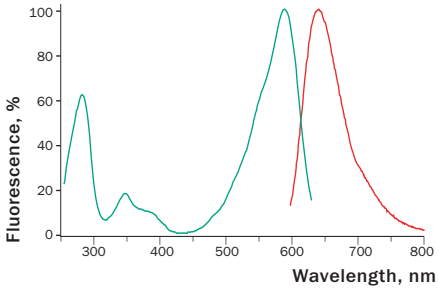
Far-red fluorescent proteins t-HcRed

- Far-red fluorescence
- Suitability to generate fusions

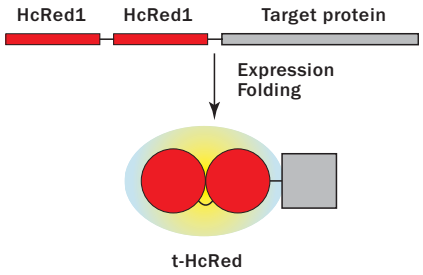
Description

Far-red fluorescent mutant HcRed was generated on the basis of chromoprotein hcriCP from sea anemone *Heteractis crispa* by random and direct mutagenesis (Gurskaya *et al.*, 2001). Tetrameric HcRed protein was then transformed into a dimeric HcRed1 mutant characterized by unique red-shifted emission spectra. To improve HcRed1 for protein labeling we generated its tandem variant t-HcRed. t-HcRed comprises two head-to-tail linked identical HcRed1 molecules. Since HcRed1 is a dimeric protein, its two covalently linked monomers form an intramolecular dimer, which can be used as a non-oligomerizing tag to be fused with proteins of interest (Fradkov *et al.*, 2002).

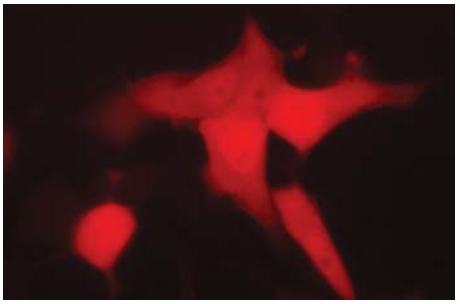
t-HcRed is a convenient reporter gene for multicolor labeling. Owing to t-HcRed is characterized by far-red fluorescence spectra, it is a suitable for tetra-color labeling as a distinct far-red color. In combination with color variants of GFP, t-HcRed can be used in fluorescence resonance energy transfer (FRET) -based techniques (Verkhusha and Lukyanov, 2004).



t-HcRed normalized excitation (green line) and emission (red line) spectra.



Generation of t-HcRed-tagged fusions.



293T cells transiently transfected with a plasmid carrying t-HcRed gene.

Main properties of t-HcRed

Characteristic	
Molecular weight,	53 kDa
Polypeptide length	469 aa
Fluorescence color	far-red
Excitation max	590 nm
Emission max	637 nm
Quantum yield	0.04
Extinction coefficient	160 000 $M^{-1}cm^{-1}$
Brightness*	6.4
Structure	monomer (intramolecular dimer)
Aggregation	no
Maturation at 37°C	slow
Photostability	medium

*Brightness is a product of extinction coefficient and quantum yield, divided by 1000.

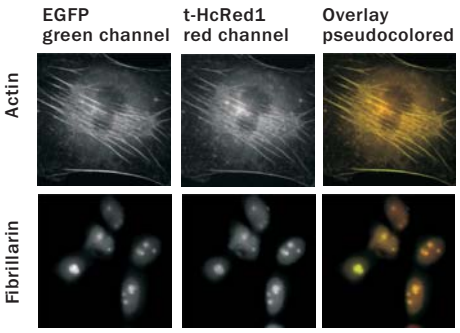
Performance and use

t-HcRed can be expressed in eukaryotic and prokaryotic cells. Mammalian cells transiently transfected with t-HcRed vector give far-red fluorescence without visible aggregation. Fluorescence is clearly detected within 24-48 hrs after transfection.

t-HcRed demonstrates successful performance in fusions with subcellular localization signals and many cellular proteins including BH3 interacting domain death agonist (BID), nucleolar protein fibrillarin, beta-acin. However, t-HcRed molecule is about two times bigger than other fluorescent tags. This may affect some t-HcRed -tagged fusions.

Recommended filter sets

Recommended Omega Optical filter sets for t-HcRed are QMAX-Red and XF102-2.



Fluorescence microscopy of cells expressing FP-tagged actin or fibrillarin.

Cells (L929 or HEK239 with actin and fibrillarin labeling, respectively) were simultaneously co-transfected with EGFP-labeled and t-HcRed-labeled targets and analysed in green and red channels.

References

Gurskaya et al., GFP-like chromoproteins as a source of far-red fluorescent proteins. FEBS Lett. 2001, 507, 16-20.

Fradkov et al., Far-red fluorescent tag for protein labeling. Biochem J. 2002, 368, 17-21.

Verkhusha VV, Lukyanov KA. The molecular properties and applications of Anthozoa fluorescent proteins and chromoproteins. Nat Biotechnol. 2004, 22(3):289-296.

Notice to Purchaser:
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