Far-red fluorescent protein mKate2

- Super bright far-red fluorescence
- Monomeric protein with successful performance in fusions
- Fast maturation, high pH-stability and photostability, low cytotoxicity
- Proven suitability to generate stably transfected cell lines
- Fluorescent signal is easily distinguished from background fluorescence
- Recommended for protein labeling, multicolor applications and whole body imaging

mKate2 is the next generation of far-red fluorescent protein TagFP635 (mKate) [D Shcherbo et al. 2007; D. Shcherbo et al. 2009]. Possessing fluorescence with excitation/emission maxima at 588 and 633 nm, mKate2 is almost 3-fold brighter than TagFP635 and is 10-fold brighter than mPlum at physiological pH 7.5. Within the optical window optimal for light penetration in living tissues, calculated brightness of mKate2 is at least 2-fold higher compared to any monomeric fluorescent protein reported to date.

mKate2 is characterized by complete and fast chromophore maturation at 37°C with maturation half-time <20 min (versus 40 min for mCherry). It is more photostable under both widefield and confocal illumination than other monomeric far-red proteins, including TagFP635, mRaspberry and mPlum. The high brightness, far-red emission spectrum, excellent pH resistance and photostability, coupled with low toxicity demonstrated in transgenic *Xenopus* laevis embryos, make mKate2 a superior fluorescent tag for imaging in living tissues.

mKate2 is mainly intended for protein labeling. Its far-red fluorescence allows easy and reliable separation from standard green fluorescent labels in dual-color high-throughput assays.

Main properties of mKate2

Characteristic	
Molecular weight, kDa	26
Polypeptide length, aa	232
Fluorescence color	far-red
Excitation maximum, nm	588
Emission maximum, nm	633
Quantum yield	0.40
Extinction coefficient, $M^{-1}cm^{-1}$	62 500
Brightness*	25.0
Brightness, % of EGFP	74
pKa	5.4
Structure	monomer
Aggregation	no
Maturation rate at 37°C	fast
Maturation half-time, min	<20
Photostability	high
Photostability, widefield**	69
Photostability, confocal**	390
Cell toxicity	not observed

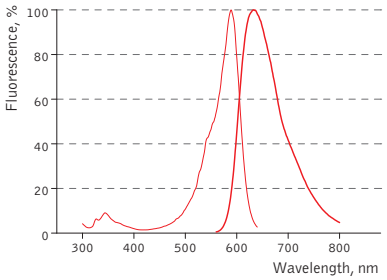
* Brightness is a product of extinction coefficient and quantum yield, divided by 1 000.

** Time to bleach 50% of fluorescent signal brightness.

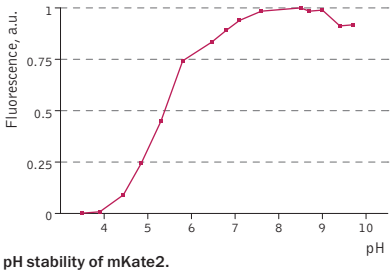
Extinction coefficient and quantum yield were measured at the physiological pH=7.5.

Performance and use

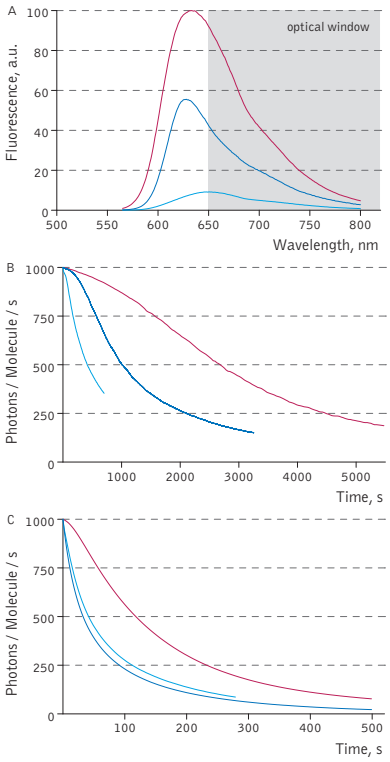
mKate2 can be easily expressed and detected in a wide range of organisms. Mammalian cells transiently transfected with mKate2 expression vectors produce bright fluorescence in 10-12 hrs after transfection. No cytotoxic effects or visible protein aggregation are observed.



mKate2 normalized excitation (thin line) and emission (thick line) spectra.
Complete mKate2 spectra in Excel format can be downloaded from the Evrogen Web site at http://www.evrogen.com



pH stability of mKate2.

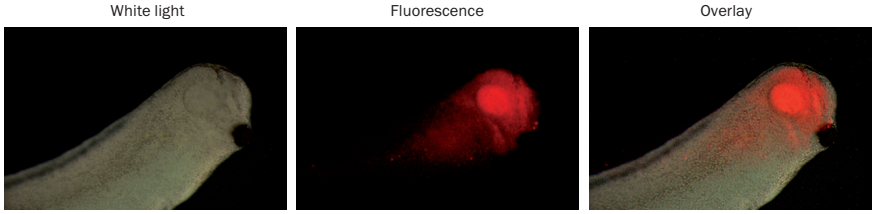


Spectral characteristics of mKate2 in comparison with selected fluorescent proteins. mKate2 - dark-red line, mRaspberry - blue line, mPlum - cyan line
(A) Emission spectra of far-red monomeric fluorescent proteins given proportionally to their calculated brightness. Scaling was applied to the area of the peak. Favorable "optical window" is shaded with gray. (B) Normalized photobleaching curves for far-red monomeric fluorescent proteins, laser scanning confocal microscopy. (C) Normalized photobleaching curves, widefield fluorescence microscopy under metal halide illumination.

mKate2 performance in fusions has been demonstrated in α-actinin, zyxin, β-actin, α-tubulin, and other models.

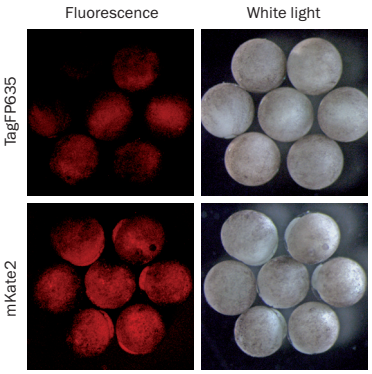
mKate2 is the superior fluorescent monomeric tag for imaging in living tissues. It has emission maximum at 635 nm optimal for deep imaging of animal tissues, and is more bright, photostable and pH-stable than other cloned far-red monomeric fluorescent proteins.

To verify the low toxicity of mKate2 in transgenic animals, mKate2 was expressed under the control of Xanf1 promoter in *Xenopus* laevis embryos. As expected, bright red fluorescence in the forehead region, including eyes, the forebrain and nasal placodes was observed.



Imaging of mKate2 in *Xenopus laevis* embryos. Expression of mKate2 under the control of Xanf1 promoter in the transgenic embryos at stage 28 is specifically localized in the forehead region, including eyes, the forebrain and nasal placodes. The embryo is shown from the right side, dorsal to the top and left. Data courtesy of Dr. A. Zaraisky, Institute of Bioorganic Chemistry, RAS (Moscow, Russia).

To compare brightness and maturation rate of TagFP635 and mKate2 *in vivo*, *Xenopus* laevis embryos were microinjected with pTagFP635-N and pmKate2-N vectors at the stage of two blastomeres. Living embryos were photographed from the animal pole side at the middle gastrula stages (10.5 hours after fertilization). As expected, embryos microinjected with pmKate2-N demonstrated superior brightness.



***In vivo* comparison of TagFP635 and mKate2 in developing *Xenopus laevis* embryos.**
Data courtesy of Dr. A. Zaraisky, Institute of Bioorganic Chemistry, RAS (Moscow, Russia).

In addition, to test in an embryonic model the performance of mKate2 in a targeting protein fusion, we generated transgenic *Xenopus* laevis embryos bearing a CMV-mKate2-zyxin fusion construct. Despite quite extensive and ubiquitous expression of mKate2-zyxin under the control of the CMV promoter, these embryos appear normal and healthy indicating that mKate2 exerts a low toxic effect on living cells in transgenic organisms. mKate2 can be used in multicolor labeling applications with blue, cyan, green, yellow, and red (orange) fluorescent dyes. High pH-stability with pKa=5.4 makes it possible to use mKate2 for imaging in acidic organelles, such as late and recycling endosomes and lysosomes.

Recommended filter sets and antibodies

mKate2 can be recognized using Anti-tRFP antibody (Cat.# AB233) available from Evrogen. Recommended Omega Optical filter sets are QMAX-Red and XF102-2. mKate2 can also be detected using Texas Red filter sets or similar.

Available variants and fusions

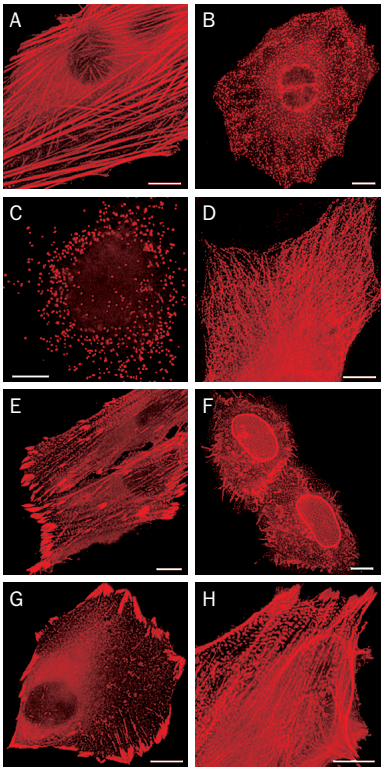
mKate2 mammalian expression vectors contain mKate2 coding sequence with codon usage optimized for high expression in mammalian cells, i.e. humanized [Haas, Park, and Seed 1996]. Humanized mKate2 can also be expressed in *E. coli* and some other heterological systems upon subcloning into appropriate vector.

The available vectors encoding mKate2 variants and fusions are listed below in the section mKate2-related products. For most updated product information, please visit Evrogen website www.evrogen.com.

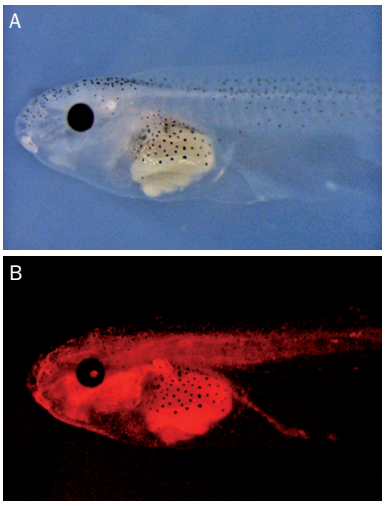
If you need mKate2 codon variant or fusion construct that is not listed on our website, please contact us at product@evrogen.com.

Licensing opportunities

Evrogen technology embodied in mKate2 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.



mKate2 use for protein labeling in mammalian cells. (A) β-actin; (B) clathrin; (C) peroxisomes; (D) α-tubulin; (E) VASP; (F) annexin (A4); (G) paxillin; (H) α-actinin. Scale bar represents 10 μm. Images from D. Shcherbo et al. 2009.



Expression of mKate2-zyxin in *Xenopus laevis* embryos. (A) White light; (B) Fluorescence. Data courtesy of Dr. A. Zaraisky, Institute of Bioorganic Chemistry, RAS (Moscow, Russia).

References

Haas, J., E. C. Park, and B. Seed (1996). Curr Biol, 6 (3): 315–324 / pmid: 8805248

Shcherbo, D et al. (2007). Nat Methods, 4 (9): 741–746 / pmid: 17721542

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

mKate2-related products

Product	Cat.#	Description	Size
mKate2 expression/source vectors			
pmKate2-C	FP181	Mammalian expression vector encoding humanized mKate2 and allowing its expression and generation of fusions to the mKate2 C-terminus	20 µg
pmKate2-N	FP182	Mammalian expression vector encoding humanized mKate2 and allowing its expression and generation of fusions to the mKate2 N-terminus	20 µg
pmKate2-actin	FP184	Mammalian expression vector encoding humanized mKate2 fused with human cytoplasmic β-actin	20 µg
pmKate2-f-mem	FP186	Mammalian expression vector encoding membrane-targeted mKate2	20 µg
pmKate2-mito	FP187	Mammalian expression vector encoding humanized mKate2 targeted to mitochondria	20 µg
pmKate2-H2B	FP311	Mammalian expression vector encoding humanized mKate2 fused with human histone H2B	20 µg
pmKate2-lyso	FP312	Mammalian expression vector encoding humanized mKate2 targeted to lysosomes	20 µg
pmKate2-peroxi	FP313	Mammalian expression vector encoding humanized mKate2 targeted to peroxisomes	20 µg
pmKate2-endo	FP314	Mammalian expression vector encoding humanized mKate2 fused with human RhoB protein	20 µg
pmKate2-EB3	FP316	Mammalian expression vector encoding humanized mKate2 fused with human EB3 protein	20 µg
pmKate2-annexin	FP321	Mammalian expression vector encoding humanized mKate2 fused with human annexin	20 µg
pmKate2-clathrin	FP322	Mammalian expression vector encoding humanized mKate2 fused with human clathrin LCB	20 µg
pmKate2-paxillin	FP323	Mammalian expression vector encoding humanized mKate2 fused with chicken paxillin	20 µg
pmKate2-ER	FP324	Mammalian expression vector encoding humanized mKate2 targeted to the endoplasmic reticulum	20 µg
Antibodies against mKate2			
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.