# Red fluorescent protein FusionRed

- Superior performance in fusions
- Low cytotoxicity
- Fast maturation, high pH-stability and photostability
- Proven suitability to generate stably transfected cell lines
- Recommended for protein labeling and long-term experiments

FusionRed is a red fluorescent protein characterized by improved performance in fusions and low toxicity [Shemiakina et al. 2012]. FusionRed lacks the residual tendency of other monomeric RFPs to dimerize at high concentration and behaves as a pure monomer at concentrations up to 10 mg/ml in HPLC analysis (Fig.2). Such "supermonomeric" properties ensure superior efficiency of FusionRed in protein labeling applications, especially in the cells with high expression level. Similarly to parental mKate2, FusionRed demonstrates fast maturation rate, high pH-stability and photostability, and significantly lower cytotoxicity than widely used mCherry [Shaner et al. 2004] and mRuby [Kredel et al. 2009].
FusionRed is mainly intended for protein labeling and long-term experiments including generation of transgenic animals.

**Main properties of FusionRed**

| Characteristic | |
|---|---|
| Molecular weight, kDa | 26 |
| Polypeptide length, aa | 232 |
| Fluorescence color | red |
| Excitation maximum, nm | 580 |
| Emission maximum, nm | 608 |
| Quantum yield | 0.19 |
| Extinction coefficient, $M^{-1}cm^{-1}$ | 94 500 |
| Brightness* | 18.0 |
| Brightness, % of EGFP | 53 |
| pKa | 4.6 |
| Structure | supermonomer** |
| Aggregation | no |
| Maturation half-time, min | 130 |
| Photostability, widefield*** | 150 |
| Photostability, confocal*** | 176 |
| Cell toxicity | not observed |

* Brightness is a product of extinction coefficient and quantum yield, divided by 1 000.
** Purified recombinant protein behaves as a pure monomer at concentrations of 10 mg/ml as verified by high-performance liquid chromatography (HPLC)
*** Time to bleach 50% of fluorescent signal brightness.

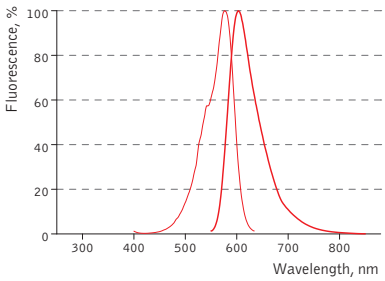



**Figure 1. FusionRed normalized excitation (thin line) and emission (thick line) spectra.**
Complete FusionRed spectra in Excel format can be downloaded from the Evrogen Web site at http://www.evrogen.com

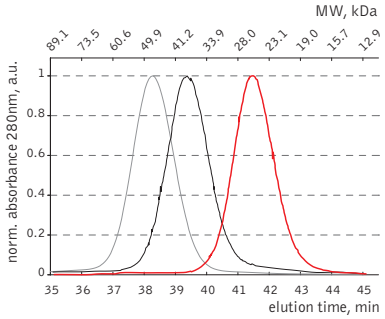



**Figure 2. HPLC analysis of FusionRed in comparison with selected fluorescent proteins loaded in a high concentration.**
FusionRed - red line, mKate2 - black line, mNeptune - gray line. Proteins were loaded at a concentration of 10 mg/ml. While HPLC demonstrates reversible dimerization of mKate2 and reveals a dimeric character for the mKate derivative, far-red fluorescent protein mNeptune [Lin et al. 2009], FusionRed behaves as a pure monomer. Data from Shemiakina et al. 2012.

### Performance and use

FusionRed can be easily expressed and detected in a wide range of organisms. Mammalian cells transiently transfected with FusionRed expression vectors produce bright fluorescence in 10-12 hours after transfection. FusionRed performance in fusions has been demonstrated in a number of models.

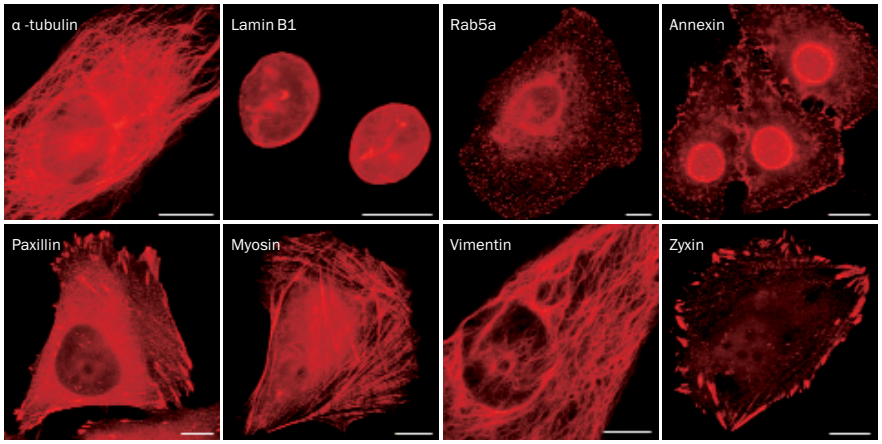



**Figure 3. FusionRed use for protein labeling in mammalian cells.** Scale bar represents 10 μ m. Images from Shemiakina et al. 2012.

### Labeling efficiency testing

The performance of FusionRed and mCherry was directly compared in HeLa CCL2 cells for the following targets: connexin-43, endosomes, vinculin, and the Golgi complex. In this experiment, the cells were transfected with each of the four target fusions, fixed, and then compared for localization efficiency, which was determined by calculating the percentage of properly expressing cells versus the total number of transfected cells. FusionRed demonstrated clear advantage in all four fusions.

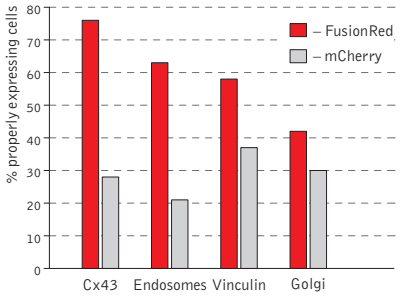



**Figure 4. FusionRed and mCherry labeling efficiency testing.** Data from Shemiakina et al. 2012

### Cytotoxicity testing in HeLa cells

The cytotoxicity of FusionRed relative to selected red fluorescent proteins and EGFP was evaluated in the following experiment: HeLa cells were transfected with appropriate vectors encoding EGFP or one of the following red fluorescent proteins: mRuby, FusionRed, mKate2 or mCherry. Next, the EGFP-expressing cells were mixed with those expressing one of the RFPs, resulting in 4 separate cell mixtures: EGFP and mRuby, EGFP and FusionRed, EGFP and mKate2, and EGFP and mCherry. 48 hours after transfection, the green-to-red cell ratios were calculated utilizing flow cytometry and each of the cell mixtures were then plated into 3 plates. After additional 92 hour incubation, the green-to-red cell ratios were recalculated. Because only living cells were counted for this experiment, the difference between the ratios before and after the incubation can be assumed to accurately reflect RFP toxicity versus EGFP. mRuby exhibited a more than 10-fold higher cytotoxicity level compared to EGFP, while the remaining RFPs were almost as cytotoxic as EGFP in this experiment (Fig. 5).

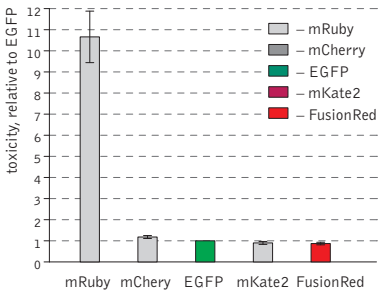
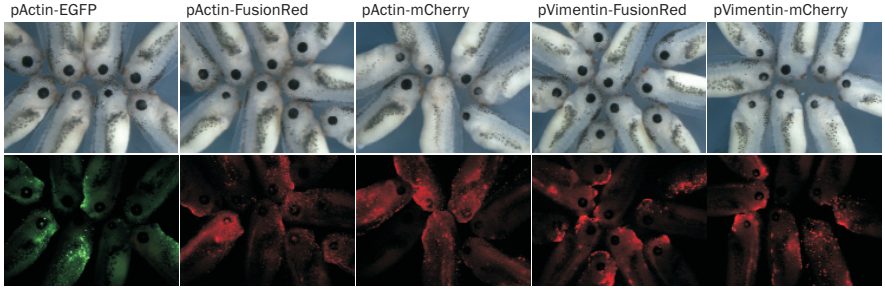



**Figure 5. Protein cytotoxicity testing in HeLa cells.** Data from Shemiakina et al. 2012

### Cytotoxicity testing in *Xenopus* laevis tadpoles

The eye formation is a complex multistage process that depends on many mechanisms, any disturbance of which may result in an abnormal phenotype. Here eye development was used as a sensitive readout of possible abnormal course of embryogenesis caused by expression of EGFP, FusionRed, and mCherry fusions (Fig. 6). In these experiments, actin and vimentin fusions of EGFP and FusionRed were only slightly more toxic than the uninjected controls, while expression of mCherry fusions led to notable decrease of the average eye size. Moreover, in many cases (80% of tadpoles with the reduced eye size), the reduced eye phenotype was accompanied by varying degree of an unclosed optic fissure, indicating an abnormal eye development (Fig. 7).




Stage 41 Xenopus laevis tadpoles expressing fluorescent protein fusions. Top panels: white light; bottom panels: fluorescence. Data from Shemiakina et al. 2012.

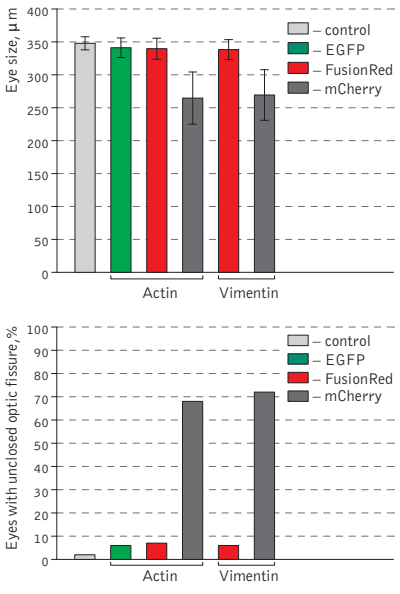



**Figure 7.** Top graph: Mean values of eye size of Xenopus laevis embryos injected with plasmids expressing different fluorescent protein fusions.
Bottom graph: Percentage of reduced eyes with unclosed optic fissure. Data from Shemiakina et al. 2012

### Long-term expression

The excellent suitability of FusionRed for long-term experiments was proved both in experiments with stably transfected cell lines and in transgenic animals. In addition to its low cytotoxicity, FusionRed does not show abnormal lysosomal localization typical for many fluorescent proteins in long-term expression.

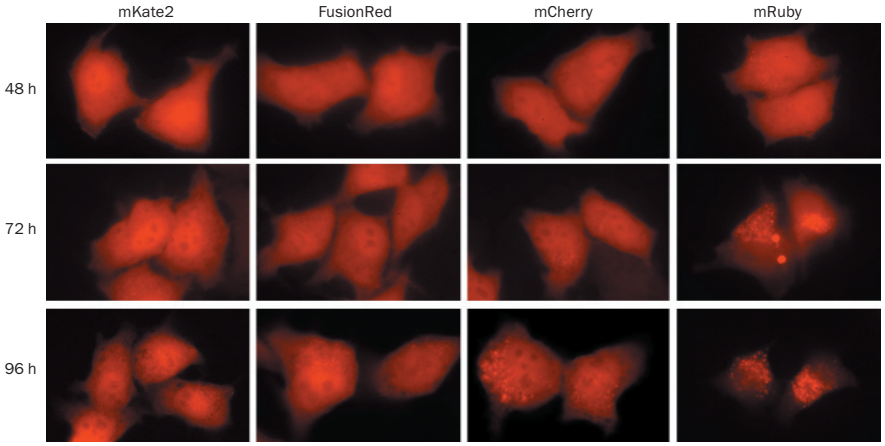



**Figure 8.** Being expressed in HeLa cells, both mKate2 and FusionRed remain evenly localized in cytoplasm after 96 hours of cultivation, while mCherry and mRuby show abnormal lysosomal localization. Data from Shemiakina et al. 2012.

### Recommended filter sets and antibodies

FusionRed can be recognized using Anti-tRFP antibody (Cat.# AB233-AB234) available from Evrogen.

FusionRed can be detected using TRITC filter set or similar. Recommended Omega Optical filter sets are QMAX-Red and XF102-2.

### Available variants and fusions

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

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

If you need FusionRed 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 FusionRed 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., E. C. Park, and B. Seed (1996). Curr Biol, 6 (3): 315–324 / pmid: 8805248

Kredel, S. et al. (2009). PLoS One, 4 (2): e4391 / pmid: 19194514

Lin, MZ et al. (2009). Chem Biol, 16 (11): 1169–79 / pmid: 19942140

Shaner, NC et al. (2004). Nat Biotechnol, 22 (12): 1567–1572 / pmid: 15558047

Shemiakina, II. et al. (2012). Nat Commun, 3: 1204 / pmid: 23149748


### FusionRed-related products

| Product | Cat.# | Description | Size |
|---|---|---|---|
| FusionRed expression/source vectors | | | |
| pFusionRed-C | FP411 | Mammalian expression vector encoding humanized FusionRed and allowing its expression and generation of fusions to the FusionRed C-terminus | 20 μg |
| pFusionRed-N | FP412 | Mammalian expression vector encoding humanized FusionRed and allowing its expression and generation of fusions to the FusionRed N-terminus | 20 μg |
| pFusionRed-CD151 | FP415 | Mammalian expression vector encoding humanized FusionRed fused with human CD151 | 20 μg |
| pFusionRed-f-mem | FP418 | Mammalian expression vector encoding membrane-targeted FusionRed | 20 μg |
| pFusionRed-Golgi | FP419 | Mammalian expression vector encoding humanized FusionRed fused with human Golgi targeting sequence (GTS) | 20 μg |
| pFusionRed-ER | FP420 | Mammalian expression vector encoding humanized FusionRed targeted to the endoplasmic reticulum | 20 μg |

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| Product | Cat.# | Description | Size |
|---|---|---|---|
| pFusionRed-H2B | FP421 | Mammalian expression vector encoding humanized FusionRed fused with human histone H2B | 20 µg |
| pFusionRed-endo | FP427 | Mammalian expression vector encoding humanized FusionRed fused with human RhoB protein | 20 µg |
| pFusionRed-MAP4 | FP428 | Mammalian expression vector encoding humanized FusionRed fused with microtubule binding domain of mouse microtubule-associated protein 4 (MAP4) | 20 µg |
| pFusionRed-PDHA1 | FP430 | Mammalian expression vector encoding humanized FusionRed fused with human pyrovate dehydrogenase (lipoamide) α 1 (PDHA1) | 20 µg |
| pFusionRed-Rab5a | FP431 | Mammalian expression vector encoding humanized FusionRed fused with human Ras-related protein Rab-5A | 20 µg |
| pFusionRed-tubulin | FP433 | Mammalian expression vector encoding humanized FusionRed fused with human α-tubulin | 20 µg |
| pFusionRed-cadherin | FP434 | Mammalian expression vector encoding humanized FusionRed fused with human VE-cadherin | 20 µg |
| pFusionRed-CDC42 | FP435 | Mammalian expression vector encoding humanized FusionRed fused with human CDC42 | 20 µg |
| pFusionRed-B | FP438 | Bacterial expression vector; source of the FusionRed coding sequence | 20 µg |
| Antibodies against FusionRed | | | |
| 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.