

Red fluorescent protein TagRFP

- Bright red (orange) fluorescence
- Monomeric protein with successful performance in fusions
- Fast maturation, high pH-stability
- Proven suitability to generate stably transfected cell lines
- Recommended for protein labeling, acidic organelle labeling, FRET applications

TagRFP is a monomeric red (orange) fluorescent protein generated from the wild-type RFP from sea anemone *Entacmaea quadricolor* [Merzlyak et al. 2007]. It possesses bright fluorescence with excitation/emission maxima at 555 and 584 nm, respectively. TagRFP is about three times brighter than mCherry protein [Shaner et al. 2004], which makes it the brightest monomeric red fluorescent protein available so far.

TagRFP is mainly intended for protein labeling and FRET [Shcherbo et al. 2009] applications. It can also be used for cell and organelle labeling and for tracking the promoter activity.

Main properties of TagRFP

Characteristic	
Molecular weight, kDa	27
Polypeptide length, aa	237
Fluorescence color	red (orange)
Excitation maximum, nm	555
Emission maximum, nm	584
Quantum yield	0.48
Extinction coefficient, $M^{-1}cm^{-1}$	100 000
Brightness*	48.0
Brightness, % of EGFP	148
pKa	3.8
Structure	monomer
Aggregation	no
Maturation rate at 37°C	fast
Maturation half-time, min	100
Photostability	medium
Photostability, widefield***	48
Photostability, confocal***	125
Cell toxicity	not observed

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

*** Time to bleach 50% of fluorescent signal brightness.

Performance and use

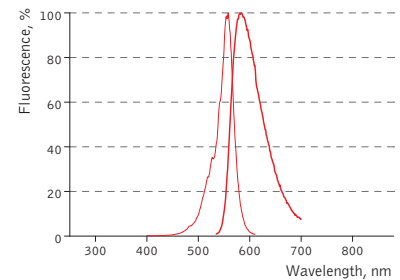
TagRFP can be easily expressed and detected in a wide range of organisms. Mammalian cells transiently transfected with TagRFP expression vectors produce bright fluorescence in 10-12 hrs after transfection. No cytotoxic effects or visible protein aggregation are observed. High pH-stability with $pK_a=3.8$ makes it possible to use TagRFP for imaging in acidic organelles, such as late and recycling endosomes and lysosomes.

TagRFP performance in protein fusions has been demonstrated in fibrillarin, vinculin, zyxin, β -actin, α -tubulin, and other models.

TagRFP suitability to generate stably transfected cells has been proven by Marinpharm company. Cell lines expressing TagRFP are commercially available.

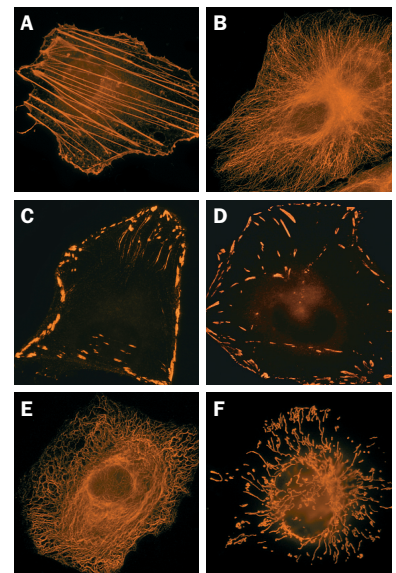
TagRFP can be used in multicolor labeling applications with blue, cyan, green, yellow, and far-red fluorescent dyes.

TagRFP is ideally suitable for use as an acceptor for FRET with Evrogen green fluorescent protein TagGFP2. The calculated Forster distance ($R^0 = 5.7$ nm) for the TagGFP2-TagRFP pair is one of



TagRFP normalized excitation (thin line) and emission (thick line) spectra.

Complete TagRFP spectra in Excel format can be downloaded from the Evrogen Web site at <http://www.evrogen.com>



TagRFP use for cell and protein labeling.

(A) HeLa cells expressing TagRFP fusion with β -actin; (B) HeLa cells expressing TagRFP fusion with α -tubulin; (C) HeLa cells expressing TagRFP fusion with zyxin; (D) HeLa cells expressing TagRFP fusion with vinculin; (E) HeLa cells expressing TagRFP fusion with keratin; (F) HeLa cells expressing TagRFP targeted to mitochondria.

the largest among the values reported. At the same time, since TagGFP2 and TagRFP emission peaks are spaced by as much as 78 nm, the emission signal for these two proteins can be easily separated in any imaging system. High pH-stability of the both proteins allows using this pair for imaging in acidic organelles.

The excellent performance of TagRFP in FRET application was demonstrated both *in vitro* and *in vivo* on the example of FRET-based apoptosis reporter Casper3-GR [Shcherbo et al. 2009].

Recommended filter sets and antibodies

TagRFP can be recognized using Anti-tRFP antibody (Cat.# AB233-AB234) available from Evrogen.

Recommended Omega Optical filter sets are QMAX-Yellow, XF108-2, XF101-2, and XF111-2. TagRFP can also be detected using TRITC filter set or similar.

Available variants and fusions

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

TagRFP-AS codon usage is optimized for expression in *Arabidopsis* and *Saccharomyces*.

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

If you need 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 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. et al. (1996). *Curr Biol*, 6 (3): 315–324 / pmid: 8805248
- Merzlyak, EM et al. (2007). *Nat Methods*, 4 (7): 555–557 / pmid: 17572680
- Shaner, NC et al. (2004). *Nat Biotechnol*, 22 (12): 1567–1572 / pmid: 15558047
- Shcherbo, D et al. (2009). *BMC Biotechnology*, 9: 24 / pmid: 19321010

TagRFP-related products

Product	Cat.#	Description	Size
TagRFP expression/source vectors			
pTagRFP-C	FP141	Mammalian expression vector encoding humanized TagRFP and allowing its expression and generation of fusions to the TagRFP C-terminus	20 µg
pTagRFP-N	FP142	Mammalian expression vector encoding humanized TagRFP and allowing its expression and generation of fusions to the TagRFP N-terminus	20 µg
pTagRFP-actin	FP144	Mammalian expression vector encoding humanized TagRFP fused with human cytoplasmic β -actin	20 µg
pTagRFP-tubulin	FP145	Mammalian expression vector encoding humanized TagRFP fused with human α -tubulin	20 µg
pTagRFP-mito	FP147	Mammalian expression vector encoding humanized TagRFP targeted to mitochondria	20 µg
Gateway® TagRFP-AS-C	FP148	Gateway® entry clone for generation of fusions to the C-terminus of TagRFP; transfer of the construct encoding TagRFP or its fusion into Gateway® destination vectors; TagRFP codon usage is optimized for expression in <i>Arabidopsis</i> and <i>Saccharomyces</i>	20 µg
Gateway® TagRFP-AS-N	FP149	Gateway® entry clone for generation of fusions to the N-terminus of TagRFP; transfer of the construct encoding TagRFP or its fusion into Gateway® destination vectors; TagRFP codon usage is optimized for expression in <i>Arabidopsis</i> and <i>Saccharomyces</i>	20 µg
pTagRFP-actinin	FP360	Mammalian expression vector encoding humanized TagRFP fused with human α -actinin	20 µg
pTagRFP-integrin	FP361	Mammalian expression vector encoding humanized TagRFP fused with human α -V-integrin	20 µg
pTagRFP-Cx26	FP362	Mammalian expression vector encoding humanized TagRFP fused with rat connexin 26	20 µg
pTagRFP-Cx32	FP363	Mammalian expression vector encoding humanized TagRFP fused with human connexin 32	20 µg
pTagRFP-Cx43	FP364	Mammalian expression vector encoding humanized TagRFP fused with rat connexin 43	20 µg
pTagRFP-EB3	FP365	Mammalian expression vector encoding humanized TagRFP fused with human EB3 protein	20 µg
pTagRFP-FAK	FP366	Mammalian expression vector encoding humanized TagRFP fused with chicken focal adhesion kinase	20 µg

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Product	Cat.#	Description	Size
pTagRFP-Golgi	FP367	Mammalian expression vector encoding humanized TagRFP fused with human Golgi targeting sequence (GTS)	20 µg
pTagRFP-H2B	FP368	Mammalian expression vector encoding humanized TagRFP fused with human histone H2B	20 µg
pTagRFP-keratin	FP369	Mammalian expression vector encoding humanized TagRFP fused with human cytokeratin-18	20 µg
pTagRFP-laminB1	FP370	Mammalian expression vector encoding humanized TagRFP fused with human lamin B1	20 µg
pTagRFP-profilin	FP371	Mammalian expression vector encoding humanized TagRFP fused with mouse profilin	20 µg
pTagRFP-vinculin	FP372	Mammalian expression vector encoding humanized TagRFP fused with human vinculin	20 µg
pTagRFP-zyxin	FP373	Mammalian expression vector encoding humanized TagRFP fused with human zyxin	20 µg
Antibodies against TagRFP			
Anti-tRFP	AB233	Rabbit polyclonal antibody against TurboRFP, TurboFP602, TurboFP635, TurboFP650,	100 µg
	AB234	NirFP, TagBFP, TagRFP, FusionRed, TagFP635, mKate2 and PA-TagRFP	200 µg

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

Notice to Purchaser:

TagRFP-related materials (also referred to as "Products") are intended for research use only.

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MSDS information is available at <http://www.evrogen.com/MSDS.shtml>