Green fluorescent protein TurboGFP

- Bright green fluorescence
- Fast maturation at a wide range of temperatures
- High pH-stability and photostability
- Proven suitability to generate stably transfected cell lines
- Destabilized variant is available
- Recommended for gene expression analysis and cell and organelle labeling

TurboGFP is an improved variant of the green fluorescent protein CopGFP cloned from copepod *Pontellina plumata* (Arthropoda; Crustacea; Maxillopoda; Copepoda) [Shagin et al. 2004]. It possesses bright green fluorescence (excitation/ emission max = 482/ 502 nm) that is visible earlier than fluorescence of other green fluorescent proteins.
TurboGFP 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 TurboGFP variant allows accurate analysis of rapid and/or transient events in gene regulation.

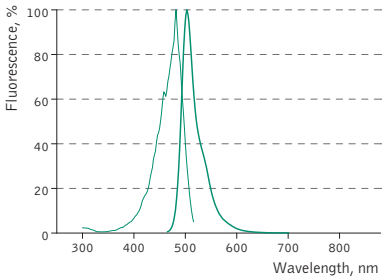
Main properties of TurboGFP

Characteristic	
Molecular weight, kDa	26
Polypeptide length, aa	232
Fluorescence color	green
Excitation maximum, nm	482
Emission maximum, nm	502
Quantum yield	0.53
Extinction coefficient, $M^{-1}cm^{-1}$	70 000
Brightness*	37.1
Brightness, % of EGFP	112
pKa	5.2
Structure	dimer
Aggregation	no
Maturation rate at 37°C	super fast
Photostability	high
Cell toxicity	not observed

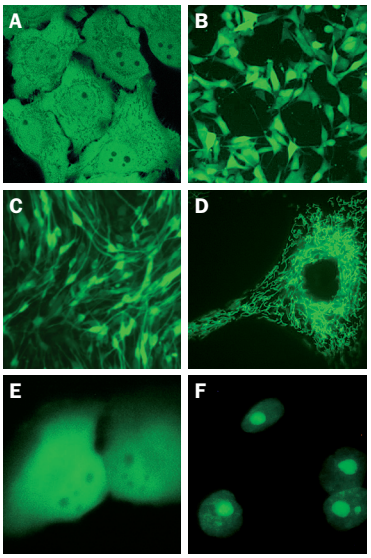
* Brightness is a product of extinction coefficient and quantum yield, divided by 1 000.

Performance and use

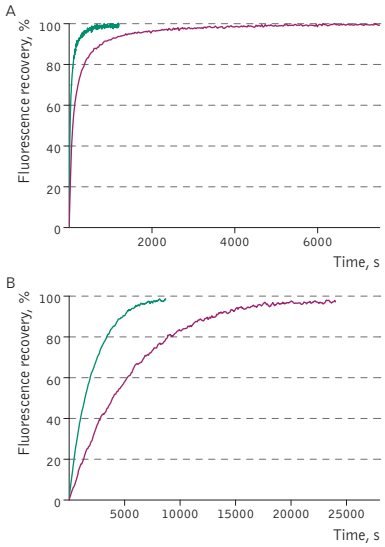
TurboGFP can be expressed and detected in a wide range of organisms including cold-blooded animals. Mammalian cells transiently transfected with TurboGFP expression vectors give bright fluorescent signals in 8-10 hrs after transfection. No cytotoxic effects or visible protein aggregation are observed. TurboGFP can be used in multicolor labeling applications with blue, true-yellow, red, and far-red fluorescent dyes.
TurboGFP suitability to generate stably transfected cells has been proven by Marinpharm company. Cell lines expressing TurboGFP are commercially available.
Despite its dimeric structure, TurboGFP performs well in some fusions. However, for protein labeling applications we recommend using specially optimized monomeric TagFPs.
TurboGFP maturation kinetics: TurboGFP allows monitoring the activity from early promoters. It matures noticeably faster than EGFP and most other fluorescent proteins. This difference in performance is illustrated here using both *in vitro* analysis of TurboGFP and EGFP refolding and maturation kinetics and *in vivo* examination of the developing *Xenopus* embrios expressing either TurboGFP or EGFP.



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



TurboGFP expression in mammalian cells.
(A) Transiently transfected HeLa cells expressing TurboGFP in cytoplasm; (B) stably transfected M3-mouse melanoma cells expressing TurboGFP in cytoplasm; (C) stably transfected C2C12 mouse myoblasts expressing TurboGFP in cytoplasm; (D) stably transfected HeLa cells expressing mitochondria-targeted TurboGFP; (E) stably transfected HeLa cells expressing TurboGFP-BID fusion; (F) stably transfected HeLa cells expressing TurboGFP-fibrillarin fusion.
Photographs of stably transfected cell lines were kindly provided by Dr. Christian Petzelt (Marinpharm).



Comparison of EGFP (violet lines) and TurboGFP (green lines) refolding and maturation speed *in vitro* [Evdokimov et al. 2006]. Normalized fluorescence recovery plots are shown. (A) Refolding kinetics; (B) chromophore maturation kinetics.

Refolding and maturation kinetics of GFPs *in vitro*

	EGFP	Venus	SYFP2	TurboGFP
Refolding half-time, s	90.6	46.2	69.3	11.0
Maturation half-time, s	3915	4076	3300	1468
kox 10^{-4} s^{-1}	1.77	1.70	2.10	4.72
Reference	Evdokimov et al. 2006	Kremers et al. 2006	Kremers et al. 2006	Evdokimov et al. 2006

Samples of fluorescent proteins were heated to 95°C in denaturation solution (8 M urea, 1 mM DTT) for 4 min. Refolding reactions were initiated upon 100-fold dilution into the renaturation buffer (35 mM KCl, 2 mM $MgCl_2$, 50 mM Tris pH 7.5, 1 mM DTT). In maturation assay, 5 mM freshly dissolved dithionite was added to the denaturation solution [Reid and Flynn 1997]. Due to the instability of dithionite at high temperatures, to provide for complete chromophore reduction the sample was cooled to 25°C and the addition of 5 mM dithionite followed by heating to 5°C were repeated. Protein refolding and maturation were followed by measuring the recovery of fluorescence using Varian Cary Eclipse Fluorescence Spectrophotometer, chamber temperature maintained at 25°C. Maturation rate constants (kox) were determined by computer-fitting the kinetic data to the first order exponential decay (Origin 6.0).

Recommended filter sets and antibodies

TurboGFP can be recognized using Anti-TurboGFP(d) (Cat.# AB513-AB514) antibody available from Evrogen.

TurboGFP can be detected using common fluorescence filter sets for EGFP, FITC, and other green dyes. Recommended Omega Optical filter sets are QMAX-Green, XF100-2, XF100-3, (XF115-2), and XF116-2.

Available variants and fusions

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

Destabilized TurboGFP variant (TurboGFP-dest1) is produced by addition of 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. TurboGFP-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, TurboGFP-dest1 can be used to measure changes in gene expression.

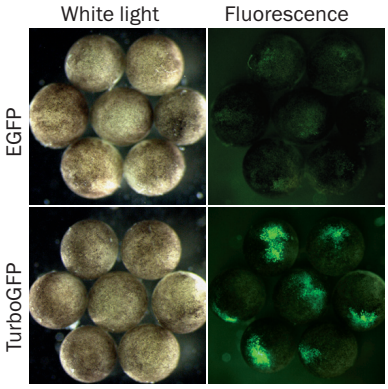
The available vectors encoding TurboGFP variants and fusions are listed below in the section TurboGFP-related products. For most updated product information, please visit Evrogen website www.evrogen.com.

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

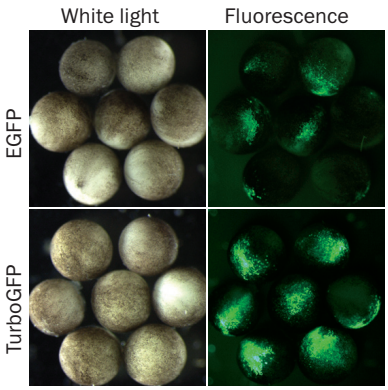
Licensing opportunities

Evrogen technology embodied in TurboGFP 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.

Midgastrula stage



Late gastrula stage



***In vivo* comparison of TurboGFP and EGFP maturation in developing *Xenopus* embryos.** Vectors encoding TurboGFP and EGFP under the control of CMV promoter were microinjected into animal poles of *Xenopus* embryos at the stage of two blastomeres. Living embryos were then photographed from the animal pole at the middle and late gastrula stages.

Experimental data were presented by Dr. A. Zaraisky, Institute of Bioorganic Chemistry, RAS (Moscow, Russia).

References

Evdokimov et al. (2006). EMBO Rep, 7 (10): 1006–1012 / pmid: 16936637

Haas et al. (1996). Curr Biol, 6 (3): 315–324 / pmid: 8805248

Kremers et al. (2006). Biochemistry, 45 (21): 6570–6580 / pmid: 16716067

Li et al. (1998). J Biol Chem, 273 (52): 34970–34975 / pmid: 9857028

Reid and Flynn (1997). Biochemistry, 36 (22): 6786–6791 / pmid: 9184161

Shagin et al. (2004). Curr Biol, 21 (5): 841–850 / pmid: 14963095

TurboGFP-related products

Product	Cat.#	Description	Size
TurboGFP expression/source vectors			
pTurboGFP-C	FP511	Mammalian expression vector encoding humanized TurboGFP and allowing its expression and generation of fusions to the TurboGFP C-terminus	20 μg
pTurboGFP-N	FP512	Mammalian expression vector encoding humanized TurboGFP and allowing its expression and generation of fusions to the TurboGFP N-terminus	20 μg
pTurboGFP-B	FP513	Bacterial expression vector; source of the TurboGFP coding sequence	20 μg
pTurboGFP-PRL	FP515	Promoterless vector encoding humanized TurboGFP and designed for monitoring of activity of different promoters and promoter/enhancer combinations	20 μg
pTurboGFP-mito	FP517	Mammalian expression vector encoding humanized TurboGFP targeted to mitochondria	20 μg
pTurboGFP-PRL-dest1	FP518	Promoterless vector encoding destabilized TurboGFP and designed for monitoring of activity of different promoters and promoter/enhancer combinations	20 μg
pTurboGFP-dest1	FP519	Mammalian expression vector encoding destabilized TurboGFP for its expression and generation of fusions to the TurboGFP-dest1 N-terminus	20 μg

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Product	Cat.#	Description	Size
Gateway® TurboGFP-C	FP521	Gateway® entry clone for generation of fusions to the C-terminus of humanized TurboGFP; transfer of the constract encoding TurboGFP or its fusion into Gateway® destination vectors	20 μg
Gateway® TurboGFP-N	FP522	Gateway® entry clone for generation of fusions to the N-terminus of humanized TurboGFP; transfer of the constract encoding TurboGFP or its fusion into Gateway® destination vectors	20 μg
Recombinant protein			
rTurboGFP	FP552	Purified recombinant bright green fluorescent protein	100 μg
Antibodies against TurboGFP			
Anti-TurboGFP(d)	AB513	Rabbit polyclonal antibody against TurboGFP and CopGFP	100 μg
	AB514		200 μg

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