

Green fluorescent protein TurboGFP

- Bright green fluorescence
- Extra fast maturation at a wide range of temperatures
- Proven suitability to generate stably transfected cell lines
- Destabilized version is available
- Recommended for gene expression analysis, cell and organelle labeling

Description

TurboGFP is an improved variant of the green fluorescent protein CopGFP cloned from copepod *Pontellina plumata* (Arthropoda; Crustacea; Maxillopoda; Copepoda) (Shagin *et al.*, 2002). 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

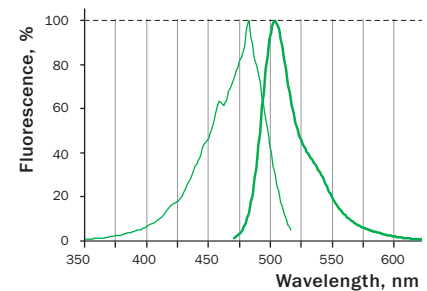
Characteristic	
Molecular weight	26 kDa
Polypeptide length	232 aa
Fluorescence color	green
Excitation max	482 nm
Emission max	502 nm
Quantum yield	0.53
Extinction coefficient	70 000 M ⁻¹ cm ⁻¹
Brightness*	37.1
Brightness % of EGFP	112
pKa	5.2
Structure	dimer
Aggregation	no
Maturation rate at 37°C	superfast
Photostability	high

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

Performance and use

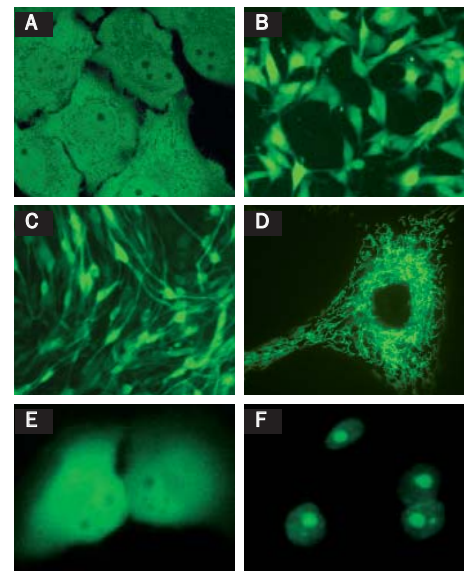
TurboGFP can be expressed and detected in a wide range of organisms including cold-blooded animals. Mammalian cells transiently transfected with TurboGFP expression vectors give bright fluorescent signals within 8-10 hrs after transfection. No cell toxic effects and visible protein aggregation are observed.

Despite its dimeric structure, TurboGFP is suitable for generation of fusions (successful result has been obtained in mitochondria, BID protein, and fib-



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 www.evrogen.com/TurboGFP.shtml



TurboGFP expression in mammalian cells.

A — Whole-cell expression in transiently transfected HeLa cells; B — in stably transfected M3 mouse melanoma cells; C — in stably transfected C2C12 mouse myoblast cells; D — mitochondrial TurboGFP expression in stably transfected HeLa cells; E — TurboGFP-BID fusion expression in transiently transfected HeLa cells; F — TurboGFP-fibrillarin expression in transiently transfected HeLa cells. Images of stably transfected cell lines were kindly provided by Dr. Christian Petzelt (Marinpharm).

rillar models); however, we recommend that you use specially optimized monomeric reporters for protein labeling applications. Please see “TagFPs” section (at www.evrogen.com/TagFPs.shtml) to select a reporter for such purposes.

TurboGFP suitability to generate stably transfected cells has been proven by Marinpharm company (www.marinpharm.com). Various cell lines expressing TurboGFP are commercially available.

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

Fast maturation rate: TurboGFP allows monitoring of early promoters. It matures noticeably faster than EGFP and most other fluorescent proteins. This difference in performance has been demonstrated using both *in vitro* analysis of TurboGFP and EGFP refolding and maturation kinetics and *in vivo* examination of the developing *Xenopus* embryos expressing either TurboGFP or EGFP.

Refolding and maturation kinetics of TurboGFP and other fluorescent proteins *in vitro*

Fluorescent protein	Refolding half-time (s)	Maturation half-time (s)	k _{ox} (10 ⁴ s ⁻¹)	Reference
EGFP	90.6	3915	1.77	Evdokimov <i>et al.</i> , 2006
Venus	46.2	4076	1.70	Kremers <i>et al.</i> , 2006
SYFP2	69.3	3300	2.10	Kremers <i>et al.</i> , 2006
TurboGFP	11.0	1468	4.72	Evdokimov <i>et al.</i> , 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 MgCl₂, 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). Because of the instability of dithionite at high temperatures, to enable complete chromophore reduction, the sample was cooled to 25°C and the addition of 5 mM dithionite followed by heating to 95°C were repeated. Protein refolding and maturation were followed by measuring the recovery of fluorescence using Varian Cary Eclipse Fluorescence Spectrophotometer, with chamber temperature maintained at 25°C. Maturation rate constants (k_{ox}) were determined by computer-fitting the kinetic data to the first-order exponential decay (Origin 6.0).

In vivo examination of developing *Xenopus* embryos microinjected with vectors comprising either TurboGFP or EGFP under the control of CMV promoter showed bright fluorescence of TurboGFP immediately after midblastula transition, when gene expression is activated. At the same time, EGFP was practically invisible at this developmental stage. This example clearly demonstrates that TurboGFP is a better tool to study expression in rapidly developing embryos at early stages.

Available variants and fusions

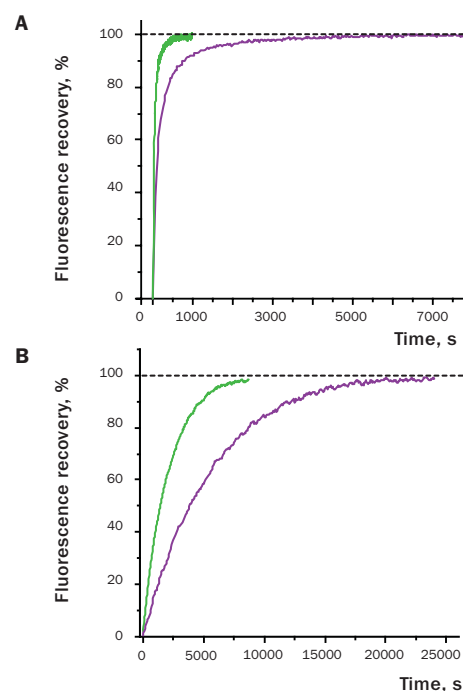
TurboGFP codon usage is optimized for high expression in mammalian cells (Haas *et al.*, 1996), but it can be successfully expressed in other heterologous systems.

TurboGFP-mito fusion

A mitochondrial targeting sequence (MTS) is linked to the TurboGFP 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 green fluorescent labeling of mitochondria.

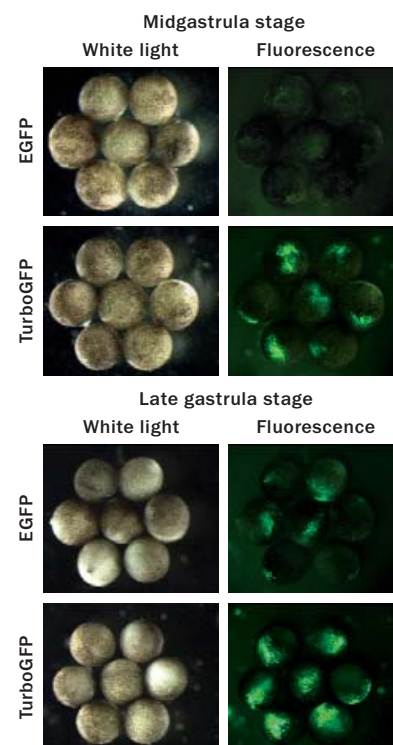
Destabilized TurboGFP variant (TurboGFP-dest1)

TurboGFP-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. TurboGFP-dest1 retains spectral



Comparison of EGFP (violet lines) and TurboGFP (green lines) refolding and maturation speed *in vitro*.

Normalized fluorescence recovery plots are shown. A — refolding kinetics; B — chromophore maturation kinetics.



***In vivo* comparison of TurboGFP and EGFP maturation in developing *Xenopus* embryos.**

Living embryos were then photographed from the animal pole at the middle and late gastrula stages. Experimental data were provided by Dr. A. Zaráisky (Moscow, Russia).

properties of the initial protein, but has shorter half-life (approximately 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 monitor rapid and temporal events in gene regulation.

Recommended filter sets and antibodies

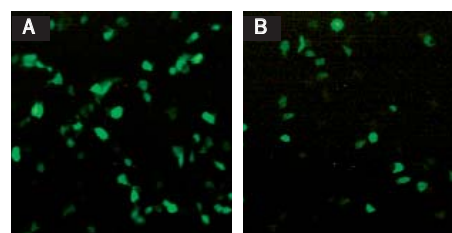
TurboGFP can be detected using common fluorescence filter sets for EGFP, FITC, and other green dyes. Recommended Omega Optical filter sets are QMAX-Green, XF100-2, XF100-3, XF115-2, and XF116-2.

Antibody against TurboGFP (Cat.# AB513-AB514) is available from Evrogen.

TurboGFP 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.



Fluorescence intensities of cells expressing TurboGFP-dest1 rapidly decrease in response to cycloheximide (CHX).

Mammalian cells expressing TurboGFP-dest1 under the control of CMV promoter were treated with CHX. After 1.5 hours CHX treatment, fluorescence intensity of cells was greatly reduced.

A — no CHX treatment; B — after CHX treatment.

References

- Evdokimov *et al.* (2006) *EMBO Rep.* 7(10): 1006-1012.
 Haas *et al.* (1996) *Curr. Biol.* 6: 315–324.
 Kremers *et al.* (2006) *Biochemistry* 45: 6570-6580.
 Li *et al.* (1998) *J. Biol. Chem.* 273:34970-34975.
 Reid and Flynn (1997) *Biochemistry* 36: 6786-6791.
 Rizzuto *et al.* (1989) *J. Biol. Chem.* 264: 10595–10600.
 Rizzuto *et al.* (1995) *Curr. Biol.* 5: 635–642.
 Shagin *et al.* (2004) *Mol. Biol. Evol.* 21(5): 841-850.

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 humanized TurboGFP coding sequence	20 µg
pTurboGFP-PRL	FP515	Promoterless expression vector encoding humanized TurboGFP and designed for monitoring transcription from different promoters and promoter/enhancer combinations	20 µg
pTurboGFP-PRL-dest1	FP518	Promoterless vector encoding destabilized TurboGFP and designed for monitoring transcription from 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
pTurboGFP-mito	FP517	Mammalian expression vector encoding humanized TurboGFP targeted to mitochondria	20 µg

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

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

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