evrogen

Fluorescent reporters for *in vivo* cell labeling and monitoring of promoter activity

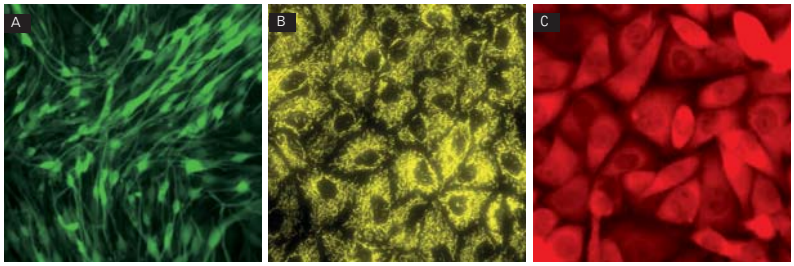
Evrogen TurboColors are superbright and fast maturing fluorescent proteins, specially recommended for applications requiring fast appearance of bright fluorescence, including cell and organelle labeling or tracking promoter activity. Far-red marker TurboFP635 is ideal for whole body imaging applications.

Protein/Characteristics	TurboGFP	TurboYFP	TurboRFP	TurboFP602	TurboFP635	TurboFP650
Fluorescence color	green	yellow	red (orange)	true-red	far-red	near-infrared
Excitation max	482 nm	525 nm	553 nm	574 nm	588 nm	592 nm
Emission max	502 nm	538 nm	574 nm	602 nm	635 nm	650 nm
Quantum yield	0.53	0.53	0.67	0.35	0.34	0.24
Extinction coefficient ($M^{-1}cm^{-1}$)	70 000	105 000	92 000	74 400	65 000	65 000
Brightness*	37.1	55.7	61.6	26.0	22.1	15.6
Brightness, % of EGFP	112	169	187	79	67	47
pKa	5.2	5.9	4.4	4.7	5.5	5.7

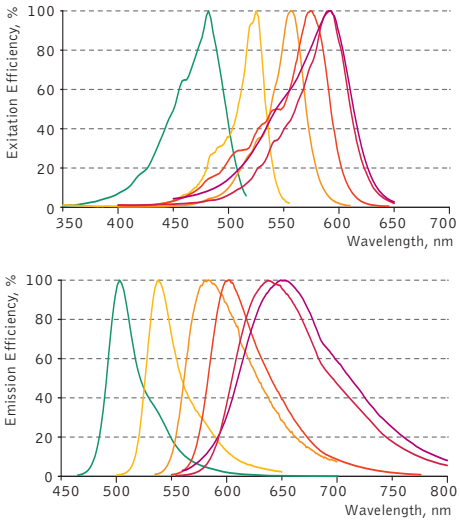
* Brightness is a product of extinction coefficient and quantum yield, divided by 1000.

Bright labels of cells and cell organelles

TurboFPs possess bright stable fluorescence allowing monitoring of cells over extended periods of time. Despite their dimeric structure, TurboFPs are suitable for generation of fusions with subcellular localization signals targeting the reporters to desired cell compartments. Stable cell lines expressing TurboFPs are available.



Expression of TurboFPs in stably transfected mammalian cell lines. (A) - TurboGFP, C2C12 myoblast cells, (B) - mitochondria-targeted PhiYFP*, PtK2 cells, (C) - TurboFP635, T24 cells.
* PhiYFP is a variant of TurboYFP optimized for stable expression.
Images of stably transfected cell lines were kindly provided by Dr. Christian Petzelt (Marinpharm).



TurboFPs normalized exitation/emission spectra

Perfect reporters of gene expression

TurboFPs mature noticeably faster than many other fluorescent proteins, allowing monitoring of gene expression from early promoters. The example below shows *in vivo* examination of the developing *Xenopus* embrios expressing either TurboGFP or EGFP. Destabilized protein variants (*-dest1) allow accurate analysis of rapid and/or transient events in gene regulation.

***In vivo* comparison of TurboGFP and EGFP maturation in developing *Xenopus* embryos.** Vectors expressing the respective fluorescent proteins 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).

Suitable markers for whole body imaging

For deep imaging of animal tissues, the optical window favorable for light penetration is in near-infrared wavelengths, which requires proteins with emission spectra in the far-red wavelengths. TurboFP635 (scientific name Katushka) has emission maxima at 635 nm and is more bright, photostable and pH-stable than other cloned far-red fluorescent proteins. Superiority of TurboFP635 for whole-body imaging has been demonstrated by direct comparison with other red and far-red fluorescent proteins (Shcherbo *at al.* Nat Methods. (2007) 4:741-746).

DsRed-Express and TurboFP635 expression in *Xenopus laevis*.
Transgenic 2.5 months intact animals expressing TurboFP635 and DsRed-Express under the control of cardiac actin promoter are shown from the dorsal side. TurboFP635 (on the right) is clearly visible in the whole body, while DsRed-Express (on the left) is not. This experiment clearly demonstrates the advantage of longer wavelength emission of TurboFP635 for the whole body imaging. Leica MZFLIII fluorescent stereomicroscope, excitation filter 546/10; emission filter 565LP.

Available vectors

Vector	Cat#
Bacterial expression vectors	
pTurboGFP-B	FP513
pTurboYFP-B	FP613
pTurboRFP-B	FP233
Mammalian expression vectors	
pTurboGFP-C	FP511
pTurboRFP-C	FP231
pTurboFP635-C	FP721
pTurboFP650-C	FP731
pTurboGFP-N	FP512
pTurboYFP-N	FP612
pTurboRFP-N	FP232
pTurboFP602-N	FP712
pTurboFP635-N	FP722
pTurboFP650-N	FP732
Vectors for labeling of mitochondria	
pTurboGFP-mito	FP517
pTurboRFP-mito	FP237
pTurboFP602-mito	FP717
Promoterless vectors	
pTurboGFP-PRL	FP515
pTurboRFP-PRL	FP235
pTurboFP602-PRL	FP715

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