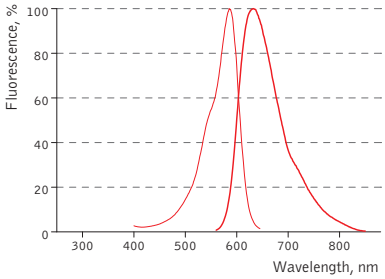
Far-red fluorescent protein Katushka2S

- Super bright far-red fluorescence
- High signal-to-noise ratio, fast maturation
- Recommended for whole body imaging

Katushka2S is the next generation of far-red fluorescent protein TurboFP635 (Katushka) [Luker et al. 2015; Shcherbo et al. 2009; Gurskaya et al. 2011]. Katushka2S exhibits highest brightness, fastest maturation and superior signal-to-noise ratio compared to TurboFP635 and many other available far-red fluorescent proteins: E2-Crimson, mNeptune, mNeptune2.5, mCardinal, TurboFP650 and NirFP [Luker et al. 2015], which makes it the protein of choice for visualization within living tissues. Katushka2S can also be used together with iRFP720 [Luker et al. 2015] for dual color whole body imaging.

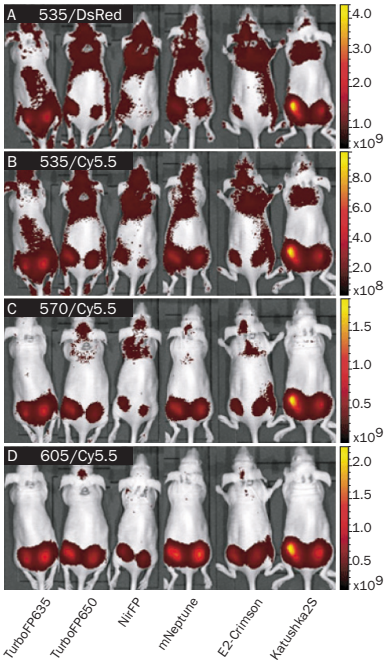


Katushka2S normalized excitation (thin line) and emission (thick line) spectra.
Complete Katushka2S spectra in Excel format can be downloaded from the Evrogen Web site at http://www.evrogen.com

Main properties of Katushka2S

Characteristic	
Molecular weight, kDa	26
Polypeptide length, aa	235
Fluorescence color	far-red
Excitation maximum, nm	588
Emission maximum, nm	633
Quantum yield	0.44
Extinction coefficient, $M^{-1}cm^{-1}$	67 000
Brightness*	29.5
Brightness, % of EGFP	89
pKa	5.4
Structure	dimer
Aggregation	no
Maturation rate at 37°C	super fast
Photostability,widefield (sec)	72
Cell toxicity	not observed

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

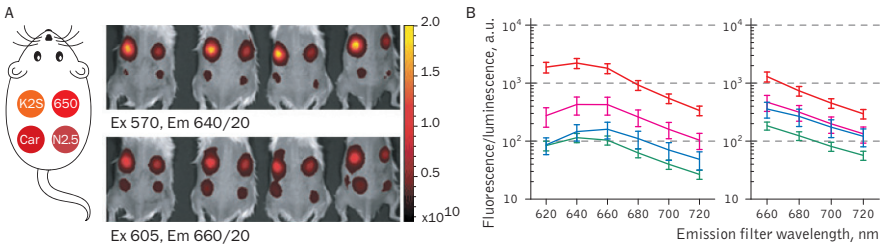


Whole-mouse imaging with IVIS Lumina II system (PerkinElmer). Representative fluorescence images of nude mice injected into the gluteal muscle with HEK293FT cells transiently expressing mNeptune, E2-Crimson, Katushka2S, TurboFP635, TurboFP650 and NirFP captured with indicated excitation (35-nm bandwidth centered at the indicated wavelength) and emission (DsRed 575-650 nm and Cy5.5 695-770 nm) filter combination. Pseudocolor scale bar: radiant efficiency (photons/s)/(μW/cm^2). Note that scale bar differ for images with different excitation and emission filters. Images from [Luker et al. 2015]

Performance and use

Mammalian cells transiently transfected with Katushka2S expression vectors produce bright fluorescence in 12 hrs after transfection. No cytotoxic effects or visible protein aggregation are observed.

Superior performance of Katushka2S in whole-body imaging was demonstrated using mouse xenograft model. HEK293FT cells transiently transfected with plasmids encoding different far-red fluorescent proteins were implanted into mice intramuscularly or subcutaneously. The cells were co-transfected with firefly luciferase to normalize the transfection efficiency and total numbers of injected cells. Katushka2S produced higher fluorescence signal and better signal-to-noise ratio than other far-red fluorescence proteins analyzed at various excitation and emission channels.



Whole-mouse imaging with IVIS Spectrum system (PerkinElmer).

(A) Representative fluorescence images of mice injected in backs with HEK293FT cells transiently expressing Katushka2S (K2S), TurboFP650 (650), mCardinal (Car) and mNeptune2.5(N2.5). captured with indicated excitation (35-nm bandwidth centered at the indicated wavelength) and emission (640/20 and 660/20) filter combination. Pseudocolor scale bar: radiant efficiency (photons/s/cm^2/sr)/(μW/cm^2).

(B) Graphs display mean values ±SEM for fluorescence radiant efficiency normalized to luciferase photon flux for each implant (n=4 per condition) for 570-nm (left) and 605-nm (right) excitation and listed emission filters. Fluorescent proteins are depicted by the following colors: Katushka2S (red), TurboFP650 (pink), mCardinal (blue) and mNeptune2.5 (green).

Images and data from [Luker et al. 2015]

Katushka2S was also compared with one of the best phytochrome photoreceptors iRFP720. Katushka2S produced brighter fluorescence intensity at its optimum conditions as compared with the best output from iRFP720. At the same time, both proteins showed comparable signal-to-noise ratios at the optimum wavelengths for excitation and emission since autofluorescence from mouse tissue was lower in the channel optimal for iRFP720.

Note: *Katushka2S demonstrates lower photostability than its parental variant TurboFP635. It may limit Katushka2S utility in cell culture experiments, but not in whole body imaging, where scattering and absorption of light naturally limit excitation power [Leblond et al. 2010].*

Recommended filter sets and antibodies

Katushka2S can be recognized using Anti-tRFP antibody (Cat.# AB233) available from Evrogen. Recommended Omega Optical filter sets are QMAX-Red and XF102-2. Katushka2S can also be detected using Texas Red filter sets or similar.

For IVIS Lumina II imaging system the highest fluorescence signal for Katushka2S is observed with the following settings:

In cell culture: "Cy5.5" (695-770 nm) channel using 570/35nm excitation

In whole body imaging: "Cy5.5" (695-770 nm) channel using 605/35nm excitation

Available variants and fusions

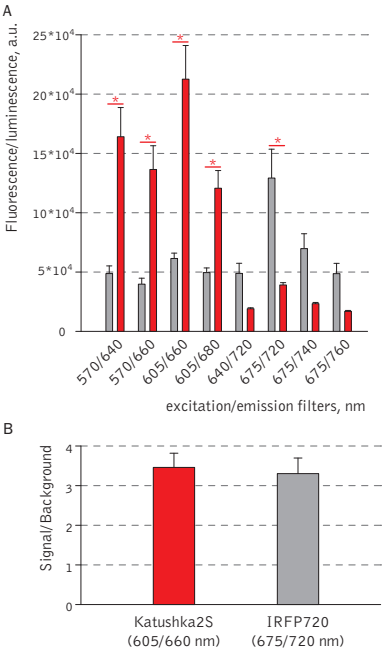
Katushka2S mammalian expression vectors contain Katushka2S coding sequence with codon usage optimized for high expression in mammalian cells, i.e. humanized [Haas et al. 1996]. Humanized Katushka2S can also be expressed in *E. coli* and some other heterological systems upon subcloning into appropriate vector.

The available vectors encoding Katushka2S variants and fusions are listed below in the section Katushka2S-related products. For most updated product information, please visit Evrogen website www.evrogen.com.

If you need Katushka2S 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 Katushka2S 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 signals acquired with IVIS Spectrum system (PerkinElmer). Fluorescence signals normalized to corresponding firefly luciferase luminescence in mice injected in backs with HEK293FT cells transiently expressing Katushka2S or iRFP70.

(A) Pairwise comparisons of the signals from Katushka2S (red) and iRFP720 (grey) at different excitation/emission wavelengths. * p<0.0005

(B) Signal-to-noise ratios at optimal excitation and emission wavelengths for each protein.

References

Gurskaya, NG. et al. (2011). Bioorg Khim, 37 (3): 425–428 / pmid: 21899059

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

Leblond, F. et al. (2010). J Photochem Photobiol B, 98 (1): 77–94 / pmid: 20031443

Luker, KE. et al. (2015). Sci Rep, 5: 10332 / pmid: 26035795

Shcherbo, D. et al. (2009). Biochemical Journal, 418 (3): 567–574 / pmid: 19143658

Katushka2S-related products

Product	Cat.#	Description	Size
Katushka2S expression/source vectors			
pKatushka2S-C	FP761	Mammalian expression vector encoding humanized Katushka2S and allowing its expression and generation of fusions to the Katushka2S C-terminus	20 μg
pKatushka2S-N	FP762	Mammalian expression vector encoding humanized Katushka2S and allowing its expression and generation of fusions to the Katushka2S N-terminus	20 μg
pKatushka2S-B	FP763	Bacterial expression vector; source of the Katushka2S coding sequence	20 μg

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Product	Cat.#	Description	Size
Antibodies against Katushka2S			
Anti-tRFP	AB233	Rabbit polyclonal antibody against TurboRFP, TurboFP602, TurboFP635, Katushka2S, TurboFP650, NirFP, TagBFP, TagRFP, FusionRed, TagFP635, mKate2 and PA-TagRFP	100 μg

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