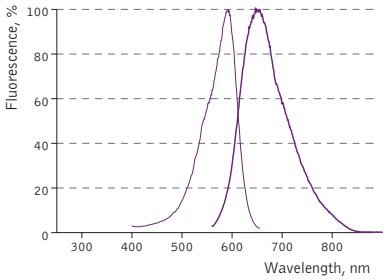
Near-infrared fluorescent protein TurboFP650

- Bright near-infrared fluorescence
- Fast maturation, high photostability
- Fluorescent signal is easily distinguished from background fluorescence
- Recommended for whole body imaging and multicolor applications

TurboFP650 (scientific name eqFP650) is a red-shifted variant of TurboFP635 (Katushka) [Shcherbo et al. 2010]. TurboFP650 is characterized by a strong bathochromic shift, with excitation and emission peaks at 592 nm and 650 nm, respectively. It is currently the brightest fluorescent protein with emission maxima above 635 nm.

TurboFP650 demonstrates fast maturation at 37°C and a high pH-stability and photostability. The protein does not show residual short wavelength fluorescence of intermediate or alternative chromophore forms, in contrast to E2-Crimson [Strack et al. 2009], which exhibits a second bright blue emission peak, and mNeptune [Lin et al. 2009], which has a pronounced green peak. TurboFP650 is specially recommended for whole body imaging and multicolor applications.

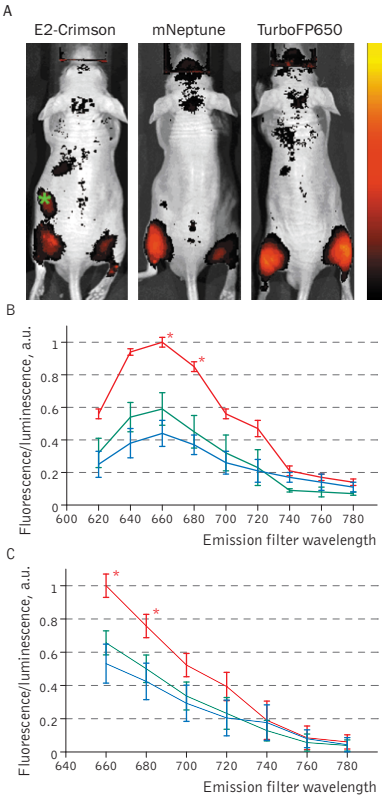


TurboFP650 normalized excitation (thin line) and emission (thick line) spectra.
Complete TurboFP650 spectra in Excel format can be downloaded from the Evrogen Web site at http://www.evrogen.com

Main properties of TurboFP650

Characteristic	
Molecular weight, kDa	26
Polypeptide length, aa	234
Fluorescence color	near-infrared
Excitation maximum, nm	592
Emission maximum, nm	650
Quantum yield	0.24
Extinction coefficient, $M^{-1}cm^{-1}$	65 000
Brightness*	15.6
Brightness, % of EGFP	47
Extinction coefficient, $M^{-1}cm^{-1}$ at 635 nm	4 300
Quantum yield in infrared (700-900 nm)	0.07
Brightness in infrared**	0.3
pKa	5.7
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.
** Brightness in infrared is a product of extinction coefficient at 635 nm, quantum yield and emission fraction between 700 nm and 900 nm, divided by 1000.



Whole-mouse imaging with IVIS Spectrum system (Caliper). (A) Representative fluorescence reflectance images (excitation filter, 605/30 nm and emission filter, 660/20 nm) of mice injected intramuscularly with HEK 293T cells expressing E2-Crimson, mNeptune or TurboFP650. Green asterisk denotes background fluorescence in mice injected with E2-Crimson cells. The color bar indicates radiant efficiency × 10^{-6}; minimum is 0.001, and maximum is 0.006. (B,C) Fluorescence efficiency from cell implants imaged with 570/30 nm (B) or 605/30 nm (C) excitation filters and various emission filters, normalized to photons from firefly luciferase to control for transfection efficiency and numbers of implanted cells. Means ± s.e.m. are shown (n = 6-10 per point). *P < 0.05 (Student's t-test) for TurboFP650 relative to other proteins. Red line - TurboFP650, green line - mNeptune, blue line - E2-Crimson. Image and data from Shcherbo et al. 2010.

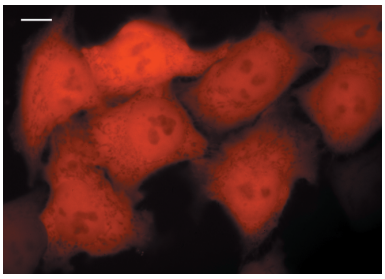
Performance and use

TurboFP650 can be easily visualized within living tissues. Mammalian cells transiently transfected with TurboFP650 expression vectors produce bright fluorescence in 14 hrs after transfection. No cytotoxic effects or visible protein aggregation are observed.

Superior performance of TurboFP650 in whole-body imaging was demonstrated using mouse xenograft model. HEK 293T cells transiently transfected with a plasmids encoding either TurboFP635, TurboFP650, NirFP, mNeptune or E2-Crimson were injected intramuscularly into the gluteal region of mice. The cells were co-transfected with firefly luciferase plasmid to normalize the transfection efficiency and total numbers of injected cells. Imaging of cell implants and quantification at various emission wavelengths showed higher fluorescence from TurboFP650 at two excitation wavelengths.

Despite its dimeric structure, TurboFP650 can be used in some fusions. However, for protein

labeling applications we recommend using specially optimized monomeric TagFPs. TurboFP650 can be used in multicolor labeling applications with blue, cyan, green, yellow, and red (orange) fluorescent dyes.



HeLa cells transiently transfected with pTurboFP650-N vector. Widefield Leica AFLX 6000 microscope, 63x objective, after 3 days of incubation. Scale bar, 10 μm. Images from Shcherbo et al. 2010.

Recommended filter sets and antibodies

TurboFP650 can be recognized using Anti-tRFP antibody (Cat.# AB233) available from Evrogen.
The optimal excitation/emission ranges for TurboFP650 visualization are:
excitation: 550-610 nm
emission: 620-800 nm
Therefore, many common filter sets used for visualization of red and far-red fluorescent proteins, Texas Red, Allophycocyanin and Cy5 (wide excitation), can be used with TurboFP650 as well. The recommended filter sets for gathering the maximal signal from TurboFP650 alone: Chroma Technology Corp.: 11010v2 Yellow; 11007v2 Wide Green
Semrock : LF594/LP-A (especially with 594 nm laser excitation); LF561/LP-A (especially with 561 nm laser excitation);
Omega Optical: XF102-2, XF40-2
The recommended filter sets for spectral separation with orange-red fluorescent proteins*, such as TurboRFP or TagRFP:
Chroma Technology Corp.: 41024 Cy5 Longpass Emission, 49006 ET - Cy5
Semrock: Cy5-4040A, Cy5-4040B, LF594/LP-A
Omega Optical: XF110-2

* The final choice of the filter set should be made basing on the spectral characteristics of the second fluorescent protein.

Available variants and fusions

TurboFP650 mammalian expression vectors contain TurboFP650 coding sequence with codon usage optimized for high expression in mammalian cells, i.e. humanized [Haas et al. 1996]. Humanized TurboFP650 can also be expressed in *E. coli* and some other heterological systems upon subcloning into appropriate vector.
The available vectors encoding TurboFP650 are listed below in the section TurboFP650-related products. For most updated product information, please visit Evrogen website www.evrogen.com. If you need TurboFP650 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 TurboFP650 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
Lin, MZ et al. (2009). Chem Biol, 16 (11): 1169–79 / pmid: 19942140
Shcherbo, D et al. (2010). Nat Methods, 7 (10): 827–9 / pmid: 20818379
Strack, RL et al. (2009). Biochemistry, 48 (35): 8279–81 / pmid: 19658435

TurboFP650-related products

Product	Cat.#	Description	Size
TurboFP650 expression/source vectors			
pTurboFP650-C	FP731	Mammalian expression vector encoding humanized TurboFP650 and allowing its expression and generation of fusions to the TurboFP650 C-terminus	20 μg
pTurboFP650-N	FP732	Mammalian expression vector encoding humanized TurboFP650 and allowing its expression and generation of fusions to the TurboFP650 N-terminus	20 μg
Antibodies against TurboFP650			
Anti-tRFP	AB233	Rabbit polyclonal antibody against TurboRFP, TurboFP602, TurboFP635, TurboFP650, NirFP, TagBFP, TagRFP, FusionRed, TagFP635, mKate2 and PA-TagRFP	100 μg

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