

Red fluorescent protein TurboRFP

- Superbright red (orange) fluorescence
- Fast maturation
- Fluorescent signal is easily distinguished from background fluorescence
- Destabilized version is available
- Recommended for gene expression analysis, cell and organelle labeling

Description

TurboRFP is a red fluorescent protein (excitation/emission maxima are 553 and 574 nm, respectively) derived from sea anemone *Entacmaea quadricolor* (Merzlyak *et al.*, 2007). Possessing high photostability and pH stability, TurboRFP is more than twice brighter than DsRed2. Fast TurboRFP maturation makes it clearly detectable in mammalian cells as early as within 8-10 hrs after transfection.

TurboRFP is mainly intended for applications where fast appearance of bright fluorescence is crucial. It is specially recommended for cell and organelle labeling and tracking the promoter activity. Destabilized TurboRFP variant allows accurate analysis of rapid and/or transient events in gene regulation.

Main properties of TurboRFP

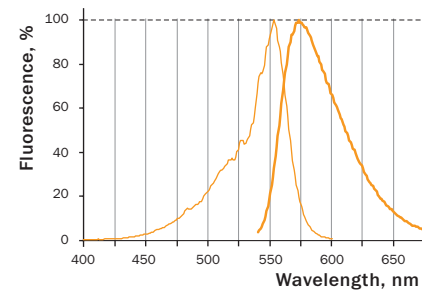
Characteristic	
Molecular weight	26 kDa
Polypeptide length	231 aa
Fluorescence color	red (orange)
Excitation max	553 nm
Emission max	574 nm
Quantum yield	0.67
Extinction coefficient	92 000 M ⁻¹ cm ⁻¹
Brightness*	61.6
Brightness % of EGFP	187
pKa	4.4
Structure	dimer
Aggregation	no
Maturation rate at 37°C	superfast
Photostability	high

*Brightness is a product of extinction coefficient and quantum yield, divided by 1000.

Performance and use

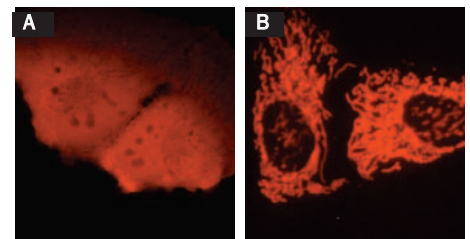
TurboRFP can be expressed and detected in a wide range of organisms. Mammalian cells transiently transfected with TurboRFP expression vectors give bright fluorescent signals in 8-10 hrs after transfection. No cell toxic effects and visible protein aggregation are observed.

Despite its dimeric structure, TurboRFP demonstrates successful performance in fusions with subcellular localization signals and many cellular pro-



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

Complete TurboRFP spectra in Excel format can be downloaded from the Evrogen Web site at www.evrogen.com/TurboRFP.shtml



TurboRFP use for cell and organelle labeling.

A — Fluorescent microscopy of mammalian cells expressing cytoplasmic TurboRFP; B — fluorescent microscopy of mammalian cells expressing TurboRFP fusion with mitochondrial targeting signal. Images made from HeLa cells 24 hrs after transfection.

teins. However, we recommend that you use specially optimized monomeric TagFPs for protein labeling applications.

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

Comparison of TurboRFP, DsRed2, and DsRed-Express maturation in mammalian cells

To compare maturation of TurboRFP and DsRed-related proteins, HeLa cells were transiently transfected with mammalian expression vectors comprising TurboRFP, DsRed2, or DsRed-Express fluorescent proteins under the control of CMV promoter. The DNA concentrations were equalized before transfection. Cells were photographed using fluorescent microscope after different periods of cultivation. Faster appearance of bright fluorescence was detected in the case of TurboRFP. In addition, unlike DsRed-related proteins, no abnormal Golgi-like localization of TurboRFP was observed within 7 days after transfection.

Available variants and fusions

TurboRFP codon usage is optimized for high expression in mammalian cells (Haas *et al.*, 1996), but it can be successfully expressed in many other heterologous systems.

TurboRFP-mito fusion

A mitochondrial targeting sequence (MTS) is linked to the TurboRFP N-terminus. MTS was derived from the subunit VIII of human cytochrome C oxidase (Rizzuto *et al.*, 1989; Rizzuto *et al.*, 1995). When expressed in mammalian cells, this variant provides red fluorescent labeling of mitochondria.

Destabilized TurboRFP variant (TurboRFP-dest1)

Destabilized TurboRFP variant (TurboRFP-dest1) is produced by fusing the initial protein with PEST amino acid sequence encoded by region 422-461 of mouse ornithine decarboxylase gene (Li *et al.*, 1998). This sequence targets the protein to degradation and enables a rapid protein turnover. TurboRFP-dest1 retains spectral properties of the initial protein, but has shorter half-life (approximately 1-2 hrs) as measured by the analysis of fluorescence intensity of cells treated with a protein synthesis inhibitor, cycloheximide. Because of rapid turnover, TurboRFP-dest1 can be used to measure changes in gene expression.

Recommended filter sets and antibodies

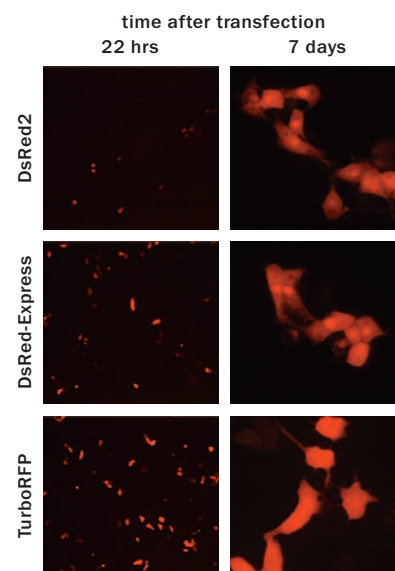
Recommended Omega Optical filter sets are QMAX-Yellow, XF108-2, XF101-2, and XF111-2. TurboRFP can also be detected using TRITC filter set or similar.

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

TurboRFP licensing opportunities

Evrogen technology embodied in TurboRFP 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.



Fluorescent microscopy of mammalian cells expressing DsRed2, DsRed-Express, and TurboRFP.

TurboRFP gives the brightest signal 22 hrs after transfection; DsRed2 and DsRed-Express show abnormal Golgi-like localization 7 days after transfection, whereas TurboRFP localizes evenly in cytosol.

References

- Haas *et al.* (1996) *Curr. Biol.* 6: 315–324.
- Li *et al.* (1998) *J. Biol. Chem.* 273: 34970-34975.
- Merzlyak *et al.* (2007) *Nat. Methods.* 4(7): 555-557.
- Rizzuto *et al.* (1989) *J. Biol. Chem.* 264: 10595–10600.
- Rizzuto *et al.* (1995) *Curr. Biol.* 5: 635–642.

TurboRFP-related products

Product	Cat.#	Description	Size
TurboRFP expression/source vectors			
pTurboRFP-C	FP231	Mammalian expression vector encoding humanized TurboRFP and allowing TurboRFP expression and generation of fusions to the TurboRFP C-terminus	20 µg
pTurboRFP-N	FP232	Mammalian expression vector encoding humanized TurboRFP and allowing TurboRFP expression and generation of fusions to the TurboRFP N-terminus	20 µg
pTurboRFP-B	FP233	Bacterial expression vector; source of humanized TurboRFP coding sequence	20 µg
pTurboRFP-PRL	FP235	Promoterless mammalian expression vector encoding humanized TurboRFP and designed for monitoring transcription from different promoters and promoter/enhancer combinations	20 µg
pTurboRFP-PRL-dest1	FP238	Promoterless vector encoding destabilized TurboRFP and designed for monitoring transcription from different promoters and promoter/enhancer combinations	20 µg
pTurboRFP-dest1	FP239	Mammalian expression vector encoding destabilized TurboRFP for its expression and generation of fusions to the TurboRFP-dest1 N-terminus	20 µg
pTurboRFP-mito	FP237	Mammalian expression vector encoding humanized TurboRFP targeted to mitochondria	20 µg
Antibodies against TurboRFP			
Anti-tRFP antibody	AB233	Rabbit polyclonal antibody against TurboRFP, TurboFP602,	100 µg
	AB234	TurboFP635, TagBFP, TagRFP, TagFP635, and mKate2	200 µg

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

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