 photons per spot at 25 ± 5 nm precision.



Microscopy images of cells expressing the fusion protein td-EosFP-Paxillin, which accumulates in focal adhesions
PALM **(A, C)** and Sum Wide Field Microscopy **(B, D)**. In **(C)** and **(D)** zoomed-in views of a focal adhesion complex of the indicated area of **(A)** and **(B)** are shown. Scale bars are 5 µm **(A, B)** and 0.5 µm **(C, D)**. The profiles in **(B)** and **(D)** are along the indicated arrows. For better comparison, the profile of PALM **(C)** is also displayed in the sum wide field image **(D)** in light orange (Carl Zeiss Microscopy GmbH).

Methods

Transfection and Sample Preparation

- NIH 3T3 fibroblast cells are cultivated in a well of a six-well plate with Dulbecco's Modified Eagle Medium (DMEM) + 10 % Fetal Bovine Serum (FBS) to 80 % confluence.
- 3 µg of ptd-EosFP-Paxillin vector are diluted in 250 µl of Opti-MEM I (Life Technologies) and mixed gently.
- The transfection reagent (e.g., *Trans*IT®-2020, Mirus, or Lipofectamine 2000, Life Technologies) is mixed gently before use. 5 µl are diluted in 250 µl Opti-MEM I, mixed gently, and incubated for 5 min at room temperature.
- After incubation, the diluted plasmid is combined with the diluted transfection reagent, mixed gently, and incubated for at least 20 min (not longer than 6 hours) at room temperature.
- The cells are washed once with PBS (Phosphate Buffered Saline, pH 7.4) and covered with Opti-MEM I.
- The "plasmid transfection reagent complexes" are added to the cells and mixed gently by rocking the plate back and forth. Then, cells are incubated at 37 °C in a CO_2 incubator overnight.
- The medium is exchanged with culture medium (DMEM + 10 % FBS) and incubated for 36 h in total (starting time point: adding "plasmid transfection reagent complexes").
- During incubation, cells are trypsinized, centrifuged, and resuspended in culture medium, and seeded onto fibronectin coated (10 µg/ml, Sigma-Aldrich) dishes with glass bottom (e.g., Imaging Dish CG, MoBiTec, or Glass Bottom Culture Dish, MatTek Corporation). This is followed by at least 2 h incubation.
- After incubation, cells are fixed by prewarmed 4 % paraformaldehyde in PBS for 10 min, followed by washing and permeabilizing with PBS + 0.1 % TritonX-100 for 5 min. Then cells are washed 2-times with ddH_2O for 5 min.
- 80 nm gold particles are diluted 1:10 in ddH_20 and treated by ultrasound for at least 15 min.
- The cells are incubated with the gold solution for 10 min, washed once with ddH_2O, and analyzed by 2D-PALM and Sum Wide Field Microscopy
- For long-term storage (approx. 6 months), all liquid is sucked off, cells are dried overnight, and stored at room temperature in the dark.
- Rehydration is done with PBS buffer. Samples can be dried and rehydrated up to 5-times. Before drying again, PBS buffer is sucked off and cells are washed 3-times with ddH_2O.

Imaging Conditions

Power density:	TIRF field
Objective:	alpha Plan-Apochromat 100x/1.46 Oil DIC M27 ELYRA PS.1
Camera:	EMCCD Andor 897
Integration time:	50 ms
Laser lines:	561 nm 20 % and 405 nm up of 0.5 %
Time series:	10000 frames

References

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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.

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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-FLPC1011	ptd-EosFP-Paxillin, FLAG®-tagged, 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.		

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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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