Photoactivatable red fluorescent protein PA-TagRFP

- Monomer, successful performance in fusions

- Non-fluorescent before photoactivation

- Irreversible photoactivation to a red fluorescent form by UV-violet light irradiation

- High brightness and photostability

- Recommended for super-resolution imaging

PA-TagRFP is a photoactivatable mutant of the bright monomeric red fluorescent protein TagRFP [Subach et al. 2010]. PA-TagRFP is capable of irreversible photoconversion from non-fluorescent to red fluorescent form (with excitation/emission maxima at 562 nm and 595 nm, respectively) in response to UV-violet light irradiation.

High brightness, photostability and monomeric nature of PA-TagRFP make it an excellent protein tag for both conventional microscopy and super-resolution PALM imaging techniques [Subach et al. 2010].

PA-TagRFP normalized excitation (thin line) and emission (thick line) spectra.
Complete PA-TagRFP spectra in Excel format can be downloaded from the Evrogen Web site at http://www.evrogen.com

Main properties of PA-TagRFP

Characteristic	before / after photoactivation
Fluorescence color	NO / red
Excitation maximum, nm	- / 562
Emission maximum, nm	- / 595
Quantum yield	nd / 0.38
Extinction coefficient, $M^{-1}cm^{-1}$	nd / 66 000
Brightness*	0 / 25.1
pKa	nd / 5.3
Activating light	UV-violet (e.g. 390-420 nm)
Calculated contrast, fold	540
Structure	monomer
Cell toxicity	not observed
Aggregation	no
Maturation rate at 37°C	fast
Molecular weight, kDa	27
Polypeptide length, aa	237

* Brightness is a product of extinction coefficient and quantum yield, divided by 1 000.

PA-TagRFP use for protein labeling in mammalian cells. Microscopic images of HeLa cells transiently transfected with PA-TagRFP-tagged fusions after the photoactivation: (A) α-tubulin; (B) histone H2B.
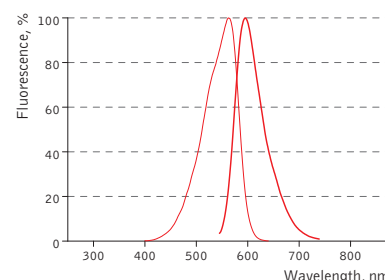
Performance and use

PA-TagRFP can be easily expressed and detected in a wide range of organisms. Mammalian cells transiently transfected with PA-TagRFP expression vectors produce bright fluorescence upon UV-activation of PA-TagRFP in 10-12 hrs after transfection. No cytotoxic effects or visible protein aggregation are observed.

PA-TagRFP performance in protein fusions has been demonstrated in α-tubulin, histone H2B and other models.
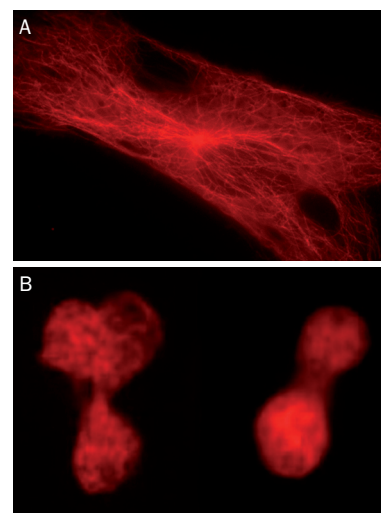
PA-TagRFP use in PALM imaging techniques

High brightness, photostability and absence of initial fluorescence signal from PA-TagRFP make it a protein tag of choice for super resolution two-color PALM/single-particle tracking PALM imaging techniques. The excellent performance of PA-TagRFP in two-color single-particle tracking PALM experiments was demonstrated for several PA-TagRFP-tagged and PAGFP-tagged fusions in live COS-7 cells [Subach et al. 2010].

An example for the tracking of PA-TagRFP-tagged epidermal growth factor receptor (EGFR-PATagRFP) and PAGFP-tagged vesicular stomatitus virus G protein tsO45 (VSVG-PAGFP) in live COS-7 cells by two-color single-particle tracking PALM is shown below.
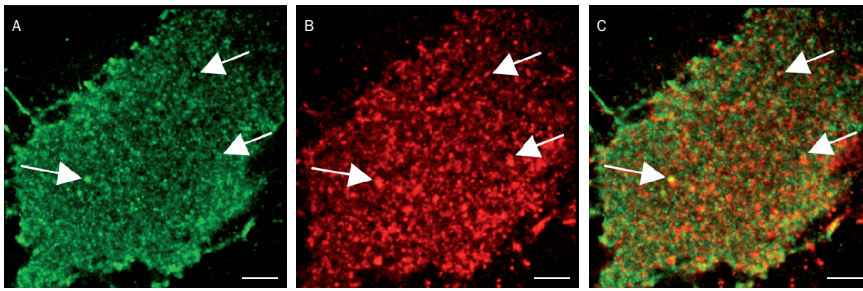
(A,B) The separate and (C) merged distribution of VSVG-PAGFP (green) and EGFR-PATagRFP (red) in PALM images. Arrows indicate areas of apparent colocalization between the VSVG and EGFR molecules. Scale bars are $2\mu m$.
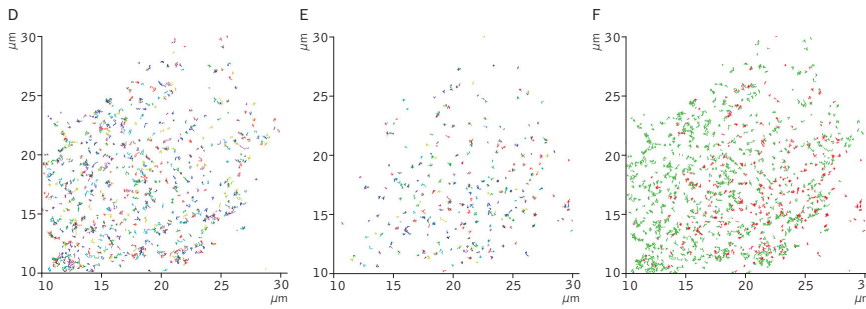
(D,E) Tracks of VSVG-PAGFP and EGFR-PATagRFP molecules lasting longer than 0.7 sec are plotted. Approximately 1635 VSVG molecules were tracked along with 627 EGFR molecules. (F) VSVG-PAGFP (green) and EGFR-PATagRFP (red) tracks are merged.

Recommended antibodies, filter sets and laser lines

PA-TagRFP can be recognized using Anti-tRFP antibody (Cat.# AB233) available from Evrogen. PA-TagRFP is non-fluorescent before light activation. Upon UV-violet irradiation the protein irreversibly converts to its red fluorescent form.

PA-TagRFP can be activated during both widefield imaging (e.g. the Arc-lamp irradiation, 100xoil objective, 390-420 nm, 10-50 mW/cm^2) and confocal laser scanning imaging (e.g. 405 nm laser line, estimated < 2.5 W/cm^2 at the sample). Maximal efficiency of photoactivation for PA-TagRFP is observed at 390-420 nm. The photoactivation efficiency drops dramatically with the wavelength increasing above 420 nm.

The source of irradiation, irradiation time and intensity of activating UV-violet light must be individually adjusted for particular instrumentation and intended application.

TRITC filter set or similar can be used for visualization of activated PA-TagRFP. Omega Optical filter sets QMAX-Red and XF174 are recommended.

Available variants and fusions

PA-TagRFP mammalian expression vectors contain PA-TagRFP coding sequence with codon usage optimized for high expression in mammalian cells, i.e. humanized [Haas, Park, and Seed 1996]. Humanized PA-TagRFP can also be expressed in *E. coli* and some other heterological systems upon subcloning into appropriate vector.

The available vectors encoding PA-TagRFP variants and fusions are listed below in the section PA-TagRFP-related products. For most updated product information, please visit Evrogen website www.evrogen.com.

If you need PA-TagRFP codon variant or fusion construct that is not listed on our website, please contact us at product@evrogen.com.

Licensing opportunities

Evrogen technology embodied in PA-TagRFP is available for expanded and commercial use with an adaptable licensing program. Benefits from flexible and market driven license options are offered for upgrade and novel development of products and applications. For licensing information, please contact Evrogen at license@evrogen.com.

References

Haas, J., E. C. Park, and B. Seed (1996). Curr Biol, 6 (3): 315–324 / pmid: 8805248

Subach, FV et al. (2010). J Am Chem Soc, 132 (18): 6481–91 / pmid: 20394363

PA-TagRFP-related products

Product	Cat.#	Description	Size
PA-TagRFP expression/source vectors			
pPA-TagRFP-C	FP811	Mammalian expression vector encoding humanized PA-TagRFP and allowing its expression and generation of fusions to the PA-TagRFP C-terminus	20 µg
pPA-TagRFP-N	FP812	Mammalian expression vector encoding humanized PA-TagRFP and allowing its expression and generation of fusions to the PA-TagRFP N-terminus	20 µg
pPA-TagRFP-tubulin	FP814	Mammalian expression vector encoding humanized PA-TagRFP fused with human α-tubulin	20 µg
pPA-TagRFP-H2B	FP815	Mammalian expression vector encoding humanized PA-TagRFP fused with human histone H2B	20 µg
Antibodies against PA-TagRFP			
Anti-tRFP	AB233	Rabbit polyclonal antibody against TurboRFP, TurboFP602, TurboFP635, TurboFP650, NirFP, TagBFP, TagRFP, FusionRed, TagFP635, mKate2 and PA-TagRFP	100 µg

Please contact your local distributor for exact prices and delivery information.