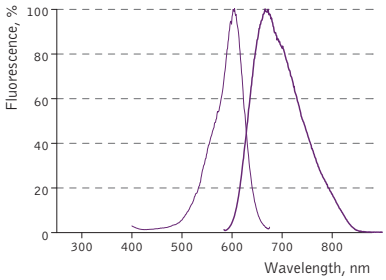
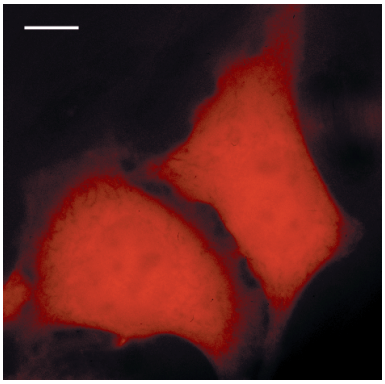
Near-infrared fluorescent protein NirFP

- Near-infrared fluorescence with emission maximum at 670 nm
- Extremely high photostability
- Fluorescent signal is easily distinguished from background fluorescence
- Recommended for multicolor applications

NirFP (scientific name eqFP670) is a red-shifted variant of TurboFP635 (Katushka) [Shcherbo et al. 2010]. NirFP is characterized by a strong bathochromic shift, with excitation and emission peaks at 605 nm and 670 nm, respectively. It is currently the most red-shifted fluorescent protein available, with approximately half of emission falling in the infrared part of the spectrum. The brightness of NirFP in the 700 - 900 nm region upon excitation at 635 nm is about 4 times higher than the brightness of TurboFP635 and 1.6 times higher than the brightness of TurboFP650. 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. NirFP is characterized by high pH stability and extremely high photostability that should allow for accumulation of the fluorescent signal over long exposure times.
NirFP is recommended for multicolor applications. It can also be used for whole body imaging utilizing long wavelengths for excitation (e.g., 633 or 635 nm laser lines).



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



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

Main properties of NirFP

Characteristic	
Molecular weight, kDa	26
Polypeptide length, aa	234
Fluorescence color	near-infrared
Excitation maximum, nm	605
Emission maximum, nm	670
Quantum yield	0.06
Extinction coefficient, $M^{-1}cm^{-1}$	70 000
Brightness*	4.2
Brightness, % of EGFP	13
Extinction coefficient, $M^{-1}cm^{-1}$ at 635 nm	15 700
Quantum yield in infrared (700-900 nm)	0.03
Brightness in infrared**	0.47
pKa	4.5
Structure	dimer
Aggregation	no
Photostability	super 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.

Performance and use

NirFP can be easily visualized within living tissues. Mammalian cells transiently transfected with NirFP expression vectors produce fluorescence in 48 hrs after transfection. No cytotoxic effects or visible protein aggregation are observed.
Despite its dimeric structure, NirFP can be used in some fusions. However, for protein labeling applications we recommend using specially optimized monomeric TagFPs.
NirFP can be used in multicolor labeling applications with blue, cyan, green, yellow, and red (orange) fluorescent dyes.

Recommended filter sets and antibodies

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

The optimal excitation/emission ranges for NirFP visualization are:
excitation: 560-620 nm
emission: 630-850 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 NirFP as well.
The recommended filter sets for gathering the maximal signal from NirFP alone:
Chroma Technology Corp.: 11010v2 Yellow, 41024 Cy5 Longpass Emission
Semrock : LF594/LP-A (especially with 594 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

NirFP mammalian expression vectors contain NirFP coding sequence with codon usage optimized for high expression in mammalian cells, i.e. humanized [Haas et al. 1996]. Humanized NirFP can also be expressed in *E. coli* and some other heterological systems upon subcloning into appropriate vector.
The available vectors encoding NirFP are listed below in the section NirFP-related products. For most updated product information, please visit Evrogen website www.evrogen.com.
If you need NirFP 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 NirFP 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

NirFP-related products

Product	Cat.#	Description	Size
NirFP expression/source vectors			
peNirFP-N	FP743	Mammalian expression vector encoding humanized NirFP and allowing its expression and generation of fusions to the NirFP N-terminus	20 µg
Antibodies against NirFP			
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.