2010 – 2011

Basic Fluorescent Proteins

Photoactivatable Fluorescent Proteins

Fluorescent Biosensors

Photosensitizer

Antibodies

Nucleic Acid Research Kits

Molecular Biology Services

evrogen

Mission

Evrogen is an innovation-driven company founded by scientists dedicated to making successful ideas widely available as efficient products and services for life science research. Established in 2000 and constantly growing, **Evrogen** invents, develops and offers novel technologies and means in the field of molecular and cell biology.

Scientists ourselves, we aim at maintaining quality and efficiency of our solutions and providing all our expertise to meet customer needs.

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Contents

Basic Fluorescent Proteins

Eight available colors for *in vivo* labeling, from blue to far-red

Easy detection by flow cytometry or fluorescent microscopy

No cofactors, substrate addition or chemical staining required

Special optimization for different applications, including:

- Labeling of cells, cell organelles and proteins of interest
- Gene expression analysis
- Multicolor labeling
- FRET-based studies of protein interaction
- Whole body imaging

Easy evaluation and adaptable license program for commercial use

Contents

Overview

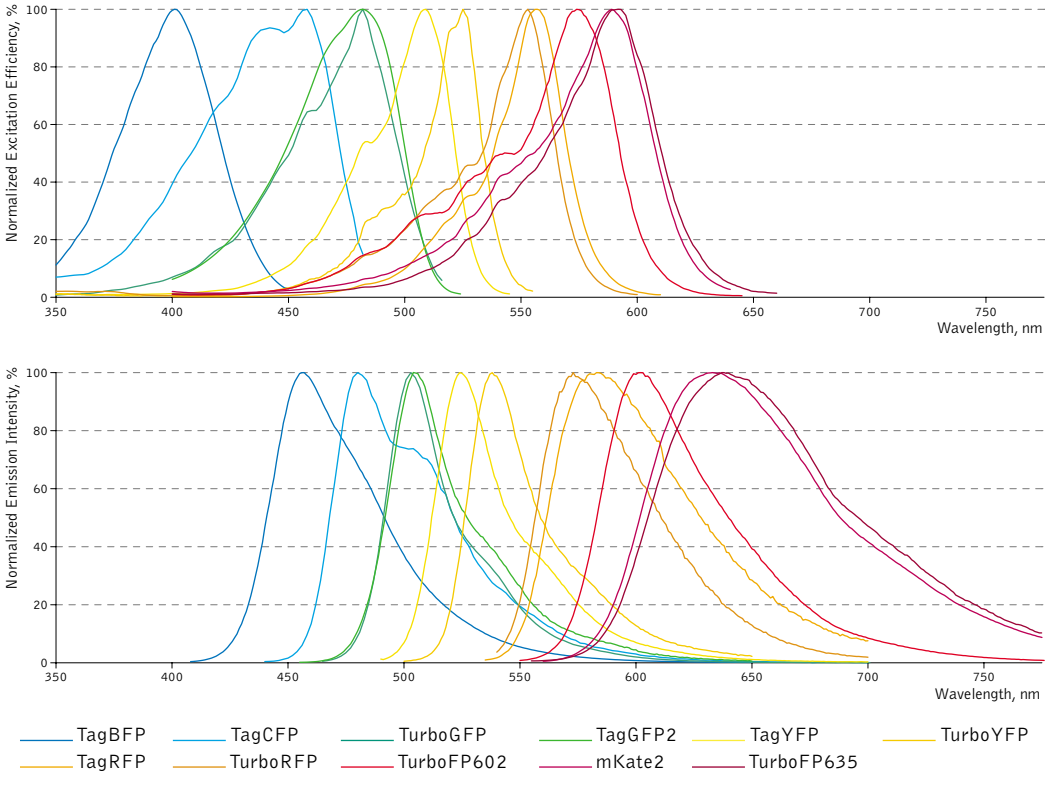
Evrogen offers a collection of bright fluorescent proteins (FPs) for wide range of applications in the field of live-cell assays. Evrogen fluorescent proteins can be used for *in vivo* protein localization and interaction studies; analysis of promoter activity in live cells; tracking subcellular organelles; labeling to identify and isolate specific populations of cells; generation of stably transfected cell lines, and more. Ranging in color from blue to far-red, Evrogen FPs allow visualization of multiple events simultaneously by both fluorescent microscopy and flow-cytometry.

All Evrogen FPs are improved by mutagenesis and codon usage optimization for high expression level in mammalian cells and fast maturation at 37°C. Variants with codon usage optimized for expression in other heterological systems are available or can be generated by request. Evrogen FPs have been used successfully in prokaryotes, yeasts, animals, and plants. The proteins possess bright stable fluorescence allowing monitoring of target cells or proteins over extended period of time. No addition of cofactors or substrates is required for FP detection.

Evrogen technologies embodied in basic FPs are available for expanded and commercial use with an adaptable licensing program.

Whole body imaging in transgenic *Xenopus laevis* using Evrogen far-red fluorescent protein TurboFP635. Photographs were kindly provided by Dr. A. Zaraisky (Institute of Bioorganic Chemistry RAS, Moscow, Russia).

Spectral diversity of Evrogen basic FPs



Basic fluorescent proteins available

Protein	Color	Ex/Em, nm	Brightness, % of EGFP	pKa	Photostability	Structure	M.W., kDa	Filter Sets
TagFPs								
TagBFP	blue	402/457	99	2.7	high	monomer	26	Omega XF119-2, QMAX-Blue; Semrock DAPI-5060B
TagCFP	cyan	458/480	64	4.7	high	monomer	27	common sets for ECFP, e.g. Omega XF114-2 and XF130-2
TagGFP2	green	483/506	105	5.0	high	monomer	27	common sets for EGFP, FITC, e.g. Omega QMAX-Green, XF100-2, XF100-3, XF115-2, and XF116-2
TagYFP	yellow	508/524	94	5.5	high	monomer	27	Omega XF104-3, XF105-2; C.T. 41028 Yellow GFP BP (10C/Topaz)
TagRFP	red (orange)	555/584	148	3.8	medium	monomer	27	TRITC filter sets, e.g. Omega QMAX-Yellow, XF108-2, XF101-2, XF111-2
mKate2	far red	588/633	74	5.4	high	monomer	26	Texas Red filter sets, e.g. Omega QMAX-Red, XF102-2
TurboFPs								
TurboGFP	green	482/502	112	5.2	high	dimer	26	common sets for EGFP, FITC, e.g. Omega QMAX-Green, XF100-2, XF100-3, XF115-2, and XF116-2
TurboYFP	yellow	525/538	169	5.9	high	dimer	26	Omega XF104-3; C.T. 42003 (ZsYellow1)
TurboRFP	red (orange)	553/574	187	4.4	high	dimer	26	TRITC filter sets, e.g. Omega QMAX-Yellow, XF108-2, XF101-2, XF111-2
TurboFP602	red	574/602	79	4.7	medium	dimer	26	Omega QMAX-Red
TurboFP635	far red	588/635	67	5.5	high	dimer	26	Texas Red filter sets, e.g. Omega QMAX-Red, XF102-2

M.W. - molecular weight; Ex/Em - excitation/emission maxima

C.T. - Chroma Technology Corp. (www.chroma.com); Omega - Omega Optical (www.omegafilters.com); Semrock (www.semrock.com)

Evrogen FPs are divided into subgroups according to their properties and recommended applications:

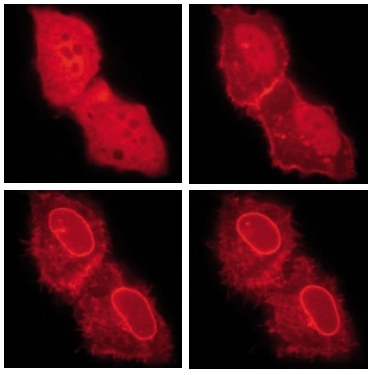
TagFPs (see page 11)

The group comprises monomeric fluorescent proteins specially optimized for protein localization and interaction studies. Successful TagFPs performance in protein labeling applications was validated in various models including highly oligomerizing cellular proteins like α-tubulin, β-actin, vinculin, zyxin, etc.

TurboFPs (see page 26)

The group comprises dimeric fluorescent proteins specially recommended for applications requiring fast appearance of bright fluorescence, including cell labeling and tracking promoter activity. Despite their dimeric structure, TurboFPs can be fused with subcellular localization signals for labeling of cellular organelles.

Evrogen offers various source and expression vectors encoding fluorescent proteins alone or in fusion with cellular proteins and localization signals. All available vector types are described in details in the section "Expression and source vectors" on page 39. Custom optimization of Evrogen vectors and proteins for your particular needs is available upon request (please see page 135 for details).



Spinning disk confocal imaging of mKate2 fused to human annexin A4 in HeLa cells during translocation from the cytoplasm to the plasma and nuclear membranes upon induction with ionomycin.

Performance of basic fluorescent proteins in different applications

Protein	Cell labeling	Fusion generation	Promoter activity testing	Whole body imaging	Acidic organelle labeling	Generation of stably transfected cell lines
TagFPs						
TagBFP	+++	++++	++++	-	++++	not tested
TagCFP	+++	+++	+++	-	+++	proved
TagGFP2	++++	++++	++++	+	++	proved**
TagYFP	+++	+++	+++	+	++	proved
TagRFP	++++	++++	+++	+++	++++	proved
mKate2	+++	++++	++++	++++	++	proved**
TurboFPs						
TurboGFP	++++	+	++++	+	++	proved
TurboYFP	++++	++	++++	+	+	not tested*
TurboRFP	++++	+	++++	+++	+++	not tested
TurboFP602	+++	+	+++	+++	+++	proved
TurboFP635	+++	+	++++	++++	++	proved

The performance is estimated basing on reporters properties. Not all reporters have been tested experimentally in each application.

* Being overexpressed in long-term culture of cells with high expression levels, TurboYFP shows slight tendency to aggregate. It might limit TurboYFP use in such experimental systems. Please use PhiYFP or PhiYFPm proteins for stable expression (see page 30)

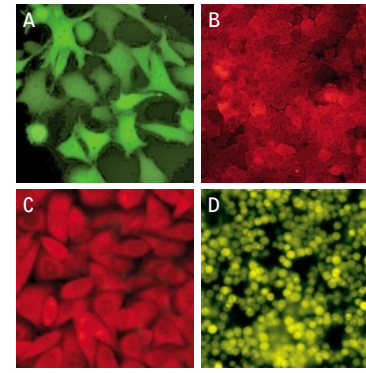
** The suitability for stable cell lines generation was proved for TagGFP and TagFP635, the parental variants of TagGFP2 and mKate2.

Cell labeling

Super bright fluorescence and absence of cytotoxic effects make Evrogen fluorescent proteins ideal for labeling living cells. Because of distinctive spectra, Evrogen FPs can be readily multiplexed, i.e. combined for the simultaneous detection of several events in a cell population. All proteins from Evrogen collection are suitable for cell labeling. We recommend TurboFPs since it is not necessary to use monomeric fluorescent proteins for this application, while the dimeric FPs often provide brighter and more stable signal.

Recommended products for cell labeling:

Product(s)	Pages
TurboFPs	26



Cell labeling using Evrogen TurboFPs.
(A) Stably transfected H-TG cells expressing TurboGFP; (B) stably transfected U-2-OS cells expressing TurboFP602; (C) stably transfected T-24 cells expressing TurboFP635; (D) stably transfected W-PY cells expressing PhiYFP*.
Photographs of stably transfected cell lines were kindly provided by Dr. Christian Petzelt (Marinpharm). *PhiYFP is a parental version of TurboYFP.

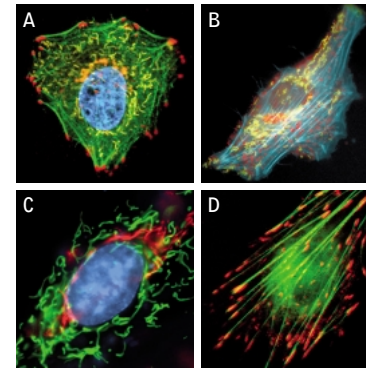
Labeling of cellular proteins and organelles

Monomeric TagFPs are the optimal choice for generation of fusions with proteins and subcellular localization signals. Successful performance of TagFPs in protein labeling applications was validated in various models including highly oligomerizing cellular proteins like cytoplasmic β-actin, α-tubulin, vinculin, zyxin, etc.

Evrogen mammalian expression vectors comprise convenient multiple cloning sites allowing easy generation of fusions of interest. Ready-to-use subcellular localization vectors for fluorescent labeling of various cellular organelles and proteins are available.

Recommended products for labeling of proteins and subcellular structures:

Product(s)	Pages
TagFPs	11
Ready-to use subcellular localization vectors	50



Multicolor labeling of subcellular structures in transiently transfected mammalian cells using Evrogen TagFPs.
(A) TagBFP-histone H2B fusion (blue), TagGFP-actin fusion (green), mitochondria-targeted PhiYFP (yellow), golgi-targeted TagRFP (orange), mKate2-zyxin fusion (red); (B) TagCFP-actin fusion (cyan), mitochondria-targeted PhiYFP (yellow), and mKate2-clathrin fusion (red) in HeLa cells; (C) TagRFP-cytokeratin 14 fusion (red) and mitochondria-targeted TagGFP2 (green) in REF3 cells with Hoechst staining (blue); (D) TagGFP2-actin fusion (green) and mKate2-zyxin fusion (red) in REF52 cells.

Generation of stably transfected cell lines

Most Evrogen fluorescent proteins have been successfully tested in stable transfection experiments. Various cell lines expressing Evrogen FPs are commercially available from Marinpharm GmbH (www.marinpharm.com).

Acidic organelle labeling

Many Evrogen fluorescent proteins are characterized by high pH stability, the most stable are TagBFP (pKa=2.7) and TagRFP (pKa=3.8). The resistance to low pH makes it possible to use these reporters for imaging in acidic organelles, such as late and recycling endosomes and lysosomes.

Recommended products for acidic organelle labeling:

Product(s)	Pages
TagBFP	12
TagRFP	20

FRET applications

Ranging in color from blue to far-red, Evrogen fluorescent proteins can be used in fluorescence resonance energy transfer (FRET) applications as donors and acceptors of fluorescence. TagFPs are perfect for *in vivo* protein interaction studies by FRET due to their improved performance in fusions. TagBFP-TagGFP2 and TagGFP2-TagRFP pairs show the highest FRET efficiency among the tested TagFPs combinations and compare favorably to other available FRET pairs of monomeric fluorescent proteins.

Recommended products for FRET application:

Product(s)	Pages
TagBFP	12
TagGFP2	16
TagRFP	20

Recommended FRET pairs are:

donor, ex/em	acceptor, ex/em
TagBFP ex/em: 402/457	TagGFP2 ex/em: 483/506
TagGFP2 ex/em: 483/506	TagRFP ex/em: 555/584

Monitoring of promoter activity

Early detection of the promoter activity onset requires reporters providing for maximally bright and fast appearing signal. All TurboFPs and some TagFPs (namely TagBFP, TagGFP2, and mKate2) perfectly meet these requirements demonstrating superior brightness and maturation speed. The monitoring of rapid changes in gene regulation can be done using the destabilized TurboFPs variants characterized by short protein half-life.

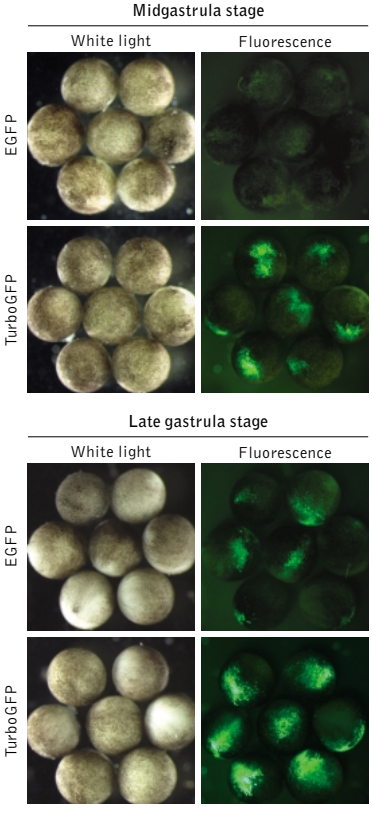
Evrogen offers promoterless vectors encoding unmodified and destabilized TurboFPs. In each vector, multiple cloning sites (MCS) located upstream of the reporter sequence can be used to clone a promoter or a promoter/enchancer combination of interest. Destabilized variants are generated by fusion of residues 422-461 of mouse ornithine decarboxylase (MODC) to the TurboFPs C-termini. MODC region contains a PEST amino acid sequence that targets the protein for degradation and provides for rapid protein turnover [Li et al. 1998]. Destabilized TurboFPs retain fluorescent properties of the native proteins and have a half-life of approximately 1-1.5 hrs, as measured by fluorescence intensity of cells treated with the protein synthesis inhibitor, cycloheximide.

Recommended products for *in vivo* testing promoter activity:

Product(s)	Pages
TurboFPs	26
TagBFP	12
TagGFP2	16
mKate2	23
Promoterless vectors	52

Whole body imaging

Deep tissue imaging using the fluorescent proteins allows direct and non-invasive observation of the biological processes inside the living organisms. Importantly, main photon absorbers within the visual spectrum in animal tissues are melanin and hemoglobin. Wavelengths longer than 1100 nm are absorbed by water. In addition, light-scattering intensity drops off as the wavelength increases.

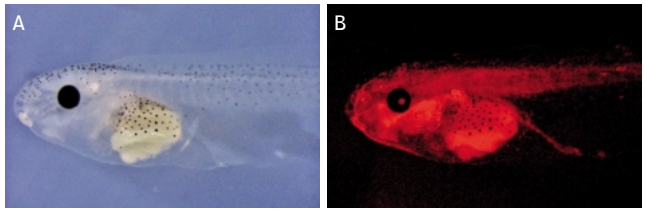


***In vivo* comparison of TurboGFP and EGFP maturation in developing *Xenopus* embryos.**
Vectors expressing the respective fluorescent proteins under the control of CMV promoter were microinjected into animal poles of *Xenopus* embryos at the stage of two blastomeres. Living embryos were then photographed from the animal pole at the middle and late gastrula stages. Experimental data were presented by Dr. A. Zaraisky (Institute of Bioorganic Chemistry RAS, Moscow, Russia).

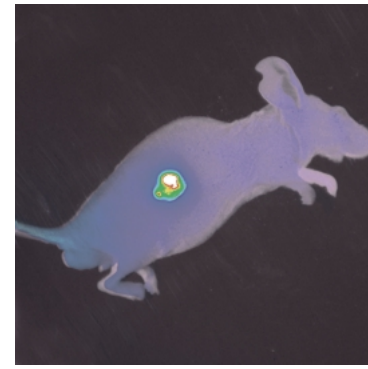
The "optical window" for the visualization in living tissues is approximately 650-1100 nm. Within this optical window, TurboFP635 and mKate2 are the brightest fluorescent proteins available so far. Experimental studies show that the signal coming from TurboFP635 located deep inside tissue is about 45 times stronger than the signal from EGFP and 2 times stronger than the signal from mRaspberry, the closest far-red fluorescent protein tested [Deliolanis et al. 2008]. Together with excellent photostability and fast maturation it makes TurboFP635 and mKate2 the proteins of choice for whole body imaging.

Recommended products for whole body imaging:

Product(s)	Pages
TurboFP635	37
mKate2	23



Expression of mKate2-zyxin in *Xenopus laevis* embryos.
To test in an embryonic model the performance of mKate2 in a targeting protein fusion, transgenic *Xenopus laevis* embryos were generated bearing a mKate2-zyxin fusion construct under the control of the CMV promoter. Despite quite extensive and ubiquitous expression of mKate2-zyxin, these embryos appear normal and healthy indicating that mKate2 exerts a low toxic effect on living cells in transgenic organisms. (A) White light; (B) fluorescence. Images from Shcherbo et al. 2009.



Melanoma implant expressing TurboFP635 in mouse xenograft model.
KODAK *In-Vivo* Imaging System FX. Image was kindly provided by ChemDiv Inc.

REFERENCES

Deliolanis et al. (2008). J. Biomed. Opt., 13 (4): 044008 / pmid: 19021336

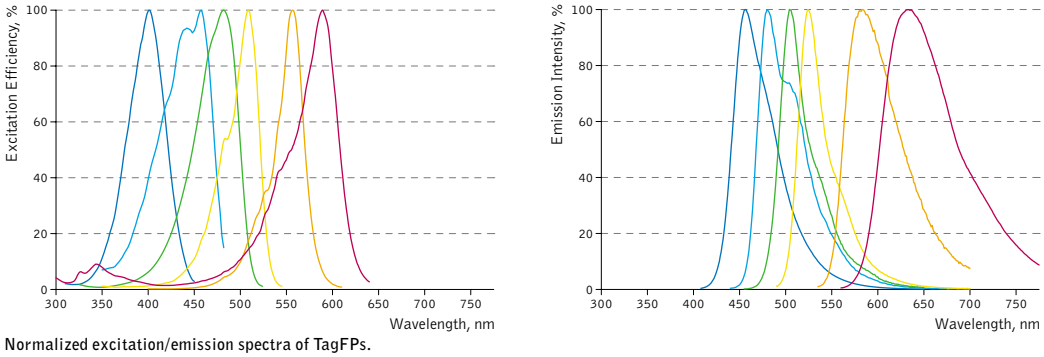
Li et al. (1998). J Biol Chem, 273 (52): 34970–34975 / pmid: 9857028

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

TagFPs

Monomeric tags for protein labeling

TagFPs are monomeric fluorescent proteins specially designed for generation of fusions. Six available colors allow multi-color labeling of different cellular proteins for protein localization and interaction studies.



Normalized excitation/emission spectra of TagFPs.
TagBFP - blue line, TagCFP - cyan line, TagGFP2 - green line, TagYFP - yellow line, TagRFP - orange line, mKate2 - dark-red line.

Main properties of TagFPs:

Protein	TagBFP	TagCFP	TagGFP2	TagYFP	TagRFP	mKate2
Molecular weight, kDa	26	27	27	27	27	26
Polypeptide length, aa	233	239	238	239	237	232
Fluorescence color	blue	cyan	green	yellow	red (orange)	far-red
Excitation maximum, nm	402	458	483	508	555	588
Emission maximum, nm	457	480	506	524	584	633
Quantum yield	0.63	0.57	0.60	0.62	0.48	0.40
Extinction coefficient, $M^{-1}cm^{-1}$	52 000	37 000	56 500	50 000	100 000	62 500
Brightness*	32.8	21.1	33.9	31.0	48.0	25.0
Brightness, % of EGFP	99	64	105	94	148	74
pKa	2.7	4.7	5.0	5.5	3.8	5.4
Structure	monomer	monomer	monomer	monomer	monomer	monomer
Aggregation	no	no	no	no	no	no
Maturation rate at 37°C	fast	fast	fast	fast	fast	fast
Photostability	high	high	high	high	medium	high
Cell toxicity	not observed	not observed	not observed	not observed	not observed	not observed

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

Blue fluorescent protein TagBFP

- Bright blue fluorescence
- Monomeric protein with successful performance in fusions
- Fast maturation, high photostability
- Extremely high pH-stability
- Recommended for protein labeling, acidic organelle labeling, FRET applications

TagBFP (scientific name mTagBFP) is a monomeric blue fluorescent protein generated by site-specific and random mutagenesis of TagRFP [Subach et al. 2008]. TagBFP possesses bright blue fluorescence with excitation/emission maxima at 402 and 457 nm, characterized by high photostability and extremely high pH-stability.

Compared to EBFP2 [Ai et al. 2007], TagBFP is more then 1.8 times brighter, much more pH-stable and has twice shorter maturation half-time at 37°C. Narrow fluorescence emission peak of TagBFP provides for accurate and easy spectral separation with cyan and green fluorescent proteins and makes it a preferable tag for multicolor labeling.

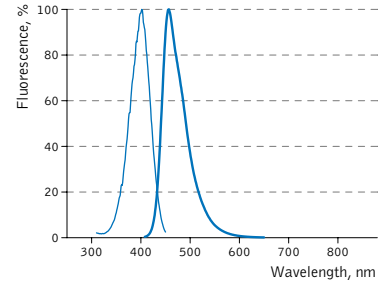
Good overlap between the emission spectrum of TagBFP and the absorbance spectra of TagGFP2 (page 16) allows using these two proteins as a FRET pair. The calculated Forster distance (R_0 = 5.25 nm) for the TagBFP / TagGFP2 pair is larger than those reported for the standard ECFP-EYFP and mCyPet-mYPet pairs (R_0 = 4.86 nm and 4.93 nm correspondingly), suggesting that TagBFP / TagGFP2 is one of the best among available FRET pairs of monomeric fluorescent proteins. High efficiency of TagBFP as a FRET donor was demonstrated in living cells by Subach et al. [Subach et al. 2008].

TagBFP can be easily expressed and detected in a wide range of organisms. Mammalian cells transiently transfected with TagBFP expression vectors

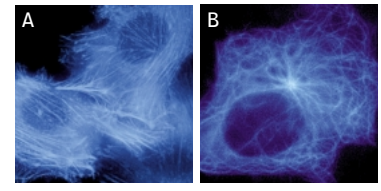
Main properties of TagBFP

Characteristic	
Molecular weight, kDa	26
Polypeptide length, aa	233
Fluorescence color	blue
Excitation maximum, nm	402
Emission maximum, nm	457
Quantum yield	0.63
Extinction coefficient, $M^{-1}cm^{-1}$	52 000
Brightness*	32.8
Brightness, % of EGFP	99
pKa	2.7
Structure	monomer
Aggregation	no
Maturation rate at 37°C	fast
Photostability	high
Cell toxicity	not observed

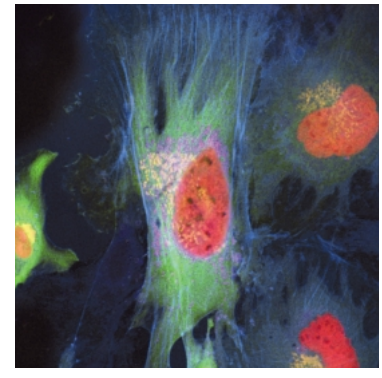
*Brightness is a product of extinction coefficient and quantum yield, divided by 1000.



TagBFP normalized excitation (thin line) and emission (thick line) spectra.
Complete TagBFP spectra in Excel format can be downloaded from the Evrogen Web site at www.evrogen.com / support / FP-tech.shtml



HeLa cells expressing TagBFP fusion proteins.
(A) Confocal microscopy of TagBFP fusion with β-actin in transiently transfected HeLa cells; (B) confocal microscopy of TagBFP fusion with cytoplasmic α-tubulin in transiently transfected HeLa cells.



TagBFP use in multicolor labeling of mammalian cells. TagYFP - tagged α-tubulin (green), TagCFP - tagged β-actin (cyan), mitochondria-targeted TagBFP (magenta), Golgi-targeted TagRFP(yellow), and TagFP635-H2B fusion (red).

give bright fluorescent signals within 10-12 hrs after transfection. No cytotoxic effects or visible protein aggregation are observed.

TagBFP performance in fusions has been demonstrated in the β-actin and α-tubulin models.

TagBFP can be used in multicolor labeling applications with other fluorescent proteins of green, yellow, red, and far-red colors.

High pH-stability with pKa=2.7 makes it possible to use TagBFP for imaging in acidic organelles, such as late and recycling endosomes and lysosomes.

Recommended filter sets and antibodies

TagBFP can be recognized using Anti-tRFP antibody (Cat.# AB231-AB232) available from Evrogen.

The protein can be detected using common fluorescence filter sets for BFP, DAPI, and other blue dyes.

Recommended filter sets are: XF119-2*, QMAX-Blue*, XF131, XF06, XF13-2, XF03, XF11, XF129-2, XF05-2 (Omega Optical); DAPI-5060B* and DAPI-1160A (Semrock); 31037, 31041, 31016*, 31021, 31000v2, 1009v2, 31013v2, 11005v2, 31047 (Chroma Technology Corp.).
* - preferred filter sets

REFERENCES

Ai et al. (2007). Biochemistry, 46 (20): 5904–5910 / pmid: 17444659

Subach et al. (2008). Chemistry & Biology, 15 (10): 1116–1124 / pmid: 18940671

TagBFP-related products

Product	Cat.#	Description	Size	Page(s)
TagBFP expression/source vectors				
pTagBFP-C	FP171	Mammalian expression vector encoding humanized TagBFP and allowing its expression and generation of fusions to the TagBFP C-terminus	20 μg	43
pTagBFP-N	FP172	Mammalian expression vector encoding humanized TagBFP and allowing its expression and generation of fusions to the TagBFP N-terminus	20 μg	45
pTagBFP-actin	FP174	Mammalian expression vector encoding humanized TagBFP fused with human cytoplasmic β-actin	20 μg	50
pTagBFP-tubulin	FP175	Mammalian expression vector encoding humanized TagBFP fused with human α-tubulin	20 μg	50
pTagBFP-H2B	FP176	Mammalian expression vector encoding humanized TagRFP fused with human histone H2B	20 μg	51
Vector sets				
Fusion Blue	FPF20	Mammalian expression vectors encoding TagBFP for its expression and fusion generation: pTagBFP-N and pTagBFP-C	20 μg each	45, 43
Antibodies against TagBFP				
Anti-tRFP	AB231 AB232	Rabbit polyclonal antibody against TurboRFP, TurboFP602, TurboFP635, TagBFP, TagRFP, TagFP635, and mKate2	100 μg 200 μg	104

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

Cyan fluorescent protein TagCFP

- Bright cyan fluorescence
- Monomeric protein with successful performance in fusions
- Fast maturation, high pH-stability and photostability
- Proven suitability to generate stably transfected cell lines
- Recommended for protein labeling

TagCFP is a cyan monomeric protein generated on the basis of the wild-type GFP-like protein from jellyfish *Aequorea macrodactyla* [Xia et al. 2002]. It possesses bright fluorescence with excitation/emission maxima at 458 and 480 nm, respectively. TagCFP is significantly brighter than commonly used ECFP.

TagCFP is mainly intended for protein labeling in protein localization and interaction studies. It can also be used for cell and organelle labeling and for tracking the promoter activity.

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

TagCFP performance in fusions has been demonstrated in human cytoplasmic β-actin, α-tubulin, and mitochondria models.

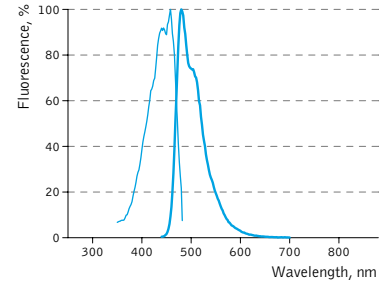
TagCFP suitability to generate stably transfected cells has been proven by Marinpharm company. A cell line expressing TagCFP fusion with mitochondrial targeting sequence (MTS) is commercially available.

TagCFP can be used in multicolor labeling applications with green, yellow, red, and far-red fluorescent dyes.

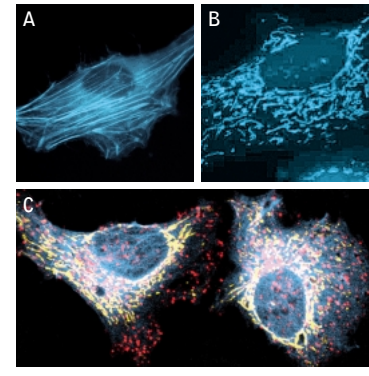
Main properties of TagCFP

Characteristic	
Molecular weight, kDa	27
Polypeptide length, aa	239
Fluorescence color	cyan
Excitation maximum, nm	458
Emission maximum, nm	480
Quantum yield	0.57
Extinction coefficient, $M^{-1}cm^{-1}$	37 000
Brightness*	21.1
Brightness, % of EGFP	64
pKa	4.7
Structure	monomer
Aggregation	no
Maturation rate at 37°C	fast
Photostability	high
Cell toxicity	not observed

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



TagCFP normalized excitation (thin line) and emission (thick line) spectra.
Complete TagCFP spectra in Excel format can be downloaded from the Evrogen Web site at www.evrogen.com / support / FP-tech.shtml



Expression of TagCFP fusions in mammalian cells. (A) Transiently transfected HeLa cells expressing TagCFP-tagged β-actin; (B) stably transfected U-205 cells expressing mitochondria-targeted TagCFP; (C) TagCFP use in multicolor labeling of HeLa cells: TagCFP-tagged α-tubulin (cyan), TagFP635-clathrin fusion (red), mitochondria-targeted TagYFP (yellow). Image was kindly provided by Michael W. Davidson (Florida State University).

Recommended filter sets and antibodies

TagCFP can be recognized using Anti-Tag(CGY)FP antibody (Cat.# AB121-AB122) available from Evrogen.

TagCFP can be detected using fluorescence filter sets for ECFP and the similar. Recommended Omega Optical filter sets are XF114-2 and XF130-2.

REFERENCES

Xia et al. (2002). Mar Biotechnol (NY), 4 (2): 155–162 / pmid: 14961275

TagCFP-related products

Product	Cat.#	Description	Size	Page(s)
TagCFP expression/source vectors				
pTagCFP-C	FP111	Mammalian expression vector encoding humanized TagCFP and allowing its expression and generation of fusions to the TagCFP C-terminus	20 μg	43
pTagCFP-N	FP112	Mammalian expression vector encoding humanized TagCFP and allowing its expression and generation of fusions to the TagCFP N-terminus	20 μg	45
pTagCFP-actin	FP114	Mammalian expression vector encoding humanized TagCFP fused with human cytoplasmic β-actin	20 μg	50
pTagCFP-tubulin	FP115	Mammalian expression vector encoding humanized TagCFP fused with human α-tubulin	20 μg	50
pTagCFP-mito	FP117	Mammalian expression vector encoding humanized TagCFP targeted to mitochondria	20 μg	47
Vector sets				
Fusion Cyan	FPF11	Mammalian expression vectors encoding TagCFP for its expression and fusion generation: pTagCFP-N and pTagCFP-C	20 μg each	43, 45
Antibodies against TagCFP				
Anti-Tag(CGY)FP	AB121 AB122	Rabbit polyclonal antibody against TagCFP, TagGFP, TagYFP, PS-CFP2, and EGFP	100 μg 200 μg	101

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

Green fluorescent protein TagGFP2

- Bright green fluorescence
- Monomeric protein with successful performance in fusions
- Fast maturation, high pH-stability and photostability
- Proven suitability to generate stably transfected cell lines
- Recommended for protein labeling and FRET applications

TagGFP2 (scientific name mTagGFP) is the improved variant of TagGFP, the mutant of the *Aequorea macrodactyla* GFP-like protein [Xia et al. 2002, Subach et al. 2008]. TagGFP2 possesses bright green fluorescence with excitation/emission maxima at 483 and 506 nm, respectively.

TagGFP2 matures 1.6-fold faster than TagGFP and is characterized by the improved performance in fusions. Compared to EGFP, TagGFP2 provides about the same brightness of fluorescence but is significantly more pH stable. TagGFP2 is specially optimized for expression at 37°C.

Because of monomeric nature, TagGFP2 is mainly intended for protein localization studies and expression in long-term cell cultures. In FRET applications, TagGFP2 can be used as a donor for red fluorescent protein TagRFP (see page 20) or as an acceptor for blue fluorescent protein TagBFP (see page 12).

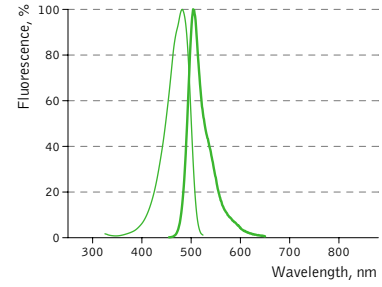
TagGFP2 can be easily expressed and detected in a wide range of organisms. Mammalian cells transiently transfected with TagGFP2 expression vectors give bright fluorescent signals within 10-12 hrs after transfection. No cytotoxic effects or visible protein aggregation are observed.

TagGFP2 performance in fusions has been demonstrated in the β-actin, α-tubulin and mitochondria-targeting signal models. It can be used in multicolor labeling applications with cyan, yellow, red, and far-red fluorescent dyes.

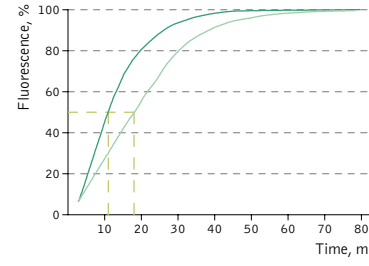
Main properties of TagGFP2

Characteristic	
Molecular weight, kDa	27
Polypeptide length, aa	238
Fluorescence color	green
Excitation maximum, nm	483
Emission maximum, nm	506
Quantum yield	0.6
Extinction coefficient, $M^{-1}cm^{-1}$	56 500
Brightness*	33.9
Brightness, % of EGFP	105
pKa	5.0
Structure	monomer
Aggregation	no
Maturation rate at 37°C	fast
Photostability	high
Cell toxicity	not observed

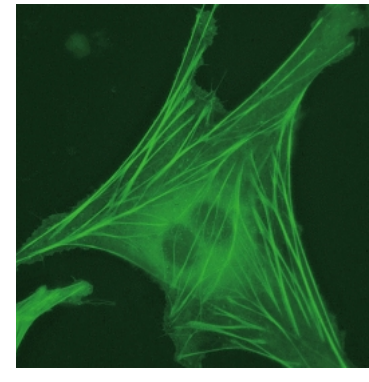
*Brightness is a product of extinction coefficient and quantum yield, divided by 1000.



TagGFP2 normalized excitation (thin line) and emission (thick line) spectra.
Complete TagGFP2 spectra in Excel format can be downloaded from the Evrogen Web site at www.evrogen.com / support / FP-tech.shtml



Maturation curves for TagGFP2 and parental TagGFP.
Color dashed lines indicate maturation half-times of 11 min and 18 min for TagGFP2 (dark green curve) and TagGFP (light green curve), respectively. Recording of protein maturation was started when about 7% from their maximal fluorescence has been detected. Time point "0" was defined using an approximation of the beginning of the maturation curves with straight lines.
Data from Subach et al. 2008.



Transiently transfected REF-52 cells expressing TagGFP2-tagged β-actin.

Recommended filter sets and antibodies

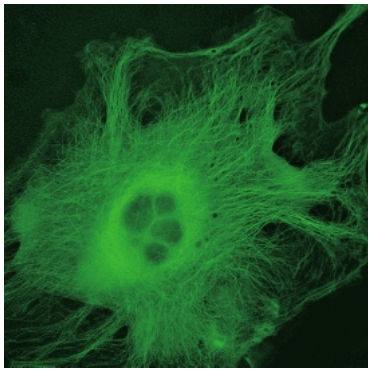
The protein can be recognized using Anti-Tag(CGY)FP antibody (Cat.# AB121-AB122) available from Evrogen.

TagGFP2 can be detected using common fluorescence filter sets for EGFP, FITC, and other green dyes. Recommended Omega Optical filter sets are QMAX-Green, XF100-2, XF100-3, (XF115-2), and XF116-2.

REFERENCES

Subach et al. (2008). Chemistry & Biology, 15 (10): 1116–1124 / pmid: 18940671

Xia et al. (2002). Mar Biotechnol (NY), 4 (2): 155–162 / pmid: 14961275



Transiently transfected REF-52 cells expressing TagGFP2-tagged α-tubulin.

TagGFP2-related products

Product	Cat.#	Description	Size	Page(s)
TagGFP2 expression/source vectors				
pTagGFP2-C	FP191	Mammalian expression vector encoding humanized TagGFP2 and allowing its expression and generation of fusions to the TagGFP2 C-terminus	20 μg	43
pTagGFP2-N	FP192	Mammalian expression vector encoding humanized TagGFP2 and allowing its expression and generation of fusions to the TagGFP2 N-terminus	20 μg	45
pTagGFP2-actin	FP194	Mammalian expression vector encoding humanized TagGFP2 fused with human cytoplasmic β-actin	20 μg	50
pTagGFP2-tubulin	FP195	Mammalian expression vector encoding humanized TagGFP2 fused with human α-tubulin	20 μg	50
pTagGFP2-mito	FP197	Mammalian expression vector encoding humanized TagGFP2 targeted to mitochondria	20 μg	47
Vector sets				
Fusion Green	FPF22	Mammalian expression vectors encoding TagGFP2 for its expression and fusion generation: pTagGFP-N and pTagGFP-C	20 μg each	43, 45
Antibodies against TagGFP2				
Anti-Tag(CGY)FP	AB121 AB122	Rabbit polyclonal antibody against TagCFP, TagGFP, TagGFP2, TagYFP, PS-CFP2, and EGFP	100 μg 200 μg	101

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

Yellow fluorescent protein TagYFP

- Bright yellow fluorescence
- Monomeric protein with successful performance in fusions
- Fast maturation, high pH-stability and photostability
- Proven suitability to generate stably transfected cell lines
- Recommended for protein labeling

TagYFP is a monomeric yellow fluorescent protein developed on the basis of GFP-like protein from jellyfish *Aequorea macrodactyla* [Xia et al. 2002]. TagYFP possesses single excitation maximum at 508 nm, and emission maximum at 524 nm. TagYFP is more pH stable than EYFP.

TagYFP is mainly intended for protein labeling in protein localization and interaction studies. It can also be used for cell and organelle labeling and for tracking the promoter activity, although TurboYFP and Phi-Yellow proteins are preferable for such applications because they mature faster and give brighter fluorescent signal (see page 30).

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

TagYFP performance in fusions has been demonstrated in human cytoplasmic β-actin and α-tubulin models. An expected pattern of fluorescence has been obtained in each case.

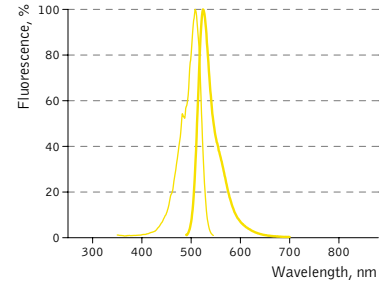
TagYFP suitability to generate stably transfected cells has been proven by Marinpharm company. Cell lines expressing TagYFP fusions are commercially available.

TagYFP can be used in multicolor labeling applications with blue, cyan, green, red, and far-red fluorescent dyes.

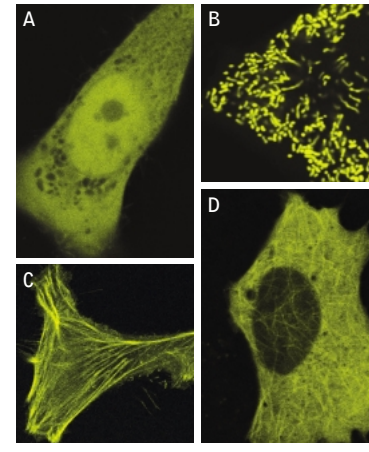
Main properties of TagYFP

Characteristic	
Molecular weight, kDa	27
Polypeptide length, aa	239
Fluorescence color	yellow
Excitation maximum, nm	508
Emission maximum, nm	524
Quantum yield	0.62
Extinction coefficient, $M^{-1}cm^{-1}$	50 000
Brightness*	31
Brightness, % of EGFP	94
pKa	5.5
Structure	monomer
Aggregation	no
Maturation rate at 37°C	fast
Photostability	high
Cell toxicity	not observed

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



TagYFP normalized excitation (thin line) and emission (thick line) spectra.
Complete TagYFP spectra in Excel format can be downloaded from the Evrogen Web site at www.evrogen.com / support / FP-tech.shtml



TagYFP expression in mammalian cells.
(A) Confocal microscopy of cytoplasmic TagYFP expression in transiently transfected human HeLa cells; (B) confocal microscopy of mitochondria-targeted TagYFP expression in transiently transfected HeLa cells; (C) confocal microscopy of TagYFP fusion with the cytoplasmic β-actin in transiently transfected 3T3 cells; (D) confocal microscopy of TagYFP fusion with the α-tubulin in transiently transfected 3T3 cells.

Recommended filter sets and antibodies

The protein can be recognized using Anti-Tag(CGY)FP antibody (Cat.# AB121-AB122) available from Evrogen.

Recommended Omega Optical filter sets for TagYFP are XF104-3 and XF105-2. It can also be detected using Chroma Technology Corp. filter set 41028 Yellow GFP BP (10C/Topaz) or the similar.

REFERENCES

Xia et al. (2002). Mar Biotechnol (NY), 4 (2): 155–162 / pmid: 14961275

TagYFP-related products

Product	Cat.#	Description	Size	Page(s)
TagYFP expression/source vectors				
pTagYFP-C	FP131	Mammalian expression vector encoding humanized TagYFP and allowing its expression and generation of fusions to the TagYFP C-terminus	20 μg	43
pTagYFP-N	FP132	Mammalian expression vector encoding humanized TagYFP and allowing its expression and generation of fusions to the TagYFP N-terminus	20 μg	45
pTagYFP-actin	FP134	Mammalian expression vector encoding humanized TagYFP fused with human cytoplasmic β-actin	20 μg	50
pTagYFP-tubulin	FP135	Mammalian expression vector encoding humanized TagYFP fused with human α-tubulin	20 μg	50
pTagYFP-mito	FP137	Mammalian expression vector encoding humanized TagYFP targeted to mitochondria	20 μg	47
Vector sets				
Fusion Yellow	FPF13	Mammalian expression vectors encoding TagYFP for its expression and fusion generation: pTagYFP-C and pTagYFP-N	20 μg each	43, 45
Antibodies against TagYFP				
Anti-Tag(CGY)FP	AB121 AB122	Rabbit polyclonal antibody against TagCFP, TagGFP, TagYFP, PS-CFP2, and EGFP	100 μg 200 μg	101

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

Red fluorescent protein TagRFP

- Bright red (orange) fluorescence
- Monomeric protein with successful performance in fusions
- Fast maturation, high pH-stability
- Proven suitability to generate stably transfected cell lines
- Recommended for protein labeling, acidic organelle labeling, FRET applications

TagRFP is a monomeric red (orange) fluorescent protein generated from the wild-type RFP from sea anemone *Entacmaea quadricolor* [Merzlyak et al. 2007]. It possesses bright fluorescence with excitation/emission maxima at 555 and 584 nm, respectively. TagRFP is about three times brighter than mCherry protein [Shaner et al. 2004], which makes it the brightest monomeric red fluorescent protein available so far.

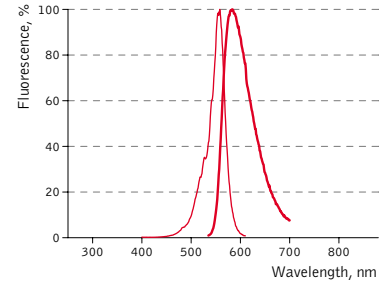
TagRFP is mainly intended for protein labeling. It can also be used for cell and organelle labeling and for tracking the promoter activity.

Another application of TagRFP is its use as an acceptor for FRET in pair with green fluorescent proteins. The traditional cyan and yellow FRET partners exhibit several substantial drawbacks limiting their utility, such as relatively low dynamic range (donor/acceptor emission ratio change) and difficulties with spectral separation. Using of TagRFP as an acceptor for Evrogen green fluorescent protein TagGFP2 (page 16) ensures higher FRET efficiency and more reliable spectral separation of the donor and acceptor fluorescence. Shifting the wavelengths towards the red part of the spectrum reduces input of cellular autofluorescence. High molar extinction coefficient of TagRFP along with high fluorescence quantum yield of TagGFP2 and excellent overlap of TagGFP2 emission and TagRFP excitation spectra result in highly effective FRET between these fluorescent proteins. The calculated Forster distance (R_0 = 5.7 nm) for the TagGFP2 / TagRFP pair is one of the largest

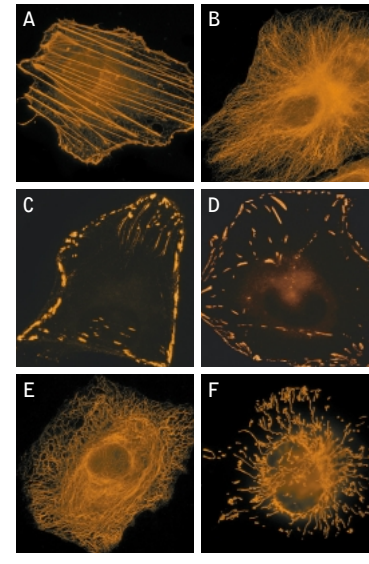
Main properties of TagRFP

Characteristic	
Molecular weight, kDa	27
Polypeptide length, aa	237
Fluorescence color	red (orange)
Excitation maximum, nm	555
Emission maximum, nm	584
Quantum yield	0.48
Extinction coefficient, $M^{-1}cm^{-1}$	100 000
Brightness*	48.0
Brightness, % of EGFP	148
pKa	3.8
Structure	monomer
Aggregation	no
Maturation rate at 37°C	fast
Photostability	medium
Cell toxicity	not observed

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



TagRFP normalized excitation (thin line) and emission (thick line) spectra.
Complete TagRFP spectra in Excel format can be downloaded from the Evrogen Web site at www.evrogen.com / support / FP-tech.shtml



TagRFP use for cell and protein labeling.
(A) HeLa cells expressing TagRFP fusion with β-actin; (B) HeLa cells expressing TagRFP fusion with α-tubulin; (C) HeLa cells expressing TagRFP fusion with zyxin; (D) HeLa cells expressing TagRFP fusion with vinculin; (E) HeLa cells expressing TagRFP fusion with keratin; (F) HeLa cells expressing TagRFP targeted to mitochondria.

among the values reported. At the same time, since TagGFP2 and TagRFP emission peaks are spaced by as much as 78 nm, the emission signal for these two proteins can be easily separated in any imaging system. High pH-stability of the both proteins allows using this pair for imaging in acidic organelles. As an additional advance, TagRFP and TagGFP2 proteins derive from different marine sources and therefore luck the ability to form heterodimers. It ensures zero background for FRET analysis that may not be the case for weakly dimerizing FRET pairs consisting of highly homological fluorescent proteins. The excellent performance of TagRFP in FRET application was demonstrated both *in vitro* and *in vivo* on the example of FRET-based apoptosis reporter CaspeR3-GR (see page 86 of this catalogue and [Shcherbo et al. 2009]).

TagRFP can be easily expressed and detected in a wide range of organisms. It becomes clearly detectable in mammalian cells as early as within 10-12 hrs after transfection. No cytotoxic effects or visible protein aggregation are observed.

TagRFP performance in protein fusions has been demonstrated in fibrillarin, vinculin, zyxin, β-actin, α-tubulin, and other models.

TagRFP suitability to generate stably transfected cells has been proven by Marinpharm company. Cell lines expressing TagRFP fusions are commercially available.

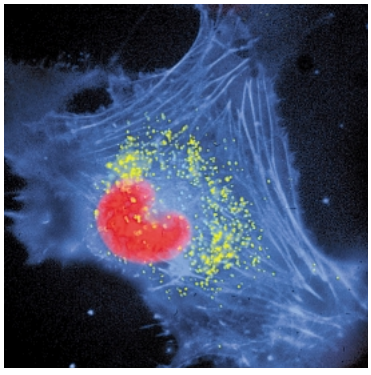
TagRFP can be used in multicolor labeling applications with other fluorescent proteins of blue, cyan, green, yellow, and far-red colors.

High pH-stability with pKa=3.8 makes it possible to use TagRFP for imaging in acidic organelles, such as late and recycling endosomes and lysosomes.

Recommended filter sets and antibodies

TagRFP can be recognized using Anti-tRFP antibody (Cat.# AB231-AB232) available from Evrogen.

Recommended Omega Optical filter sets are QMAX-Yellow, XF108-2, XF101-2, and XF111-2. TagRFP can also be detected using TRITC filter set or similar.



TagRFP application for multicolor labeling. Transiently transfected HeLa cells expressing TagBFP fusion with β-actin (blue), peroxisomes-targeted PhiYFP (yellow), and TagRFP fusion with histon H2B (red).

REFERENCES

Merzlyak et al. (2007). Nat Methods, 4 (7): 555–557 / pmid: 17572680

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

Shcherbo et al. (2009). BMC Biotechnology, 9: 24 / pmid: 19321010

TagRFP-related products

Product	Cat.#	Description	Size	Page(s)
TagRFP expression/source vectors				
pTagRFP-C	FP141	Mammalian expression vector encoding humanized TagRFP and allowing its expression and generation of fusions to the TagRFP C-terminus	20 μg	43
pTagRFP-N	FP142	Mammalian expression vector encoding humanized TagRFP and allowing its expression and generation of fusions to the TagRFP N-terminus	20 μg	45
pTagRFP-actin	FP144	Mammalian expression vector encoding humanized TagRFP fused with human cytoplasmic β-actin	20 μg	50
pTagRFP-tubulin	FP145	Mammalian expression vector encoding humanized TagRFP fused with human α-tubulin	20 μg	50
pTagRFP-mito	FP147	Mammalian expression vector encoding humanized TagRFP targeted to mitochondria	20 μg	47
pTagRFP-actinin	FP360	Mammalian expression vector encoding humanized TagRFP fused with human α-actinin	20 μg	50

Product	Cat.#	Description	Size	Page(s)
pTagRFP-integrin	FP361	Mammalian expression vector encoding humanized TagRFP fused with human α-V-integrin	20 μg	50
pTagRFP-Cx26	FP362	Mammalian expression vector encoding humanized TagRFP fused with rat connexin 26	20 μg	51
pTagRFP-Cx32	FP363	Mammalian expression vector encoding humanized TagRFP fused with human connexin 32	20 μg	51
pTagRFP-Cx43	FP364	Mammalian expression vector encoding humanized TagRFP fused with rat connexin 43	20 μg	51
pTagRFP-EB3	FP365	Mammalian expression vector encoding humanized TagRFP fused with human EB3 protein	20 μg	50
pTagRFP-FAK	FP366	Mammalian expression vector encoding humanized TagRFP fused with chicken focal adhesion kinase	20 μg	50
pTagRFP-Golgi	FP367	Mammalian expression vector encoding humanized TagRFP fused with human Golgi targeting sequence (GTS)	20 μg	47
pTagRFP-H2B	FP368	Mammalian expression vector encoding humanized TagRFP fused with human histone H2B	20 μg	51
pTagRFP-keratin	FP369	Mammalian expression vector encoding humanized TagRFP fused with human cytokeratin-18	20 μg	50
pTagRFP-laminB1	FP370	Mammalian expression vector encoding humanized TagRFP fused with human lamin B1	20 μg	51
pTagRFP-profilin	FP371	Mammalian expression vector encoding humanized TagRFP fused with mouse profilin	20 μg	50
pTagRFP-vinculin	FP372	Mammalian expression vector encoding humanized TagRFP fused with human vinculin	20 μg	50
pTagRFP-zyxin	FP373	Mammalian expression vector encoding humanized TagRFP fused with human zyxin	20 μg	51
Gateway® TagRFP-AS-C	FP148	Gateway® entry clone for generation of fusions to the C-terminus of TagRFP; transfer of the construct encoding TagRFP or its fusion into Gateway® destination vectors; TagRFP codon usage is optimized for expression in *Arabidopsis* and *Saccharomyces*	20 μg	40
Gateway® TagRFP-AS-N	FP149	Gateway® entry clone for generation of fusions to the N-terminus of TagRFP; transfer of the construct encoding TagRFP or its fusion into Gateway® destination vectors; TagRFP codon usage is optimized for expression in *Arabidopsis* and *Saccharomyces*	20 μg	41
Vector sets				
Fusion Red	FPF14	Mammalian expression vectors encoding TagRFP for its expression and fusion generation: pTagRFP-C and pTagRFP-N	20 μg each	43, 45
Antibodies against TagRFP				
Anti-tRFP	AB231 AB232	Rabbit polyclonal antibody against TurboRFP, TurboFP602, TurboFP635, TagBFP, TagRFP, TagFP635, and mKate2	100 μg 200 μg	104

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Far-red fluorescent protein mKate2

- Super bright far-red fluorescence
- Monomeric protein with successful performance in fusions
- Fast maturation, high pH-stability and photostability
- 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) [Shcherbo et al. 2007; Shcherbo et al. 2009]. It 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.

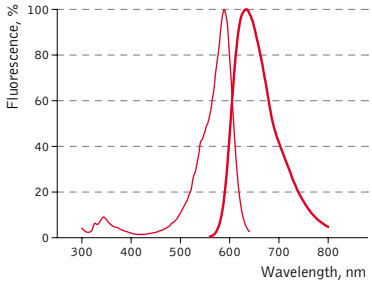
mKate2 is mainly intended for protein labeling. Its far-red fluorescence allows easy and reliable separation from standard green fluorescent labels in dual-color high-throughput assays.

mKate2 can be easily expressed and detected in a wide range of organisms. It becomes clearly detectable in mammalian cells as early as 10-12 hrs

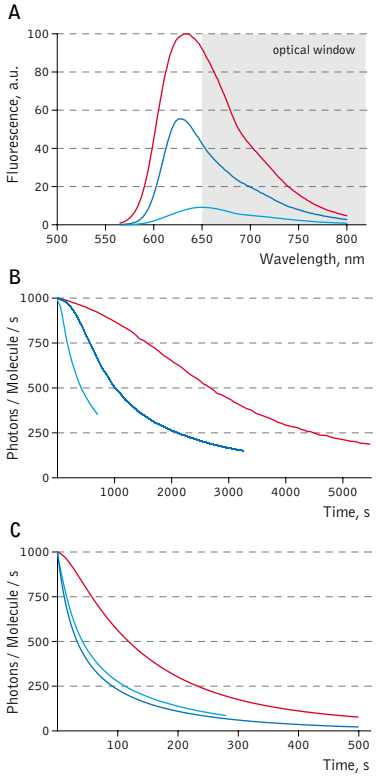
Main properties of mKate2

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
Photostability	high
Cell toxicity	not observed

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



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 www.evrogen.com / support / FP-tech.shtml

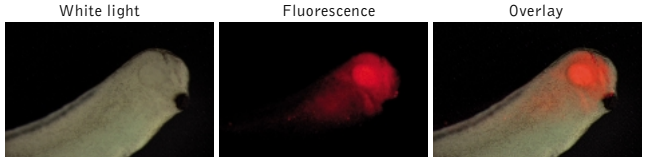


Spectral characteristics of mKate2 in comparison with selected fluorescent proteins. mKate2 - dark-red line, mRaspberry - blue line, mPlum - cyan line (A) Emission spectra of far-red monomeric fluorescent proteins given proportionally to their calculated brightness. Scaling was applied to the area of 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.

after transfection. No cytotoxic effects or visible protein aggregation are observed.

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

mKate2 can be used in multicolor labeling applications with blue, cyan, green, yellow, and red (orange) fluorescent proteins.

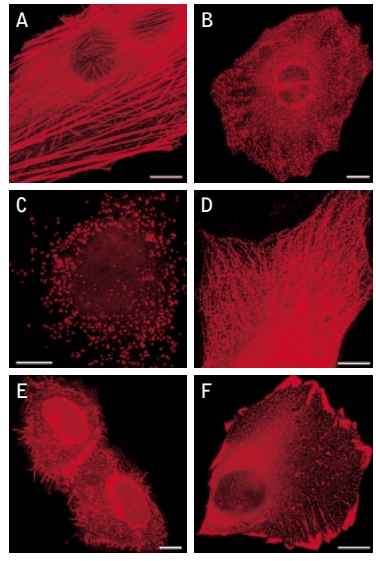


Imaging of mKate2 in *Xenopus laevis* embryos. Expression of mKate2 under the control of Xanf1 promoter in the transgenic embryos at stage 28 is specifically localized in the forehead region, including eyes, the forebrain and nasal placodes. The embryo is shown from the right side, dorsal to the top and left. Images from Shcherbo et al. 2009.

Recommended filter sets and antibodies

mKate2 can be recognized using Anti-tRFP antibody (Cat.# AB231-AB232) 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.



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

REFERENCES

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

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

mKate2-related products

Product	Cat.#	Description	Size	Page(s)
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	43
pmKate2-N	FP182	Mammalian expression vector encoding humanized mKate2 and allowing its expression and generation of fusions to the mKate2 N-terminus	20 μg	45
pmKate2-actin	FP184	Mammalian expression vector encoding humanized mKate2 fused with human cytoplasmic β-actin	20 μg	50
pmKate2-tubulin	FP185	Mammalian expression vector encoding humanized mKate2 fused with human α-tubulin	20 μg	50
pmKate2-f-mem	FP186	Mammalian expression vector encoding membrane-targeted mKate2	20 μg	48
pmKate2-mito	FP187	Mammalian expression vector encoding humanized mKate2 targeted to mitochondria	20 μg	47
pmKate2-laminB1	FP310	Mammalian expression vector encoding humanized mKate2 fused with human lamin B1	20 μg	51
pmKate2-H2B	FP311	Mammalian expression vector encoding humanized mKate2 fused with human histone H2B	20 μg	51
pmKate2-lyso	FP312	Mammalian expression vector encoding humanized mKate2 targeted to lysosomes	20 μg	49

Product	Cat.#	Description	Size	Page(s)
pmKate2-peroxi	FP313	Mammalian expression vector encoding humanized mKate2 targeted to peroxisomes	20 μg	48
pmKate2-endo	FP314	Mammalian expression vector encoding humanized mKate2 fused with human RhoB protein	20 μg	49
pmKate2-zyxin	FP315	Mammalian expression vector encoding humanized mKate2 fused with human zyxin	20 μg	50
pmKate2-EB3	FP316	Mammalian expression vector encoding humanized mKate2 fused with human EB3 protein	20 μg	50
pmKate2-actinin	FP317	Mammalian expression vector encoding humanized mKate2 fused with human α-actinin	20 μg	50
pmKate2-vimentin	FP318	Mammalian expression vector encoding humanized mKate2 fused with human vimentin	20 μg	50
pmKate2-keratin	FP319	Mammalian expression vector encoding humanized mKate2 fused with human cytokeratin-18	20 μg	50
pmKate2-profilin	FP320	Mammalian expression vector encoding humanized mKate2 fused with mouse profilin	20 μg	50
pmKate2-annexin	FP321	Mammalian expression vector encoding humanized mKate2 fused with human annexin A4	20 μg	51
pmKate2-clathrin	FP322	Mammalian expression vector encoding humanized mKate2 fused with human clathrin light chain LCB	20 μg	51
pmKate2-paxillin	FP323	Mammalian expression vector encoding humanized mKate2 fused with chicken paxillin	20 μg	50
pTagFP635-vinculin	FP388	Mammalian expression vector encoding humanized TagFP635* fused with human vinculin	20 μg	50
pTagFP635-Cx26	FP382	Mammalian expression vector encoding humanized TagFP635* fused with rat connexin 26	20 μg	51
pTagFP635-Cx32	FP383	Mammalian expression vector encoding humanized TagFP635* fused with human connexin 32	20 μg	51
pTagFP635-Cx43	FP384	Mammalian expression vector encoding humanized TagFP635* fused with rat connexin 43	20 μg	51
Vector sets				
Fusion Far-Red	FPF25	Mammalian expression vectors encoding mKate2 for its expression and fusion generation: pmKate2-C and pmKate2-N	20 μg each	43, 45
Antibodies against mKate2				
Anti-tRFP	AB231 AB232	Rabbit polyclonal antibody against TurboRFP, TurboFP602, TurboFP635, TagBFP, TagRFP, TagFP635 and mKate2	100 μg 200 μg	104

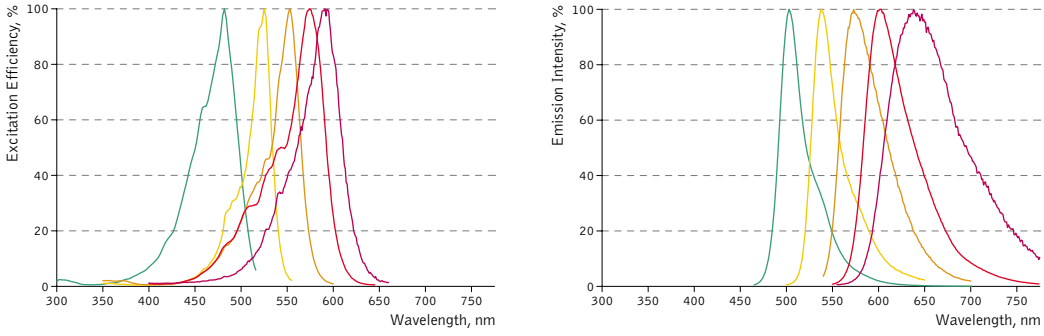
Please contact your local distributor for exact prices and delivery information.

TagFP635* - the parental variants of mKate2.

TurboFPs

Bright reporters for cell labeling

TurboFPs are proteins of different colors that are recommended for use in applications where fast appearance of bright fluorescence is crucial (e.g. for tracking the promoter activity), and for cell and organelle labeling.



Normalized excitation/emission spectra of TurboFPs.
TurboGFP - green line, TurboYFP - yellow line, TurboRFP - orange line, TurboFP602 - red line, TurboFP635 - dark-red line.

Main properties of TurboFPs:

Protein	TurboGFP	TurboYFP	TurboRFP	TurboFP602	TurboFP635
Molecular weight, kDa	26	26	26	26	26
Polypeptide length, aa	232	234	231	231	231
Fluorescence color	green	yellow	red (orange)	true-red	far-red
Excitation maximum, nm	482	525	553	574	588
Emission maximum, nm	502	538	574	602	635
Quantum yield	0.53	0.53	0.67	0.35	0.34
Extinction coefficient, $M^{-1}cm^{-1}$	70 000	105 000	92 000	74 400	65 000
Brightness*	37.1	55.7	61.6	26.0	22.1
Brightness, % of EGFP	112	169	187	79	67
pKa	5.2	5.9	4.4	4.7	5.5
Structure	dimer	dimer	dimer	dimer	dimer
Aggregation	no	at high concentrations	no	no	no
Maturation rate at 37°C	super fast	super fast	super fast	fast	super fast
Photostability	high	high	high	medium	high
Cell toxicity	not observed	at high concentrations	not observed	not observed	not observed

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

Green fluorescent protein TurboGFP

- Bright green fluorescence
- Fast maturation at a wide range of temperatures
- High pH-stability and photostability
- Proven suitability to generate stably transfected cell lines
- Destabilized variant is available
- Recommended for gene expression analysis and cell and organelle labeling

TurboGFP is an improved variant of the green fluorescent protein CopGFP cloned from copepod *Pontellina plumata* (Arthropoda; Crustacea; Maxillopoda; Copepoda) [Shagin et al. 2004]. It possesses bright green fluorescence (excitation/ emission max = 482/ 502 nm) that is visible earlier than fluorescence of other green fluorescent proteins.

TurboGFP is mainly intended for applications where fast appearance of bright fluorescence is crucial. It is specially recommended for cell and organelle labeling and tracking the promoter activity. Destabilized TurboGFP variant allows accurate analysis of rapid and/or transient events in gene regulation.

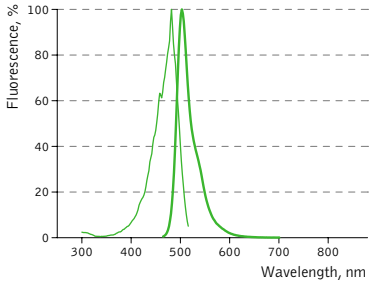
TurboGFP can be expressed and detected in a wide range of organisms including cold-blooded animals. Mammalian cells transiently transfected with TurboGFP expression vectors give bright fluorescent signals within 8-10 hrs after transfection. No cytotoxic effects or visible protein aggregation are observed.

TurboGFP suitability to generate stably transfected cells has been proven by Marinpharm company. Various cell lines expressing TurboGFP are commercially available.

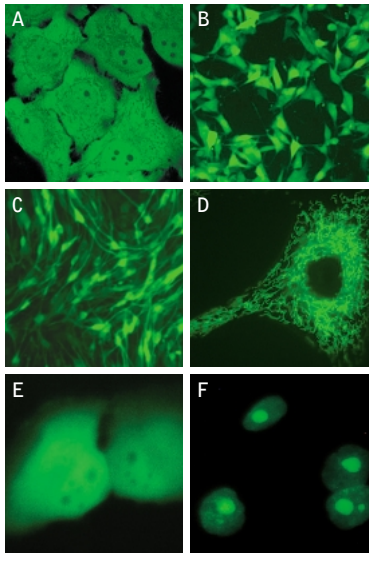
Main properties of TurboGFP

Characteristic	
Molecular weight, kDa	26
Polypeptide length, aa	232
Fluorescence color	green
Excitation maximum, nm	482
Emission maximum, nm	502
Quantum yield	0.53
Extinction coefficient, $M^{-1}cm^{-1}$	70 000
Brightness*	37.1
Brightness, % of EGFP	112
pKa	5.2
Structure	dimer
Aggregation	no
Maturation rate at 37°C	super fast
Photostability	high
Cell toxicity	not observed

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



TurboGFP normalized excitation (thin line) and emission (thick line) spectra.
Complete TurboGFP spectra in Excel format can be downloaded from the Evrogen Web site at www.evrogen.com / support / FP-tech.shtml



TurboGFP expression in mammalian cells.
(A) Transiently transfected HeLa cells expressing TurboGFP in cytoplasm; (B) stably transfected M3-mouse melanoma cells expressing TurboGFP in cytoplasm; (C) stably transfected C2C12 mouse myoblasts expressing TurboGFP in cytoplasm; (D) stably transfected HeLa cells expressing mitochondria-targeted TurboGFP; (E) stably transfected HeLa cells expressing TurboGFP-BID fusion; (F) stably transfected HeLa cells expressing TurboGFP-fibrillarin fusion.
Photographs of stably transfected cell lines were kindly provided by Dr. Christian Petzelt (Marinpharm).

Refolding and maturation kinetics of GFPs *in vitro*

	EGFP	Venus	SYFP2	TurboGFP
Refolding half-time, s	90.6	46.2	69.3	11.0
Maturation half-time, s	3915	4076	3300	1468
kox 10^{-4} s^{-1}	1.77	1.70	2.10	4.72
Reference	Evdokimov et al. 2006	Kremers et al. 2006	Kremers et al. 2006	Evdokimov et al. 2006

Samples of fluorescent proteins were heated to 95°C in denaturation solution (8 M urea, 1 mM DTT) for 4 min. Refolding reactions were initiated upon 100-fold dilution into the renaturation buffer (35 mM KCl, 2 mM $MgCl_2$, 50 mM Tris pH 7.5, 1 mM DTT). In maturation assay, 5 mM freshly dissolved dithionite was added to the denaturation solution [Reid and Flynn 1997]. Due to the instability of dithionite at high temperatures, to provide for complete chromophore reduction the sample was cooled to 25°C and the addition of 5 mM dithionite followed by heating to 5°C were repeated. Protein refolding and maturation were followed by measuring the recovery of fluorescence using Varian Cary Eclipse Fluorescence Spectrophotometer, chamber temperature maintained at 25°C. Maturation rate constants (kox) were determined by computer-fitting the kinetic data to the first order exponential decay (Origin 6.0).

Despite its dimeric structure, TurboGFP performs well in some fusions. However, for protein labeling applications we recommend using specially optimized monomeric TagFPs (see page 11).

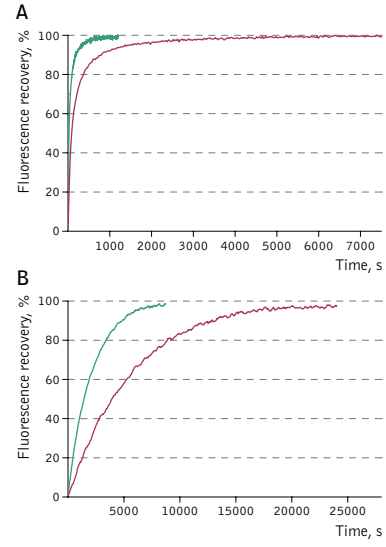
TurboGFP maturation kinetics: TurboGFP allows monitoring the activity from early promoters. It matures noticeably faster than EGFP and most other fluorescent proteins. This difference in performance is illustrated here using both *in vitro* analysis of TurboGFP and EGFP refolding and maturation kinetics (see table above) and *in vivo* examination of the developing *Xenopus* embrios expressing either TurboGFP or EGFP (see page 9).

TurboGFP can be used in multicolor labeling applications with blue, cyan, green, red, and far-red fluorescent dyes.

Recommended filter sets and antibodies

TurboGFP can be recognized using Anti-TurboGFP (Cat.# AB511-AB512) and Anti-TurboGFP(d) (Cat.# AB513-AB514) antibodies available from Evrogen.

TurboGFP can be detected using common fluorescence filter sets for EGFP, FITC, and other green dyes. Recommended Omega Optical filter sets are QMAX-Green, XF100-2, XF100-3, (XF115-2), and XF116-2.



Comparison of EGFP (violet lines) and TurboGFP (green lines) refolding and maturation speed *in vitro* [Evdokimov et al. 2006].
Normalized fluorescence recovery plots are shown. (A) Refolding kinetics; (B) chromophore maturation kinetics.

REFERENCES

Evdokimov et al. (2006). EMBO Rep, 7 (10): 1006–1012 / pmid: 16936637

Kremers et al. (2006). Biochemistry, 45 (21): 6570–6580 / pmid: 16716067

Reid and Flynn (1997). Biochemistry, 36 (22): 6786–6791 / pmid: 9184161

Shagin et al. (2004). Curr Biol, 21 (5): 841–850 / pmid: 14963095

TurboGFP-related products

Product	Cat.#	Description	Size	Page(s)
TurboGFP expression/source vectors				
pTurboGFP-C	FP511	Mammalian expression vector encoding humanized TurboGFP and allowing its expression and generation of fusions to the TurboGFP C-terminus	20 μg	43
pTurboGFP-N	FP512	Mammalian expression vector encoding humanized TurboGFP and allowing its expression and generation of fusions to the TurboGFP N-terminus	20 μg	45
pTurboGFP-B	FP513	Bacterial expression vector; source of the TurboGFP coding sequence	20 μg	42

Product	Cat.#	Description	Size	Page(s)
pTurboGFP-PRL	FP515	Promoterless vector encoding humanized TurboGFP and designed for monitoring activity of different promoters and promoter/enhancer combinations	20 µg	52
pTurboGFP-PRL-dest1	FP518	Promoterless vector encoding destabilized TurboGFP and designed for monitoring activity of different promoters and promoter/enhancer combinations	20 µg	52
pTurboGFP-dest1	FP519	Mammalian expression vector encoding destabilized TurboGFP for its expression and generation of fusions to the TurboGFP-dest1 N-terminus	20 µg	45
Gateway® TurboGFP-C	FP521	Gateway® entry clone for generation of fusions to the C-terminus of humanized TurboGFP; transfer of the construct encoding TurboGFP or its fusion into Gateway® destination vectors	20 µg	40
Gateway® TurboGFP-N	FP522	Gateway® entry clone for generation of fusions to the N-terminus of humanized TurboGFP; transfer of the construct encoding TurboGFP or its fusion into Gateway® destination vectors	20 µg	41
pTurboGFP-mito	FP517	Mammalian expression vector encoding humanized TurboGFP targeted to mitochondria	20 µg	47
Vector sets				
Promoter-tracker 3-colors	FPP15	Promoterless vectors pTurboYFP-PRL, pTurboGFP-PRL, and pTurboRFP-PRL	20 µg each	52
Promoter-tracker Green	FPP03	Promoterless vectors pTurboGFP-PRL, pTurboGFP-PRL-dest1, control vector pTurboGFP-dest1	20 µg each	52,45
Mito-tracker	FPM01	Mammalian expression vectors for fluorescent labeling of mitochondria: pTurboGFP-mito, pPhi-Yellow-mito, and pKindling-Red-mito	20 µg each	47,69
Recombinant protein				
rTurboGFP	FP552	Purified recombinant TurboGFP	100 µg	54
Antibodies against TurboGFP				
Anti-TurboGFP	AB511 AB512	Rabbit polyclonal antibody against non-denatured TurboGFP	100 µg 200 µg	98
Anti-TurboGFP(d)	AB513 AB514	Rabbit polyclonal antibody against denatured TurboGFP	100 µg 200 µg	99

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

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The CMV promoter is covered under U.S. Patents 5,168,062 and 5,385,839, and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242.

Yellow fluorescent protein TurboYFP

- Super bright true-yellow fluorescence
- Emission wavelength is ideally positioned between those of green and red fluorescent proteins
- Fast maturation, high pH-stability and photostability
- Destabilized variant is available
- Recommended for gene expression analysis and cell and organelle labeling

TurboYFP is an enhanced variant of the yellow fluorescent protein PhiYFP from jellyfish *Phialidium sp.* [Shagin et al. 2004]. It possesses super-bright yellow fluorescence with emission maximum at 538 nm and is ideally positioned between green and red fluorescent proteins, allowing easy separation of these markers by flow cytometry using common channels of detection and a single laser excitation line. Compared with PhiYFP, TurboYFP matures faster in mammalian cells.

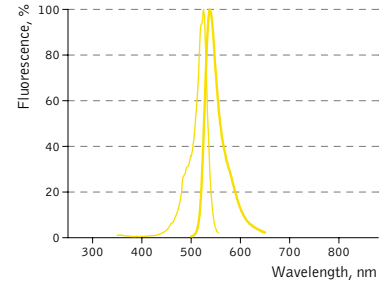
TurboYFP is mainly intended for applications where fast appearance of bright fluorescence is crucial. It is specially recommended for cell labeling and tracking the promoter activity. Destabilized TurboYFP variant allows accurate analysis of rapid and/or transient events in gene regulation.

Mammalian cells transiently transfected with TurboYFP expression vectors give bright fluorescense within 8-10 hrs after transfection. Being overexpressed in long-term culture of cells with high expression levels, TurboYFP shows slight tendency to aggregate. Therefore we recommend that you use parental PhiYFP and PhiYFP-m proteins for long-term expression and organelle labeling. Suitability of these proteins for stable transfection was demonstrated by Marinpharm (Germany).

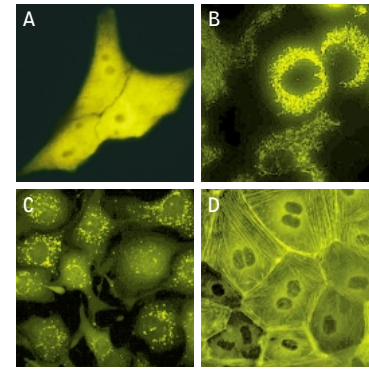
Main properties of TurboYFP

Characteristic	TurboYFP	PhiYFP	PhiYFP-m
Molecular weight, kDa	26	26	26
Polypeptide length, aa	234	234	234
Fluorescence color	yellow	yellow	yellow
Excitation maximum, nm	525	525	525
Emission maximum, nm	538	537	537
Quantum yield	0.53	0.40	0.39
Extinction coefficient, $M^{-1}cm^{-1}$	105 000	130 000	124 000
Brightness*	55.7	52.0	48.4
Brightness, % of EGFP	169	158	147
pKa	5.9	6.0	6.0
Structure	dimer	dimer	dimer
Aggregation	at high concentrations	no	no
Maturation rate at 37°C	super fast	fast	fast
Photostability	high	high	high
Cell toxicity	not observed	not observed	not observed

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



TurboYFP normalized excitation (thin line) and emission (thick line) spectra.
Complete TurboYFP spectra in Excel format can be downloaded from the Evrogen Web site at www.evrogen.com / support / FP-tech.shtml



TurboYFP and PhiYFP expression in mammalian cells. (A) Whole-cell expression in HeLa cells; (B-D) stably transfected mammalian cells expressing PhiYFP-tagged fusions: (B) mitochondria-targeted PhiYFP in 3T3 mouse fibroblasts; (C) T24 human bladder carcinoma cells expressing peroxisome-targeted PhiYFP-m; (D) PhiYFP-m fusion with β-actin in PtK rat kangaroo cells.
Images (B-D) were kindly provided by Dr. Christian Petzelt (Marinpharm).

Despite there dimeric structure, TurboYFP and PhiYFPs perform well in some fusions. Please note, that PhiYFP can be used for generation of fusions to its N-terminus, whereas PhiYFP-m is optimized to generate fusions to its C-terminus. PhiYFP can not be used to generate C-terminal fusions. For protein labeling applications we recommend using specially optimized monomeric TagFPs (see page 11).

TurboYFP can be used in multicolor labeling applications with blue, cyan, green, red, and far-red fluorescent dyes.

Recommended filter sets and antibodies

TurboYFP, PhiYFP and PhiYFP-m can be recognized using Anti-PhiYFP (Cat.# AB601-AB602) and Anti-PhiYFP(d) (Cat.# AB603-AB604) antibodies available from Evrogen.

TurboYFP can be detected using Omega Optical filter set XF104-3 or Chroma Technology Corp. filter set 42003 ("ZsYellow1").

REFERENCES

Shagin et al. (2004). Curr Biol, 21 (5): 841–850 / pmid: 14963095

TurboYFP and PhiYFP-related products

Product	Cat.#	Description	Size	Page(s)
TurboYFP expression/source vectors				
pTurboYFP-C	FP611	Mammalian expression vector encoding humanized TurboYFP and allowing its expression and generation of fusions to the TurboYFP C-terminus	20 μg	43
pTurboYFP-N	FP612	Mammalian expression vector encoding humanized TurboYFP and allowing its expression and generation of fusions to the TurboYFP N-terminus	20 μg	45
pTurboYFP-B	FP613	Bacterial expression vector; source of the TurboYFP coding sequence	20 μg	42
pTurboYFP-PRL	FP615	Promoterless vector encoding humanized TurboYFP and designed for monitoring activity of different promoters and promoter/enhancer combinations	20 μg	52
pTurboYFP-PRL-dest1	FP618	Promoterless vector encoding destabilized TurboYFP and designed for monitoring activity of different promoters and promoter/enhancer combinations	20 μg	52
pTurboYFP-dest1	FP619	Mammalian expression vector encoding destabilized TurboYFP for its expression and generation of fusions to the TurboYFP-dest1 N-terminus	20 μg	45
PhiYFP expression/source vectors				
pPhi-Yellow-C	FP601	Mammalian expression vector encoding humanized PhiYFP-m and allowing its expression and generation of fusions to the PhiYFP-m C-terminus	20 μg	43
pPhi-Yellow-N	FP602	Mammalian expression vector encoding humanized PhiYFP and allowing its expression and generation of fusions to the PhiYFP N-terminus	20 μg	45
pPhi-Yellow-B	FP603	Bacterial expression vector; source of the PhiYFP coding sequence	20 μg	42
pPhi-Yellow-PRL	FP604	Promoterless vector encoding humanized PhiYFP and designed for monitoring activity of different promoters and promoter/enhancer combinations	20 μg	52
pPhi-Yellow-PRL-dest1	FP605	Promoterless vector encoding destabilized PhiYFP-m and designed for monitoring activity of different promoters and promoter/enhancer combinations	20 μg	52

Product	Cat.#	Description	Size	Page(s)
pPhi-Yellow-peroxi	FP606	Mammalian expression vector encoding humanized PhiYFP-m targeted to peroxisomes	20 μg	48
pPhi-Yellow-mito	FP607	Mammalian expression vector encoding humanized PhiYFP targeted to mitochondria	20 μg	47
pPhi-Yellow-dest1	FP608	Mammalian expression vector encoding destabilized PhiYFP-m for its expression and generation of fusions to the PhiYFP-m-dest1 N-terminus	20 μg	45
Vector sets				
Promoter-tracker 3-colors	FPP15	Promoterless vectors pTurboYFP-PRL, pTurboGFP-PRL, and pTurboRFP-PRL	20 μg each	52,52,52
Promoter-tracker Yellow	FPP14	Promoterless vectors pTurboYFP-PRL, pTurboYFP-PRL-dest1, control vector pTurboYFP-dest1	20 μg each	52,52,45
Mito-tracker	FPM01	Mammalian expression vectors for fluorescent labeling of mitochondria: pTurboGFP-mito, pPhi-Yellow-mito, and pKindling-Red-mito	20 μg each	47,47,69
Recombinant protein				
rPhiYFP	FP651	Purified recombinant PhiYFP	100 μg	54
Antibodies against TurboYFP and PhiYFP				
Anti-PhiYFP	AB601 AB602	Rabbit polyclonal antibody against non-denatured PhiYFP, PhiYFP-m, and TurboYFP	100 μg 200 μg	102
Anti-PhiYFP(d)	AB603 AB604	Rabbit polyclonal antibody against denatured PhiYFP, PhiYFP-m, and TurboYFP	100 μg 200 μg	103

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

Red (orange) fluorescent protein TurboRFP

- Super bright red (orange) fluorescence
- Fast maturation, high pH-stability
- Destabilized variant is available
- Recommended for gene expression analysis and cell and organelle labeling

TurboRFP is a red (orange) fluorescent protein derived from sea anemone *Entacmaea quadricolor* [Merzlyak et al. 2007]. TurboRFP is more than twice brighter than DsRed2. Fast TurboRFP maturation makes it clearly detectable in mammalian cells as early as within 8-10 hrs after transfection. In addition, unlike DsRed proteins TurboRFP shows no abnormal Golgi-like localization in long-term cell culture.

TurboRFP is mainly intended for applications where fast appearance of bright fluorescence is crucial. It is specially recommended for cell and organelle labeling and tracking the promoter activity. Destabilized TurboRFP variant allows accurate analysis of rapid and/or transient events in gene regulation.

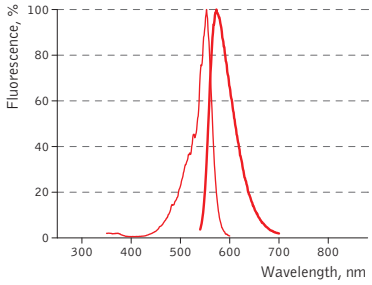
TurboRFP can be expressed and detected in a wide range of organisms. Mammalian cells transiently transfected with TurboRFP expression vectors give bright fluorescent signals within 8-10 hrs after transfection. No cytotoxic effects or visible protein aggregation are observed.

Despite its dimeric structure, TurboRFP performs well in some fusions. However, for protein labeling applications we recommend using specially optimized monomeric TagFPs (see page 11).

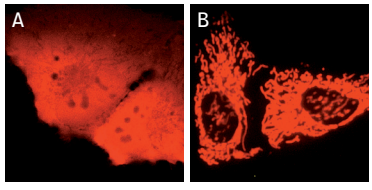
Main properties of TurboRFP

Characteristic	
Molecular weight, kDa	26
Polypeptide length, aa	231
Fluorescence color	red (orange)
Excitation maximum, nm	553
Emission maximum, nm	574
Quantum yield	0.67
Extinction coefficient, $M^{-1}cm^{-1}$	92 000
Brightness*	61.6
Brightness, % of EGFP	187
pKa	4.4
Structure	dimer
Aggregation	no
Maturation rate at 37°C	super fast
Photostability	high
Cell toxicity	not observed

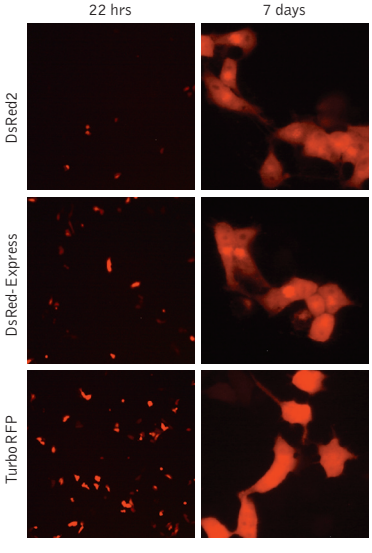
*Brightness is a product of extinction coefficient and quantum yield, divided by 1000.



TurboRFP normalized excitation (thin line) and emission (thick line) spectra.
Complete TurboRFP spectra in Excel format can be downloaded from the Evrogen Web site at www.evrogen.com / support / FP-tech.shtml



TurboRFP use for cell and organelle labeling.
(A) Fluorescent microscopy of mammalian cells expressing cytoplasmic TurboRFP; (B) Fluorescent microscopy of mammalian cells expressing TurboRFP fusion with mitochondrial targeting signal.
Images made from HeLa cells 24 hrs after transfection.



Fluorescent microscopy of Hela cells expressing TurboRFP, DsRed2, and DsRed-Express. TurboRFP gives the brightest signal 22 hrs after transfection; DsRed2 and DsRed-Express show abnormal Golgi-like localization 7 days after transfection, whereas TurboRFP localizes evenly in the cytosol.

TurboRFP can be used in multicolor labeling applications with blue, cyan, green, yellow, and far-red fluorescent dyes.

Recommended filter sets and antibodies

TurboRFP can be recognized using Anti-tRFP antibody (Cat.# AB231-AB232) available from Evrogen.

Recommended Omega Optical filter sets are QMAX-Yellow, XF108-2, XF101-2, and XF111-2. TurboRFP can also be detected using TRITC filter set or similar.

REFERENCES

Merzlyak et al. (2007). Nat Methods, 4 (7): 555–557 / pmid: 17572680

TurboRFP-related products

Product	Cat.#	Description	Size	Page(s)
TurboRFP expression/source vectors				
pTurboRFP-C	FP231	Mammalian expression vector encoding humanized TurboRFP and allowing its expression and generation of fusions to the TurboRFP C-terminus	20 μg	43
pTurboRFP-N	FP232	Mammalian expression vector encoding humanized TurboRFP and allowing its expression and generation of fusions to the TurboRFP N-terminus	20 μg	45
pTurboRFP-B	FP233	Bacterial expression vector; source of the TurboRFP coding sequence	20 μg	42
pTurboRFP-PRL	FP235	Promoterless vector encoding humanized TurboRFP and designed for monitoring activity of different promoters and promoter/enhancer combinations	20 μg	52
pTurboRFP-mito	FP237	Mammalian expression vector encoding humanized TurboRFP targeted to mitochondria	20 μg	47
pTurboRFP-PRL-dest1	FP238	Promoterless vector encoding destabilized TurboRFP and designed for monitoring activity of different promoters and promoter/enhancer combinations	20 μg	52
pTurboRFP-dest1	FP239	Mammalian expression vector encoding destabilized TurboRFP for its expression and generation of fusions to the TurboRFP-dest1 N-terminus	20 μg	45
Vector sets				
Promoter-tracker 3-colors	FPP15	Promoterless vectors pTurboYFP-PRL, pTurboGFP-PRL, and pTurboRFP-PRL	20 μg each	52,52,52
Antibodies against TurboRFP				
Anti-tRFP	AB231 AB232	Rabbit polyclonal antibody against TurboRFP, TurboFP602, TurboFP635, TagBFP, TagRFP, TagFP635, and mKate2	100 μg 200 μg	104

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

Red fluorescent protein TurboFP602

- Bight true-red fluorescence
- Fast maturation, high pH-stability
- Proven suitability to generate stably transfected cell lines
- Recommended for gene expression analysis and cell and organelle labeling in an autofluorescent environment

TurboFP602 is a red-shifted variant of the red fluorescent protein TurboRFP from sea anemone *Entacmaea quadricolor* [Merzlyak et al. 2007]. TurboFP602 possesses true-red fluorescence, optimal for detection via most popular filter sets, and is easily distinguished from background signals. TurboFP602 exhibits fast maturation and high pH stability.

TurboFP602 is mainly intended for applications where fast appearance of true-red fluorescence is crucial. It is specially recommended for cell and organelle labeling and for tracking the promoter activity in autofluorescent tissues.

TurboFP602 can be expressed and detected in a wide range of organisms. Mammalian cells transiently transfected with TurboFP602 expression vectors give bright fluorescent signals within 8-12 hrs after transfection. No cytotoxic effects or visible protein aggregation are observed.

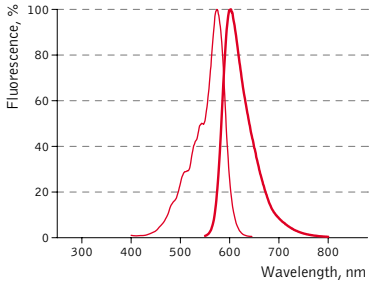
TurboFP602 suitability to generate stably transfected cells has been proven by Marinpharm company. Cell lines expressing TurboFP602 are commercially available.

Despite its dimeric structure, TurboFP602 performs well in some fusions. However, for protein labeling applications we recommend using specially optimized monomeric TagFPs (see page 11).

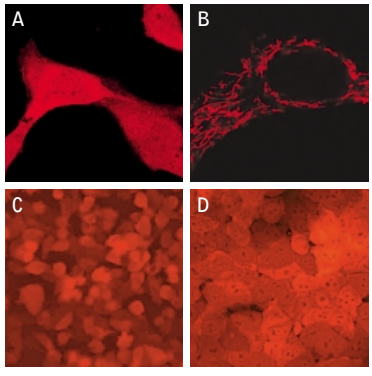
Main properties of TurboFP602

Characteristic	
Molecular weight, kDa	26
Polypeptide length, aa	231
Fluorescence color	true-red
Excitation maximum, nm	574
Emission maximum, nm	602
Quantum yield	0.35
Extinction coefficient, $M^{-1}cm^{-1}$	74 400
Brightness*	26.0
Brightness, % of EGFP	79
pKa	4.7
Structure	dimer
Aggregation	no
Maturation rate at 37°C	fast
Photostability	medium
Cell toxicity	not observed

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



TurboFP602 normalized excitation (thin line) and emission (thick line) spectra.
Complete TurboFP602 spectra in Excel format can be downloaded from the Evrogen Web site at www.evrogen.com / support / FP-tech.shtml



TurboFP602 expression in mammalian cells.
(A) Transiently transfected HeLa cells; (B) transiently transfected HeLa cells expressing mitochondria-targeted TurboFP602; (C) stably transfected human melanoma MelJuso cell line; (D) stably transfected human osteosarcoma U-2-OS cell line.
Photographs of stably transfected cell lines were provided by Dr. Christian Petzelt (Marinpharm).

TurboFP602 can be used in multicolor labeling applications with blue, cyan, green, and yellow fluorescent dyes.

REFERENCES

Merzlyak et al. (2007). Nat Methods, 4 (7): 555–557 / pmid: 17572680

TurboFP602-related products

Product	Cat.#	Description	Size	Page(s)
TurboFP602 expression/source vectors				
pTurboFP602-C	FP711	Mammalian expression vector encoding humanized TurboFP602 and allowing its expression and generation of fusions to the TurboFP602 C-terminus	20 μg	43
pTurboFP602-N	FP712	Mammalian expression vector encoding humanized TurboFP602 and allowing its expression and generation of fusions to the TurboFP602 N-terminus	20 μg	45
pTurboFP602-B	FP713	Bacterial expression vector; source of the TurboFP602 coding sequence	20 μg	42
pTurboFP602-PRL	FP715	Promoterless vector encoding humanized TurboFP602 and designed for monitoring activity of different promoters and promoter/enhancer combinations	20 μg	52
pTurboFP602-mito	FP717	Mammalian expression vector encoding humanized TurboFP602 targeted to mitochondria	20 μg	47
Antibodies against TurboFP602				
Anti-tRFP	AB231 AB232	Rabbit polyclonal antibody against TurboRFP, TurboFP602, TurboFP635, TagBFP, TagRFP, TagFP635, and mKate2	100 μg 200 μg	104

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

Far-red fluorescent protein TurboFP635

- Super bright far-red fluorescence
- Fast maturation, high photostability
- Proven suitability to generate stably transfected cell lines
- Fluorescent signal is easily distinguished from background fluorescence
- Recommended for cell and organelle labeling in autofluorescent environment, multicolor applications and whole body imaging

TurboFP635 (scientific name Katushka) is a far-red mutant of the red fluorescent protein from sea anemone *Entacmaea quadricolor* [Shcherbo et al. 2007]. Possessing excitation/emission maxima at 588/635 nm, TurboFP635 is 7 to 10-fold brighter compared to the spectrally close HcRed [Gurskaya et al. 2001] or mPlum [Wang et al. 2004]. TurboFP635 is characterized by fast maturation and a high pH-stability and photostability. The unique characteristics of TurboFP635 make it the protein of choice for visualization within living tissues and dual-color high-throughput assays.

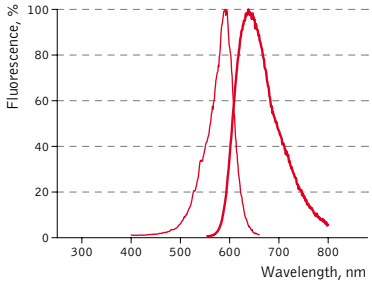
TurboFP635 is mainly intended for applications where fast appearance of far-red fluorescence is crucial. It is specially recommended for whole body imaging, cell and organelle labeling, and for tracking the promoter activity in auto-fluorescent tissues.

TurboFP635 can be easily expressed and detected in a wide range of organisms. It can be easy visualised within living tissues. Mammalian cells transiently transfected with TurboFP635 expression vectors give bright fluorescent signals within 10-12 hours after transfection. No cytotoxic effects or visible protein aggregation are observed.

Main properties of TurboFP635

Characteristic	
Molecular weight, kDa	26
Polypeptide length, aa	231
Fluorescence color	far-red
Excitation maximum, nm	588
Emission maximum, nm	635
Quantum yield	0.34
Extinction coefficient, $M^{-1}cm^{-1}$	65 000
Brightness*	22.1
Brightness, % of EGFP	67
pKa	5.5
Structure	dimer
Aggregation	no
Maturation rate at 37°C	super fast
Photostability	high
Cell toxicity	not observed

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



TurboFP635 normalized excitation (thin line) and emission (thick line) spectra.
Complete TurboFP635 spectra in Excel format can be downloaded from the Evrogen Web site at www.evrogen.com / support / FP-tech.shtml

DsRed-Express and TurboFP635 expression in transgenic *Xenopus laevis*.
Transgenic 2.5 months living animals expressing TurboFP635 and DsRed-Express under the control of cardiac actin promoter are shown from the dorsal side. TurboFP635 (on the right) is excellently visible in the whole body, while DsRed-Express (on the left) can be hardly visualized. This experiment clearly demonstrates the advantage of longer wavelength emission of TurboFP635 for the whole body imaging. Leica MZFLIII fluorescent stereomicroscope, excitation filter 546/10; emission filter 565LP. Image from Shcherbo et al. 2007.

Despite its dimeric structure, TurboFP635 performs well in some fusions. However, for protein labeling applications we recommend using specially optimized monomeric TagFPs (see page 11).

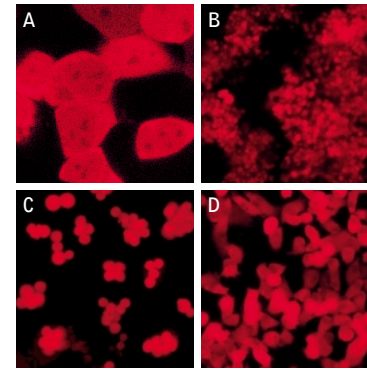
TurboFP635 suitability to generate stably transfected cells has been proven by Marinpharm company. Various cell lines expressing TurboFP635 are commercially available.

TurboFP635 can be used in multicolor labeling applications with blue, cyan, green, yellow and red (orange) fluorescent proteins.

Recommended filter sets and antibodies

TurboFP635 can be recognized using Anti-tRFP antibody (Cat.# AB231-AB232) available from Evrogen.

Recommended Omega Optical filter sets are QMAX-Red and XF102-2. TurboFP635 can also be detected using Texas Red filter sets or similar.



TurboFP635 expression in mammalian cells.
(A) Transiently transfected Phoenix cells; (B) stably transfected WALKER 256 rat tumor cells; (C) stably transfected mouse Ehrlich-Ascites cells; (D) stably transfected metastasizing melanoma MelJuSo cells.
Photographs of stably transfected cell lines were provided by Dr. Christian Petzelt (Marinpharm).

REFERENCES

Gurskaya et al. (2001). FEBS Lett, 507 (1): 16–20 / pmid: 11682051

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

Wang et al. (2004). Proc Natl Acad Sci U S A, 101 (48): 16745–16749 / pmid: 15556995

TurboFP635-related products

Product	Cat.#	Description	Size	Page(s)
TurboFP635 expression/source vectors				
pTurboFP635-C	FP721	Mammalian expression vector encoding humanized TurboFP635 and allowing its expression and generation of fusions to the TurboFP635 C-terminus	20 μg	43
pTurboFP635-N	FP722	Mammalian expression vector encoding humanized TurboFP635 and allowing its expression and generation of fusions to the TurboFP635 N-terminus	20 μg	45
Antibodies against TurboFP635				
Anti-tRFP	AB231 AB232	Rabbit polyclonal antibody against TurboRFP, TurboFP602, TurboFP635, TagBFP, TagRFP, TagFP635, and mKate2	100 μg 200 μg	104

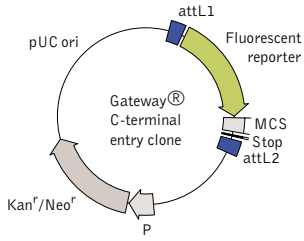
Please contact your local distributor for exact prices and delivery information.

Basic FPs: related products

Expression/source vectors

Vector group	Description	Subtypes	Details	Page
Gateway® entry clones	The vectors allow generation of fusions with the fluorescent proteins and easy transfer of the reporter sequence into Gateway® destination vectors (Invitrogen) for expression in various heterological systems	Gateway® C-terminal entry clone	MCS are located downstream of the fluorescent protein sequence allowing fusion generation to the reporter C-terminus	40
		Gateway® N-terminal entry clone	MCS are located upstream of the fluorescent protein sequence allowing fusion generation to the reporter N-terminus	41
Bacterial expression vectors	The vectors allow easy excision of the fluorescent protein sequence for subcloning and can be also used for fluorescent protein expression in prokaryotic cells			42
Basic mammalian expression vectors	The vectors allow generation of fusions with the fluorescent proteins and expression of these fusions or fluorescent proteins alone in eukaryotic (mammalian) cells under the control of early CMV promoter ($P_{CMV\ IE}$)	C-terminal mammalian expression vectors	MCS are located downstream of the fluorescent protein sequence allowing fusion generation to the reporter C-terminus	43
		N-terminal mammalian expression vectors	MCS are located upstream of the fluorescent protein sequence allowing fusion generation to the reporter N-terminus	45
Subcellular localization vectors	Ready-to-use vectors for labeling of cellular organelles and proteins	Mitochondria localization	Vectors for fluorescent labeling of mitochondria	47
		Golgi apparatus localization	Vectors for fluorescent labeling of Golgi apparatus	47
		Peroxisome localization	Vectors for fluorescent labeling of peroxisomes	48
		Plasma membrane localization	Vectors for fluorescent labeling of plasma membrane	48
		Endosomes localization	Vectors for fluorescent labeling of endosomes	49
		Lysosomes localization	Vectors for fluorescent labeling of lysosomes	49
		Protein localization vectors	Vectors for labeling of cytoskeletal and adhesion proteins (α-actinin, α-V-integrin, α-tubulin, β-actin, EB3 protein, focal adhesion kinase, cytokeratin-18, profilin, vinculin, zyxin), nuclear proteins (histone H2B, lamin B1), gap junction proteins (connexin 26, connexin 32, connexin 43), and vesicular transport protein clathrin	50
Promoterless vectors	The vectors comprise fluorescent reporter coding sequence with multiple cloning sites (MCS) at the 5'-end allowing cloning of a functional promoter			52

Gateway® C-terminal entry clone



For vector sequence, please visit our Web site at
http://www.evrogen.com/support/vector-info.shtml

Vector type	Gateway® entry clone
Reporter(s)	TurboGFP, TagRFP-AS
Promoter	No
Host cells	prokaryotic
Selection	kanamycin
Replication	pUC ori
Use	Generation of fusions to the C-terminus of the fluorescent protein; transfer of the construct encoding fluorescent protein or its fusion into Gateway® destination vectors

Product	Cat.#	Reporter	Codon usage	Color	Size
Gateway® TagRFP-AS-C	FP148	TagRFP	*Arabidopsis* and *Saccharomyces*	red (orange)	20μg
Gateway® TurboGFP-C	FP521	TurboGFP	mammalian	green	20μg

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

Multiple cloning sites (MCS)

Gateway® TagRFP-AS-C vector MCS

TagRFP → . . . TCC.GGA.CTC.AGA.TCT.CGA.GCT.CAA.GCT.TCG.AAT.TCT.GCA.GTC.GAC.GGT.ACC.GCG.GGC.CCG.GGA.TCC.ACC.GGA.TCT.AGG.TAA.CTG.AAC.C . . .

Bgl II (BspE I); Sac I (Xho I); EcoR I (Hind III); Sal I (Pst I); Sac II (Kpn I); Sma I/Xma I (Apa I); BamH I; STOPs; AttL 2 site

Gateway® TurboGFP-C vector MCS

TurboGFP → . . . AGA.TCT.CGA.GCT.CAA.GCT.TCG.AAT.TCT.GCA.GTC.GAC.GGT.ACC.GCG.GGC.CCG.GGA.TCC.ACC.GGA.TCT.AGG.TAA.CTG.AAC.C . . .

Sac I (Xho I); EcoR I (Hind III); Sal I (Pst I*); Sac II (Kpn I); Sma I/Xma I (Apa I*); BamH I; STOP; AttL 2 site

* — not unique sites.

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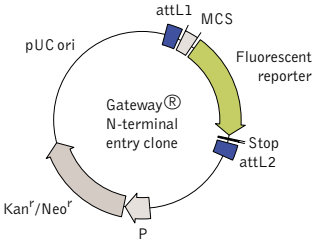
Gateway® N-terminal entry clone



For vector sequence, please visit our Web site at http://www.evrogen.com/support/vector-info.shtml

Vector type	Gateway® entry clone
Reporter(s)	TurboGFP, TagRFP-AS
Promoter	No
Host cells	prokaryotic
Selection	kanamycin
Replication	pUC ori
Use	Generation of fusions to the N-terminus of the fluorescent protein; transfer of the construct encoding fluorescent protein or its fusion into Gateway® destination vectors

Product	Cat.#	Reporter	Codon usage	Color	Size
Gateway® TagRFP-AS-N	FP149	TagRFP	*Arabidopsis* and *Saccharomyces*	red (orange)	20μg
Gateway® TurboGFP-N	FP522	TurboGFP	mammalian	green	20μg

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

Multiple cloning sites (MCS)

Gateway® TagRFP-AS-N vector MCS

attL 1 site — Bgl II — Sac I — EcoR I — Sal I — Sac II — Sma I/Xma I — Age I — TagRFP

... AGG.CTG.CTA.GCG.CTA.CCG.GAC.TCA.GAT.CTC.GAG.CTC.AAG.CTT.CGA.ATT.CTG.CAG.TCG.ACG.GTA.CCG.CGG.GCC.CGG.GAT.CCA.CCG.GTC.GCC.ACC.ATG.G...

Afe I — Xho I — Hind III — Pst I — Kpn I — Apa I — BamH I — Nco I*

Gateway® TurboGFP-N vector MCS

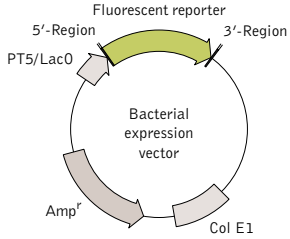
attL 1 site — Bgl II — Sac I — EcoR I — Sal I — Sac II — Sma I/Xma I — Age I — TurboGFP

... AGG.CTG.CTA.GCG.CTA.CCG.GAC.TCA.GAT.CTC.GAG.CTC.AAG.CTT.CGA.ATT.CTG.CAG.TCG.ACG.GTA.CCG.CGG.GCC.CGG.GAT.CCA.CCG.GTC.GCC.ACC.ATG.G...

Afe I — Xho I — Hind III — Pst I* — Kpn I — Apa I* — BamH I — Nco I*

* — not unique sites.

Bacterial expression vectors



For vector sequence, please visit our Web site at http://www.evrogen.com/support/vector-info.shtml

Vector type	bacterial expression vector
Reporter(s)	TurboGFP, TurboYFP, PhiYFP, TurboRFP, TurboFP602
Reporter codon usage	mammalian
Promoter	T5 promoter/lac operator
Host cells	prokaryotic
Selection	ampicillin
Replication	ColE1 ori
Use	Source of the reporter coding sequence; reporter expression in bacterial cells

Product	Cat.#	Reporter	Color	Size
pTurboRFP-B	FP233	TurboRFP	red (orange)	20 μg
pTurboGFP-B	FP513	TurboGFP	green	20 μg
pPhi-Yellow-B	FP603	PhiYFP	yellow	20 μg
pTurboYFP-B	FP613	TurboYFP	yellow	20 μg
pTurboFP602-B	FP713	TurboFP602	red	20 μg

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

pTurboGFP-B vector 5' Region

RBS ATG.AGA.GGA.TCG.GGA.TCC.GAG.A... (TurboGFP)
BamH I

pTurboGFP-B vector 3' Region

...TGA.AGC.TT... (STOP)
Hind III

pTurboYFP-B vector 5' Region

RBS ATG.AGA.GGA.TCG.GGA.TCC.ATG.A... (TurboYFP)
BamH I

pTurboYFP-B vector 3' Region

...TGA.AAG.CTT... (STOP)
Hind III

pPhi-Yellow-B vector 5' Region

RBS ATG.AGA.GGA.TCG.GGA.TCC.A... (PhiYFP)
BamH I

pPhi-Yellow-B vector 3' Region

...TGA.AGC.TT... (STOP)
Hind III

pTurboRFP-B vector 5' Region

RBS ATG.AGA.GGA.TCG.GGA.TCC.ATG.A... (TurboRFP)
BamH I

pTurboRFP-B vector 3' Region

...TGA.AGC.TT... (STOP)
Hind III

pTurboFP602-B vector 5' Region

RBS ATG.AGA.GGA.TCG.GGA.TCC.ATG.G... (BamH I; TurboFP602)
Nco I*

pTurboFP602-B vector 3' Region

...TGA.AGC.TT... (STOP)
Hind III

* — not unique sites.

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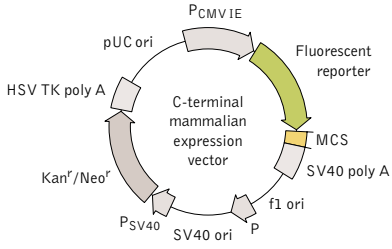
C-terminal mammalian expression vectors



For vector sequence, please visit our Web site at http://www.evrogen.com/support/vector-info.shtml

Vector type	mammalian expression vector
Reporter(s)	TagBFP, TagCFP, TagGFP2, TagYFP, TagRFP, mKate2, TurboGFP, TurboYFP, PhiYFP-m, TurboRFP, TurboFP602, TurboFP635
Reporter codon usage	mammalian
Promoter	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin; eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori; eukaryotic - SV40 ori
Use	Reporter expression in mammalian cells; generation of fusions to the reporter C-terminus

Product	Cat.#	Reporter	Color	Size
pTagCFP-C	FP111	TagCFP	cyan	20μg
pTagYFP-C	FP131	TagYFP	yellow	20μg
pTagRFP-C	FP141	TagRFP	red (orange)	20μg
pTagBFP-C	FP171	TagBFP	blue	20μg
pmKate2-C	FP181	mKate2	far-red	20μg
pTagGFP2-C	FP191	TagGFP2	green	20μg
pTurboRFP-C	FP231	TurboRFP	red (orange)	20μg
pTurboGFP-C	FP511	TurboGFP	green	20μg
pPhi-Yellow-C	FP601	PhiYFP-m	yellow	20μg
pTurboYFP-C	FP611	TurboYFP	yellow	20μg
pTurboFP602-C	FP711	TurboFP602	red	20μg
pTurboFP635-C	FP721	TurboFP635	far-red	20μg

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

Multiple cloning sites (MCS)

pTagCFP-C vector MCS

TagCFP → ... TCC.GGA.CTC.AGA.TCT.CGA.GCT.CAA.GCT.TCG.AAT.TCT.GCA.GTC.GAC.GGT.ACC.GCG.GGC.CCG.GGA.TCC.ACC.GGA.TCT.AGA.TAA.CTG.ATC.A ...
Xho I | Hind III | Pst I | Kpn I | Apa I | BamH I | STOPs
BspE I | Bgl II | Sac I | EcoR I | Sal I | Sac II* | Sma I/Xma I | Xba I# | Bcl I#

pTagYFP-C vector MCS

TagYFP → ... TCC.GGA.CTC.AGA.TCT.CGA.GCT.CAA.GCT.TCG.AAT.TCT.GCA.GTC.GAC.GGT.ACC.GCG.GGC.CCG.GGA.TCC.ACC.GGA.TCT.AGA.TAA.CTG.ATC.A ...
Xho I | Hind III | Pst I* | Kpn I | Apa I | BamH I | STOPs
BspE I | Bgl II | Sac I | EcoR I | Sal I | Sac II* | Sma I/Xma I | Xba I# | Bcl I#

pTagRFP-C vector MCS

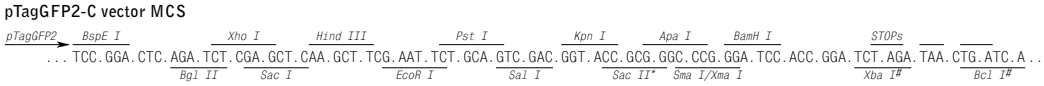
TagRFP → ... TCC.GGA.CTC.AGA.TCT.CGA.GCT.CAA.GCT.TCG.AAT.TCT.GCA.GTC.GAC.GGT.ACC.GCG.GGC.CCG.GGA.TCC.ACC.GGA.TCT.AGA.TAA.CTG.ATC.A ...
Xho I | Hind III | Pst I | Kpn I | Apa I | BamH I | STOPs
BspE I | Bgl II | Sac I | EcoR I | Sal I | Sac II | Sma I/Xma I | Xba I# | Bcl I#

pTagBFP-C vector MCS

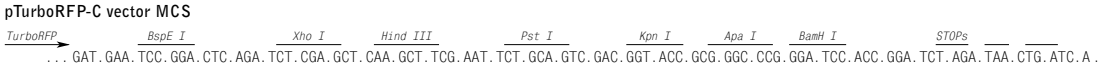
TagBFP → ... TCC.GGA.CTC.AGA.TCT.CGA.GCT.CAA.GCT.TCG.AAT.TCT.GCA.GTC.GAC.GGT.ACC.GCG.GGC.CCG.GGA.TCC.ACC.GGA.TCT.AGA.TAA.CTG.ATC.A ...
Xho I | Hind III* | Pst I | Kpn I | Apa I | BamH I | STOPs
BspE I | Bgl II | Sac I | EcoR I | Sal I | Sac II | Sma I/Xma I | Xba I# | Bcl I#

pmKate2-C vector MCS

pTagGFP2-C vector MCS

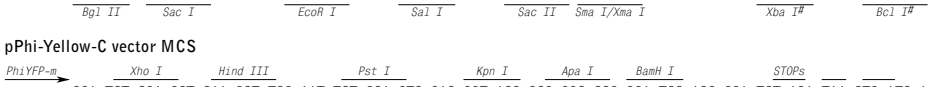


pTurboRFP-C vector MCS

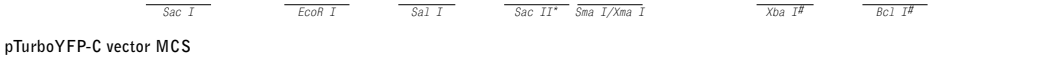


pTurboGFP-C vector MCS

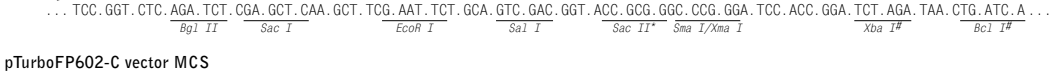
pPhi-Yellow-C vector MCS



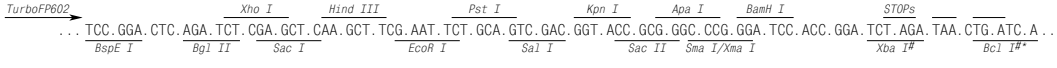
pTurboYFP-C vector MCS



pTurboFP602-C vector MCS



pTurboFP635-C vector MCS



* — not unique sites.

\# — sites are blocked by *dam* methylation. If you wish to digest the vector with these enzymes, you will need to transform the vector into a *dam*$^-$ host and make fresh DNA.

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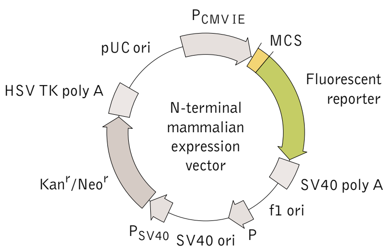
MATERIAL SAFETY DATA SHEET INFORMATION: To the best of our knowledge, these products do not require a Material Safety Data Sheet. However, all the properties of these products (and, if applicable, each of their components) have not been thoroughly investigated. Therefore, we recommend that you use gloves and eye protection, and wear a laboratory coat when working with these products.

N-terminal mammalian expression vectors



For vector sequence, please visit our Web site at http://www.evrogen.com/support/vector-info.shtml

*-dest1 - fluorescent proteins fused with protein degradation sequence.

Vector type	mammalian expression vector
Reporter(s)	TagBFP, TagCFP, TagGFP, TagYFP, TagRFP, mKate2, TurboGFP, TurboYFP, PhiYFP, TurboRFP, TurboFP602, TurboFP635, TurboGFP-dest1*, TurboYFP-dest1*, PhiYFP-m-dest1*, TurboRFP-dest1*
Reporter codon usage	mammalian
Promoter	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin; eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori; eukaryotic - SV40 ori
Use	Reporter expression in mammalian cells; generation of fusions to the reporter N-terminus

Product	Cat.#	Reporter	Color	Size
pTagCFP-N	FP112	TagCFP	cyan	20μg
pTagYFP-N	FP132	TagYFP	yellow	20μg
pTagRFP-N	FP142	TagRFP	red (orange)	20μg
pTagBFP-N	FP172	TagBFP	blue	20μg
pmKate2-N	FP182	mKate2	far-red	20μg
pTagGFP2-N	FP192	TagGFP2	green	20μg
pTurboRFP-N	FP232	TurboRFP	red (orange)	20μg
pTurboRFP-dest1	FP239	TurboRFP-dest1	red (orange)	20μg
pTurboGFP-N	FP512	TurboGFP	green	20μg
pTurboGFP-dest1	FP519	TurboGFP-dest1	green	20μg
pPhi-Yellow-N	FP602	PhiYFP	yellow	20μg
pPhi-Yellow-dest1	FP608	PhiYFP-dest1	yellow	20μg
pTurboYFP-N	FP612	TurboYFP	yellow	20μg
pTurboYFP-dest1	FP619	TurboYFP-dest1	yellow	20μg
pTurboFP602-N	FP712	TurboFP602	red	20μg
pTurboFP635-N	FP722	TurboFP635	far-red	20μg

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

Multiple cloning sites (MCS)

pTagCFP-N vector MCS

Afe I, Xho I, Hind III, Pst I, Kpn I, Apa I, BamH I, TagCFP →

... GCT. AGC. GCT. ACC. GGA. CTC. AGA. TCT. CGA. GCT. CAA. GCT. TCG. AAT. TCT. GCA. GTC. GAC. GGT. ACC. GCG. GGC. CCG. GGA. TCC. ACC. GGT. CGC. CAC. *CAT. GA* ...

Nhe I, Bgl II, Sac I, EcoR I, Sal I, Sac II*, Sma I/Xma I, Age I

pTagYFP-N vector MCS

Nhe I, Bgl II, Sac I, EcoR I, Sal I, Sac II*, Sma I/Xma I, Age I, TagYFP →

... GCT. AGC. GCT. ACC. GGA. CTC. AGA. TCT. CGA. GCT. CAA. GCT. TCG. AAT. TCT. GCA. GTC. GAC. GGT. ACC. GCG. GGC. CCG. GGA. TCC. ACC. GGT. CGC. CAC. *CAT. GG* ...

Afe I, Xho I, Hind III, Pst I*, Kpn I, Apa I, BamH I, Nco I*

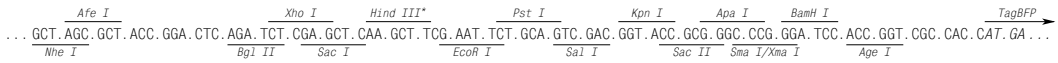
pTagRFP-N vector MCS

Nhe I, Bgl II, Sac I, EcoR I, Sal I, Sac II, Sma I/Xma I, Age I, TagRFP →

... GCT. AGC. GCT. ACC. GGA. CTC. AGA. TCT. CGA. GCT. CAA. GCT. TCG. AAT. TCT. GCA. GTC. GAC. GGT. ACC. GCG. GGC. CCG. GGA. TCC. ACC. GGT. CGC. CAC. *CAT. GG* ...

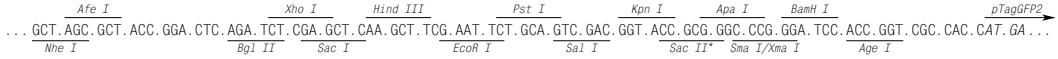
Afe I, Xho I, Hind III, Pst I, Kpn I, Apa I, BamH I, Nco I*

pTagBFP-N vector MCS

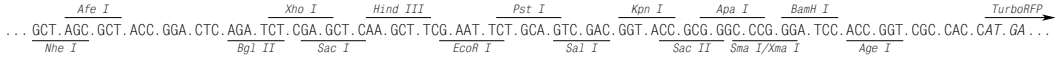


pmKate2-N vector MCS

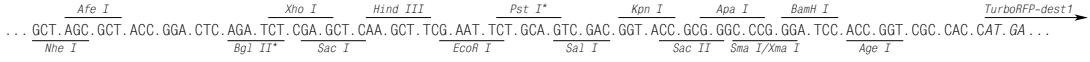
pTagGFP2-N vector MCS



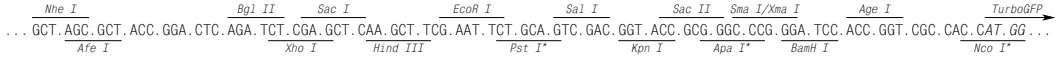
pTurboRFP-N vector MCS



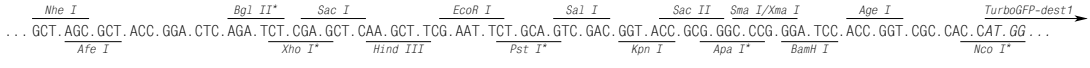
pTurboRFP-dest1 vector MCS



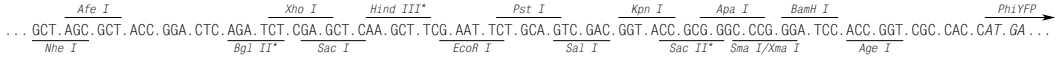
pTurboGFP-N vector MCS



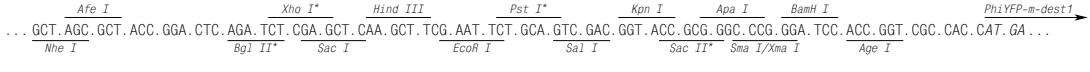
pTurboGFP-dest1 vector MCS



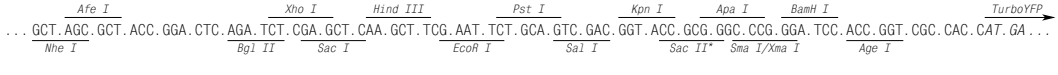
pPhi-Yellow-N vector MCS



pPhi-Yellow-dest1 vector MCS



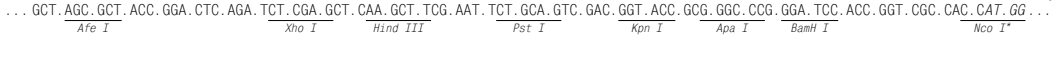
pTurboYFP-N vector MCS



pTurboYFP-dest1 vector MCS

pTurboFP602-N vector MCS

pTurboFP635-N vector MCS



* — not unique sites.

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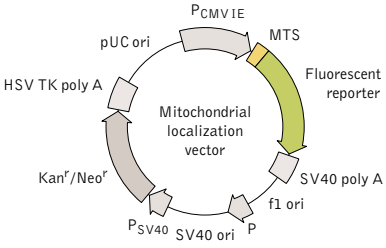
Mitochondria localization vectors



For vector sequence, please visit our Web site at http://www.evrogen.com/support/vector-info.shtml

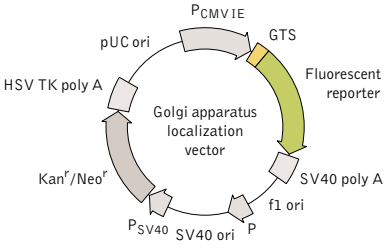
MTS - Mitochondrial targeting sequence

Vector type	mammalian expression vector
Reporter(s)	TagCFP, TagGFP2, TagYFP, TagRFP, TurboGFP, PhiYFP, TurboRFP, TurboFP602
Reporter codon usage	mammalian
Promoter	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin; eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori; eukaryotic - SV40 ori
Use	Fluorescent labeling of mitochondria

Product	Cat.#	Reporter	Color	Size
pTagCFP-mito	FP117	TagCFP	cyan	20μg
pTagYFP-mito	FP137	TagYFP	yellow	20μg
pTagRFP-mito	FP147	TagRFP	red (orange)	20μg
pmKate2-mito	FP187	mKate2	far-red	20μg
pTagGFP2-mito	FP197	TagGFP2	green	20μg
pTurboRFP-mito	FP237	TurboRFP	red (orange)	20μg
pTurboGFP-mito	FP517	TurboGFP	green	20μg
pPhi-Yellow-mito	FP607	PhiYFP	yellow	20μg
pTurboFP602-mito	FP717	TurboFP602	red	20μg

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

Golgi apparatus localization vectors



For vector sequence, please visit our Web site at http://www.evrogen.com/support/vector-info.shtml

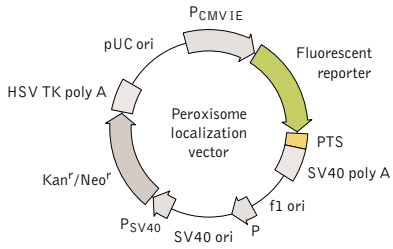
GTS - Golgi targeting sequence

Vector type	mammalian expression vector
Reporter(s)	TagRFP
Reporter codon usage	mammalian
Promoter	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin; eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori; eukaryotic - SV40 ori
Use	Fluorescent labeling of Golgi apparatus

Product	Cat.#	Reporter	Color	Size
pTagRFP-Golgi	FP367	TagRFP	red (orange)	20μg

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

Peroxisome localization vectors



For vector sequence, please visit our Web site at
http://www.evrogen.com/support/vector-info.shtml

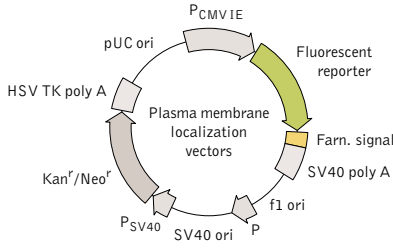
PTS - Peroximal targeting signal

Vector type	mammalian expression vector
Reporter(s)	PhiYFP-m
Reporter codon usage	mammalian
Promoter	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin; eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori; eukaryotic - SV40 ori
Use	Fluorescent labeling of peroxisomes

Product	Cat.#	Reporter	Color	Size
pmKate2-peroxi	FP313	mKate2	far-red	20 μg
pPhi-Yellow-peroxi	FP606	PhiYFP	yellow	20 μg

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

Plasma membrane localization vectors



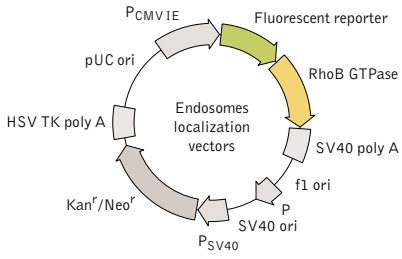
For vector sequence, please visit our Web site at
http://www.evrogen.com/support/vector-info.shtml

Vector type	mammalian expression vector
Reporter	mKate2
Reporter codon usage	mammalian
Promoter	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori
Use	Far-red fluorescent labeling of plasma membrane

Product	Cat.#	Reporter	Color	Size
pmKate2-f-mem vector	FP186	mKate2	far-red	20 μg

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

Endosome localization vectors



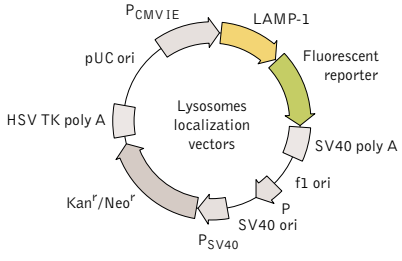
For vector sequence, please visit our Web site at http://www.evrogen.com/support/vector-info.shtml

Vector type	mammalian expression vector
Reporter	mKate2
Reporter codon usage	mammalian
Promoter	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin; eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori; eukaryotic - SV40 ori
Use	Far-red fluorescent labeling of vesicles of the endocytic pathway

Product	Cat.#	Reporter	Color	Size
pmKate2-endo vector	FP314	mKate2	far-red	20 μg

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

Lysosome localization vectors



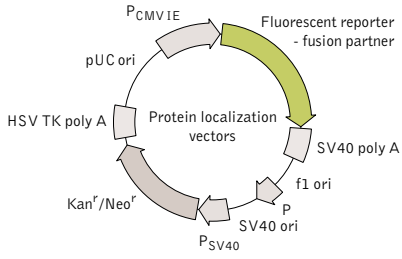
For vector sequence, please visit our Web site at http://www.evrogen.com/support/vector-info.shtml

Vector type	mammalian expression vector
Reporter	mKate2
Reporter codon usage	mammalian
Promoter	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin; eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori; eukaryotic - SV40 ori
Use	Far-red fluorescent labeling of lysosomes

Product	Cat.#	Reporter	Color	Size
pmKate2-lyso vector	FP312	mKate2	far-red	20 μg

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

Protein localization vectors



For vector sequence, please visit our Web site at http://www.evrogen.com/support/vector-info.shtml

TagFP635* - the parental variant of mKate2.

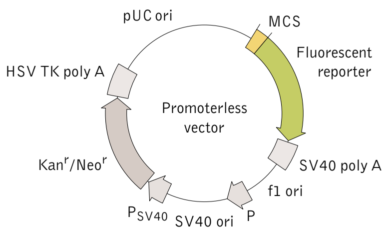
Vector type	mammalian expression vector
Reporter(s)	TagBFP, TagCFP, TagGFP2, TagYFP, TagRFP, mKate2, TagFP635*
Reporter codon usage	mammalian
Promoter	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin; eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori; eukaryotic - SV40 ori
Use	Fluorescent protein labeling in living cells

Fusion partner	Product	Cat.#	Reporter	Color	Size
Cytoskeletal and adhesion proteins					
β-actin	pTagCFP-actin	FP114	TagCFP	cyan	20μg
	pTagYFP-actin	FP134	TagYFP	yellow	20μg
	pTagRFP-actin	FP144	TagRFP	red (orange)	20μg
	pTagBFP-actin	FP174	TagBFP	blue	20μg
	pmKate2-actin	FP184	mKate2	far-red	20μg
	pTagGFP2-actin	FP194	TagGFP2	green	20μg
α-tubulin	pTagCFP-tubulin	FP115	TagCFP	cyan	20μg
	pTagYFP-tubulin	FP135	TagYFP	yellow	20μg
	pTagRFP-tubulin	FP145	TagRFP	red (orange)	20μg
	pTagBFP-tubulin	FP175	TagBFP	blue	20μg
	pmKate2-tubulin	FP185	mKate2	far-red	20μg
	pTagGFP2-tubulin	FP195	TagGFP2	green	20μg
focal adhesion kinase	pTagRFP-FAK	FP366	TagRFP	red (orange)	20μg
paxillin	pmKate2-paxillin	FP323	mKate2	far-red	20μg
profilin	pTagRFP-profilin	FP371	TagRFP	red (orange)	20μg
	pmKate2-profilin	FP320	mKate2	far-red	20μg
vimentin	pmKate2-vimentin	FP318	mKate2	far-red	20μg
vinculin	pTagRFP-vinculin	FP372	TagRFP	red (orange)	20μg
	pTagFP635-vinculin	FP388	TagFP635	far-red	20μg
α-actinin	pTagRFP-actinin	FP360	TagRFP	red (orange)	20μg
	pmKate2-actinin	FP317	mKate2	far-red	20μg
α-V-integrin	pTagRFP-integrin	FP361	TagRFP	red (orange)	20μg
EB3 protein	pmKate2-EB3	FP316	mKate2	far-red	20μg
	pTagRFP-EB3	FP365	TagRFP	red (orange)	20μg
cytokeratin-18	pmKate2-keratin	FP319	pmKate2	far-red	20μg
	pTagRFP-keratin	FP369	TagRFP	red (orange)	20μg
zyxin	pmKate2-zyxin	FP315	mKate2	far-red	20μg

	pTagRFP-zyxin	FP373	TagRFP	red (orange)	20μg
Gap junction proteins					
connexin 26	pTagRFP-Cx26	FP362	TagRFP	red (orange)	20μg
	pTagFP635-Cx26	FP382	TagFP635	far-red	20μg
connexin 32	pTagRFP-Cx32	FP363	TagRFP	red (orange)	20μg
	pTagFP635-Cx32	FP383	TagFP635	far-red	20μg
connexin 43	pTagRFP-Cx43	FP364	TagRFP	red (orange)	20μg
	pTagFP635-Cx43	FP384	TagFP635	far-red	20μg
Vesicular transport proteins					
clathrin light chain LCB	pmKate2-clathrin	FP322	pmKate2	far-red	20μg
Nuclear proteins					
histone H2B	pTagBFP-H2B	FP176	TagBFP	blue	20μg
	pTagRFP-H2B	FP368	TagRFP	red (orange)	20μg
	pmKate2-H2B	FP311	mKate2	far-red	20μg
lamin B1	pmKate2-laminB1	FP310	mKate2	far-red	20μg
	pTagRFP-laminB1	FP370	TagRFP	red (orange)	20μg
Other					
annexin	pmKate2-annexin	FP321	mKate2	far-red	20μg

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

Promoterless vectors



For vector sequence, please visit our Web site at http://www.evrogen.com/support/vector-info.shtml

*-dest1 - fluorescent proteins fused with protein degradation sequence.

Vector type	promoterless vector
Reporter(s)	TurboGFP, TurboGFP-dest1*, TurboYFP, TurboYFP-dest1*, PhiYFP-m, PhiYFP-m-dest1*, TurboRFP, TurboRFP-dest1*, TurboFP602
Reporter codon usage	mammalian
Promoter	No
Host cells	mammalian, prokaryotic
Selection	prokaryotic - kanamycin; eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori; eukaryotic - SV40 ori
Use	Monitoring of the activity of different promoters and promoter/enhancer combinations introduced to the vector MCS

Product	Cat.#	Reporter	Color	Size
pTurboRFP-PRL	FP235	TurboRFP	red (orange)	20μg
pTurboRFP-PRL-dest1	FP238	TurboRFP-dest1	red (orange)	20μg
pTurboGFP-PRL	FP515	TurboGFP	green	20μg
pTurboGFP-PRL-dest1	FP518	TurboGFP-dest1	green	20μg
pPhi-Yellow-PRL	FP604	PhiYFP	yellow	20μg
pPhi-Yellow-PRL-dest1	FP605	PhiYFP-m-dest1	yellow	20μg
pTurboYFP-PRL	FP615	TurboYFP	yellow	20μg
pTurboYFP-PRL-dest1	FP618	TurboYFP-dest1	yellow	20μg
pTurboFP602-PRL	FP715	TurboFP602	red	20μg

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

Multiple cloning sites (MCS)

pTurboRFP-PRL vector MCS

Afe I, Xho I, Hind III, Pst I, Kpn I, Apa I, BamH I, TurboRFP →
... ACT.AGC.GCT.ACC.GGA.CTC.AGA.TCT.CGA.GCT.CAA.GCT.TCG.AAT.TCT.GCA.GTC.GAC.GGT.ACC.GCG.GGC.CCG.GGA.TCC.ACC.GGT.CGC.CAC.CAT.GA ...
Bgl II, Sac I, EcoR I, Sal I, Sac II, Sma I/Xma I, Age I

pTurboRFP-PRL-dest1 vector MCS

Afe I, Xho I, Hind III, Pst I*, Kpn I, Apa I, BamH I, TurboRFP-dest1 →
... ACT.AGC.GCT.ACC.GGA.CTC.AGA.TCT.CGA.GCT.CAA.GCT.TCG.AAT.TCT.GCA.GTC.GAC.GGT.ACC.GCG.GGC.CCG.GGA.TCC.ACC.GGT.CGC.CAC.CAT.GA ...
Bgl II*, Sac I, EcoR I, Sal I, Sac II, Sma I/Xma I, Age I

pTurboGFP-PRL vector MCS

Bgl II, Sac I, EcoR I, Sal I, Sac II, Sma I/Xma I, Age I, TurboGFP →
... ACT.AGC.GCT.ACC.GGA.CTC.AGA.TCT.CGA.GCT.CAA.GCT.TCG.AAT.TCT.GCA.GTC.GAC.GGT.ACC.GCG.GGC.CCG.GGA.TCC.ACC.GGT.CGC.CAC.CAT.GG ...
Afe I, Xho I, Hind III, Pst I*, Kpn I, Apa I*, BamH I, Nco I*

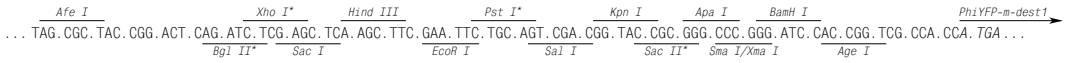
pTurboGFP-PRL-dest1 vector MCS

Bgl II*, Sac I, EcoR I, Sal I, Sac II, Sma I/Xma I, Age I, TurboGFP-dest1 →
... ACT.AGC.GCT.ACC.GGA.CTC.AGA.TCT.CGA.GCT.CAA.GCT.TCG.AAT.TCT.GCA.GTC.GAC.GGT.ACC.GCG.GGC.CCG.GGA.TCC.ACC.GGT.CGC.CAC.CAT.GG ...
Afe I, Xho I*, Hind III, Pst I*, Kpn I, Apa I*, BamH I, Nco I*

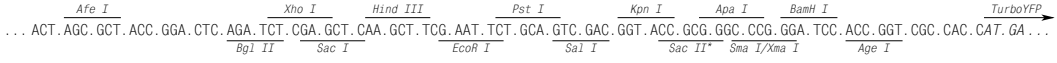
pPhi-Yellow-PRL vector MCS

Afe I, Xho I, Hind III*, Pst I, Kpn I, Apa I, BamH I, PhiYFP →
... TAG.CGC.TAC.CGG.ACT.CAG.ATC.TCG.AGC.TCA.AGC.TTC.GAA.TTC.TGC.AGT.CGA.CGG.TAC.CGC.GGG.CCC.GGG.ATC.CAC.CGG.TCG.CCA.CCA.TGA ...
Bgl II*, Sac I, EcoR I, Sal I, Sac II*, Sma I/Xma I, Age I

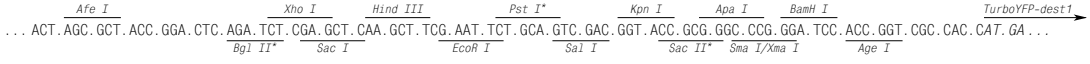
pPhi-Yellow-PRL-dest1 vector MCS



pTurboYFP-PRL vector MCS



pTurboYFP-PRL-dest1 vector MCS



pTurboFP602-PRL vector MCS

* — not unique sites.

\# — sites are blocked by *dam* methylation. If you wish to digest the vector with these enzymes, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

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MATERIAL SAFETY DATA SHEET INFORMATION: To the best of our knowledge, these products do not require a Material Safety Data Sheet. However, all the properties of these products (and, if applicable, each of their components) have not been thoroughly investigated. Therefore, we recommend that you use gloves and eye protection, and wear a laboratory coat when working with these products.

Recombinant proteins

Product	Cat.#	Reporter	Color	Size
rTurboGFP	FP552	TurboGFP	green	100μg
rPhiYFP	FP651	PhiYFP	green	100μg

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

Use

- Standard on protein gels and Western blots
- Control for fluorescence microscopy
- Calibration of fluorimeters and FACS machines
- Microinjection into cells and tissues

rTurboGFP

Recombinant TurboGFP (rTurboGFP) is a 26-kDa green fluorescent protein. It has excitation and emission spectra identical to those of the expressed TurboGFP. rTurboGFP is suitable as control reagent for TurboGFP expression using the TurboGFP expression vectors. rTurboGFP is purified from transformed *E. coli* using organic extraction and hydrophobic chromatography or metal-ion affinity chromatography (methods vary for different lots). Both methods ensure high purity of the recombinant protein and maintenance of fluorescence. The protein concentration is measured by chromophore absorption. rTurboGFP may contain 6xHis tag at its N-terminus (varying in different lots).

rPhiYFP

Recombinant PhiYFP (rPhiYFP) is a 26-kDa yellow fluorescent protein. It has excitation and emission spectra identical to those of the expressed PhiYFP. rPhiYFP is suitable as control reagent for PhiYFP expression using the PhiYFP expression vectors. rPhiYFP is purified from transformed *E. coli* using organic extraction and hydrophobic chromatography or metal-ion affinity chromatography (methods vary for different lots). Both methods ensure high purity of the recombinant protein and maintenance of fluorescence. The protein concentration is measured by chromophore absorption. rPhiYFP may contain 6xHis tag at its N-terminus (varying in different lots).

Special Fluorescent Technologies

Photoactivatable fluorescent proteins:

- Tracking of movements of individual cells, cellular organelles and protein fractions
- Monitoring of protein degradation
- Superresolution imaging

Genetically encoded biosensors:

- Real time monitoring of changes in concentration of Ca^{2+} and H_2O_2 in various subcellular compartments
- Early detection of apoptosis

Genetically encoded photosensitizer:

- Selective light-induced cell killing
- Precise light-induced inactivation of proteins

Contents

Overview

In addition to basic fluorescent proteins traditionally used as fluorescent labels (see page 97), Evrogen offers specialized fluorescent protein-based tools including:

Photoactivatable fluorescent proteins (see page 58)

Photoactivatable fluorescent proteins (PAFPs) are the genetically encoded reporters that change spectral properties in response to irradiation with specific light. The main application of PAFPs is the precise labeling and tracking of movements of individual cells, cellular organelles and protein fractions. The object of interest tagged by PAFP can be photoconverted using a beam of focused light and monitored directly within the living tissue or cell [Patterson and Lippincott-Schwartz 2002; Chudakov et al. 2004; Gurskaya et al. 2006].

Other applications utilizing photoactivatable fluorescent proteins include real-time monitoring of protein degradation at the single cell level and superresolution imaging by PALM technique [Zhang et al. 2007; Shroff et al. 2007].

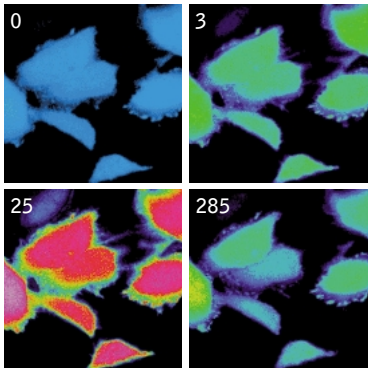
Fluorescent biosensors (see page 70)

Evrogen offers genetically-encoded fluorescent indicators for monitoring activity of caspase-3 (Casper3-BG, Casper3-GR) and changes in intracellular concentration of calcium ions (Case 12) and hydrogen peroxide (HyPer). All indicators demonstrate clearly detectable alterations in spectral properties in response to a cell event of interest [Subach et al. 2008; Shcherbo et al. 2009; Souslova et al. 2007; Belousov et al. 2006], allow precise targeting into various subcellular compartments and real time measuring of signals in natural intracellular surroundings.

Fluorescent photosensitizer (see page 87)

Photosensitizers are chromophores that generate reactive oxygen species (ROS) upon light irradiation. They can be used for precise inactivation of selected proteins in chromophore-assisted light inactivation (CALI) technique and for the light-induced cell killing, for example in photodynamic therapy.

Red fluorescent protein KillerRed is the first genetically-encoded photosensitizer [Bulina et al. 2006]. Besides KillerRed, all currently known photosensitizers are chemical compounds that must be introduced into living systems exogenously. Unlike chemical analogs, KillerRed can be directly expressed by target cells, both individually and in fusion with a target protein. It shows no cytotoxic effects before light activation. Upon green-light irradiation, KillerRed generates ROS that damage the neighboring molecules.



Ratiometric imaging of HyPer response to H_2O_2 in HeLa cells. HeLa cells expressing HyPer in cytoplasm were plated to glass bottom dishes and challenged with 180 μM H_2O_2. Images were acquired by Leica AF 6000 LX with 0.5 Hz frequency by sequential illumination of cells via CFP/YFP (excitation/emission) and YFP/YFP filters. Resulting images were obtained by dividing of YFP/YFP images to CFP/YFP images followed by pseudo coloring. Time after H_2O_2 addition (sec) is indicated at the top.

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Zhang et al. (2007). Biotechniques, 42 (4): 446–450 / pmid: 17489230

Photoactivatable Fluorescent proteins

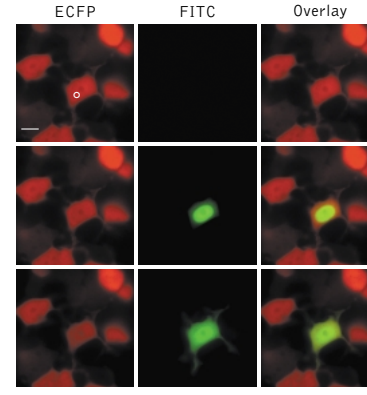
Photoactivatable fluorescent proteins (PAFPs) represent an effective tool for monitoring cellular events. These reporters are capable of pronounced changes in their spectral properties in response to irradiation with light of a specific wavelength and intensity. KFP-Red converts from a non-fluorescent (dark) to a bright fluorescent state (photoactivation), whereas PS-CFP2 changes fluorescence color (photoswitching or photoconversion).

PAFPs provide a more precise and less damaging way to study cell migration and protein movements than photobleaching techniques such as fluorescence recovery after photobleaching (FRAP) or fluorescence loss in photobleaching (FLIP). In contrast to the observation of fluorescently tagged objects by constant imaging, tracking with PAFPs do not require the continual visualization. This feature greatly extends the spatiotemporal limits of studies of biological dynamics, and reduces the photobleaching and phototoxicity problems of imaging procedures.

Main properties of Evrogen photoactivatable FPs:

	PS-CFP2	KFP-Red
	before / after activation	before / after activation
Fluorescence color	cyan / green	No / red
Excitation maximum, nm	400 / 490	580 / 580
Emission maximum, nm	468 / 511	600 / 600
Quantum yield	0.2 / 0.23	<0.001 / 0.07
Extinction coefficient, $M^{-1}cm^{-1}$	43 000 / 47 000	123 000 / 59 000
Brightness*	8.6 / 10.8	0 / 4.1
pKa	4.3 / 6.1	no data / no data
Activating light	UV-violet (360-420 nm)	green (530-560 nm)
Photoswitching	irreversible	reversible
Calculated contrast, fold	up to 2000	35 (irreversible kindling) 70 (reversible kindling)
Structure	monomer	tetramer
Cell toxicity	not observed	not observed
Aggregation	no	no
Maturation rate at 37°C	fast	medium
Molecular weight, kDa	27	26
Polypeptide length, aa	238	238

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

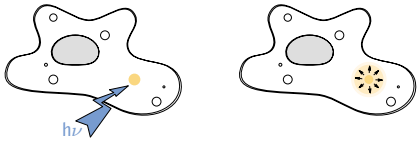


PS-CFP photoswitching in the cell nucleus
PS-CFP was uniformly expressed in mammalian cells and its subpopulation was selectively photoswitched in the nucleus of one cell. Further imaging has revealed a gradual decrease in the green signal and an increase in the cyan signal within the nucleus due to the movement of photoswitched PS-CFP through nuclear pores into the cytoplasm and of non-photoswitched protein in the reverse way. This resulted in their equilibration through the cell within several minutes. Signals in ECFP and FITC channels are shown in red and green, respectively. Circle outlines the photoswitched region. Scale bar, 10 μg.
PS-CFP is a parental version of PS-CFP2.

Applications of photoactivatable FPs

Protein tracking

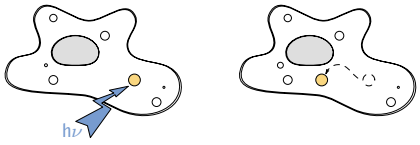
Recomended protein:
PS-CFP2



Parameters determined:
- Movement rate and direction,
- Diffusion coefficient,
- Mobile and immobile fractions,
- Time parameters of compartmental residency and exchange between compartments,
- Rate of turnover.

Organelle tracking

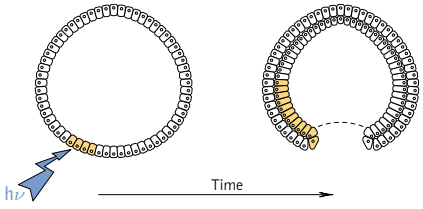
Recomended proteins:
PS-CFP2, KFP-Red



Parameters determined:
- Movement rate and direction,
- Rate of content interchange,
- Fission and fusion events.

Cell tracking

Recomended proteins:
PS-CFP2, KFP-Red



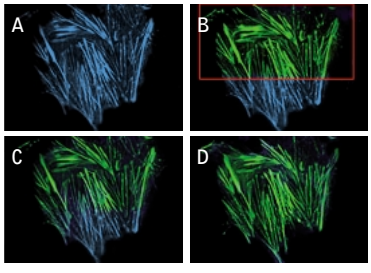
Parameters determined:
- Movement rate and direction,
- Cell localization,
- Rate of cell division,
- Shape and volume of cells.

Three levels of spatio-temporal labeling with photoactivatable fluorescent proteins.
A focused beam of light (blue arrows) is used to activate photoactivatable fluorescent proteins (orange zones) in a region of interest in a cell, tissue or organism. Migration of the labeled object (protein, organelle or cell) can then be monitored over time. A number of qualitative and quantitative parameters can be determined in each case.

Monitoring of cell migration: PAFPs provide an unique opportunity for non-invasive labeling and tracking the movements of specific cells in living organisms and tissues. The obvious examples include studying of embryogenesis, metastasis and tumor formation, the migration of small parasites within a host, and the taxis reactions of free unicellular organisms. Use example is described in [Chudakov et al. 2003] and on page 66 of this catalog.

Tracking of protein and organelle movement: Monomeric PS-CFP2 is proved to be suitable for generation of fusions. It makes it the ideal photoactivatable tags for selective labeling of the proteins and cellular organelles and tracking their movements in the living cells. Examples of use are described in [Chudakov et al. 2003; Chudakov et al. 2007] and on page 62 of this catalog.

Monitoring protein turnover: Another application of monomeric PAFPs is the careful determination of protein half-life. In the method proposed, cells are transfected with a construct coding for target protein fused with a PAFP. A steady-state concentration of the fusion protein and corresponding fluorescent signal (e.g. cyan) depends on protein synthesis and maturation rates as well as protein degradation rate. After photoconversion of the PAFP in a whole cell, a pool of distinct fluorescent molecules (e.g. green) appears. This process is independent on the synthesis and maturation of the new PAFP molecules. Thus, the decay of the activated fluorescence directly corresponds



Photoconversion of a PS-CFP2 fusion with human β-actin. 405-nanometer diode laser was used for imaging and conversion, argon-ion 488-nanometer spectral line was used for imaging and tracking of the photoconverted protein. (A) PS-CFP2 fusion with β-actin was expressed in opossum kidney epithelial cells. A portion of the actin cytoskeletal network (B; red box) was then illuminated at 405-nanometers with 40-percent laser power. After 10 minutes (C), the photoconverted actin has begun to be incorporated into filaments outside the region of interest, and at 30 minutes (D) much of the cytoskeletal network is labeled with the optical highlighter. The image is reproduced from MicroscopyU web site http://www.microscopyu.com with kind permission of Michael W. Davidson (Florida State University).

to the degradation of the PAFP-tagged protein. Time-lapse imaging of the activated signal allows for quantification of degradation process in real-time at the single cell level [Zhang et al. 2007].

Superresolution imaging: Photoactivated localization microscopy (PALM) using PAFPs allows imaging of intracellular proteins at nanometer spatial resolution. Two-color PALM utilizing spectrally distinct PAFPs fused to the proteins of interest is used for determination of ultrastructural relationship between these proteins. It has been demonstrated that cyan-to-green photoactivatable PS-CFP2 is perfectly suitable for such application [Shroff et al. 2007].

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Zhang et al. (2007). Biotechniques, 42 (4): 446–450 / pmid: 17489230

Cyan-to-green photoswitchable fluorescent protein PS-CFP2

- Monomer, succesful perfomance in fusions
- Irreversible photoconversion from a cyan to a green fluorescent form
- High contrast of photoconversion
- High pH stability allowing labeling of acidic organelles
- Recommended for tracking cell, organelle, and protein movement, monitoring the protein turnover and superresolution imaging

PS-CFP2 is an improved mutant of the photoswitchable monomeric fluorescent protein PS-CFP [Chudakov et al. 2004]. PS-CFP2 exhibits faster maturation and a brighter fluorescence both before and after photoswitching than its parental variant.

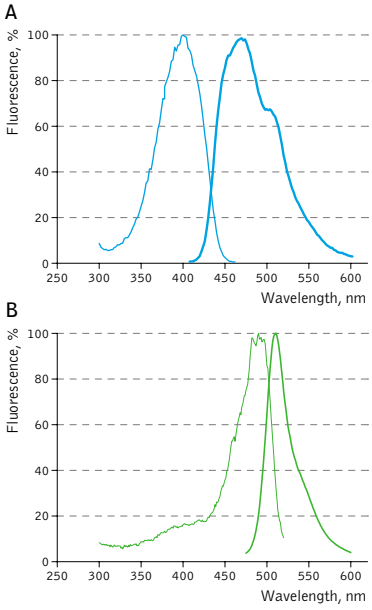
PS-CFP2 is capable of irreversible photoconversion from cyan to green fluorescent form in response to 405 nm light irradiation. It is recommended for real-time *in vivo* tracking movement of individual cells, organelles, and protein fractions [Chudakov et al. 2007]. It can also be applied for monitoring of the protein turnover at the single cell level [Zhang et al. 2007] and superresolution imaging by PALM [Shroff et al. 2007]. In addition, PS-CFP2 can be used as a routine cyan fluorescent tag (excitation maximum at 400 nm and emission maximum at 468 nm) at moderate excitation intensities and as a donor in FRET applications [Souslova and Chudakov 2006].

PS-CFP2 can be easily expressed and detected in a wide range of organisms. Mammalian cells transiently transfected with PS-CFP2 expression vectors display an evenly distributed cyan signal within 10-12 hrs after transfection. No cytotoxic effects are observed.

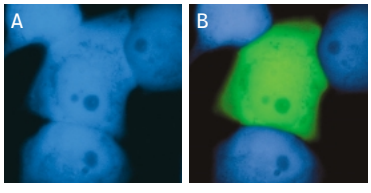
Main properties of PS-CFP2

Characteristic	before / after photoactivation
Fluorescence color	cyan / green
Excitation maximum, nm	400 / 490
Emission maximum, nm	468 / 511
Quantum yield	0.2 / 0.23
Extinction coefficient, $M^{-1}cm^{-1}$	43 000 / 47 000
Brightness*	8.6 / 10.8
pKa	4.3 / 6.1
Activating light	UV-violet (e.g. 405 nm)
Photoswitching	irreversible
Calculated contrast, fold	up to 2000
Structure	monomer
Cell toxicity	not observed
Aggregation	no
Maturation rate at 37°C	fast
Molecular weight, kDa	27
Polypeptide length, aa	238

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



PS-CFP2 normalized excitation (thin line) and emission (thick line) spectra.
(A) before activation; (B) after activation. Complete PS-CFP2 spectra in Excel format can be downloaded from the Evrogen Web site at www.evrogen.com / support / FP-tech-pa.shtml



PS-CFP2 photoconversion in transiently transfected mammalian cells.
Central cell expressing PS-CFP2 was irradiated by intense 405 nm light that resulted in PS-CFP2 photoswitching. Before photoswitching no detectable green fluorescence at FITC excitation was seen in cells expressing PS-CFP2. In contrast, high-level signal was observed in cyan channel. Upon irradiation with a 10-15 micro Joules (about 20-30 W/cm^2) violet dye laser (405 nm) for a few seconds a fluorescence increase of more than 300-fold was observed in FITC channel. (A) before photoconversion; (B) after photoconversion.

PS-CFP2 successful performance has been proven in many fusions including that with cytoplasmic β-actin, BH3 interacting domain death agonist (BID), nucleolar protein fibrillarin, and dopamine transporter (hDAT).

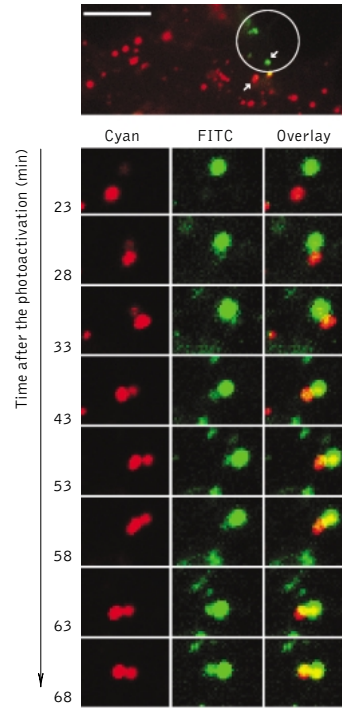
High pH stability: Before photoactivation, PS-CFP2 exhibits a high pH stability with a pKa of 4.3. No changes were observed either in the shape or in the amplitude of fluorescence spectra within a pH range of 5.0 and 9.0. This makes it possible to target PS-CFP2 to acidic organelles such as endosomes and lysosomes. After photoswitching, PS-CFP2 has a pKa of 6.1, similar to that of other GFP-like proteins with a phenolate anion chromophore (e.g. EGFP).

High contrast of photoconversion: In response to intense 400 nm light irradiation, PS-CFP2 undergoes irreversible photoconversion expressed in a decrease in cyan fluorescence and appearance of a 490 nm excitation peak with emission maximum at 511 nm. After complete photoconversion, green fluorescence of PS-CFP2 increases more than 400 times, whereas the level of cyan fluorescence drops more than 5.5 times lower. Thus, the increase in the green-to-cyan fluorescence ratio accounts for more than a 2000-fold contrast. Considerable decrease of cyan fluorescence during PS-CFP2 photoconversion provides a molecular tool to simultaneously track both the movement of the photoactivated protein and its replacement with the non-activated form.

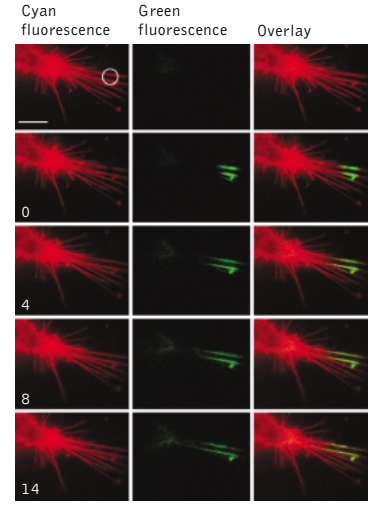
Suitability for tracking protein traffic has been demonstrated on example of PS-CFP (the parental variant of PS-CFP2) fused with the human dopamine transporter, hDAT [Chudakov et al. 2004]. PS-CFP-tagged hDAT was expressed in HEK293 cells. As expected, the fusion protein was localized in the cellular membranes. Then PS-CFP-hDAT was selectively photoswitched in the middle parts of two filopodia by short pulse of 404 nm laser irradiation. High contrast of photoconversion allowed monitoring hDAT movement precisely within thin filopodia in the vicinity of a big non-switched PS-CFP-hDAT pool at the filopodia base. At the same time, a decrease in the cyan fluorescence during photoswitching allowed monitoring non-switched PS-CFP-hDAT molecules entering the activated region.

When expressed heterologously, hDAT is capable of endocytosis. To test whether early endosomes are able to exchange cargo proteins such as hDAT, PS-CFP-hDAT fusion was selectively photoswitched in several endosomes. Then the endosomes (both photolabeled and intact) were monitored within the whole cell for more than an hour. They exhibited fast and rather chaotic intracellular movement. Eventually two endosomes drew together to form a doublet. One of them contained photoswitched PS-CFP-hDAT and soon after their contact PS-CFP-hDAT mutual exchange between the endosomes occurred: cyan fluorescence of the activated endosome recovered while green fluorescence of the non-switched endosome grew.

PS-CFP2 use in superresolution imaging: The performance of PS-CFP2 in photoactivated localization microscopy (PALM) was demonstrated in [Shroff et al. 2007]. Using PS-CFP2 as a second label in two-color PALM it was shown that paxillin and zyxin proteins, which seem co-localized when viewed by conventional TIRF, in fact form separate nanoscale clusters.



PS-CFP-hDAT interchange between two endosomes. Signals in cyan and FITC channels are shown in red and green pseudocolors, respectively. Circle outlines the photoswitched region. Arrows point to the endosomes tracked. Scale bar, 10 μm.



Tracking of PS-CFP-hDAT fusion within filopodia of HEK293 cells. Signals in cyan and FITC channels are shown in red and green pseudocolors, respectively. Circle outlines the photoswitched region. Scale bar, 10 μm. Time after the photoactivation (min) is indicated on left.

Recommended antibodies, filter sets and laser lines

PS-CFP2 can be recognized using Anti-Tag(CGY)FP antibody (Cat.# AB121-AB122) available from Evrogen.

Visualization before photoswitching: Before activation, PS-CFP2 produces cyan fluorescence with excitation and emission maxima at 400 and 468 nm, respectively. Standard levels of excitation do not cause significant photoswitching of cyan or photobleaching of green fluorescence.

PS-CFP2 excitation spectrum is absolutely different from that of common cyan fluorescent proteins, such as TagCFP, ECFP or Cerulean. Therefore, common CFP filter sets are not optimal for PS-CFP2 visualization and photoactivation.

Recommended filter sets are: XF119-2*, XF131, XF06, XF03, XF11, XF129-2, XF05-2 (Omega Optical); DAPI-5060B* and DAPI-1160A (Semrock); 31037, 31041, 31016, 31021, 31000v2, 1009v2, 31013v2, 11005v2, 31047 (Chroma Technology Corp.).
* - preferred filter sets

Photoswitching: PS-CFP2 undergoes irreversible photoconversion (in response to intense 405 nm light irradiation) expressed in a decrease in cyan fluorescence and appearance of a 490 nm excitation peak with emission maximum at 511 nm.

Visualization after photoswitching: Standard GFP filter sets (e.g. Omega Optical XF100-2) can be used for visualization of green fluorescence of photoactivated PS-CFP2. Importantly, the filter should not pass excitation light with wavelengths shorter than 450 nm to avoid cross-excitation of non-photoactivated PS-CFP2.

The detailed protocol is published in Chudakov et al. 2007.

REFERENCES

Chudakov et al. (2004). Nat Biotechnol, 22 (11): 1435–1439 / pmid: 15502815

Chudakov et al. (2007). Nat Protoc, 2 (8): 2024–2032 / pmid: 17703215

Shroff et al. (2007). Proc Natl Acad Sci USA, 104 (51): 20308–20313 / pmid: 18077327

Souslova and Chudakov (2006). Microsc Res Tech, 69 (3): 207–209 / pmid: 16538627

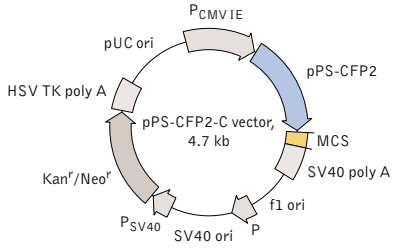
Zhang et al. (2007). Biotechniques, 42 (4): 446–450 / pmid: 17489230

PS-CFP2-related products

Product	Cat.#	Description	Size	Page(s)
PS-CFP2 expression/source vectors				
pPS-CFP2-C	FP801	Mammalian expression vector encoding humanized PS-CFP2 and allowing its expression and generation of fusions to the PS-CFP2 C-terminus	20 μg	64
pPS-CFP2-N	FP802	Mammalian expression vector encoding humanized PS-CFP2 and allowing its expression and generation of fusions to the PS-CFP2 N-terminus	20 μg	64
Antibodies against PS-CFP2				
Anti-Tag(CGY)FP	AB121 AB122	Rabbit polyclonal antibody against TagCFP, TagGFP, TagGFP2, TagYFP, PS-CFP2, and EGFP	100 μg 200 μg	101

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

pPS-CFP2-C vector



For vector sequence, please visit our Web site at http://www.evrogen.com/support/vector-info.shtml

Vector type	mammalian expression vector
Reporter(s)	PS-CFP2
Reporter codon usage	mammalian
Promoter	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori; eukaryotic - SV40 ori
Use	Reporter expression in mammalian cells; generation of fusions to the reporter C-terminus

Product	Cat.#	Reporter	Color	Size
pPS-CFP2-C	FP801	PS-CFP2	cyan-to-green	20μg

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

pPS-CFP2-C vector MCS

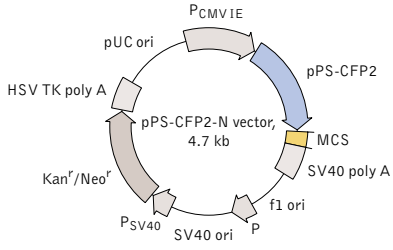
PS-CFP2 → BspE I | Xho I* | Hind III | Pst I | Kpn I | Apa I | BamH I | STOPs

... TCC.GGA.CTC.AGA.TCT.CGA.GCT.CAA.GCT.TCG.AAT.TCT.GCA.GTC.GAC.GGT.ACC.GCG.GGC.CCG.GGA.TCC.ACC.GGA.TCT.AGA.TAA.CTG.ATC.A ...

Bgl II | Sac I | EcoR I | Sal I | Sac II | Sma I/Xma I | Xba I# | Bcl I#

* — not unique sites.
\# — sites are blocked by *dam* methylation. If you wish to digest the vector with these enzymes, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

pPS-CFP2-N vector



For vector sequence, please visit our Web site at http://www.evrogen.com/support/vector-info.shtml

Vector type	mammalian expression vector
Reporter(s)	PS-CFP2
Reporter codon usage	mammalian
Promoter	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori; eukaryotic - SV40 ori
Use	Reporter expression in mammalian cells; generation of fusions to the reporter N-terminus

Product	Cat.#	Reporter	Color	Size
pPS-CFP2-N	FP802	PS-CFP2	cyan-to-green	20μg

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

pPS-CFP2-N vector MCS

Afe I | Xho I* | Hind III | Pst I | Kpn I | Apa I | BamH I | PS-CFP2 →

... GCT.AGC.GCT.ACC.GGA.CTC.AGA.TCT.CGA.GCT.CAA.GCT.TCG.AAT.TCT.GCA.GTC.GAC.GGT.ACC.GCG.GGC.CCG.GGA.TCC.ACC.GGT.CGC.CAC.CAT.GA ...

Nhe I | Bgl II | Sac I | EcoR I | Sal I | Sac II | Sma I/Xma I | Age I

* — not unique sites.

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The CMV promoter is covered under U.S. Patents 5,168,062 and 5,385,839, and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242.
MATERIAL SAFETY DATA SHEET INFORMATION: To the best of our knowledge, these products do not require a Material Safety Data Sheet. However, all the properties of these products (and, if applicable, each of their components) have not been thoroughly investigated. Therefore, we recommend that you use gloves and eye protection, and wear a laboratory coat when working with these products.

Kindling red fluorescent protein KFP-Red

- Reversible or irreversible photoactivation
- Activated by green light that does not damage cells and tissues
- Quenching by blue light
- Recommended for tracking cells and cellular organelle movements

KFP-Red (also referred to as KFP1) is a photoactivatable GFP-like protein generated on the basis of *Anemonia sulcata* chromoprotein, asFP595 [Lukyanov et al. 2000; [Chudakov et al. 2003*a*; Chudakov et al. 2003*b*]. KFP-Red switches from a non-fluorescent to a red fluorescent form (with excitation/emission maxima at 580 nm and 600 nm, respectively) under the exposure to intense green light irradiation.

KFP-Red can be used for *in vivo* monitoring cell and cellular organelle movement. It was successfully expressed and tested in various experimental models, including bacteria, *Xenopus* embryo, and cultured mammalian cells.

Like other Anthozoa GFP-like proteins, KFP-Red is a tetramer. This restricts the wide use of KFP-Red as a fusion partner for cellular proteins.

Reversible or irreversible kindling: Depending on the kindling light intensity KFP-Red can be photoactivated reversibly or irreversibly allowing the monitoring of both short- and long-term cell processes.

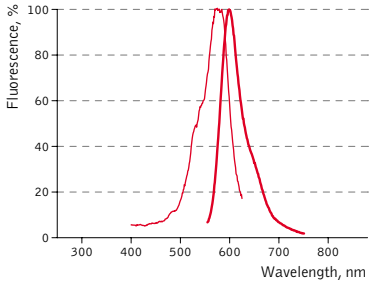
A reversibly kindled KFP-Red relaxes to the initial non-fluorescent form ($t_{1/2}$ = 50 sec.), or can be quenched instantly by blue light (430-490 nm). Reversible kindling results in about 70 times increase of the red fluorescence intensity comparing to unkindled protein.

Reversible kindling and quenching can be repeated many times.

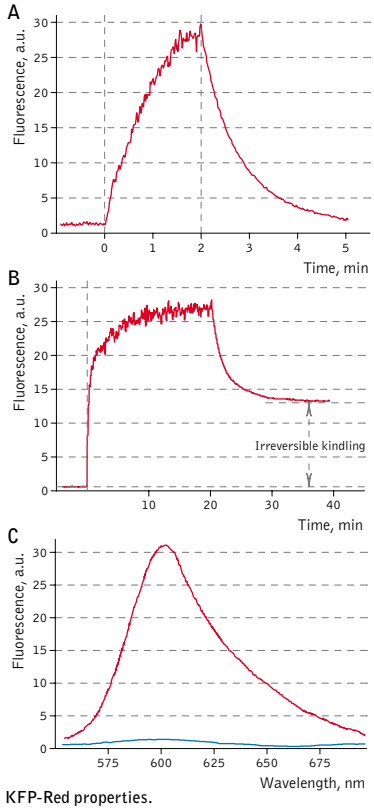
Main properties of KFP-Red

Characteristic	before / after photoactivation
Fluorescence color	No / red
Excitation maximum, nm	580 / 580
Emission maximum, nm	600 / 600
Quantum yield	<0.001 / 0.07
Extinction coefficient, $M^{-1}cm^{-1}$	123 000 / 59 000
Brightness*	0 / 4.1
Activating light	green (530-560 nm)
Photoswitching	reversible
Calculated contrast, fold	35-70
Structure	tetramer
Cell toxicity	not observed
Aggregation	no
Maturation rate at 37°C	medium
Molecular weight, kDa	26
Polypeptide length, aa	238
Possible limitations	Limited applicability for fusions generation

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



KFP-Red normalized excitation (thin line) and emission (thick line) spectra.
Complete KFP-Red spectra in Excel format can be downloaded from the Evrogen Web site at www.evrogen.com / support / FP-tech-pa.shtml



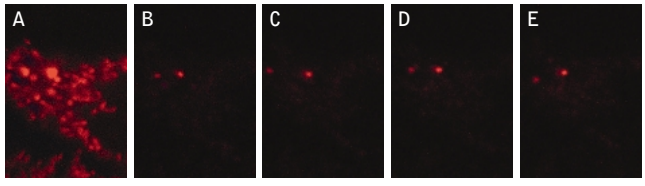
KFP-Red properties.
(A) KFP-Red reversible kindling and relaxation kinetics. Zero time is set at the commencement of irradiation with kindling light (532 nm laser light, 1% power). Kindling irradiation was stopped after 2 minutes. (B) KFP-Red irreversible kindling. Zero time is set at the commencement of irradiation with kindling light (532 nm laser light, 20% power). Kindling irradiation was stopped after 20 min. (C) Irreversibly kindled (red line) and "unkindled" (blue line) KFP-Red fluorescence spectra and brightness ratio.

An irreversibly kindled KFP-Red gives stable red fluorescence which is at least 35 times brighter than that of the protein before kindling. An irreversibly kindled KFP-Red remains stable and brightly fluorescent for more than 72 hrs in living cells and for at least a year in protein samples.

An irreversibly kindled KFP-Red can be partially quenched by blue light, but then it restores its brightness within several minutes. Therefore, in some applications, blue light can be used to quench a reversibly kindled KFP-Red, whereas an irreversibly kindled KFP-Red remains fluorescent.

Application of KFP-Red to track cell migration was demonstrated using embryonic fate mapping as an example. Xenopus embryos were taken at the stage of two blastomeres and KFP-Red mRNA was microinjected into the animal poles of both blastomeres. At the early neurula stage, a round-shaped group of cells within the neural plate was kindled irreversibly. Irradiated cells became brightly fluorescent and their migration in the developing embryo was monitored. Longitudinal extension accompanied by transversal convergence of the labeled group of cells was visible after the first two hours after kindling. At the end of neurulation, the labeled spot appeared as a thin stripe on the surface of the left neural fold.

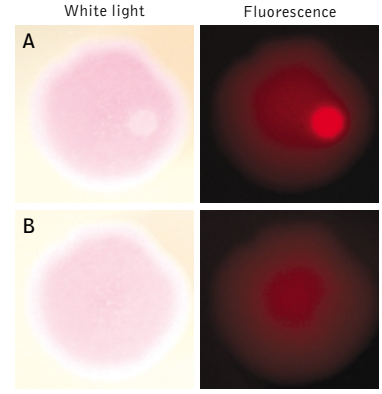
KFP-Red suitability for tracking movement of cell organelles was demonstrated on PC12 cells transfected with a mitochondria-targeted KFP-Red expressing vector. After 25 hours of incubation, mitochondria remained non-fluorescent (no kindling observed) upon irradiation using a 1% power scanning green laser (HeNe laser line 543 nm, 1 mW, once per 10 seconds; the number of scans is not limited). After several scans with a 5-10% power laser, mitochondria became brightly fluorescent and were observed using a 1% power laser for several minutes. Brief irradiation (about 20 seconds in fast mode) with a 30% power green laser light induced irreversible kindling of KFP-Red in mitochondria within the irradiated field. Irreversibly kindled mitochondria were monitored.



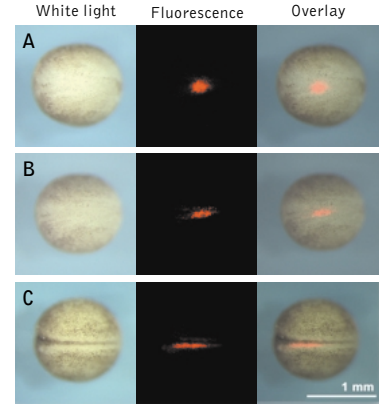
Monitoring of mitochondrial movement using KFP-Red in PC12 cells.
(A) Reversibly and irreversibly kindled mitochondria. Irradiation with weak blue laser light caused instantaneous quenching of reversibly kindled mitochondria, while the irreversibly kindled mitochondria (compare A and B) remained fluorescent; (B-E) Irreversibly kindled mitochondria tracking using a 1% power green laser.

Recommended filter sets and laser lines

KFP-Red is non-fluorescent before light activation. Upon green-light irradiation, the protein kindles to its red fluorescent form. Green light of low intensity (e.g. 1% power scanning green laser, HeNe laser line 543 nm, 1 mW, scan per 10 seconds; the number of scans is not limited) does not cause kindling and may be used as excitation light for KFP-Red visualization.



Reversible photoactivation of KFP-Red in *E. coli*.
The round-shaped part of the *E. coli*. colony expressing KFP-Red was irreversibly kindled with intense green light. This region fluoresces brightly, while its absorption is low. After several minutes, the kindled protein relaxed to the non-fluorescent state, while its absorption recovered. (A) immediately after kindling; (B) five minutes later.



Monitoring of cell migration during *Xenopus* neural plate development using KFP-Red.
(A) At the early neurula stage, a round-shaped group of cells within the neural plate was irreversibly "kindled"; (B) longitudinal extension of the labeled group of cells after two hours after kindling; (C) thin stripe of the labeled cells at the end of neurulation.
Experimental data were presented by Dr. A. Zaraisky (Institute of Bioorganic Chemistry RAS, Moscow, Russia).

Scanning with about 5-10% power laser results in reversible kindling of KFP-Red. More intensive-light irradiation is required for irreversible KFP-Red kindling (e.g. irradiation for 20 seconds in fast mode with a 30% power green laser light induces irreversible kindling of KFP-Red in mitochondria within the irradiated field). Irradiation with weak blue laser light causes instantaneous quenching of reversibly kindled KFP-Red, whereas for the irreversibly kindled KFP-Red, quenching is not so pronounced.

TRITC filter set or similar can be used for visualization of activated KFP-Red. Omega Optical filter sets QMAX-Red and XF174 are recommended.

Kindling effect depends on temperature. Light intensity required for kindling goes down when the temperature decreases and goes up when the temperature rises. This property can be used to achieve kindling at lower light intensities by sample cooling.

REFERENCES

Chudakov et al. (2003*a*). J Biol Chem, 278 (9): 7215–7219 / pmid: 12496281

Chudakov et al. (2003*b*). Nat Biotechnol, 21 (2): 191–194 / pmid: 12524551

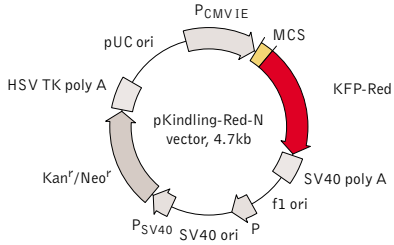
Lukyanov et al. (2000). J Biol Chem, 275 (34): 25879–25882 / pmid: 10852900

KFP-Red-related products

Product	Cat.#	Description	Size	Page(s)
KFP-Red expression/source vectors				
pKindling-Red-N	FP301	Mammalian expression vector encoding humanized KFP-Red and allowing its expression and generation of fusions to the KFP-Red N-terminus	20 μg	68
pKindling-Red-B	FP302	Bacterial expression vector; source of the KFP-Red coding sequence	20 μg	68
pKindling-Red-mito	FP401	Mammalian expression vector encoding humanized KFP-Red targeted to mitochondria	20 μg	69
Vector sets				
Mito-tracker vector set	FPM01	Mammalian expression vectors for fluorescent labeling of mitochondria: pTurboGFP-mito (FP517), pPhi-Yellow-mito(FP607), and pKindling-Red-mito(FP517)	20 μg each	47,69
Recombinant protein				
rKFP-Red	FP351	Purified recombinant kindling red fluorescent protein	100 μg	69

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

pKindling-Red-N vector



For vector sequence, please visit our Web site at http://www.evrogen.com/support/vector-info.shtml

Vector type	mammalian expression vector
Reporter(s)	KFP-Red
Reporter codon usage	mammalian
Promoter	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori; eukaryotic - SV40 ori
Use	Reporter expression in mammalian cells; generation of fusions to the reporter N-terminus

Product	Cat.#	Reporter	Color	Size
pKindling-Red-N	FP301	KFP-Red	no-to-red	20μg

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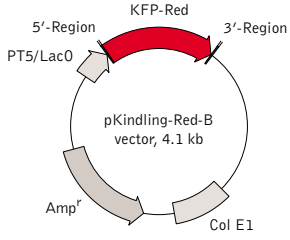
Multiple cloning site (MCS)

Nhe I / Afe I — Bgl II / Xho I — Sac I / Hind III — EcoR I / Pst I — Sal I / Kpn I — Sac II / Apa I — Sma I/Xma I / BamH I — Age I — KFP-Red / Nco I*

```
... GCT.AGC.GCT.ACC.GGA.CTC.AGA.TCT.CGA.GCT.CAA.GCT.TCG.AAT.TCT.GCA.GTC.GAC.GGT.ACC.GCG.GGC.CCG.GGA.TCC.ACC.GGT.CGC.CAC.CAT.GG...
```

* — not unique sites.

pKindling-Red-B vector



For vector sequence, please visit our Web site at http://www.evrogen.com/support/vector-info.shtml

Vector type	bacterial expression vector
Reporter(s)	KFP-Red
Reporter codon usage	mammalian
Promoter	T5 promoter/lac operator
Host cells	prokaryotic
Selection	ampicillin
Replication	ColE1 ori
Use	Source of the reporter coding sequence; reporter expression in bacterial cells

Product	Cat.#	Reporter	Color	Size
pKindling-Red-B	FP302	KFP-Red	no-to-red	20μg

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

5' Region

BamH I / Nco I* — KFP-Red

```
RBS ATG.AGA.GGA.TCG.GGA.TCC.ATG.G...
```

3' Region

STOP / Hind III

```
... TGA.AGC.TT ...
```

* — not unique sites.

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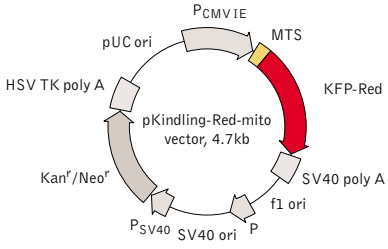
The CMV promoter is covered under U.S. Patents 5,168,062 and 5,385,839, and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242.

MATERIAL SAFETY DATA SHEET INFORMATION: To the best of our knowledge, these products do not require a Material Safety Data Sheet. However, all the properties of these products (and, if applicable, each of their components) have not been thoroughly investigated. Therefore, we recommend that you use gloves and eye protection, and wear a laboratory coat when working with these products.

pKindling-Red-mito vector



For vector sequence, please visit our Web site at http://www.evrogen.com/support/vector-info.shtml

MTS - Mitochondrial targeting sequence

Vector type	mammalian expression vector
Reporter(s)	KFP-Red
Reporter codon usage	mammalian
Promoter	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori; eukaryotic - SV40 ori
Use	fluorescent labeling of mitochondria

Product	Cat.#	Reporter	Color	Size
pKindling-Red-mito	FP401	KFP-Red	no-to-red	20μg

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

rKFP-Red recombinant protein

Product	Cat.#	Reporter	Color	Size
rKFP-Red	FP351	KFP-Red	no-to-red	100μg

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

Use

- Microinjection into cells and tissues
- Control for fluorescence microscopy
- Calibration of fluorimeters and FACS machines
- Standard on protein gels and Western blots

Description

Recombinant KFP-Red (rKFP-Red) is a 26-kDa photoactivatable colored non-fluorescent protein. It has spectral properties identical to those of the expressed KFP-Red.

rKFP-Red can be kindled by green light. Irreversibly kindled purified rKFP-Red retains red fluorescence for many months and can be used as a standard on protein gels and Western blots; control for fluorescence microscopy and for calibration of fluorometeres and FACS machines. Moreover, rKFP-Red may be microinjected into cells and tissues of interest, kindled, and used as a marker of these particular objects.

rKFP-Red is purified from transformed *E. coli* using acetone precipitation.

Storage: at +4°C in the dark place (before photoactivation).

Fluorescent biosensors

Sensor	Intended use	Detection	Advantages	Page
HyPer	Monitoring changes in intracellular H_2O_2 concentration	Rise in H_2O_2 concentration leads to decrease in reporter excitation peak at 420 nm and proportional increase in excitation peak at 500 nm	Ratiometric measurement; High specificity and sensitivity; Capability of targeting the sensor to the specific cell compartments	71
Case12	Monitoring changes in intracellular Ca^{2+} concentration	Rise in Ca^{2+} concentration leads to significant increase of the reporter brightness	High dynamic range; Relatively high pH stability; Capability of targeting the sensor to the specific cell compartments	76
Casper3-BG	Monitoring caspase-3 activity in living cells; Early detection of apoptosis	Activation of caspase-3 leads to elimination of FRET between TagBFP and TagGFP2, resulting in the decrease of green and increase of blue fluorescence	High sensitivity	81
Casper3-GR	Monitoring caspase-3 activity in living cells; Early detection of apoptosis	Activation of caspase-3 leads to elimination of FRET between TagGFP and TagRFP, resulting in the decrease of red and increase of green fluorescence	High sensitivity; Proven suitability for FLIM-based screenings	84

Hydrogen peroxide sensor HyPer

- Ratiometric detection of intracellular H_2O_2 level changes
- High selectivity and sensitivity, no artifactual ROS generation
- Direct expression in cells, easy targeting to various subcellular compartments
- No exogenous chemical compounds required
- Recommended for monitoring H_2O_2 production inside living cells

Reactive oxygen species (ROS) are tightly involved in normal cell functions as well as in development of a wide variety of pathologies. Commonly used for ROS detection, dichlorofluorescein (DCF) derivatives have several serious disadvantages: they are not specific (i.e. they are sensitive to multiple types of ROS); they cannot be targeted to specific intracellular compartments; and, most importantly, they can produce ROS upon light exposure, which results in artifactual ROS generation and signal amplification.

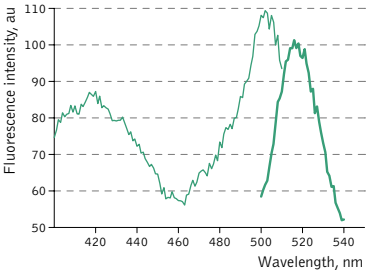
HyPer is the first fully genetically encoded fluorescent sensor capable of detecting intracellular hydrogen peroxide (H_2O_2), one of the main ROS generated by cells [Belousov et al. 2006]. Developed on the basis of yellow fluorescent protein inserted into the regulatory domain of *E. coli* protein OxyR (OxyR-RD) [Choi et al. 2001], HyPer demonstrates submicromolar affinity to hydrogen peroxide and is insensitive to other oxidants tested, such as superoxide, oxidized glutathione, nitric oxide, and peroxinitrite. HyPer does not cause artifactual ROS generation and can be used for detection of fast changes of H_2O_2 concentration in different cell compartments under various physiological and pathological conditions.

Without H_2O_2 HyPer has two excitation peaks with maxima at 420 nm and 500 nm, and one emission peak with maximum at 516 nm. Upon exposure to H_2O_2, the excitation peak at 420 nm decreases proportionally to the increase in the peak at 500 nm, allowing ratiometric measurement of H_2O_2. Similarly to wild-type OxyR, oxidized HyPer can be reduced inside cells.

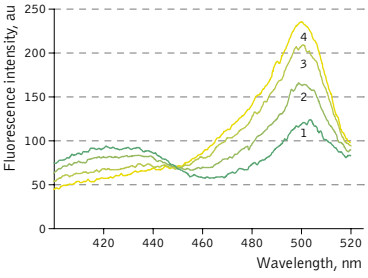
Main properties of HyPer

Characteristic	
Emission maximum, nm	516
Excitation maximum, nm	420 and 500
Fluorescence color	green
Polypeptide length, aa	478
Molecular weight, kDa	52
Specificity	H_2O_2
Sensitivity	submicromolar H_2O_2 concentrations
pKa	8.5
Structure	monomer
Aggregation	no
Maturation rate at 37°C	fast

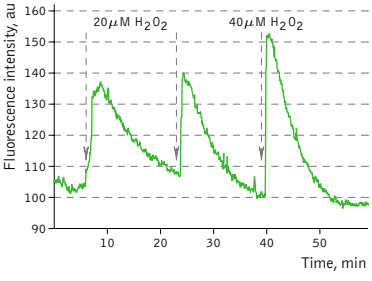
*Brightness is a product of extinction coefficient and quantum yield, divided by 1000.



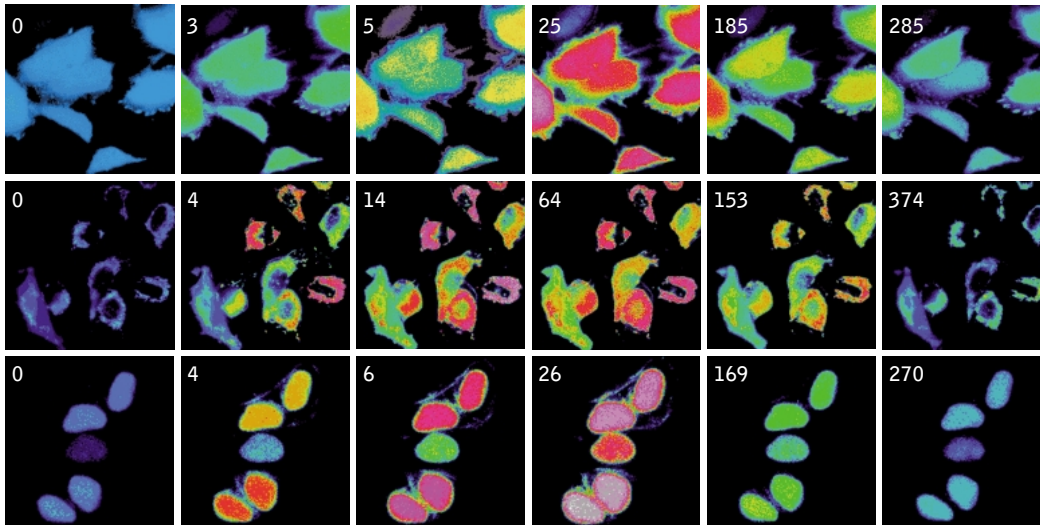
HyPer normalized excitation (thin line) and emission (thick line) spectra.
Complete HyPer spectra in Excel format can be downloaded from the Evrogen Web site at www.evrogen.com/support/FP-tech-biosensors.shtml



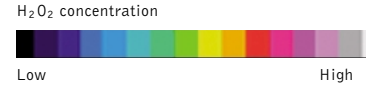
Changes in the excitation spectrum of isolated HyPer in response to H_2O_2 addition.
Changes in the excitation spectrum of isolated HyPer in response to H_2O_2 addition. Trace 1 - without H_2O_2; trace 2 - 25 nM H_2O_2; trace 3 - 100 nM H_2O_2; trace 4 - 250 nM H_2O_2. Emission was measured at 530 nm.



Kinetics of fluorescence (excitation at 490 nm, emission at 530 nm) of HyPer in *E. coli* cell suspension in the presence of 50 U/ml catalase in response to three successive additions of hydrogen peroxide.



Monotoring of cell response of mammalian cells to hydrogen peroxide addition using HyPer directed to various cellular compartments. HeLa cells expressing HyPer localized in cytoplasm (top line), mitochondria (medium line) and nucleus (bottom line) were plated to glass bottom dishes and challenged with 180 μM H_2O_2. Images were acquired by Leica AF 6000 LX with 0.5 Hz frequency by sequential illumination of cells via CFP/YFP (excitation/emission) and YFP/YFP filters. Resulting images were obtained by dividing of YFP/YFP images to CFP/YFP images followed by pseudocoloring. Time after H_2O_2 addition (sec) is indicated at the top of each image.



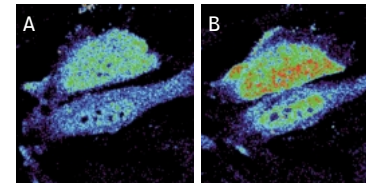
Violet and blue excitation light should be applied for monitoring HyPer green emission changes caused by intracellular H_2O_2 production. Excitation light intensity must be individually determined for a particular biological system and instrumentation used.

HyPer can be directly expressed by target cells individually or in fusion with a specific localization signal. It successfully folds and remains highly sensitive to hydrogen peroxide both in bacteria and in mammalian cells. HyPer suitability to generate stably transfected cells has been proven by Marinpharm company. Various cell lines expressing HyPer are commercially available.

HyPer effectiveness has been demonstrated on various models including detection of low concentrations of H_2O_2 generated upon physiological stimulation of mammalian cells by growth factors [Belousov et al. 2006, Markvicheva et al. 2009], monitoring of H_2O_2 production during Apo2L/TRAIL-induced apoptosis [Belousov et al. 2006], demonstration of hydrogen peroxide mediated insulin-induced calcium increase in skeletal muscle cells [Espinosa et al. 2009], and visualization of a tissue-scale gradient of hydrogen peroxide mediating rapid wound detection in zebrafish [Niethammer et al. 2009].

Recommended filter sets

Recommended Omega Optical filter sets for HyPer are QMAX-Green, XF100-2, and XF100-3. It can also be detected using Chroma Technology Corp. filter set 41001 FITC/ RSGFP/ Bodipy/ Fluo 3/ DiO or the similar.



Imaging of H_2O_2 induction in HeLa cells stimulated with EGF.
Pseudocolored images of HeLa cells expressing HyPer in cytoplasm at the time point of (A) and 2 h after (B) EGF addition.

REFERENCES

Belousov et al. (2006). Nat Methods, 3 (4): 281–286 / pmid: 16554833

Choi et al. (2001). Cell, 105 (1): 103–113 / pmid: 11301006

Espinosa et al. (2009). J Biol Chem, 284 (4): 2568–2575 / pmid: 19028699

Markvicheva et al. (2009). Methods Mol Biol., 476: 76–83 / pmid: 19253046

Niethammer et al. (2009). Nature, : doi:10.1038 / nature08119 / pmid: 19494811

HyPer-related products

Product	Cat.#	Description	Size	Page(s)
HyPer expression/source vectors				
pHyPer-cyto	FP941	Mammalian expression vector allowing HyPer expression in cytosol under the control of CMV promoter	20 µg	74
pHyPer-dMito	FP942	Mammalian expression vector encoding mitochondria-targeted HyPer	20 µg	74
pHyPer-nuc	FP944	Mammalian expression vector encoding nuclear-targeted HyPer	20 µg	75
Gateway® HyPer-AS	FP943	Gateway® entry clone for transfer of HyPer into Gateway® destination vectors; HyPer codon usage is optimized for expression in *Arabidopsis* and *Saccharomyces*	20 µg	75
Vector sets				
HyPer	FPS01	pHyPer-cyto and pHyPer-dMito vectors encoding cytosolic and mitochondria-targeted forms of HyPer	20 µg earch	74

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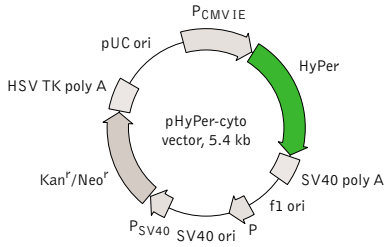
pHyPer-cyto vector



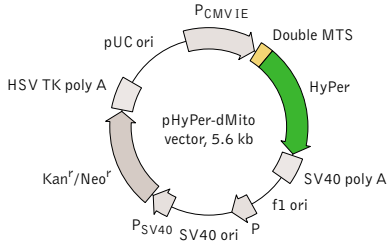
For vector sequence, please visit our Web site at http://www.evrogen.com/support/vector-info.shtml

Vector type	mammalian expression vector
Reporter	HyPer
Reporter codon usage	mammalian / *E. coli*
Promoter for HyPer	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori
Use	HyPer expression in mammalian cells under the control of CMV promoter; source of HyPer coding sequence

Product	Cat.#	Reporter	Color	Size
pHyPer-cyto vector	FP941	HyPer	green	20 μg

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

pHyPer-dMito vector



For vector sequence, please visit our Web site at http://www.evrogen.com/support/vector-info.shtml

MTS - Mitochondrial targeting sequence

Vector type	mammalian expression vector
Reporter	HyPer
Reporter codon usage	mammalian / *E. coli*
Promoter for HyPer	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori
Use	Expression of mitochondria-targeted HyPer in mammalian cells under the control of CMV promoter; source of mitochondria-targeted HyPer coding sequence

Product	Cat.#	Reporter	Color	Size
pHyPer-dMito vector	FP942	HyPer	green	20 μg

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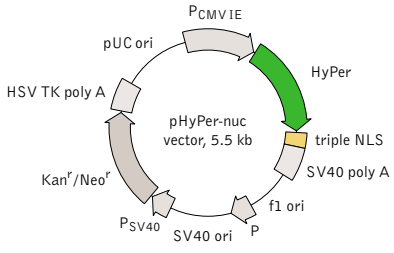
pHyPer-nuc vector



For vector sequence, please visit our Web site at http://www.evrogen.com/support/vector-info.shtml

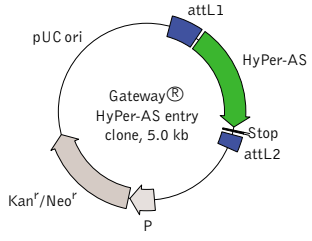
NLS - nuclear localization signal

Vector type	mammalian expression vector
Reporter	HyPer
Reporter codon usage	mammalian / *E. coli*
Promoter for HyPer	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori
Use	Expression of nuclear-targeted fluorescent hydrogen peroxide sensor HyPer in mammalian cells under the control of CMV promoter; source of nuclear-targeted HyPer coding sequence

Product	Cat.#	Reporter	Color	Size
pHyPer-nuc vector	FP944	HyPer	green	20 μg

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

Gateway® HyPer-AS entry clone



For vector sequence, please visit our Web site at http://www.evrogen.com/support/vector-info.shtml

Vector type	Gateway® entry clone
Reporter	HyPer
Reporter codon usage	*Arabidopsis* and *Saccharomyces*
Promoter for HyPer	No
Host cells	prokaryotic
Selection	kanamycin
Replication	pUC ori
Use	Transfer of HyPer codon variant optimized for expression in plants and yeast into Gateway® destination vectors

Product	Cat.#	Reporter	Color	Size
Gateway® HyPer-AS entry clone	FP943	HyPer	green	20 μg

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

Calcium ion sensor Case12

- High dynamic range detection of intracellular Ca^{2+} level changes
- High selectivity and sensitivity, relatively high pH stability
- Fast maturation, high brightness of fluorescent response
- Direct expression in cells, easy targeting to various subcellular compartments
- No exogenous chemical compounds required
- Recommended for monitoring changes in Ca^{2+} concentration inside living cells

Case12 is a high dynamic range genetically encoded fluorescent sensor for direct measurement of changes of intracellular Ca^{2+} under various physiological and pathological conditions [Souslova et al. 2007]. The sensor is sensitive to changes of calcium concentration in a physiological range from a hundred nanomoles to micromoles with a high signal-to-noise ratio. Binding of Ca^{2+} is fast and reversible, allowing monitoring of high-frequency Ca^{2+} oscillations. In response to Ca^{2+} concentration rise, Case12 shows up to 12-fold increase of fluorescence brightness. Fluorescence of Case12 is characterized by single excitation/emission maxima peaked at 491/516 nm.

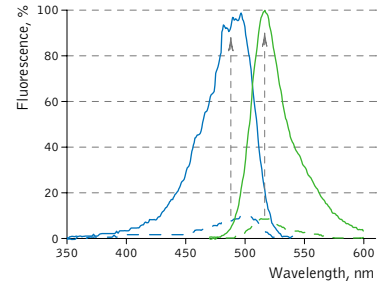
The common weak point of conventional calcium sensors is their low pH stability. For example, pKa (meaning of pH at which fluorescence brightness is 50% of maximum) for Pericams reaches as high as 8.0. Therefore, at physiological pH (7.2-7.5) such sensors exhibit low brightness and dynamic range [Nagai et al. 2001]. In contrast, the pKa of Case12 is 7.2 (in the presence of 10 μM Ca^{2+}) close to that reported for G-CaMP [Nakai et al. 2001]. This relatively high pH stability makes Case12 well suitable for *in vivo* use.

Case12 is characterized by fast maturation at 37°C. It can be directly expressed by target cells, both individually and in fusion with a specific localization signal. Case12 suitability to generate stably transfected cells has been proven by Marinpharm company. Various cell lines expressing Case12 are commercially available.

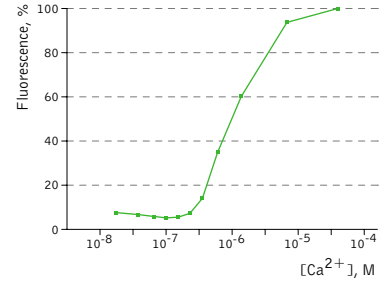
Main properties of Case12

Characteristic	
Emission maximum, nm	516
Excitation maximum, nm	491
Fluorescence color	green
Polypeptide length, aa	415
Molecular weight, kDa	46.4
Specificity	Ca^{2+}
Kd for Ca^{2+}	1 μM
pKa	7.2
Structure	monomer
Aggregation	no
Maturation rate at 37°C	fast

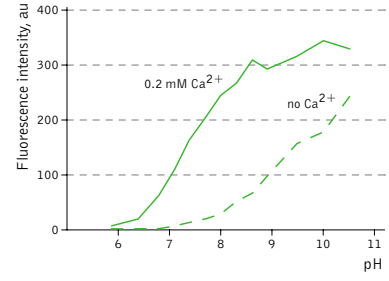
*Brightness is a product of extinction coefficient and quantum yield, divided by 1000.



Case12 normalized excitation (blue line) and emission (green line) spectra without Ca^{2+} (dashed lines) and in the presence of 1 mM of Ca2+ (solid lines)[Souslova et al. 2007].
Case12 shows multi-fold brightness increase of fluorescence in the response to 1 mM Ca^{2+}. Complete Case12 spectra in Excel format can be downloaded from the Evrogen Web site at www.evrogen.com/support/FP-tech-biosensors.shtml



Ca^{2+} titration curves [Souslova et al. 2007].
The apparent Kd for Ca^{2+} binding was found to be 1 μM, which lies within the physiological range of Ca^{2+} concentrations.



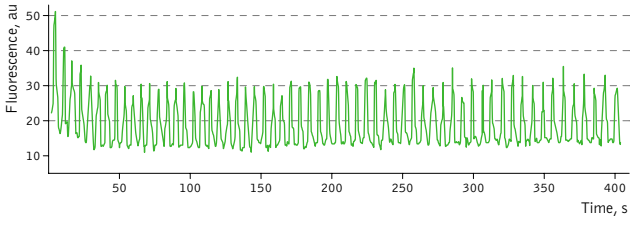
Dependence of Case12 fluorescence on pH in the presence (solid line) and in the absence (dashed line) of Ca^{2+} [Souslova et al. 2007].

Monitoring changes in green emission of Case12 in response to intracellular changes of Ca^{2+} concentration should be carried out by excitation by blue light (488 nm laser line or standard GFP filter set). Emission can be collected at approximately 500-540 nm. Intensity of excitation light should be individually determined for particular biological system and instrumentation. In general, we recommend that you minimize excitation light intensity and duration.

Note: Yellow fluorescent core of Case12 undergoes partial photoconversion to a dark state upon irradiation with blue light. It means that an apparent "bleaching" effect occurs at the beginning of time series imaging of cells expressing Case12 protein. Unlike the real bleaching, in the case of Case12, signal drops to the level of dynamic equilibrium between fluorescent and dark state of the chromophore, and then remains stable.

Maximum dynamic range in HeLa cells: HeLa cells transfected with Case12 show relatively weak green fluorescence. Addition of 20 μM calcium ionophore A23187, allowing calcium to enter cells (2 mM Ca^{2+} in the medium), resultes in 5-6-fold increase in green fluorescence brightness. Subsequent addition of 20 mM EGTA removes Ca^{2+} and decreases the fluorescence signal close to baseline level, with the final contrast of 11-12-fold.

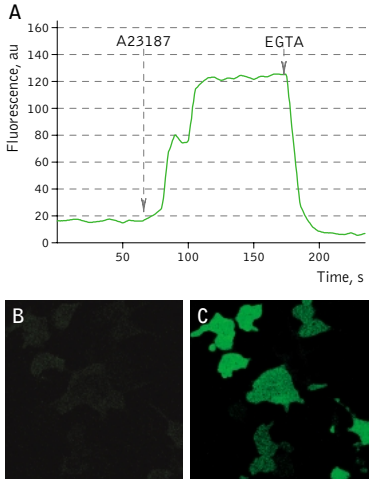
Monitoring of Ca^{2+} changes under physiological conditions: Mammalian cells expressing Case12 display a nice high dynamic range response upon addition of ATP at a final concentration of 100 μM. This experiment clearly showes that Case12 fluorescence response to Ca^{2+} oscillations is fast and reversible. It also demonstrates that the sensor responds to changes in Ca^{2+} concentration in living cells in the nanomolar range.



Fluorescence changes of human melanoma-derived M21 cells expressing Case12 in response to 100 μM ATP. Images were captured every 0.294 sec on the confocal microscope.

Compatibility with existing filter sets

We recommend standard GFP filter sets. Appropriate Omega Optical filter sets for Case12 are QMAX-Green, XF100-2 and XF100-3. It can also be detected using Chroma Technology Corp. filter sets 41001, 41017, 41020, 41025 or similar.



Testing Case12 in living cells [Souslova et al. 2007].
(A) Typical response of HeLa cells expressing Case12 to calcium ionophore A23187. (B, C) HeLa cells expressing Case12 shown before (B) and after (C) ionophore addition.
Leica microscope DM IRE2, confocal TCS-SP2, objective HCX-PL-APO-63x/1.40-0.60/OIL.

REFERENCES

Nagai et al. (2001). Proc Natl Acad Sci U S A, 98 (6): 3197–3202 / pmid: 11248055

Nakai et al. (2001). Nat Biotechnol, 19 (2): 137–141 / pmid: 11175727

Souslova et al. (2007). BMC Biotechnol, 7 (1): 7366–7375 / pmid: 17603870

Case12-related products

Product	Cat.#	Description	Size	Page(s)
Case12 expression/source vectors				
pCase12-Cyto	FP991	Mammalian expression vector allowing Case12 expression in cytosol under the control of CMV promoter	20 μg	79
pCase12-mito	FP992	Mammalian expression vector encoding mitochondria-targeted Case12	20 μg	80
pCase12-mem	FP993	Mammalian expression vector encoding Case12 targeted to cell membrane	20 μg	80

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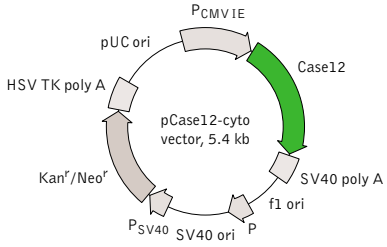
pCase12-cyto vector



For vector sequence, please visit our Web site at http://www.evrogen.com/support/vector-info.shtml

Vector type	mammalian expression vector
Reporter	Case12
Reporter codon usage	mammalian
Promoter for Case12	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori
Use	Expression of fluorescent Ca^{2+} sensor Case12 in mammalian cells under the control of CMV promoter; source of Case12 coding sequence

Product	Cat.#	Reporter	Color	Size
pCase12-cyto vector	FP991	Case12	green	20 μg

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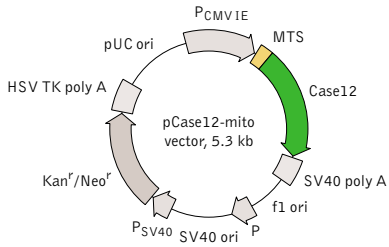
pCase12-mito vector



For vector sequence, please visit our Web site at
http://www.evrogen.com/support/vector-info.shtml

MTS - Mitochondrial targeting sequence

Vector type	mammalian expression vector
Reporter	Case12
Reporter codon usage	mammalian
Promoter for Case12	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori
Use	Expression of mitochondria-targeted fluorescent Ca^{2+} sensor Case12 in mammalian cells under the control of CMV promoter; source of mitochondria-targeted Case12 coding sequence

Product	Cat.#	Reporter	Color	Size
pCase12-mito vector	FP992	Case12	green	20 μg

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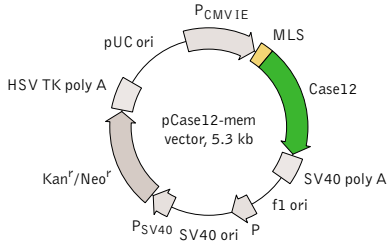
pCase12-mem vector



For vector sequence, please visit our Web site at
http://www.evrogen.com/support/vector-info.shtml

MLS - Membrane localization signal

Vector type	mammalian expression vector
Reporter	Case12
Reporter codon usage	mammalian
Promoter for Case12	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori
Use	Expression of membrane-targeted fluorescent Ca^{2+} sensor Case12 in mammalian cells under the control of CMV promoter; source of membrane-targeted Case12 coding sequence

Product	Cat.#	Reporter	Color	Size
pCase12-mem vector	FP993	Case12	green	20 μg

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Caspase-3 apoptosis sensor Casper3-BG

- Early detection of Caspase-3 activity onset
- High sensitivity
- Direct expression in cells
- No exogenous chemical compounds required
- Recommended for early detection of apoptosis

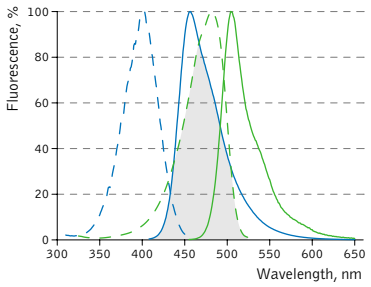
Casper3-BG is a FRET based sensor that can be used for detection of caspase-3 mediated apoptosis in living cells. The sensor consists of blue and green fluorescent proteins, TagBFP (see page 12) and TagGFP2 (see page 16), connected by the linker containing caspase-3 cleavage sequence DEVD. Good overlap between the emission spectrum of TagBFP and the absorbance spectra of TagGFP2 ensures efficient FRET between these proteins. The activation of caspase-3 during apoptosis leads to cleavage of DEVD sequence and elimination of FRET that can be detected as a decrease in green emission of TagGFP2 and a simultaneous increase in blue emission of TagBFP.

TagBFP / TagGFP2 pair is superior to other BFP / GFP pairs. The calculated Forster distance and FRET efficiency for TagBFP / TagGFP2 pair is larger than those reported for the standard ECFP / EYFP and mCyPet / mYPet pairs. Moreover, TagBFP and TagGFP2 proteins lack the ability to form heterodimers, which results in more than 6-fold lower background for FRET analysis than in case of weakly dimerizing FRET pairs, such as ECFP / EYFP [Subach et al. 2008].

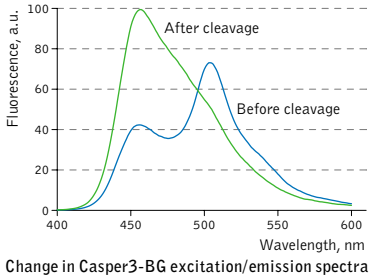
Main properties of Casper3-BG

Characteristic	
Calculated Forster distance R_0	5.25
FRET efficiency E	0.57
Specificity	caspase-3 activity
Response	elimination of FRET
Polypeptide length, aa	490
Molecular weight, kDa	55
FRET donor	TagBFP
Fluorescence color	blue
Excitation maximum, nm	402
Emission maximum, nm	457
Brightness, % of EGFP	99
pKa	2,7
FRET acceptor	TagGFP2
Fluorescence color	green
Excitation maximum, nm	483
Emission maximum, nm	506
Brightness, % of EGFP	105
pKa	5.0

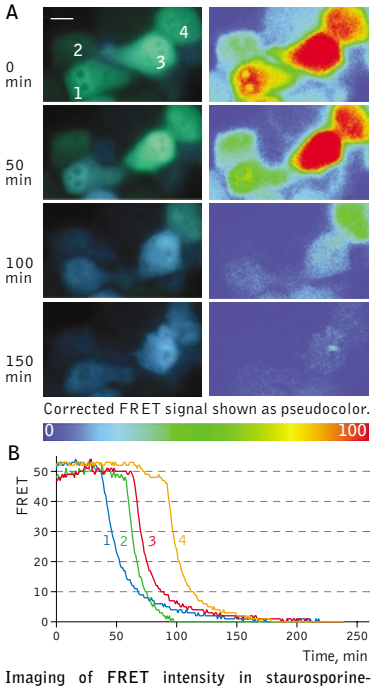
*Brightness is a product of extinction coefficient and quantum yield, divided by 1000.



Excitation (dashed lines) and emission (solid lines) spectra of TagBFP (blue) and TagGFP2 (green) are shown individually. Spectral overlap is filled with gray.



Change in Casper3-BG excitation/emission spectra upon the cleavage of DEVD sequence *in vitro*.



Imaging of FRET intensity in staurosporine-treated HeLa cells: (A) Fluorescent images of the cells after staurosporine treatment (left). The corrected FRET signals are shown as pseudocolor images (right). Scale bar, 10 mm. (B) time course of corrected FRET normalized per donor fluorescence observed in four cells indicated in (A).

The excellent performance of Casper3-BG sensor has been demonstrated *in vivo* on the example of HeLa cells staurosporine-induced apoptosis [Subach et al. 2008]. The two-filter method of sensitized FRET measurements [Gordon et al. 1998] on a pixel-by-pixel basis was applied, as described in [Galperin, E et al. 2004]. The initial mean FRET efficiency *in vivo* normalized to donor fluorescence was 51.5%.

Following 40-80 min exposure to 1 mM staurosporine, the FRET gradually dropped to zero before the shrinking of cells characteristic to apoptosis. The large FRET efficiency of the TagBFP / TagGFP2 pair enabled the detection of even weak proteolitic activity in each cell at the beginning of apoptosis, when only a fraction of the substrate was cleaved.

Recommended filter sets

The set of filters from Chroma (403/12 nm exciter, part #74673, 457/50 nm emitter, part #66974, and dichroic mirror, part #86100) or similar.

REFERENCES

Galperin, E et al. (2004). Nat Methods, 1 (3): 209217 / pmid: 15782196

Gordon et al. (1998). Biophys J, 74 (5): 2702–2013 / pmid: 9591694

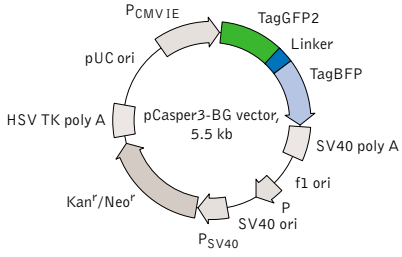
Subach et al. (2008). Chemistry & Biology, 15 (10): 1116–1124 / pmid: 18940671

Casper3-BG-related products

Product	Cat.#	Description	Size	Page(s)
pCasper3-BG	FP970	Mammalian expression vector encoding Casper3-BG	20 μg	83

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

pCasper3-BG vector



For vector sequence, please visit our Web site at http://www.evrogen.com/support/vector-info.shtml

Linker - caspase-3 cleavage sequence

Vector type	mammalian expression vector
Reporter	Casper3-BG
Reporter codon usage	mammalian
Promoter	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori
Use	Expression of fluorescent caspase-3 apoptosis sensor Casper3-BG in mammalian cells under the control of CMV promoter; source of Casper3-BG coding sequence

Product	Cat.#	Reporter	Color	Size
pCasper3-BG vector	FP970	Casper3-BG	blue/green	20 μg

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

Caspase-3 apoptosis sensor Casper3-GR

- Early detection of Caspase-3 activity onset
- High sensitivity
- Direct expression in cells
- No exogenous chemical compounds required
- Proven suitability for FLIM-based screenings
- Recommended for early detection of apoptosis

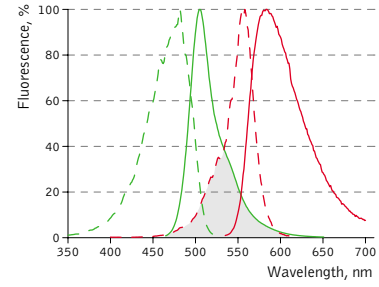
Casper3-GR is a FRET based sensor that can be used for detection of caspase-3 mediated apoptosis in living cells. The sensor consists of green and red fluorescent proteins TagGFP and TagRFP connected by the linker containing caspase-3 cleavage sequence, DEVD. The high fluorescence quantum yield of TagGFP along with the high molar extinction coefficient of TagRFP and excellent overlap of donor emission and acceptor excitation spectra result in highly effective FRET between these fluorescent proteins. The activation of caspase-3 during apoptosis leads to cleavage of DEVD sequence and elimination of FRET that can be detected as decrease in the red emission of TagRFP and a simultaneous increase in green emission of TagGFP.

The calculated Forster distance (R_0 = 5.7 nm) for TagGFP / TagRFP pair is one of the largest among the values reported. At the same time, since TagGFP and TagRFP emission peaks are spaced by as much as 79 nm, the emission signal for these two proteins can be easily separated in any imaging system. As an additional advantage, shifting the wavelengths toward

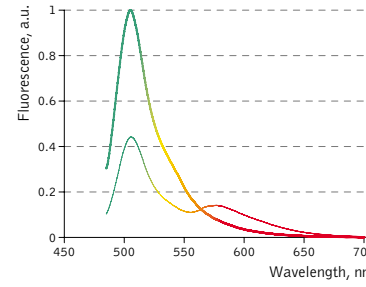
Main properties of Casper3-GR

Characteristic	
Calculated Forster distance R_0	5.70
FRET efficiency E	0.50
Specificity	caspase-3 activity
Response	elimination of FRET
Polypeptide length, aa	484
Molecular weight, kDa	54
FRET donor	TagGFP
Fluorescence color	green
Excitation maximum, nm	482
Emission maximum, nm	505
Brightness, % of EGFP	104
pKa	4,7
FRET acceptor	TagRFP
Fluorescence color	red
Excitation maximum, nm	555
Emission maximum, nm	584
Brightness, % of EGFP	148
pKa	3,8

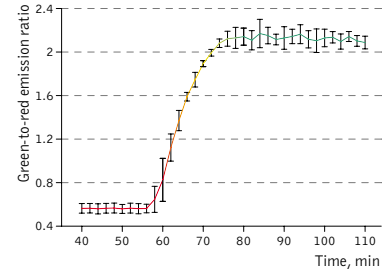
*Brightness is a product of extinction coefficient and quantum yield, divided by 1000.



Excitation (dashed lines) and emission (solid lines) spectra of TagGFP (green) and TagRFP (red) are shown individually. Spectral overlap is filled with gray.



Emission spectra of Casper3-GR before (thin line) and after digestion by Caspase-3 (thick line).



Green-to-red emission ratio change of Casper3-GR upon staurosporine-induced apoptosis. Approximately 40-50 min after staurosporine infusion, cells demonstrated pronounced changes in fluorescence signal ratio. Emission ratio shown for 5 cells, time point aligned to the median of ratio changes, individual for each cell. Excitation at 488 nm, emission was detected at 500-530 nm and 560-600 nm.

the red part of the spectrum (comparing to traditional cyan and yellow FRET partners) reduces input of cellular autofluorescence.

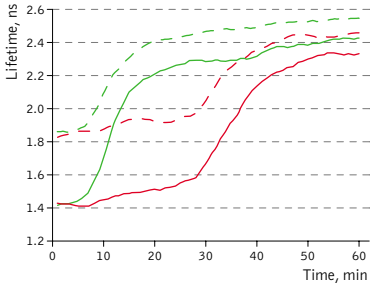
The activation of caspase-3 during apoptosis leads to cleavage of DEVD sequence and elimination of FRET that can be detected as decrease in red emission of TagRFP and a simultaneous increase in green emission of TagGFP. Direct monitoring of the donor/acceptor emission ratio demonstrated up to 5-fold ratio changes upon cleavage by recombinant caspase 3 *in vitro*. The increase in donor fluorescence intensity was at least 2-fold corresponding to a FRET efficiency of at least 50%.

The excellent performance of Casper3-GR sensor has been demonstrated *in vivo* on staurosporine-induced apoptosis of HeLa cells [Subach et al. 2008]. Living cells were monitored at 37°C with Leica SP2 confocal microscope (excitation using 488 nm laser line, emission collected at 500-530 nm and 560-650 nm). The fluorescence was evenly distributed in the cytosol and nucleus with no aggregation or non-specific localization observed. Importantly, both green and red signals were reliably stable under various irradiation conditions for hours. No reversible or irreversible fluorescence bleaching or photoconversion was observed. Approximately 30-40 min after 2 μM staurosporine infusion, cells demonstrated rapid (within 10 min) and pronounced changes in green-to-red fluorescence signal ratio, indicating activation of caspase-3. Later these cells demonstrated characteristic membrane blebbing. The average contrast in living cells (calculated as donor/acceptor emission ratio change for 5 cells, time point aligned to the median of ratio changes, individual for each cell) reached 3.8-fold.

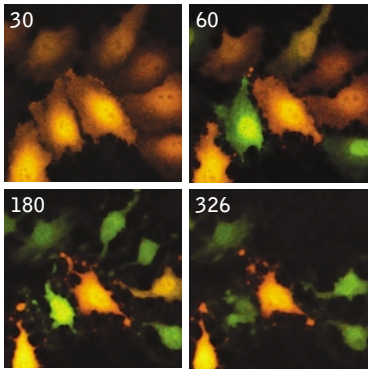
Measurement of Casper3-GR apoptosis induced FRET changes by FLIM revealed the dramatic increase of TagGFP fluorescence lifetime from 1.5 ns to 2.5 ns. The FRET efficiency of the uncleaved Casper3-GR (38% based on the phase lifetime) is among the highest measured by FLIM. Since the FRET efficiency of the cleaved substrate is zero, the dynamic range of the sensor is rather high, indicating that Casper3-GR can be successfully used for the high content FLIM based screenings on living cells.

Recommended filter sets

The excitation wavelength required to visualize FRET changes of Casper3-GR by ratio-imaging is provided by an ordinary FITC/GFP excitation filter or ubiquitous 488 nm laser line, and the two emission signals are acquired using a 500-530 nm (FITC/GFP emission filter) bandpass filter and a 560-600 nm bandpass filter (Cy3/DsRed emission filter) or a 560LP longpass filter.



TagGFP fluorescence phase lifetime (solid lines) and average modulation lifetime (dashed lines) changes for Casper3 during staurosporine-induced apoptosis. Excitation was at 488 nm and donor fluorescence emission was passed through a 500-530 nm bandpass filter.



Two channel fluorescence imaging of Casper3-GR upon staurosporine-induced apoptosis in HeLa cells. Time (in min.) is shown after staurosporine infusion.

REFERENCES

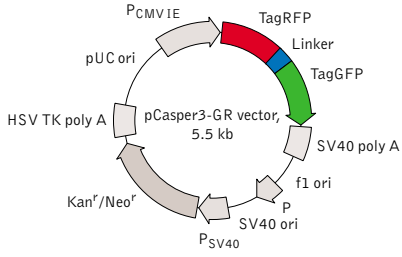
Subach et al. (2008). Chemistry & Biology, 15 (10): 1116–1124 / pmid: 18940671

Casper3-GR-related products

Product	Cat.#	Description	Size	Page(s)
pCasper3-GR	FP971	Mammalian expression vector allowing Casper3-GR expression in cytosol under the control of CMV promoter	20 μg	86

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

pCasper3-GR vector



For vector sequence, please visit our Web site at http://www.evrogen.com/support/vector-info.shtml

Linker - caspase-3 cleavage sequence

Vector type	mammalian expression vector
Reporter	Casper3-GR
Reporter codon usage	mammalian
Promoter	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori
Use	Expression of fluorescent caspase-3 apoptosis sensor Casper3-GR in mammalian cells under the control of CMV promoter; source of Casper3-GR coding sequence

Product	Cat.#	Reporter	Color	Size
pCasper3-GR vector	FP971	Casper3-GR	green/red	20 μg

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

Genetically-encoded photosensitizer KillerRed

- Light-induced production of reactive oxygen species
- Direct expression in cells, easy targeting to various subcellular compartments
- No exogenous chemical compounds required
- Not toxic before activation by green light irradiation
- Recommended for selective light-induced protein inactivation and cell killing

KillerRed is a red fluorescent protein capable of light-induced production of reactive oxygen species (ROS) [Bulina et al. 2006*a*]. It can be directly expressed both individually and in fusion with a target protein in various experimental systems, including bacteria, *Xenopus*, zebrafish, and mammalian cells. Upon green-light irradiation, KillerRed generates ROS that damage the neighboring molecules.

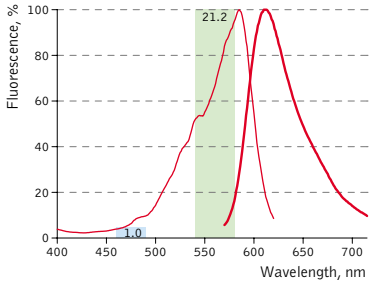
Mild illumination of cells expressing protein of interest fused to KillerRed results in precise inactivation of this protein only. Using KillerRed variants targeted to cell membrane or to mitochondria in combination with more prolonged and intensive green light irradiation it is possible to kill the selected cells in culture or inside a thin layer of transparent tissue. Intensity of green light and irradiation time must be individually determined for particular biological system and instrumentation.

KillerRed-mediated ROS production is accompanied by profound KillerRed photobleaching. The resulting cell events (cell fate after irradiation, effect on protein localization) should be monitored using another fluorescent reporter, for example a green fluorescent protein. We recommend that you use TurboGFP (page 27) for cell and organelle, or TagGFP2 (page 16) for protein labeling.

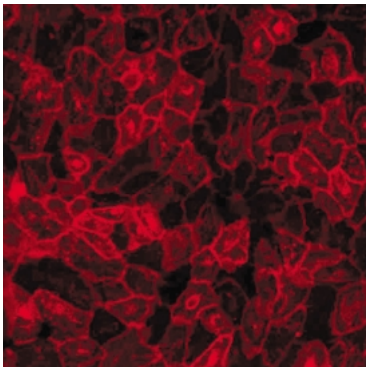
Main properties of KillerRed

Characteristic	
Molecular weight, kDa	27
Polypeptide length, aa	239
Structure	dimer
Aggregation	no
Maturation rate at 37°C	slow
Activating light	green (e.g. 540-580 nm)
Fluorescence color	red
Excitation maximum, nm	585
Emission maximum, nm	610
Quantum yield	0.25
Extinction coefficient, $M^{-1}cm^{-1}$	45 000
Brightness*	11.3

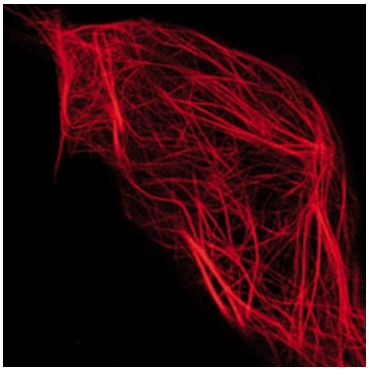
*Brightness is a product of extinction coefficient and quantum yield, divided by 1000.



KillerRed normalized excitation (thin line) and emission (thick line) spectra.
Blue and green rectangles show relative phototoxic effect from irradiation with blue (460-490 nm) and green (540-580 nm) light of 35 mW/cm^2. Numbers above the rectangles represent decrease in viable *E. coli* cells after 30-min irradiation (folds).



Expression of membrane-targeted KillerRed in stably transfected ARPE-19 cells.
Photograph of stably transfected cell line was provided by Dr. Christian Petzelt (MARINPHARM).



Transiently transfected HeLa cells expressing KillerRed fusion with Tau34.

Despite its dimerization capacity, KillerRed demonstrates successful performance in many fusions including that with mitochondrial targeted signal, cytoplasmic β-actin, fibrillarin, dopaminee transporter, Tau34, etc.

KillerRed suitability to generate stably transfected cells has been proven by Marinpharm company. Various cell lines expressing KillerRed are commercially available.

KillerRed's suitability for light-induced killing of prokaryotic cells has been demonstrated using *E. coli* XL1-Blue strain. KillerRed killed 96% of bacterial cells after 10 min and almost 100% of cells after 20 min of irradiation with white light.

KillerRed-mediated killing of eukaryotic cells: The following two ways have been found to be effective for killing the eukaryotic cells using KillerRed: (1) via an apoptotic pathway using KillerRed targeted to mitochondria, and (2) via membrane lipid oxidation using membrane-localized KillerRed. Irradiation of KillerRed localized in cell cytosol, has a weak effect on cell survival.

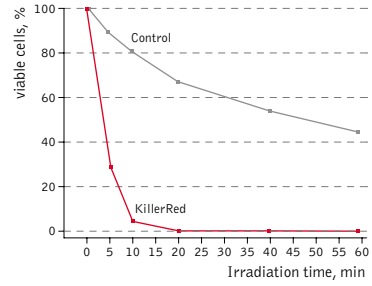
(1) Effects of KillerRed localized in mitochondria: Use of KillerRed targeted to mitochondria allows effective cell killing through an apoptotic pathway as has been demonstrated in the following experiment: HeLa cells expressing cytoplasmic TurboGFP and mitochondria-localized KillerRed-dMito were generated. 10-min irradiation of selected cells with green light resulted in profound KillerRed photobleaching. 60 min after irradiation, cells had an abnormal shape and "bubbles" typical of apoptotic pathway. These cells disrupted within the next 30-60 min.

In another experiment, nearly 100% of B16 melanoma cells expressing KillerRed targeted to mitochondria died within 45 min after 15-min of irradiation (40x objective, 535-575 nm excitation filter, 3.3 W/cm^2). It is noticeable that when preincubated with the pancaspase inhibitor zVAD-fmk (10 μM), the cells were resistant to the same green-light exposure and held their shape for at least 1.5 hours after illumination.

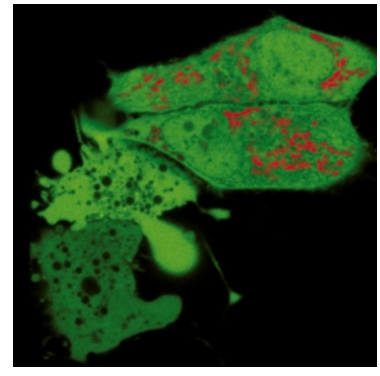
Apart from the immediate phototoxic effect, photosensitizers can mediate postponed cellular responses such as cell growth arrest or cell death via long-term apoptotic mechanism. In the experiment from [Bulina et al. 2006*a*], B16 melanoma cells expressing mitochondria-targeted KillerRed or EGFP were mixed together and irradiated by green light of low intensity (3.7x objective, 535-575 nm excitation filter, 115 mW/cm2) for 45 min. No red fluorescent cells were observed in 16 hrs after irradiation, whereas green fluorescent cells remained viable. It confirms that mitochondria-localized KillerRed can mediate cell death through long-term mechanisms in response to low-intensity illumination. This effect can be used in different applications.

(2) Effects of KillerRed localized in membrane: Comparing with the mitochondria-targeted KillerRed, irradiation of membrane-localized KillerRed causes a more effective and fast cell death within 10-30 min, presumably because of lipid oxidation [Bulina et al. 2006*b*].

Moreover, membrane-targeted KillerRed was shown to be an effective tool for the light-induced cell killing within a developing zebrafish. Zebrafish embryo was microinjected with a mixture of vectors driving expression of membrane-targeted KillerRed and a green fluorescent protein at the single- cell stage. A muscle cell expressing both proteins was irradiated with green light (40x objective, TRITC filter set, 10 min) at 48 hrs after

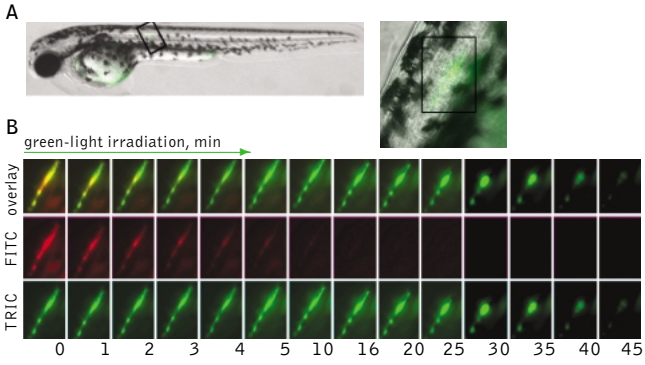


Time-course of light-induced killing of *E. coli* expressing KillerRed.
A single *E. coli* colony was picked out, diluted into 1 ml of PBS buffer and divided into two equal portions. One of them was irradiated with white light (1W/cm^2, light source Fiber-Light from Dolan-Jenner Industries, Inc) for different periods of time, whereas the other was kept in darkness. Both sample aliquots were plated onto Petri dishes at different dilutions. The number of growing colonies corresponded to the number of bacterial cells surviving after irradiation (i.e. colony forming units, CFU). CFU number for the irradiated *E. coli* portion was compared with the non-irradiated one, thus allowing estimation of the relative phototoxic effect for KillerRed. In control experiments, *E. coli* cells expressing different fluorescent and non-fluorescent proteins were used.



Confocal image of HeLa cells expressing KillerRed in mitochondria (red) and TurboGFP in cytosol (green).
Lower left cells were pre-irradiated with green light (515-560 nm, 7W/cm^2) light for ten minutes. This led to profound KillerRed photobleaching. Cells are shown 60 min after irradiation. It is clearly visible that irradiated cells have abnormal shape and "bubbles", characteristic for apoptotic cell-death pathway.

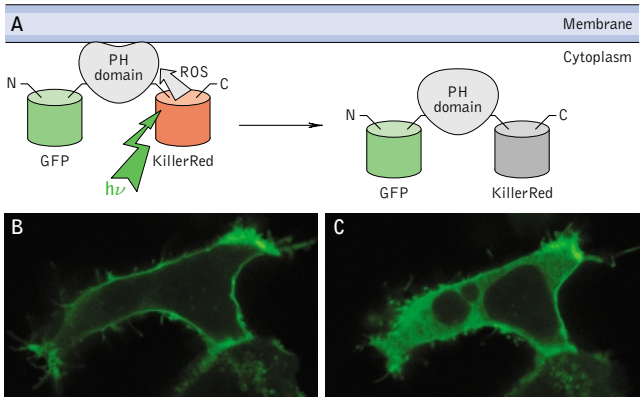
fertilization. By the end of 10-min irradiation, the cell already started to change its shape. Within 20 min after irradiation was stopped, the cell was disrupted completely. Mitochondria targeted KillerRed was shown to be of low efficiency in similar experiments.



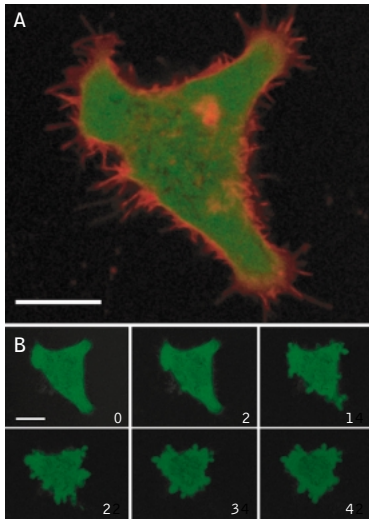
Light-induced killing of a muscle cell within a developing zebrafish embryo. (A) A region expressing membrane-targeted KillerRed and green fluorescent marker; (B) time course of light-induced killing of a muscle cell within a developing zebrafish. Fluorescence was collected using standard FITC and TRITC filter sets.

KillerRed use for protein inactivation: KillerRed use for chromophore-assisted light inactivation (CALI) of proteins has been demonstrated on the model of β-galactosidase inactivation in bacterial cells and inactivation of pleckstrin homology (PH) domain of phospholipase C δ-1 (PLC δ-1) in mammalian cells. In the first experiment, KillerRed was fused to β-galactosidase (β-gal) enzyme and expressed in *E. coli*. Effective suppression of β-gal activity was demonstrated in living *E. coli* streaks and *E. coli* cell extract.

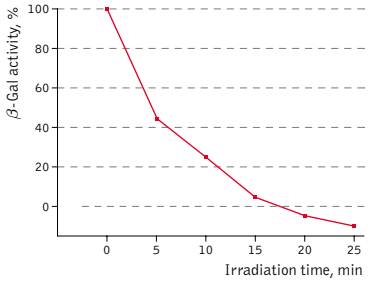
In the second experiment, a triple EGFP-PH-KillerRed fusion protein that allows both protein visualization and CALI was transiently expressed in mammalian cell line. Intracellular localization of EGFP signal was evaluated before and after CALI of the PH domain using confocal and fluorescence microscopy. In intact cells, the fusion is located predominantly at the plasma membrane because of specific affinity of PH domain to phosphatidylinositol 4,5-bisphosphate.



KillerRed-mediated light-induced inactivation of PLC δ-1 PH domain. (A) Schematic outline of the experimental system; (B,C) confocal images of a cell expressing EGFP-PH-KillerRed triple fusion (EGFP green fluorescent signal) before (B) and after (C) 10-s irradiation with green light. Note considerable increase in cytoplasmic signal.



Light-induced killing of HeLa cell using membrane-targeted KillerRed. (A) Confocal image of HeLa cells expressing membrane-localized KillerRed (red) and TurboGFP in cytosol (green); (B) time-course of cell fragmentation induced by green-light irradiation (63x objective, mercury lamp, 515-560 nm excitation filter, 7 W/cm^2) for 10 min. Numbers indicate time since irradiation, min. Scale bar, 10 μm. Figure was first published in [Bulina et al. 2006*a*].



Time-course of CALI of β-galactosidase. In the model CALI experiment KillerRed was fused to β-galactosidase (β-gal) enzyme and expressed in *E. coli*. Upon green-light irradiation (540-580 nm, 30 min, 360mW/cm^2) β-gal activity was effectively suppressed in living *E. coli* streaks. On the contrary, no effect of green light on the enzyme activity was detected in control cells containing unmodified β-gal gene. *In vitro* test showed that in *E. coli* cell extract β-gal fused to KillerRed lost 99.4% of enzymatic activity within 25 min of white light exposure (1W/cm^2), with half inactivation time of about 5 min. Irradiation of *E. coli* extracts containing unfused β-gal protein alone or β-gal mixed with KillerRed had no effect on enzyme activity. To verify specificity of KillerRed phototoxic effect, horse-radish peroxidase (HRP) was added to the sample. Upon 15 min of illumination (white light, 1W/cm^2) only 2% of HRP activity was lost, showing high specificity of the phototoxic effect.

Irradiation with intense green light led to KillerRed-mediated ROS production, PH domain damage, and fusion protein dissociation from the membrane. After 10 sec of green-light irradiation (63x objective, mercury lamp, 515-560 nm filter, 7W/cm^2), translocation of the PH domain into cytosol was clearly visible. When irradiated for a longer period of time, considerable amount of PH domain translocated into cytosol, increasing the cytoplasm-to-membrane green fluorescent signal ratio to 0.5-0.9.

In the negative control experiments, the cellular location of a DsRedExpress (Clontech) containing construct, GFP-PH-DsRedExpress, showed no dependence on green-light irradiation. Similarly, no detectable CALI of the PH domain was achieved when KillerRed was expressed in the cell separately from PH domain, in either the membrane or cytosol.

Recommended antibodies, filter sets, and activating lasers

KillerRed can be recognized using Anti-KillerRed antibody (Cat.# AB961-AB962) available from Evrogen.

Before light activation, KillerRed can be detected using TRITC filter set or similar. Recommended Omega Optical filter sets are QMAX-Red and XF174.

KillerRed phototoxicity is induced by green-light irradiation at 540-580 nm and depends on light intensity irradiation time and KillerRed concentration. Arc-lamp irradiation is strongly recommended; laser-light irradiation in confocal mode is much less efficient.

REFERENCES

Bulina et al. (2006*a*). Nat Biotechnol, 24 (1): 95–99 / pmid: 16369538

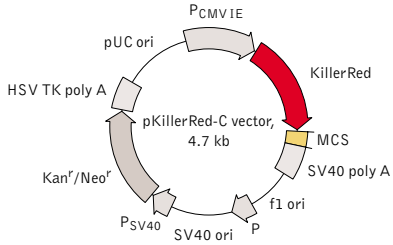
Bulina et al. (2006*b*). Nat Protoc, 1 (2): 947–953 / pmid: 17406328

KillerRed-related products

Product	Cat.#	Description	Size	Page(s)
KillerRed expression/source vectors				
pKillerRed-C	FP961	Mammalian expression vector encoding humanized KillerRed and allowing its expression and generation of fusions to the KillerRed C-terminus	20 μg	92
pKillerRed-N	FP962	Mammalian expression vector encoding humanized KillerRed and allowing its expression and generation of fusions to the KillerRed N-terminus	20 μg	92
pKillerRed-B	FP963	Bacterial expression vector; source of the KillerRed coding sequence	20 μg	93
pKillerRed-dMito	FP964	Mammalian expression vector encoding mitochondria-targeted KillerRed	20 μg	93
pKillerRed-mem	FP966	Mammalian expression vector encoding membrane-targeted KillerRed	20 μg	94
Vector sets				
Cell-Killer	FPK01	pKillerRed-dMito vector encoding mitochondria-targeted KillerRed and pTurboGFP-N vector allowing cytoplasmic expression of bright green fluorescent protein TurboGFP for monitoring cell fate	20 μg each	93, 45
Membrane-Killer	FPK02	pKillerRed-mem vector encoding membrane-targeted KillerRed and pTurboGFP-N vector allowing cytoplasmic expression of bright green fluorescent protein TurboGFP for monitoring cell fate	20 μg each	94, 45
Double-Killer	FPK03	pKillerRed-mem vector encoding membrane-targeted KillerRed, pKillerRed-dMito vector encoding mitochondria-targeted KillerRed and pTurboGFP-N vector allowing cytoplasmic expression of bright green fluorescent protein TurboGFP for monitoring cell fate	20 μg each	93,94,45
Antibodies against KillerRed				
Anti-KillerRed	AB961 AB962	Rabbit polyclonal antibody against KillerRed	100 μg 200 μg	106

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

pKillerRed-C vector



For vector sequence, please visit our Web site at http://www.evrogen.com/support/vector-info.shtml

Vector type	mammalian expression vector
Reporter	KillerRed
Reporter codon usage	mammalian
Promoter for KillerRed	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori; eukaryotic - SV40 ori
Use	KillerRed expression in mammalian cells; generation of fusions to the KillerRed C-terminus

Product	Cat.#	Reporter	Color	Size
pKillerRed-C	FP961	KillerRed	red	20 μg

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

pKillerRed-C vector MCS

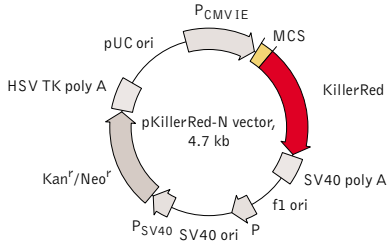
KillerRed → BspE I | Xho I | Hind III | Pst I | Kpn I | Apa I | BamH I | STOPs

... TCC.GGA.CTC.AGA.TCT.CGA.GCT.CAA.GCT.TCG.AAT.TCT.GCA.GTC.GAC.GGT.ACC.GCG.GGC.CCG.GGA.TCC.ACC.GGA.TCT.AGA.TAA.CTG.ATC.A ...

Bgl II | Sac I | EcoR I | Sal I | Sac II | Sma I/Xma I | Xba I# | Bcl I#

— sites are blocked by *dam* methylation. If you wish to digest the vector with these enzymes, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

pKillerRed-N vector



For vector sequence, please visit our Web site at http://www.evrogen.com/support/vector-info.shtml

Vector type	mammalian expression vector
Reporter	KillerRed
Reporter codon usage	mammalian
Promoter for KillerRed	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori; eukaryotic - SV40 ori
Use	KillerRed expression in mammalian cells; generation of fusions to the KillerRed N-terminus

Product	Cat.#	Reporter	Color	Size
pKillerRed-N	FP962	KillerRed	red	20 μg

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

pKillerRed-N vector MCS

Nhe I | Bgl II | Sac I | EcoR I | Sal I | Sac II | Sma I/Xma I | Age I | KillerRed →

... GCT.AGC.GCT.ACC.GGA.CTC.AGA.TCT.CGA.GCT.CAA.GCT.TCG.AAT.TCT.GCA.GTC.GAC.GGT.ACC.GCG.GGC.CCG.GGA.TCC.ACC.GGT.CGC.CAC.CAT.GG ...

Afe I | Xho I | Hind III | Pst I | Kpn I | Apa I | BamH I | Nco I*

* — not unique sites.

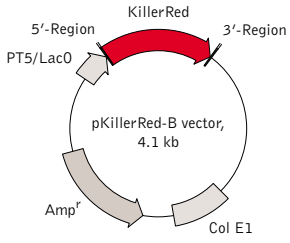
Notice to Purchaser:

Evrogen Fluorescent Protein Products (the Products) are intended for research use only. The Products are covered by Evrogen Patents and/or Patent applications pending. By use of these Products, you accept the terms and conditions of the applicable Limited Use Label License (see page 141). For license information please contact Evrogen by e-mail at license@evrogen.com.

The CMV promoter is covered under U.S. Patents 5,168,062 and 5,385,839, and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242.

MATERIAL SAFETY DATA SHEET INFORMATION: To the best of our knowledge, these products do not require a Material Safety Data Sheet. However, all the properties of these products (and, if applicable, each of their components) have not been thoroughly investigated. Therefore, we recommend that you use gloves and eye protection, and wear a laboratory coat when working with these products.

pKillerRed-B vector



For vector sequence, please visit our Web site at
http://www.evrogen.com/support/vector-info.shtml

Vector type	bacterial expression vector
Reporter	KillerRed
Reporter codon usage	mammalian
Promoter for KillerRed	T5 promoter/lac operator
Host cells	prokaryotic
Selection	ampicillin
Replication	ColE1 ori
Use	Source of the KillerRed coding sequence; KillerRed expression in bacterial cells

Product	Cat.#	Reporter	Color	Size
pKillerRed-B	FP963	KillerRed	red	20 μg

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

5' Region

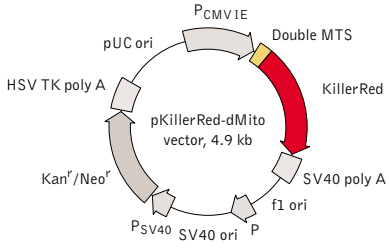
RBS ATG.AGA.GGA.TCG.GGA.TCC.ATG.G...
(BamH I; KillerRed; Nco I*)

3' Region

...TGA.AAG.CTT...
(STOP; Hind III)

* — not unique sites.

pKillerRed-dMito vector



For vector sequence, please visit our Web site at
http://www.evrogen.com/support/vector-info.shtml

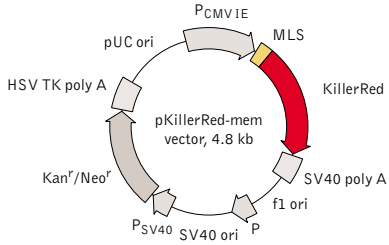
MTS - Mitochondrial targeting sequence

Vector type	mammalian expression vector
Reporter	KillerRed
Reporter codon usage	mammalian
Promoter for KillerRed	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori
Use	Expression of mitochondria-targeted KillerRed in mammalian cells under the control of CMV promoter; source of mitochondria-targeted KillerRed coding sequence

Product	Cat.#	Reporter	Color	Size
pKillerRed-dMito	FP964	KillerRed	red	20 μg

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

pKillerRed-mem vector



For vector sequence, please visit our Web site at http://www.evrogen.com/support/vector-info.shtml

MLS - Membrane localization signal

Vector type	mammalian expression vector
Reporter	KillerRed
Reporter codon usage	mammalian
Promoter for KillerRed	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori
Use	Expression of membrane-targeted KillerRed in mammalian cells under the control of CMV promoter; source of membrane-targeted KillerRed coding sequence

Product	Cat.#	Reporter	Color	Size
pKillerRed-mem	FP966	KillerRed	red	20 μg

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

Antibodies Against Fluorescent Proteins

- A collection of antibodies for the detection of Evrogen Fluorescent Proteins in different applications.

Contents

Overview

Rabbit polyclonal antibodies are available from Evrogen for immunological detection of fluorescent proteins and photosensitizer KillerRed. The antibodies were purified by affinity chromatography and can be used for Western blot, immunobloting, immunohistochemistry, and ELISA.

Target protein	Antibody	Cat. #	Pages
CopGFP	Anti-CopGFP antibody	AB501, AB502	100
Dendra2	Anti-Dendra2 antibody	AB821, AB822	105
JRed	Anti-KillerRed antibody	AB961, AB962	106
KillerRed	Anti-KillerRed antibody	AB961, AB962	106
mKate2	Anti-tRFP antibody	AB231, AB232	104
PhiYFP, PhiYFP-m	Anti-PhiYFP antibody	AB601, AB602	102
	Anti-PhiYFP(d) antibody	AB603, AB604	103
PS-CFP2	Anti-Tag(CGY)FP antibody	AB121, AB122	101
TagBFP	Anti-tRFP antibody	AB231, AB232	104
TagCFP	Anti-Tag(CGY)FP antibody	AB121, AB122	101
TagFP635	Anti-tRFP antibody	AB231, AB232	104
TagGFP, TagGFP2	Anti-Tag(CGY)FP antibody	AB121, AB122	101
TagRFP	Anti-tRFP antibody	AB231, AB232	104
TagYFP	Anti-Tag(CGY)FP antibody	AB121, AB122	101
TurboFP602	Anti-tRFP antibody	AB231, AB232	104
TurboFP635	Anti-tRFP antibody	AB231, AB232	104
TurboGFP	Anti-TurboGFP antibody	AB511, AB512	98
	Anti-TurboGFP(d) antibody	AB513, AB514	99
TurboRFP	Anti-tRFP antibody	AB231, AB232	104
TurboYFP	Anti-PhiYFP antibody	AB601, AB602	102
	Anti-PhiYFP(d) antibody	AB603, AB604	103

Anti-TurboGFP antibody

Product	Cat.#	Size
Anti-TurboGFP antibody	AB511	100 μg
	AB512	200 μg

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

Use

- Immunoblotting
- Immunohistochemistry
- ELISA

Description

Rabbit polyclonal antibody against non-denatured TurboGFP.

Specificity: The antibody has been selected to recognize non-denatured TurboGFP. It can also be used for recognizing denatured TurboGFP, but with lesser activity than Anti-TurboGFP(d) (Cat.# AB513-AB514) antibody. The antibody shows little or no cross-reactivity with other fluorescent proteins like EGFP, TagFPs, TurboRFP, CopGFP, KFP-Red and, DsRed2.

Immunogen: Full-length recombinant non-denatured TurboGFP fused to 6XHis tag.

Antibody preparation: Full-length recombinant TurboGFP fused to 6XHis tag was purified from transformed *E. coli* using metal-ion affinity chromatography. Antibodies were produced in rabbits immunized with the recombinant non-denaturated TurboGFP. Specific IgG were purified by TurboGFP affinity chromatography.

Formulation: Lyophilized from the buffer containing 0.1% mannitol, 0.1% dextran, 0.1M NaCl, 0.01M Na_2PO_4, and 0.01M $NaBO_4$; pH 7.4.

Reconstitution: Reconstitute with sterile water or 50% glycerol to a concentration of 1 mg/ml.

Storage: Lyophilized samples are stable for twelve months from date of receipt when stored at -20°C. The presence of silica gel drier is advisable.
Reconstituted with sterile water, antibody can be stored at +2 - +8°C for three months without detectable loss of activity.
Reconstituted with 50% glycerol, antibody can be stored at -20°C in a manual defrost freezer for six months without detectable loss of activity.
Aliquot antibody upon reconstitution. Avoid repeated freeze / thaw cycles.

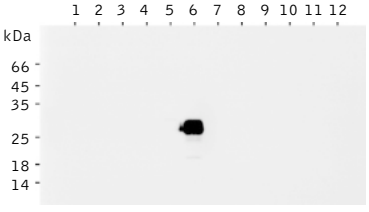
Anti-TurboGFP(d) antibody

Product	Cat.#	Size
Anti-TurboGFP(d) antibody	AB513	100 μg
	AB514	200 μg

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

Use

- Western blot
- Immunoblotting
- Immunohistochemistry
- ELISA



Western blot detection of fluorescent proteins using Anti-TurboGFP(d) antibodies.
1 - TagCFP; 2 - TagGFP; 3 - TagYFP; 4 - TagRFP; 5 - TurboFP602; 6 - TurboGFP; 7 - TurboYFP; 8 - TurboRFP; 9 - PS-CFP2; 10 - Dendra2; 11 - KillerRed; 12 - EGFP.
Recombinant proteins were purified from transformed *E. coli*. 25 ng of each protein were separated by SDS PAGE (14% acrylamide). The samples were boiled before loading. Antibody was used at a 1/10000 dilution. Secondary antibody: Goat anti-Rabbit HRP-conjugated IgG.

Description

Rabbit polyclonal antibody against denatured TurboGFP and CopGFP.

Specificity: The antibody has been selected to recognize denatured TurboGFP and CopGFP. It can also be used for recognizing non-denatured TurboGFP, but with lesser activity than corresponding Anti-TurboGFP (Cat.# AB511-AB512) antibody. The antibody shows little or no cross-reactivity with TagCFP, TagGFP, TagYFP, TagRFP, TurboFP602, TurboYFP, TurboRFP, PS-CFP2, Dendra2, KillerRed, EGFP, KFP-Red, and DsRed2.

Immunogen: Full-length recombinant denatured TurboGFP fused to 6XHis tag.

Antibody preparation: Full-length recombinant TurboGFP fused to 6XHis tag was purified from transformed *E. coli* using metal-ion affinity chromatography. Antibodies were produced in rabbits immunized with the recombinant denaturated TurboGFP. Specific IgG were purified by TurboGFP affinity chromatography.

Formulation: Lyophilized from the buffer containing 0.1% mannitol, 0.1% dextran, 0.1M NaCl, 0.01M Na_2PO_4, and 0.01M $NaBO_4$; pH 7.4.

Reconstitution: Reconstitute with sterile water or 50% glycerol to a concentration of 1 mg/ml.

Storage: Lyophilized samples are stable for twelve months from date of receipt when stored at -20°C. The presence of silica gel drier is advisable.
Reconstituted with sterile water, antibody can be stored at +2 - +8°C for three months without detectable loss of activity.
Reconstituted with 50% glycerol, antibody can be stored at -20°C in a manual defrost freezer for six months without detectable loss of activity. Aliquot antibody upon reconstitution. Avoid repeated freeze / thaw cycles.

Anti-CopGFP antibody

Product	Cat.#	Size
Anti-CopGFP antibody	AB501	100 μg
	AB502	200 μg

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

Use

- Immunoblotting
- Immunohistochemistry
- ELISA

Description

Rabbit polyclonal antibody against non-denatured CopGFP.

Specificity: The antibody has been selected to recognize non-denatured CopGFP. Heat or chemically denatured protein lacks antigen determinants. The antibody shows little or no cross-reactivity with other fluorescent proteins like EGFP, TurboGFP, KFP-Red, and DsRed2.

Immunogen: Full-length recombinant non-denatured CopGFP fused to 6XHis tag.

Antibody preparation: Full-length recombinant CopGFP fused to 6XHis tag was purified from transformed *E. coli* using metal-ion affinity chromatography. Antibodies were produced in rabbits immunized with the recombinant non-denaturated CopGFP. Specific IgG were purified by CopGFP affinity chromatography.

Formulation: Lyophilized from the buffer containing 0.1% mannitol, 0.1% dextran, 0.1M NaCl, 0.01M Na_2PO_4, and 0.01M $NaBO_4$; pH 7.4.

Reconstitution: Reconstitute with sterile water or 50% glycerol to a concentration of 1 mg/ml.

Storage: Lyophilized samples are stable for twelve months from date of receipt when stored at -20°C. The presence of silica gel drier is advisable. Reconstituted with sterile water, antibody can be stored at +2 - +8°C for three months without detectable loss of activity.
Reconstituted with 50% glycerol, antibody can be stored at -20°C in a manual defrost freezer for six months without detectable loss of activity. Aliquot antibody upon reconstitution. Avoid repeated freeze / thaw cycles.

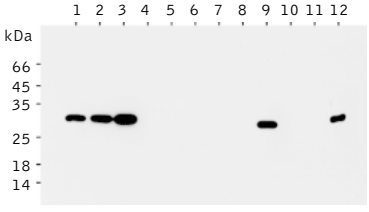
Anti-Tag(CGY)FP antibody

Product	Cat.#	Size
Anti-Tag(CGY)FP antibody	AB121	100 μg
	AB122	200 μg

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

Use

- Western blot
- Immunoblotting
- Immunohistochemistry
- ELISA



Western blot detection of fluorescent proteins using Anti-Tag(CGY)FP antibodies.
1 - TagCFP; 2 - TagGFP; 3 - TagYFP; 4 - TagRFP; 5 - TurboFP602; 6 - TurboGFP; 7 - TurboYFP; 8 - TurboRFP; 9 - PS-CFP2; 10 - Dendra2; 11 - KillerRed; 12 - EGFP.
Recombinant proteins were purified from transformed *E. coli*. 25 ng of each protein were separated by SDS PAGE (14% acrylamide). The samples were boiled before loading. Antibody was used at a 1/10000 dilution. Secondary antibody: Goat anti-Rabbit HRP-conjugated IgG.

Description

Rabbit polyclonal antibody against TagCFP, TagGFP, TagGFP2, TagYFP, PS-CFP2, and EGFP.

Specificity: The antibody has been selected to recognize both denatured and native TagCFP, TagGFP, TagGFP2, TagYFP, and PS-CFP2. The antibody also recognizes EGFP. The antibody shows little or no cross-reactivity with TagRFP, TurboGFP, TurboYFP, TurboRFP, TurboFP602, JRed, Dendra2, and KillerRed.

Immunogen: Full-length recombinant denatured TagGFP fused to 6xHis tag.

Antibody preparation: Full-length recombinant TagGFP fused to 6XHis tag was purified from transformed *E. coli* using organic extraction and hydrophobic chromatography. Antibody was produced in rabbits immunized with the recombinant denatured TagGFP and purified by TagGFP affinity chromatography.

Formulation: Lyophilized from the buffer containing 0.01M Na_2PO_4, 0.1M NaCl, 0.25 mg/ml gelatin, and 1% trehalose; pH 7.4.

Reconstitution: Reconstitute with sterile water or 50% glycerol to a concentration of 1 mg/ml.

Storage: Lyophilized samples are stable for twelve months from date of receipt when stored at -20°C. The presence of silica gel drier is advisable.
Reconstituted with sterile water, antibody can be stored at +2 - +8°C for three months without detectable loss of activity.
Reconstituted with 50% glycerol, antibody can be stored at -20°C in a manual defrost freezer for six months without detectable loss of activity.
Aliquot antibody upon reconstitution. Avoid repeated freeze / thaw cycles.

Anti-PhiYFP antibody

Product	Cat.#	Size
Anti-PhiYFP antibody	AB601	100 μg
	AB602	200 μg

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

Use

- Immunoblotting
- Immunohistochemistry
- ELISA

Description

Rabbit polyclonal antibody against non-denatured PhiYFP, PhiYFP-m, and TurboYFP.

Specificity: The antibody has been selected to recognize non-denatured TurboYFP, PhiYFP, and PhiYFP-m. Heat or chemically denatured proteins lack antigen determinants. The antibody shows little or no cross-reactivity with other fluorescent proteins like EGFP, TurboGFP, KFP-Red, and DsRed2.

Immunogen: Full-length recombinant non-denatured PhiYFP fused to 6XHis tag.

Antibody preparation: Full-length recombinant PhiYFP fused to 6XHis tag was purified from transformed *E. coli* using metal-ion affinity chromatography. Antibodies were produced in rabbits immunized with the recombinant non-denaturated PhiYFP. Specific IgG were purified by PhiYFP affinity chromatography.

Formulation: Lyophilized from the buffer containing 0.1% mannitol, 0.1% dextran, 0.1M NaCl, 0.01M Na_2PO_4, and 0.01M $NaBO_4$; pH 7.4.

Reconstitution: Reconstitute with sterile water or 50% glycerol to a concentration of 1 mg/ml.

Storage: Lyophilized samples are stable for twelve months from date of receipt when stored at -20°C. The presence of silica gel drier is advisable.
Reconstituted with sterile water, antibody can be stored at +2 - +8°C for three months without detectable loss of activity.
Reconstituted with 50% glycerol, antibody can be stored at -20°C in a manual defrost freezer for six months without detectable loss of activity.
Aliquot antibody upon reconstitution. Avoid repeated freeze / thaw cycles.

Anti-PhiYFP(d) antibody

Product	Cat.#	Size
Anti-PhiYFP(d) antibody	AB603	100 μg
	AB604	200 μg

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

Use

- Western blot
- Immunoblotting
- Immunohistochemistry
- ELISA

Western blot detection of fluorescent proteins using Anti-PhiYFP(d) antibodies.
1 - TagCFP; 2 - TagGFP; 3 - TagYFP; 4 - TagRFP; 5 - TurboFP602; 6 - TurboGFP; 7 - TurboYFP; 8 - TurboRFP; 9 - PS-CFP2; 10 - Dendra2; 11 - KillerRed; 12 - EGFP.
Recombinant proteins were purified from transformed *E. coli*. 25 ng of each protein were separated by SDS PAGE (14% acrylamide). The samples were boiled before loading. Antibody was used at a 1/10000 dilution. Secondary antibody: Goat anti-Rabbit HRP-conjugated IgG.

Description

Rabbit polyclonal antibody against denatured PhiYFP, PhiYFP-m, and TurboYFP.

Specificity: The antibody has been selected to recognize denatured TurboYFP, PhiYFP, and PhiYFP-m. It can also be used for recognizing non-denatured Phi-Yellow proteins, but with lesser activity than Anti-PhiYFP (Cat.# AB601-AB602) antibody. The antibody shows little or no cross-reactivity with TagCFP, TagGFP, TagYFP, TagRFP, TurboGFP, TurboRFP, JRed, Dendra2 and KillerRed. The antibody shows cross-reactivity with TagRFP, TurboFP602, and TurboFP635.

Immunogen: Full-length recombinant denatured PhiYFP fused to 6XHis tag.

Antibody preparation: Full-length recombinant PhiYFP fused to 6XHis tag was purified from transformed *E. coli* using metal-ion affinity chromatography. Antibodies were produced in rabbits immunized with the recombinant denaturated PhiYFP. Specific IgG were purified by PhiYFP affinity chromatography.

Formulation: Lyophilized from the buffer containing 0.1% mannitol, 0.1% dextran, 0.1M NaCl, 0.01M Na_2PO_4, and 0.01M $NaBO_4$; pH 7.4.

Reconstitution: Reconstitute with sterile water or 50% glycerol to a concentration of 1 mg/ml.

Storage: Lyophilized samples are stable for twelve months from date of receipt when stored at -20°C. The presence of silica gel drier is advisable. Reconstituted with sterile water, antibody can be stored at +2 - +8°C for three months without detectable loss of activity.
Reconstituted with 50% glycerol, antibody can be stored at -20°C in a manual defrost freezer for six months without detectable loss of activity. Aliquot antibody upon reconstitution. Avoid repeated freeze / thaw cycles.

Anti-tRFP antibody

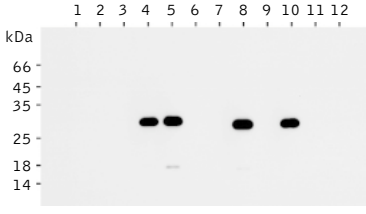
Product	Cat.#	Size
Anti-tRFP antibody	AB231	100 μg
	AB232	200 μg

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

Use

- Western blot
- Immunoblotting
- Immunohistochemistry
- ELISA

NOTE: Anti-tRFP antibody is not recognized by (at least) some of mouse monoclonal anti-rabbit antibodies. Please use goat anti-rabbit antibodies instead.



Western blot detection of fluorescent proteins using Anti-tRFP antibodies.
1 - TagCFP; 2 - TagGFP; 3 - TagYFP; 4 - TagRFP; 5 - TurboFP602; 6 - TurboGFP; 7 - TurboYFP; 8 - TurboRFP; 9 - PS-CFP2; 10 - Dendra2; 11 - KillerRed; 12 - EGFP.
Recombinant proteins were purified from transformed *E. coli*. 25 ng of each protein were separated by SDS PAGE (14% acrylamide). The samples were boiled before loading. Antibody was used at a 1/10000 dilution. Secondary antibody: Goat anti-Rabbit HRP-conjugated IgG.

Description

Rabbit polyclonal antibody against TurboRFP, TurboFP602, TurboFP635, TagBFP, TagRFP, TagFP635 and mKate2.

Specificity: The antibody has been selected to recognize both denatured and native TurboRFP, TurboFP602, TurboFP635, TagBFP, TagRFP, TagFP635 and mKate2. The antibody shows little or no cross-reactivity with TagCFP, TagGFP, TagYFP, TurboGFP, TurboYFP, JRed, PS-CFP2, KillerRed, and EGFP. The antibody shows cross-reactivity with Dendra2.

Immunogen: Full-length recombinant denatured TurboRFP.

Antibody preparation: Full-length recombinant TurboRFP was purified from transformed *E. coli* using organic extraction and hydrophobic chromatography. Antibody was produced in rabbits immunized with the recombinant denatured TurboRFP and purified by TurboRFP affinity chromatography.

Formulation: Lyophilized from the buffer containing 0.01M Na_2PO_4, 0.1M NaCl, 0.25 mg/ml gelatin, and 1% trehalose; pH 7.4.

Reconstitution: Reconstitute with sterile water or 50% glycerol to a concentration of 1 mg/ml.

Storage: Lyophilized samples are stable for twelve months from date of receipt when stored at -20°C. The presence of silica gel drier is advisable.
Reconstituted with sterile water, antibody can be stored at +2 - +8°C for three months without detectable loss of activity.
Reconstituted with 50% glycerol, antibody can be stored at -20°C in a manual defrost freezer for six months without detectable loss of activity.
Aliquot antibody upon reconstitution. Avoid repeated freeze / thaw cycles.

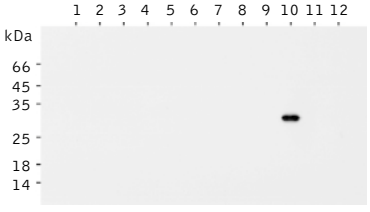
Anti-Dendra2 antibody

Product	Cat.#	Size
Anti-Dendra2 antibody	AB821	100 μg
	AB822	200 μg

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

Use

- Western blot
- Immunoblotting
- Immunohistochemistry
- ELISA



Western blot detection of fluorescent proteins using Anti-Dendra2 antibodies.
1 - TagCFP; 2 - TagGFP; 3 - TagYFP; 4 - TagRFP; 5 - TurboFP602; 6 - TurboGFP; 7 - TurboYFP; 8 - TurboRFP; 9 - PS-CFP2; 10 - Dendra2; 11 - KillerRed; 12 - EGFP.
Recombinant proteins were purified from transformed *E. coli*. 25 ng of each protein were separated by SDS PAGE (14% acrylamide). The samples were boiled before loading. Antibody was used at a 1/10000 dilution. Secondary antibody: Goat anti-Rabbit HRP-conjugated IgG.

Description

Rabbit polyclonal antibody against green-to-red photoswitchable fluorescent protein Dendra2.

Specificity: The antibody has been selected to recognize both denatured and native Dendra2. The antibody shows little or no cross-reactivity with TagCFP, TagGFP, TagYFP, TagRFP, TurboGFP, TurboYFP, TurboRFP, TurboFP602, JRed, and KillerRed.

Immunogen: Full-length recombinant denatured Dendra2.

Antibody preparation: Full-length recombinant Dendra2 was purified from transformed *E. coli* using organic extraction and hydrophobic chromatography. Antibody was produced in rabbits immunized with the recombinant denatured Dendra2 and purified by Dendra2 affinity chromatography.

Formulation: Lyophilized from the buffer containing 0.01M Na_2PO_4, 0.1M NaCl, 0.25 mg/ml gelatin, and 1% trehalose; pH 7.4.

Reconstitution: Reconstitute with sterile water or 50% glycerol to a concentration of 1 mg/ml.

Storage: Lyophilized samples are stable for twelve months from date of receipt when stored at -20°C. The presence of silica gel drier is advisable. Reconstituted with sterile water, antibody can be stored at +2 - +8°C for three months without detectable loss of activity.
Reconstituted with 50% glycerol, antibody can be stored at -20°C in a manual defrost freezer for six months without detectable loss of activity. Aliquot antibody upon reconstitution. Avoid repeated freeze / thaw cycles.

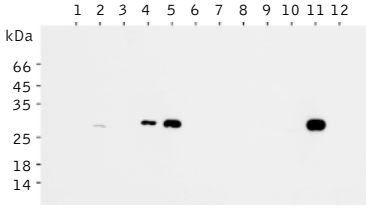
Anti-KillerRed antibody

Product	Cat.#	Size
Anti-KillerRed antibody	AB961	100 μg
	AB962	200 μg

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

Use

- Western blot
- Immunoblotting
- Immunohistochemistry
- ELISA



Western blot detection of fluorescent proteins using Anti-KillerRed antibodies.
1 - TagCFP; 2 - TagGFP; 3 - TagYFP; 4 - TagRFP; 5 - TurboFP602; 6 - TurboGFP; 7 - TurboYFP; 8 - TurboRFP; 9 - PS-CFP2; 10 - Dendra2; 11 - KillerRed; 12 - EGFP.
Recombinant proteins were purified from transformed *E. coli*. 25 ng of each protein were separated by SDS PAGE (14% acrylamide). The samples were boiled before loading. Antibody was used at a 1/10000 dilution. Secondary antibody: Goat anti-Rabbit HRP-conjugated IgG.

Description

Rabbit polyclonal antibody against KillerRed and red fluorescent protein JRed.

Specificity: The antibody has been selected to recognize both denatured and native KillerRed and JRed. The antibody shows little or no cross-reactivity with TagCFP, TagYFP, TurboGFP, TurboYFP, TurboRFP. The antibody shows cross-reactivity with TagGFP, TagRFP, TurboFP602, and TurboFP635.

Immunogen: Full-length recombinant denatured KillerRed.

Antibody preparation: Full-length recombinant KillerRed was purified from transformed *E. coli* using organic extraction and hydrophobic chromatography. Antibody was produced in rabbits immunized with the recombinant denatured KillerRed and purified by KillerRed affinity chromatography.

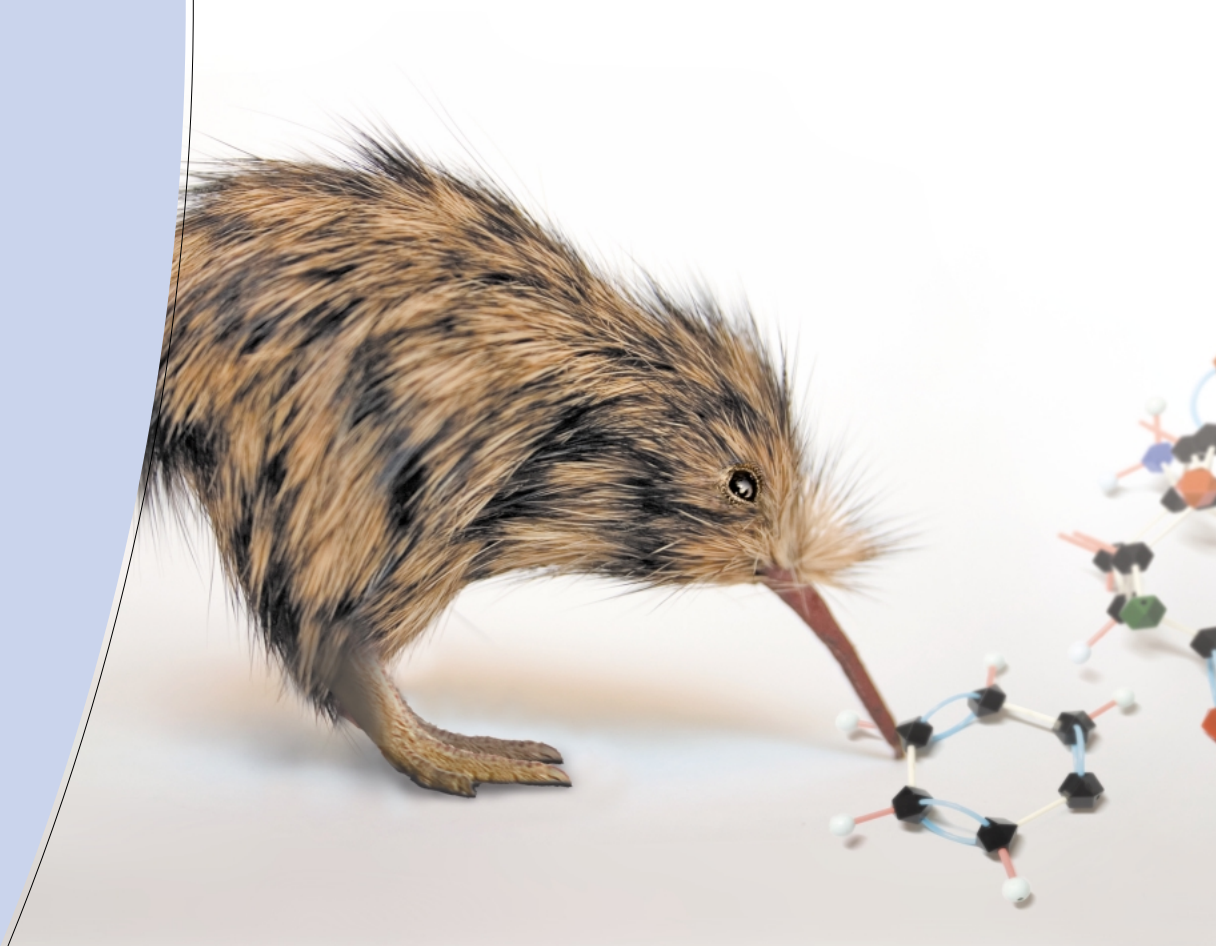
Formulation: Lyophilized from the buffer containing 0.01M Na_2PO_4, 0.1M NaCl, 0.25 mg/ml gelatin, and 1% trehalose; pH 7.4.

Reconstitution: Reconstitute with sterile water or 50% glycerol to a concentration of 1 mg/ml.

Storage: Lyophilized samples are stable for twelve months from date of receipt when stored at -20°C. The presence of silica gel drier is advisable.
Reconstituted with sterile water, antibody can be stored at +2 - +8°C for three months without detectable loss of activity.
Reconstituted with 50% glycerol, antibody can be stored at -20°C in a manual defrost freezer for six months without detectable loss of activity. Aliquot antibody upon reconstitution. Avoid repeated freeze / thaw cycles.

Nucleic Acid Research Kits

- Kits for cDNA synthesis, amplification, and normalization



Contents

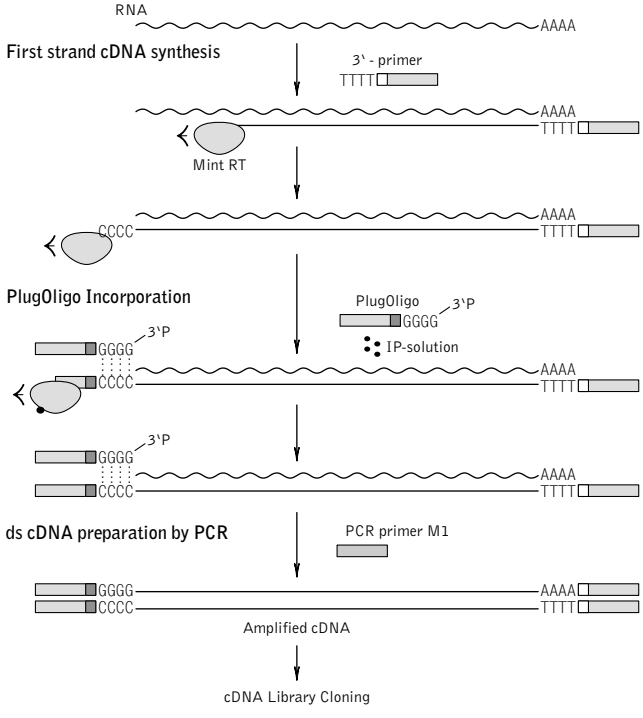
Mint cDNA synthesis kits

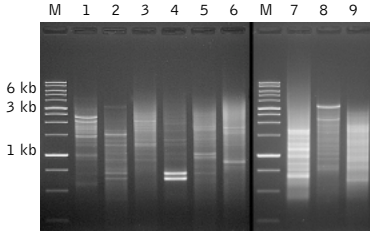
- Fast cDNA synthesis protocol
- High content of full length transcripts
- Low background
- Small starting amount of poly(A)+ or total RNA required
- Compatibility with Trimmer cDNA normalization kits

Product	Cat.#	Size
Mint cDNA synthesis kit	SK001	20 rxn
Mint-Universal cDNA synthesis kit	SK002	20 rxn

The kits are not available in certain countries. Please contact your local distributor or Evrogen before ordering. Kit components must be stored at -20°C.

Evrogen offers cDNA synthesis kits designed to generate full-length-enriched double stranded (ds) cDNA from total or poly(A)+ RNA. Depending on your particular need, you can select Mint-Universal or Mint kit, where the first allows preparation of cDNA ready for either nondirectional or directional cloning and the second allows for nondirectional cloning only.





Mint-amplified cDNA from different sources.
1 - Mouse liver; 2 - mouse skeletal muscle; 3 - mouse brain; 4 - human leucocytes; 5 - human lung; 6 - human skeletal muscle; 7 - mosquito grub; 8 - copepod *Pontella sp.*; 9 - tomato *Lycopersicon esculentum*. M - 1 kb DNA size markers, SibEnzyme, Russia.

Schematic outline of Mint cDNA synthesis. First strand cDNA synthesis starts from the 3`-end oligo(dT) primer that anneal to poly(A)+ stretch of RNA. When RT reaches the 5`-end of the mRNA, it adds several non-template nucleotides, primarily deoxycytidines, to the 3`-end of the newly synthesized first-strand cDNA [Schmidt and Mueller 1999]. This oligo(dC) stretch base pairs to complementary oligo(dG) sequence located at the 3`- end of a special deoxyribooligonucleotide called PlugOligo. RT identifies PlugOligo as an extra part of the RNA-template and continues synthesis of the first strand cDNA to the end of the oligonucleotide, thus incorporating PlugOligo sequence into the 5`-end of cDNA.
The last 3`-dG residue of the PlugOligo is a terminator nucleotide comprising 3`-phosphate group. This blocking group prevents unwanted annealing and extension of the PlugOligo. Under standard conditions, RT hardly uses PlugOligo as a template, however, our special IP-solution (solution for Incorporation of PlugOligo sequence) dramatically increases the efficiency of this process. At the last step, ds cDNA is amplified by PCR. Use of Encyclo polymerase and specially designed primers allows synthesis of full-length-enriched cDNA that is flanked by PlugOligo and 3′-end adapter sequences.

Each kit is comprised of detailed instruction and reagents for 20 cDNA synthesis reactions including a free Mint reverse transcriptase sample and a trial-size Encyclo PCR kit allowing accurate cDNA amplification. Mint kit contains adapter pair for synthesis of cDNA flanking with symmetric sequences. Mint-Universal kit comprises two pairs of adapters for synthesis of cDNA flanking with symmetric or asymmetric sequences.

Synthesized cDNA can be used in various applications including cDNA library construction; virtual Northern blot; suppression subtractive hybridization (SSH); cDNA normalization using Trimmer or Trimmer-Direct kits (Cat. NK001; NK002, please see page 112 for description).

REFERENCES

Schmidt and Mueller (1999). Nucleic Acids Res, 27 (21): e31 / pmid: 10518626

Encyclo PCR amplification kit

- High yield of PCR products from a wide variety of templates
- Suitable for difficult templates
- PCR up to 15 kb

Product	Cat.#	Size
Encyclo PCR kit	PK001	100 rxn

The kits are not available in certain countries. Please contact your local distributor or Evrogen before ordering. Kit components must be stored at -20°C.

Product description

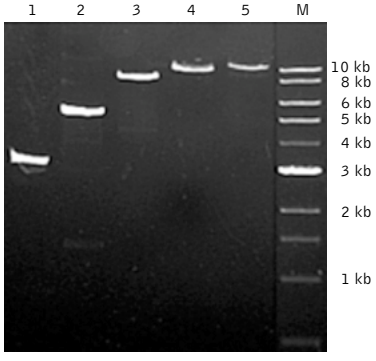
Encyclo PCR kit is suitable for most PCR applications. It is especially recommended for cDNA amplification because of optimal combination of high fidelity and processivity provided by Encyclo polymerase mix.

Evrogen Encyclo polymerase mix produces high yields of PCR products from a wide variety of templates and displays following features:

- 5'>3' DNA polymerase activity with high processivity
- Proofreading 3'>5' exonuclease activity
- Automatic hot start
- TA cloning compatibility

Encyclo buffer has been developed to facilitate the amplification of specific PCR products and to provide successful amplification of long DNA templates. Encyclo PCR kit includes a mix of high-purity deoxyribonucleotides as well as sterile PCR water. Control DNA template and primer mix enclosed can be used for positive control PCR reaction.

Kit includes components for 100 PCR reactions of 50 μl each.



PCR amplification of phage lambda DNA fragments using "Encyclo PCR kit".
1 - 2.9 kb; 2 - 5.0 kb; 3 - 8.0 kb; 4 - 10.0 kb; 5 - 15.0 kb. M - 50 ng of DNA size markers (1 kb DNA ladder, NEB).

TRIMMER
cDNA normalization kits

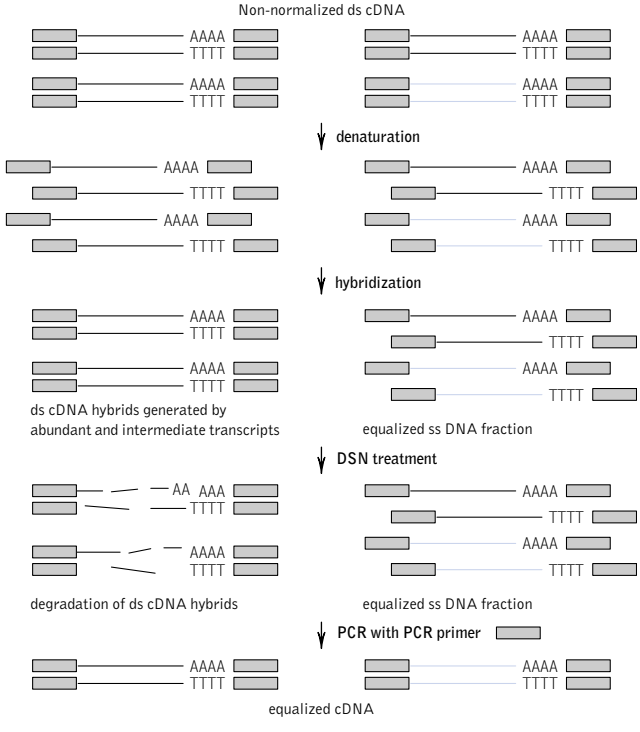
- Rapid and reliable way to remove repeated transcripts from cDNA library
- Equalization of full-length-enriched cDNA before library cloning
- Simple procedure, no physical separation steps
- Recommended for EST projects, transcriptome analysis, functional screening, and rare gene discovery

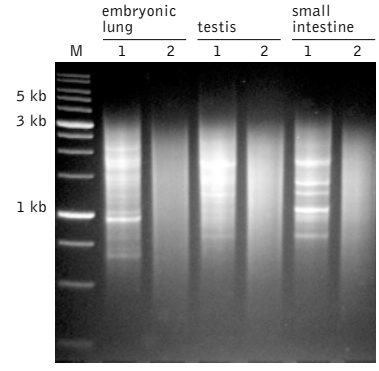
Product	Cat.#	Size
Trimmer cDNA normalization kit	NK001	10 rxn
Trimmer-Direct cDNA normalization kit	NK002	10 rxn

Please contact your local distributor for exact prices and delivery information. Kit components must be stored at -20°C. Lyophilized DSN enzyme must be stored at +4°C before reconstitution.

Evrogen offers cDNA normalization kits designed to generate full-length-enriched double stranded (ds) cDNA with equalized concentrations of different transcripts.

Depending on your particular needs, you can select Trimmer or Trimmer-Direct kit wherein the first allows preparation of normalized cDNA ready for nondirectional cloning and the second allows for directional cloning.





cDNA normalization result.
Agarose gel electrophoresis of non-normalized (lanes 1) and Trimmer-Direct-normalized (lanes 2) amplified SMART-prepared cDNA from different human tissues. M - 1 kb DNA size markers, SibEnzyme, Russia.

DSN normalization scheme.
Black lines represent abundant transcripts, blue lines - rare transcripts. Rectangle represents adapter sequence and its complement.
Evrogen normalization kits are based on a DSN-normalization technology [Zhulidov et al. 2004, [Zhulidov et al. 2005]. The method involves denaturation-reassociation of cDNA, degradation of dsDNA fraction formed by cDNA encoding abundant transcripts and PCR amplification of the equalized ssDNA fraction. The key element of this method is degradation of dsDNA fraction using Kamchatka crab duplex-specific nuclease (DSN). DSN is a termostable enzyme specific to the dsDNA [Shagin et al. 2002]. Normalization is done before cDNA cloning, and it does not include physical separation steps. Because of specific cDNA synthesis procedure, 5'n- and 3'-adapters comprise common external sequence that is used for PCR amplification of normalized cDNA fraction. A specific, suppression PCR-based approach, prevents reduction of the average cDNA length during PCR [Shagin et al. 1999].

Each kit contain a detailed instruction and reagents for 10 cDNA normalization reactions. Kits do not include materials for cDNA preparation, amplification, and size-fractionation. These materials must be purchased separately.

Starting materials: For cDNA normalization using Trimmer kit, cDNA must be prepared using one of the following cDNA synthesis kits:

- Mint cDNA synthesis kit (Evrogen Cat.# SK001, see page 109);
- Mint-Universal cDNA synthesis kit, Protocol-I (Evrogen Cat.# SK002, see page 109);
- SMART™ PCR cDNA synthesis kit (Clontech Cat.# 634902).

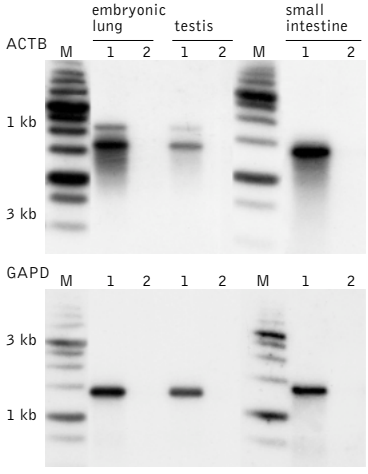
For cDNA normalization using Trimmer-Direct kit, cDNA must be prepared using one of the following cDNA synthesis kits:

- Mint-Universal cDNA synthesis kit, Protocol-II (Evrogen Cat.# SK002, see page 109);
- SMART™ cDNA Library Construction kit (Clontech Cat.# 634901)*;
- Creator™ SMART™ cDNA Library Construction kit (Clontech Cat.# 634903)*.

*Please follow instruction provided in Trimmer-Direct kit for cDNA synthesis using these kits. Please note that CDS-3M adapter (provided in Trimmer-Direct kit) must be used for cDNA preparation instead of CDS primer included into Clontech kits.

Important note for kit selection

Trimmer and Trimmer-Direct kits are based on similar technology, but utilize cDNA synthesized employing different adapters. Adapters used to synthesize cDNA for the subsequent directional cloning of the library are longer than those used to prepare cDNA for nondirectional cloning. Longer adapters leads to a reasonable decrease in the cDNA average length and often to the appearance of a low-molecular-weight fraction in the cDNA (which in turn makes it necessary to include a size-separation procedure to remove short cDNA fragments before cloning). Therefore, if directional cloning of cDNA library is not critical to your research, we recommend that you use the Trimmer kit (Cat.# NK001).



Virtual Northern blot analysis of abundant transcripts in the cDNA samples from human tissues.
1 - Non-normalized cDNA; 2 - Trimmer-Direct-normalized cDNA; ACTB - β-actin; GAPD - glyceraldehyde-3-phosphate dehydrogenase.
M - 1 kb DNA size markers, SibEnzyme, Russia.

REFERENCES

Shagin et al. (1999). Nucleic Acids Res, 27 (18): e23 / pmid: 10471753

Shagin et al. (2002). Genome Res, 12 (12): 1935–1942 / pmid: 12466298

Zhulidov et al. (2004). Nucleic Acids Res, 32 (3): e37 / pmid: 14973331

Zhulidov et al. (2005). Bioorg Khim., 31 (2): 186–194 / pmid: 15889793

Duplex-specific nuclease

- Nuclease specific to double-stranded DNA
- Thermostable
- Inhibited by EDTA
- Recommended for cDNA normalization, cDNA depletion, and for other applications requiring selective removal of dsDNA from complex nucleic acid samples

Product	Cat.#	Size
Duplex-specific nuclease	EA001	50 Units
Duplex-specific nuclease	EA002	100 Units
Duplex-specific nuclease	EA003	10 Units

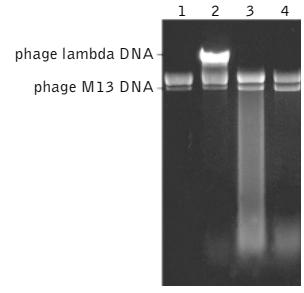
Please contact your local distributor for exact prices and delivery information. Kit components must be stored at -20°C. Lyophilized DSN enzyme must be stored at +4°C before reconstitution.

Duplex-specific nuclease (DSN) is an enzyme purified from hepatopancreas of the Kamchatka crab [Shagin et al. 2002]. DSN shows a strong preference for cleaving double-stranded DNA and DNA in DNA-RNA hybrid duplexes, compared with single-stranded DNA and RNA. Moreover, the cleavage rate of short, perfectly matched DNA duplexes by this enzyme is considerably higher than that for nonperfectly matched duplexes of the same length.

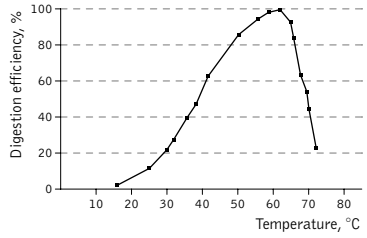
DSN acquires its enzymatic activity in the presence of Mg^{2+} ions (at least 5 mM is required for most applications) and is inhibited by EDTA. The pH and temperature optima for activity are 7-8 and 55-65°C, respectively. The nuclease is stable at a wide range of pH (from 4 to 12) and temperatures below 60°C. Moreover, 60% of DSN activity remains after incubation at 70°C for 30 min, and 40% - after incubation at 80°C. In addition, DSN is tolerant to proteinase K treatment (for 30 min at 37°C).

Each package comprises a detailed instruction, lyophilized DSN, and DSN storage and working buffers.

DNAase activity was measured using modified Kunitz assay, where unit definition was defined as: the amount of DSN added to 50 μg/ml calf thymus DNA that causes an increase of 0.001 absorbance units per minute. Activity assay was performed at 25°C, in 50 mM Tris-HCl buffer, pH 7.15, containing 5 mM $MgCl_2$.



Action of DSN on ss DNA of phage M13 and ds DNA of phage lambda.
Lanes 1, 2 - negative controls, incubation without nuclease. 1 - phage M13 DNA alone, 2 - mixture containing phage M13 and lambda DNA. Lanes 3, 4 - digestion of phage M13 and lambda DNA mixture by DSN at 70°C for 1.5 min (3) and 5 min (4).



Dependence of the DSN activity on temperature.
Activity of DNAse on ds DNA substrate was measured using Kunitz assay at different temperature.

REFERENCES

Shagin et al. (2002). Genome Res, 12 (12): 1935–1942 / pmid: 12466298

Molecular Biology Services

- cDNA preparation
- cDNA library construction
- cDNA normalization
- cDNA subtractive hybridization
- Bacterial genome subtraction
- Eukaryotic genome subtraction
- PCR/RT-PCR cloning
- Subcloning
- RACE and full-length cDNA isolation
- Genome walking
- Site-directed mutagenesis
- Gene synthesis

Contents

Overview

Evrogen offers various laboratory services to facilitate your gene discovery including

cDNA preparation and library construction services

Full-length-enriched double-stranded cDNA is prepared from poly(A)+ or total RNA. cDNA can be normalized, depleted or subtracted. Depending on your particular needs, you can obtain uncloned or cloned cDNA as well as standard, normalized, depleted or subtracted cDNA libraries.

- cDNA preparation (see page 118)
- Construction of standard cDNA libraries (see page 119)
- cDNA normalization and construction of normalized cDNA libraries (see page 120)
- cDNA depletion and construction of depleted cDNA libraries (see page 122)
- cDNA subtraction and construction of subtracted cDNA libraries (see page 124)

Genome Subtraction services

Comparison of two customer-specified genomes for the identification of unique fragments is performed by suppression subtractive hybridization (SSH). If require SSH is supplemented with mirror orientation selection.

- Bacterial genome subtraction (see page 126)
- Eukaryotic genome subtraction (see page 128)

Amplification and cloning

We provide PCR and RT-PCR cloning of known genes for array preparation and other common applications, subcloning of the target inserts into a vector of your choice, isolation of complete cDNA or genomic sequences by RACE or genome walking approaches.

- PCR cloning (see page 129)
- Subcloning (see page 130)
- RACE and full-length cDNA isolation (see page 131)
- Genome walking (see page 132)

Gene synthesis and modification

Various types of target sequence modification are available including *de-novo* gene synthesis, codon usage optimization, and introduction of any type of mutations, such as deletion, insertion, or substitution.

- Gene synthesis (see page 133)
- Site-directed mutagenesis (see page 134)

Fluorescent protein related services

We offer custom optimization of our vectors expressing fluorescent proteins in accordance with your particular needs and development of live-cell assays utilising Evrogen fluorescent technologies.

- Custom optimization of Evrogen expression vectors (see page 135)
- Custom assay development (see page 136)

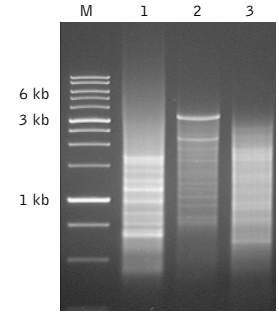
cDNA preparation service

- High-quality full-length-enriched amplified cDNA for various applications
- From limited amount of total or poly(A)+ RNA

Full-length-enriched double-stranded cDNA is produced using modified SMART technology [Matz et al. 1999; Matz 2002; Zhu et al. 2001]. The method combines cDNA synthesis and amplification and results in representative cDNA population enriched with full-length sequences even from small amounts of starting materials. Depending on research purposes, cDNA can be flanked by same (for nondirectional cloning) or different (for directional cloning) adapter sequences.

The cDNA can be used for:

- Construction of representative cDNA libraries (see page 119)
- Subtractive hybridization (see page 124)
- cDNA normalization (see page 120)
- Isolation of full-length cDNAs by RACE (see page 131)
- PCR cloning / array development (see page 129)
- Virtual Northern blot



Typical result of cDNA synthesis.
1 - mosquito grub; 2 - copepod *Pontella sp.*; 3 - tomato *Lycopersicon esculentum.* M - 1 kb DNA size marker, SibEnzyme, Russia.

Service terms and conditions

Cat.#	Service	Turnaround time
CS030	cDNA preparation	3-4 weeks

We offer DISCOUNTS for MULTIPLE ORDERS!

You provide:

1. At least 1.0 μg of total RNA or poly(A)+ RNA
   See "How to send samples to Evrogen" section for details (page 140)

You will receive:

1. Any leftover starting materials (upon request)
2. First strand cDNA (with specific adapters on both ends)
3. Amplified double-stranded cDNA (at least 1 μg)
4. PCR primers for cDNA amplification
5. cDNA preparation report

REFERENCES

Matz (2002). Methods Mol Biol, 183: 3–18 / pmid: 12136765

Matz et al. (1999). Nucleic Acids Res, 27 (6): 1558–1560 / pmid: 10037822

Zhu et al. (2001). Biotechniques, 30 (4): 892–897 / pmid: 11314272

Standard cDNA library construction service

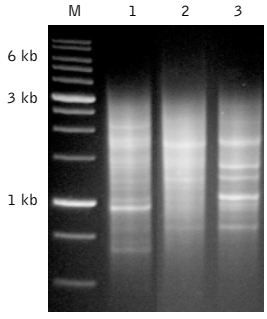
- *E. coli* libraries from high-quality full-length-enriched amplified cDNA
- From limited amount of total or poly(A)+ RNA

Full-length-enriched cDNA is usually produced using SMART technology [Zhu et al. 2001; Matz 2002]. The method combines cDNA synthesis and amplification and results in a representative cDNA population enriched with full-length sequences, even from small amounts of starting materials. After cDNA synthesis, the double stranded cDNA is size fractionated, directionally cloned into a plasmid vector from our collection using SfiIA - SfiIB, SrfI - NotI, or AscI - NotI restriction sites, and transformed into *E. coli*.

Then, the library cDNA is amplified as follows: the resulting library is arrayed in five 25x25 cm plates (approximately 10^5 colonies per plate); clones are eluted by LB/Amp medium.

As a quality control measure, a percentage of recombinant clones and average insert size is determined by gel analysis of 33 clones picked at random.

Please inquire about other variants of cDNA preparation and special vector requirements.



ds cDNA synthetized on the basis of total RNA from different human tissues.
Agarose/EtBr gel-electrophoresis result: M - 1-kb DNA size markers (SibEnzyme); lane 1 - embryonic lung; lane 2 - testis; lane 3 - small intestine.

Service terms and conditions

Cat.#	Service	Turnaround time
CS040	Standard cDNA library construction	4-6 weeks

We offer DISCOUNTS for MULTIPLE ORDERS!

You provide:

1. At least 2.0 μg of total RNA or poly(A)+ RNA
   See "How to send samples to Evrogen" section for details (page 140)

You will receive:

1. Any leftover starting material (on request)
2. Amplified double-stranded cDNA (at least 1 μg)
3. PCR primers for cDNA amplification
4. Amplified and characterized cDNA library (the library will contain at least 750 000 independent clones)
5. Service report

Extra options:
Modification of the customer-supplied vector for cloning using retriction sites described above.

REFERENCES

Matz (2002). Methods Mol Biol, 183: 3–18 / pmid: 12136765

Zhu et al. (2001). Biotechniques, 30 (4): 892–897 / pmid: 11314272

cDNA normalization service

- Significant increase of the transcriptome sequencing efficacy
- Equalization of cDNA population prior to library cloning
- Normalized cDNA is suitable for 454 sequencing
- Order processing is set up and monitored by the inventors of the technology

A great range in representation of different mRNAs in cells and tissues makes it extremely difficult to analyze rare message in cDNA libraries. Therefore, for rare transcript searching and analysis, creation of equalized (normalized) cDNA is required.

Our proprietary duplex-specific nuclease (DSN)-based normalization is a highly efficient and well-proved approach to equalize transcript concentration in a cDNA population enriched with full-length sequences [Zhulidov et al. 2004; Zhulidov et al. 2005; Bogdanova et al. 2008].

Both total or poly(A)+ RNA is suitable for cDNA synthesis. Full-length-enriched cDNA is usually produced using SMART technology [Zhu et al. 2001; Matz 2002]. After cDNA synthesis, the double-stranded cDNA is normalized using DSN-normalization method.

Depending on your particular needs, normalizad cDNA can be used for 454 sequencing (preparation of normalized cDNA over 2,0 μg is available as an extra option) or construction of normalized full-length-enriched cDNA library. Please use level descriptions at page 121 to choose the service variant you desire.

High-throughput sequencing of the normalized cDNA can be ordered at Macrogen, Korea (http://www.macrogen.com).

Service terms and conditions

You provide:

1. At least 1.0 μg of total RNA or poly(A)+ RNA
   See "How to send samples to Evrogen" section for details (page 140)

We perform:

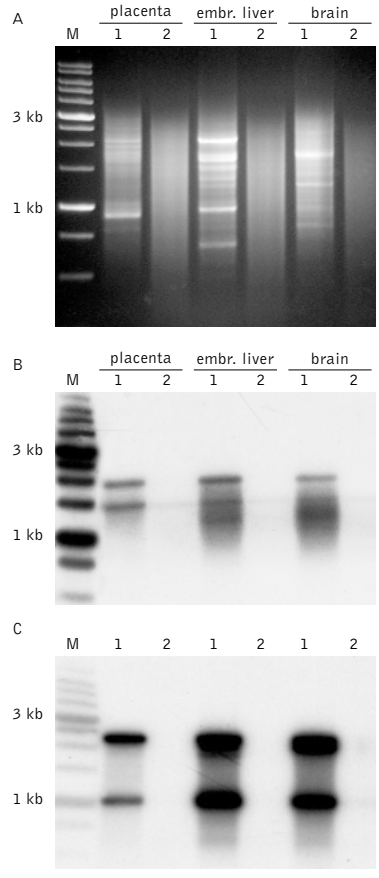
See description of available service levels on page 121

You will receive:

See list of output materials for each service level at page 121

Extra options:

1. Normalization efficiency analysis by PCR or Virtual Northern blot
2. Preparation of additional normalized cDNA up to 5-15 μg



cDNA normalization result.
(A) Agarose/EtBr gel-electrophoresis of non-normalized (1) and normalized (2) human cDNA samples;
(B, C) Concentration of abundant transcripts in these samples revealed by Virtual Northern blot. (B) - β-actin; (C) - ubiquitin C;
M - 1-kb DNA size markers (SibEnzyme); embr. - embryonic.

REFERENCES

Bogdanova et al. (2008). Mol Biosyst, 4 (3): 205–212 / pmid: 18437263

Matz (2002). Methods Mol Biol, 183: 3–18 / pmid: 12136765

Zhu et al. (2001). Biotechniques, 30 (4): 892–897 / pmid: 11314272

Zhulidov et al. (2004). Nucleic Acids Res, 32 (3): e37 / pmid: 14973331

Zhulidov et al. (2005). Bioorg Khim., 31 (2): 186–194 / pmid: 15889793

Level description for cDNA normalization service:

Procedures/Cat.#	Level 1a CS010	Level 1b CS011-1	Level 2a CS011-2a	Level 2b CS011-2b	Level 2c CS011-2c	Level 3a CS011-3a	Level 3b CS011-3b
1a. Synthesis of ds cDNA suitable for further non-directional cloning	+	–	–	–	–	–	–
1b. Synthesis of ds cDNA suitable for further directional cloning	–	+	+	+	+	+	+
2. DSN-normalization of cDNA	+	+	+	+	+	+	+
3. Ligation of normalized cDNA into an appropriate vector*	–	–	+	+	+	+	+
4. PCR quality control of 22 randomly picked clones	–	–	+	+	+	+	+
5a. Preparation of non-amplified normalized cDNA library containing at least 100 000 independent clones	–	–	–	+	–	+	–
5b. Preparation of amplified normalized cDNA library containing at least 400 000 independent clones**	–	–	–	–	+	–	+
6. Purification of plasmid DNA from the 90 clones	–	–	–	–	–	+	+
7. Single run sequencing analysis of these clones	–	–	–	–	–	+	+
Turnaround time, weeks	4-6	4-6	4-6	5-7	5-7	6-8	6-8
Output materials:							
1. Amplified non-normalized cDNA (at least 1 μg)	+	+	+	+	+	+	+
2. Normalized cDNA (at least 2 μg)	+	+	+	+	+	+	+
3. PCR primers for cDNA amplification	+	+	+	+	+	+	+
4. Service report	+	+	+	+	+	+	+
5. Normalized cDNA ligated into a vector	–	–	+	–	–	–	–
6. Non-amplified normalized cDNA library (at least 100 000 independent clones)	–	–	–	+	–	+	–
7. Amplified normalized cDNA library (at least 400 000 independent clones)	–	–	–	–	+	–	+
8. Purified plasmid DNA from the 90 clones and insert sequencing data of these 90 clones	–	–	–	–	–	+	+

We offer DISCOUNTS for MULTIPLE ORDERS!

NOTES:

*Library cloning is performed into a vector from our collection using SfiIA - SfiIB, SrfI - NotI, or AscI - NotI restriction sites. Cloning using other sites or into a customer-provided vector can be performed by agreement.

**To prepare an amplified cDNA library, the non-amplified library is arrayed in five 25x25 cm plates (approximately 10^5 colonies per plate); clones are eluted by LB/Amp medium; then the library is supplied with 17% glycerol and stored at -70°C. Preparation of the normalized amplified cDNA library containing more than 400 000 independent clones can be performed by agreement.

Confidentiality Statement:
All data and materials derived from these services are the physical and intellectual property of the purchaser and will be held strictly confidential by Evrogen.

cDNA depletion and library construction service

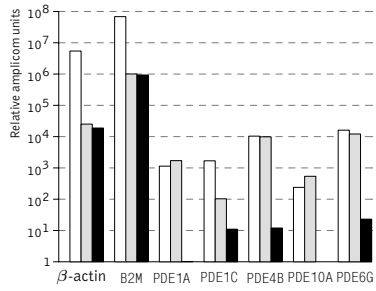
- Specific removal of already analyzed transcripts from cDNA populations
- Preparation of depleted cDNA libraries for functional screenings
- Order processing is set up and monitored by the inventors of the technology

Evrogen offers a highly efficient technology for specific removal of already analyzed transcripts from cDNA populations and preparation of depleted cDNA libraries. Analysis of the resulting depleted cDNA libraries significantly accelerates discovery of unknown genes by expression cloning.

Full-length-enriched cDNA is usually produced using SMART technology [Zhu et al. 2001; Matz 2002]. The method combines cDNA synthesis and amplification and results in representative cDNA population enriched with full-length sequences even from small amounts of starting materials.

After cDNA synthesis, the double stranded cDNA is depleted using a duplex-specific nuclease (DSN)-based method [Bogdanova et al. 2009]. The method allows specific removal of selected transcripts without loss of average cDNA size.

Depletion is accompanied by partial cDNA normalization. The depleted cDNA is size fractionated, directionally cloned into a plasmid vector from our collection (or into an appropriate customer-supplied plasmid vector), and used for preparation of depleted cDNA library.



Service terms and conditions

Cat.#	Option	Turnaround time
CS012-1	Level 1	5-7 weeks
CS012-2	Level 2	6-8 weeks

We offer DISCOUNTS for MULTIPLE ORDERS!

You provide:

1. At least 1.0 μg of of total RNA or poly(A)+ RNA
2. List of the genes to be removed; up to 40 genes (sequences or GenBank IDs are required)

See "How to send samples to Evrogen" section for details (page 140)

We perform:

Level 1

1. Ds cDNA preparation using SMART approach
2. cDNA depletion
3. PCR confirmation of the depletion

Level 2 (also includes all services provided in level 1)

4. Ligation of the depleted cDNA into an appropriate vector (cDNA is directionally cloned using SfiIA - SfiIB, SrfI - NotI, or AscI - NotI restriction sites)

REFERENCES

Bogdanova et al. (2009). Mol Biotechnol, 41 (3): 247–253 / pmid: 19127453

Matz (2002). Methods Mol Biol, 183: 3–18 / pmid: 12136765

Zhu et al. (2001). Biotechniques, 30 (4): 892–897 / pmid: 11314272

5. Transformation of the ligate into *E. coli* and preparation of amplified depleted cDNA library. For library amplification, it is arrayed in five 25x25 cm plates (approximately 10^5 colonies per plate); clones are eluted by LB/Amp medium; and the library is supplied with 17% glycerol and stored at -70°C

You will receive:

1. Any leftover starting material (on request)
2. Amplified double-stranded cDNA (at least 1 μg)
3. Depleted cDNA (at least 2 μg)
4. PCR primers for cDNA amplification
5. Amplified and characterized *E.coli* library (only for Level 2)
6. Service report

Confidentiality Statement:
All data and materials derived from these services are the physical and intellectual property of the purchaser and will be held strictly confidential by Evrogen.

cDNA subtraction and library construction service

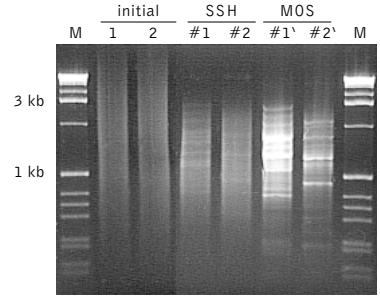
- Isolation of differentially expressed genes
- Special approach to eliminate false-positive clones
- Order processing is set up and monitored by the inventors of the SSH technology

cDNA populations are compared using well-known Supression Subtractive Hybridization (SSH) and Mirror Orientation Selection (MOS) technologies [Lukyanov et al. 1994; Diatchenko et al. 1996; Gurskaya et al. 1996; Diatchenko et al. 1999; Rebrikov et al. 2000].
SSH has been used in hundreds of model systems and is now one of the most popular molecular genetic technologies in the world that appropriate for comparison of RNA populations. MOS substantially improves the results of SSH (especially for cDNA samples of high complexity) by significant decrease in the number of false-positive clones and increase in the number of differential clones in SSH-generated libraries.

MOS is specially recommended:

- for complicated samples (e.g. brain RNA, eukaryotic genomic DNA);
- for closely related samples;
- if primary SSH PCR requires more than 30 cycles to generate visible PCR products;
- if the percentage of differentially distributed clones in your SSH-subtracted library is very low (i.e. 1-5%);
- if most of the differentially distributed clones found in your SSH-subtracted library are false positives.

Depending on your particular needs, we offer five levels of custom service. The levels differ in the intensity of work performed and may be finely tuned to exactly suit your research project. All procedures are set up and monitored by the inventors of the SSH and MOS tecnologies.



Typical results of cDNA subtraction and MOS procedure.
Lanes 1, 2 - cDNA samples 1 and 2; #1 - (sample 1 vs sample 2) subtraction; #2 - (sample 2 vs sample 1) subtraction; #1` - #1 subtraction after MOS; #2` - #2 subtraction after MOS. MOS reveals real differential bands and eliminates most of false positives. M - 1-kb DNA size markers.

Service terms and conditions

Cat.#	Level	Turnaround time
CS021-1	Level 1	3-5 weeks
CS021-2	Level 2	4-6 weeks
CS021-3	Level 3	5-7 weeks
CS021-4	Level 4	5-11 weeks
CS021-5	Level 5	5-11 weeks

We offer DISCOUNTS for MULTIPLE ORDERS!

You provide:

1. At least 1.0 μg of of total RNA or poly(A)+ RNA
See "How to send samples to Evrogen" section for details (page 140)

We perform:

Level 1

1. cDNA preparation from customer-supplied RNA
2. Subtraction in both directions (forward = A-B; reverse = B-A)

2a. MOS procedure on the subtracted samples (optional, on request)

Level 2 (also includes all services provided in level 1)

3. Ligation of subtracted cDNA into an appropriate vector

Level 3, 4 and 5 (also includes all services provided in level 1 and 2)

4. Transformation of ligates from step 3 into *E. coli*
5. Plating of the two subtracted libraries (in both directions) in 96-well plates:
   - Level 3: ONE plate of clones per library for both directions
   - Level 4: FIVE plates of clones for one direction library and ONE plate of clones for the other direction library
   - Level 5: FIVE plates of clones per library for both directions
6. Differential screening of 96-well plates with two probes to determine the percentage of differentially expressed clones in each subtracted library (probes: forward subtracted cDNA, reverse subtracted cDNA)
7. Purification of plasmid DNA from the differential clones (up to 100 clones from each direction)
8. Virtual Northern hybridization of five clones from each library to confirm differential expression
9. Sequence of differential clones found (up to 10 clones)

You will receive:

Level 1

1. Any leftover starting material (on request)
2. Amplified non-subtracted driver and tracer cDNA
3. Subtracted cDNA samples and all other cDNA samples generated during SSH
4. Primers for amplification of subtracted cDNA and SSH products
5. SSH report

Additionally for level 2

6. Transformation-ready subtracted cDNA ligated into an appropriate vector

Additionally for levels 3-5

7. Subtracted cDNA libraries
8. Description of the libraries obtained (number of clones and percentage of clones with insertions)
9. Differential screening data
10. Plates with clones used for differential screening
11. Purified plasmid DNA from the differential clones
12. Virtual Northern hybridization data

REFERENCES

Diatchenko et al. (1996). Proc Natl Acad Sci U S A, 93 (12): 6025–6030 / pmid: 8650213

Diatchenko et al. (1999). Methods Enzymol, 303: 349–380 / pmid: 10349654

Gurskaya et al. (1996). Anal Biochem, 240 (1): 90–97 / pmid: 8811883

Lukyanov et al. (1994). Bioorg Khim, 20 (6): 701–704 / pmid: 7945464

Rebrikov et al. (2000). Nucleic Acids Res, 28 (20): e90 / pmid: 11024192

Bacterial genome subtraction service

- Identification of differentially distributed sequences in bacterial strains
- Order processing is set up and monitored by the inventors of the SSH technology

Supression subtractive hybridization (SSH) is the most powerful non-sequencing approach to find genetic difference between bacterial strains [Akopyants et al. 1998]. The ability of SSH to identify nearly all major sequence differences between two closely related bacteria has been demonstrated by several independent research groups [Agron et al. 2002; Rebrikov et al. 2002; Rebrikov et al. 2003; Winstanley 2002].
Depending on your particular needs, we offer five levels of custom service. The levels differ in the intensity of work performed and may be finely tuned to exactly suit your research needs. All procedures are set up and monitored by the inventors of the SSH technique.

Service terms and conditions

Cat.#	Level	Turnaround time
CS022-1	Level 1	3-5 weeks
CS022-2	Level 2	4-6 weeks
CS022-3	Level 3	5-7 weeks
CS022-4	Level 4	5-11 weeks
CS022-5	Level 5	5-11 weeks

We offer DISCOUNTS for MULTIPLE ORDERS!

You provide:

1. Bacterial genomic DNA isolated from the two bacterial strains that you wish to compare
See "How to send samples to Evrogen" section for details (page 140)

We perform:

Level 1

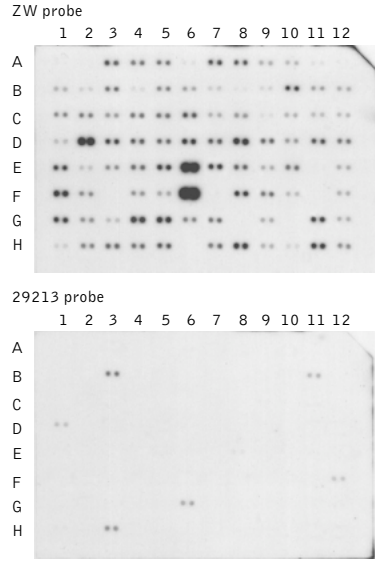
1. Subtraction in both directions (forward = A-B; reverse = B-A)

Level 2 (also includes all services provided in level 1)

2. Ligation of subtracted DNA into an appropriate vector

Level 3, 4 and 5 (also includes services provided in level 1 and 2)

3. Transformation of ligates from step 2 into *E. coli*
4. Plating of the two subtracted libraries (in both directions) in 96-well plates:
   - Level 3: ONE plate of clones per library for both directions
   - Level 4: FIVE plates of clones for one direction library and ONE plate of clones for the other direction library
   - Level 5: FIVE plates of clones per library for both directions



Typical result of differential screening for bacterial SSH-generated libraries.
DNA from the *Staphylococcus aureus* ZW strain was used as a tester and DNA from the *S. aureus* 29213 strain was used as a driver in SSH. Differential screening of ZW-specific SSH generated library with unsubtracted ZW and 29213 strain-specific probes (A and B, respectively) showed that this library contains about 95% differential clones.

REFERENCES

Agron et al. (2002). FEMS Microbiol Lett, 211 (2): 175–182 / pmid: 12076809

Akopyants et al. (1998). Proc Natl Acad Sci U S A, 95 (22): 13108–13113 / pmid: 9789049

Rebrikov et al. (2002). Ann Periodontol, 7 (1): 17–28 / pmid: 16013213

Rebrikov et al. (2003). "SSH Subtractive Cloning: Comprehensive Survey Of Genome Differences In Prokaryotes." In: *Applications of Genomics and Proteomics for Analysis of Bacterial Biological Warfare Agents.* Ed. by DelVecchio, V.G. and Krcmery, V. IOS Press, Amsterdam , Netherlands. 352:95–105.

Winstanley (2002). J Med Microbiol, 51 (6): 459–467 / pmid: 12018652

5. Differential screening of 96-well plates with two probes to determine the percentage of differentially distributed clones in each subtracted library (probes: forward subtracted DNA, reverse subtracted DNA)
6. Purification of plasmid DNA from the differential clones (up to 100 clones from each direction)
7. Virtual Northern hybridization of five clones from each library to confirm differential distribution
8. Sequence of differential clones found (up to 10 clones)

You will receive:

Level 1

1. Any leftover starting material (on request)
2. PCR-amplified subtracted DNA samples and all other DNA samples generated by SSH
3. Primers for amplification of subtracted DNA and SSH products
4. SSH report

Additionally for level 2

5. Transformation-ready subtracted DNA ligated into an appropriate vector

Additionally for levels 3-5

6. Subtracted DNA libraries
7. Description of the libraries obtained (number of clones and the percentage of clones with insertions)
8. Differential screening data
9. 96-well plates with clones used for differential screening
10. Purified plasmid DNA from the differential clones
11. Southern hybridization data

Confidentiality Statement:
All data and materials derived from these services are the physical and intellectual property of the purchaser and will be held strictly confidential by Evrogen.

Eukaryotic genome subtraction service

- Identification of differentially distributed sequences in eukaryotic genomes

Combination of Suppression Subtractive Hybridization (SSH) with Mirror Orientation Selection (MOS) allows comparison of similar eukaryotic genomes to identify genetic difference between them [Rebrikov et al. 2002].
Terms, turnaround time, and prices for eukaryotic genome subtraction service are by agreement. Please contact us via email service@evrogen.com to discuss your particular project.

Service terms and conditions

Cat.#	Option	Turnaround time
CS023	Eukaryotic genome subtraction	agreed

We offer DISCOUNTS for MULTIPLE ORDERS!

REFERENCES

Rebrikov et al. (2002). BMC Genomics, 3 (1): 15 / pmid: 12065025

PCR cloning service

- Fast cloning of DNA fragments for various applications

Evrogen offers large-scale PCR and RT-PCR cloning of known genes for array preparation and other common applications. The service includes PCR amplification of target sequences from your DNA/RNA source, cloning of PCR product(s) into the vector of your choice, and purification of the plasmid DNA with target inserts. The resulting cloned products are then confirmed by direct sequencing.

Service terms and conditions

Cat.#	Option	Turnaround time
CS031-1	Level 1	agreed
CS031-2	Level 2	agreed

We offer DISCOUNTS for MULTIPLE ORDERS!

You provide:

1. Starting materials (cDNA, RNA, genomic DNA, or plasmid DNA)
2. Vector and vector map
3. Sequence information

See "How to send samples to Evrogen" section for details (page 140)

We perform:

1. Synthesis of primers designed for PCR amplification
2. cDNA preparation (if required)
3. Amplification of the target fragment
4. Cloning of fragment into the standard pUC-based vector from our collection
5. Clone confirmation:
   - Level 1: clone confirmation by sequencing in one direction (we will select the clone that contains the target insert, but will not verify the perfect coincidence between the sequence of the insert and the customer provided sequence information)
   - Level 2: sequence verification of the perfect coincidence between the sequence of the insert and the customer provided sequence information
6. Plasmid purification of a single correct clone (from 5-10 ml culture volume)

You will receive:

1. Any leftover starting material (on request)
2. PCR primers specific to the target fragment
3. Purified plasmid DNA comprising the target fragment
4. Sequencing data of the clone selected
5. Service report

Extra options:

1. Cloning of the fragment into the bacterial vector of your choice
2. Large- or mid-scale plasmid preparation

Confidentiality Statement:

All data and materials derived from these services are the physical and intellectual property of the purchaser and will be held strictly confidential by Evrogen.

Subcloning service

- Generation of various expression constructs from your initial plasmids
- Modification of a customer-supplied constructs
- Generation of constructs for use in chimeric/fusion protein production
- Flexible service network

Evrogen offers the service to produce various expression constructs from your initial plasmid, modify an existing construct, and/or generate constructs for use in chimeric/fusion protein production. All of these procedures can be adjusted to meet your specific research needs. All products are confirmed by direct sequencing.

Service terms and conditions

Cat.#	Option	Turnaround time
CS032	Subcloning	4-6 weeks

We offer DISCOUNTS for MULTIPLE ORDERS!

You provide:

1. Insert DNA or sequence information
2. Vector and vector map

See "How to send samples to Evrogen" section for details (page 140)

We perform:

1. Isolation of the target DNA fragment: depending on your needs, we can perform (1) restriction digests to excise the target DNA fragment and isolation of the obtained fragment from low-melt agarose; (2) generation of inserts by PCR; or (3) custom DNA synthesis
2. Modification of the target DNA fragment: construction of fusions, introduction of mutations desired, etc.
3. Ligation of the fragment into an appropriate vector
4. Transformation of the ligate from step 3 into *E. coli*
5. Selection of a correct clone and clone confirmation using restriction analysis or direct sequencing
6. Plasmid purification of one correct clone from 5-10 ml culture volume

You will receive:

1. Any leftover starting material (on request)
2. Purified target insert in the selected vector
3. Service report

Extra options:

1. Mid- or large-scale plasmid preparation

Confidentiality Statement:

All data and materials derived from these services are the physical and intellectual property of the purchaser and will be held strictly confidential by Evrogen.

RACE and full-length cDNA isolation service

- Isolation of full-length cDNAs when only partial nucleotide or amino acid sequence is known

Evrogen offers isolation of full length cDNA(s) correspondent to a known fragment using Step-Out-RACE technology [Matz et al. 1999; Matz et al. 2003].
This method allows fast isolation of the 5‘- and 3‘-ends of the target transcript with no background noise. The method can be successfully applied to total RNA as well as poly(A)+ RNA and is applicable even if only a short nucleotide (30-50 bp) or protein sequence (15-30 aa) is available.

Service terms and conditions

Cat.#	Option	Turnaround time
CS033	RACE and full-length cDNA isolation	agreed

We offer DISCOUNTS for MULTIPLE ORDERS!

You provide:

1. At least 1.0 μg of of total RNA or poly(A)+ RNA
2. Sequence information

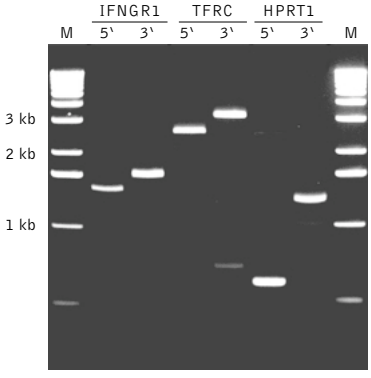
See "How to send samples to Evrogen" (page 140)

We perform:

1. cDNA preparation
2. Construction of oligonucleotide primers on the customer-supplied sequence of interest
3. Amplification of the target cDNA ends (5′- and 3′-RACE)
4. Cloning of the target cDNA ends into an appropriate vector
5. Partial sequencing of the cloned cDNA fragments
6. Construction of oligonucleotide primers to amplify the target full-length cDNA and amplification of the target full-length cDNA
7. Cloning of the target full-length cDNA into an appropriate vector and screening of the clones to select those containing target full-length cDNA inserts
8. Purification of vector DNA with the target full-length cDNA insert

You will receive:

1. Any leftover starting material (on request)
2. First strand cDNA (on request)
3. Amplified double-stranded cDNA (at least 1 μg)
4. PCR primers for cDNA amplification
5. PCR products generated by RACE (0.5 μg each, upon request)
6. Purified vector containing the target cDNA insert
7. All primers designed and synthesized during RACE
8. Service report



5‘- and 3‘- Step-Out RACE results.
Amplified cDNA was prepared from human placenta total RNA and used for 5‘- and 3‘- RACE of several marker genes: IFNGR1 - interferon gamma receptor 1; TFRC - transferrin receptor; HPRT1 - hypoxanthine phosphoribosyltransferase-1; M - 1-kb DNA size markers.

Extra options:

1. Complete sequencing of the target DNA

REFERENCES

Matz et al. (1999). Nucleic Acids Res, 27 (6): 1558–1560 / pmid: 10037822

Matz et al. (2003). Methods Mol Biol, 221: 41–49 / pmid: 12703732

Confidentiality Statement:
All data and materials derived from these services are the physical and intellectual property of the purchaser and will be held strictly confidential by Evrogen.

Genome walking service

- Cloning of genomic sequences flanking known DNA fragment

Evrogen provides rapid cloning of promoters and other upstream regulatory elements of target genes using suppression PCR-based genome walking method.

Service terms and conditions

Cat.#	Option	Turnaround time
CS035	Genome walking	agreed

We offer DISCOUNTS for MULTIPLE ORDERS!

You provide:

1. Minimum 50-bp sequence of the gene of interest
2. Minimum 3.0 μg of of genomic DNA

See "How to send samples to Evrogen" section for details (page 140)

We perform:

1. Construction of oligonucleotide primers based on the supplied sequence
2. Amplification of target gene regions
3. Cloning of target DNA regions into an appropriate vector
4. Partial sequencing of cloned DNA fragments

You will receive:

1. Any leftover starting material (on request)
2. Genome walking-generated PCR products (at least 0.5 μg each, on request)
3. Purified vector DNA with the target insert
4. All primers designed and synthesized during order processing
5. Service report

Extra options:

1. Complete sequencing of the target DNA

Confidentiality Statement:
All data and materials derived from these services are the physical and intellectual property of the purchaser and will be held strictly confidential by Evrogen.

Gene synthesis service

- Gene synthesis
- Codon usage optimization

Evrogen offers synthesis of DNA fragments 300-3 000 bp long by assembly of short oligonucleotides followed by cloning of the synthetic gene into an appropriate vector and confirmation of clone integrity by direct sequencing. In particular, Evrogen offers codon usage optimization for gene expression in various heterological systems.

Service terms and conditions

Cat.#	Fragment length	Turnaround time
CS042-1	up to 2 kb	4-7 weeks
CS042-2	more than 2 kb	6-12 weeks

We offer DISCOUNTS for MULTIPLE ORDERS!

You provide:

1. Sequence information
2. Vector and vector map (if required)

See "How to send samples to Evrogen" section for details (page 140)

We perform:

1. Oligonucleotide synthesis and assembly of the gene
2. Cloning of the target gene into an appropriate vector from our collection (cloning of the target gene into a vector of your choice is also available under the agreement)
3. Verification of the sequence by direct sequencing
4. Small-scale plasmid purification on one correct clone (5-10 ml culture volume)

You will receive:

1. Purified plasmid DNA containing the target sequence
2. Service report

Confidentiality Statement:
All data and materials derived from these services are the physical and intellectual property of the purchaser and will be held strictly confidential by Evrogen.

Site-directed mutagenesis service

Any type of mutations, such as deletion, insertion, or substitution, can be introduced into the gene you are working with. All procedures are conducted to the highest standards and confirmed by direct sequencing.

Service terms and conditions

Cat.#	Option	Turnaround time
CS041-1	1-3 mutations	agreed
CS041-2	more than 3 mutations	agreed

We offer DISCOUNTS for MULTIPLE ORDERS!

You provide:

1. Plasmid DNA with the target gene inserted
2. Plasmid map
3. Sequence of the target gene with a list of the bases to be changed

See "How to send samples to Evrogen" section for details (page 140)

We perform:

1. Gene mutagenesis
2. Sequence verification by sequencing in both directions
3. Plasmid purification of one verified clone from 5-10 ml culture

You will receive:

1. Purified plasmid DNA containing the target sequence with verified mutations
2. Service report

Extra options:

1. Cloning of the target gene to be mutated
2. Large-scale plasmid preparation

Confidentiality Statement:
All data and materials derived from these services are the physical and intellectual property of the purchaser and will be held strictly confidential by Evrogen.

Custom optimization of Evrogen expression vectors

Cat.#	Option	Turnaround time
FPS00	Custom optimization of Evrogen expression vectors	agreed

We offer DISCOUNTS for MULTIPLE ORDERS!

Optimization of Evrogen vectors expressing fluorescent proteins can be performed in accordance with your particular needs.
Terms, turnaround time, and prices for the service are by agreement.
Please contact us via email service@evrogen.com to discuss your particular project.

We offer:

1. Generation of fusions of proteins, domains or localization signals of your interest to fluorescent proteins using mammalian vectors from our collection
2. Generation of constructs for gene expression analysis using promoterless vectors encoding fluorescent proteins from our collection
3. Codon usage optimization of fluorescent proteins from our collection for expression in heterological system of your interest
4. Cloning of fluorescent protein coding sequences into specific vectors of your interest

Notice to Purchaser:
Custom-modified Evrogen vectors comprising fluorescent proteins (FP) shall be used by purchaser for research purposes only in accordance with Limited Use Label License.
Evrogen retains non-exclusive right to use the Custom-modified Evrogen FP vectors for research and commercial purposes.
Customer information of confidential nature which may be necessary for the service execution will be treated confidential and not used for other purposes by Evrogen without Customer approval.

Custom assay development

Cat.#	Option	Turnaround time
AD001	Custom assay development	agreed

We offer DISCOUNTS for MULTIPLE ORDERS!

Fluorescent proteins (FPs) act as endogenously expressed biolabels for fluorescence microscopy that makes them particularly suitable for use in various HTS/HCS-type, live-cell, drug-discovery screening assays. Red and far-red FPs, which are easily distinguished from cellular autofluorescence and from the typical fluorescence wavelength spectrum of many medicinal chemistry compounds, provide technologically useful possibilities for the design of sophisticated, novel assay formats for drug discovery.
Selection and evaluation of an appropriate FP marker along with optimization of assay parameters is a laborious and time consuming procedure.

We offer the following custom research services to assist with FP selection/assay development:

1. Making an agreed number of different constructs of interest (FP–linker–target protein)
2. Testing the constructs in an number of model cell lines to find the best ones
3. Testing the selected constructs in various cell lines to select the most appropriate cells in terms of fusion localization/redistribution
4. Making stably transfected cell lines based on the testing results above

For customers, who wish to multiplex HCS readout to achieve real multiparameter imaging, we offer the unique option to fuse protein of interest with bright monomeric red (TagRFP) and far-red (mKate2) fluorescent proteins that could be used in combination with dyes/FPs visible in UV, blue and green part of spectrum. Both proteins are perfectly fit for fusions.

Confidentiality Statement:
All data and materials derived from these services are the physical and intellectual property of the purchaser and will be held strictly confidential by Evrogen.

Support Information

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- Appendix C
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How to order

Ordering Evrogen products

Please contact your local distributor to get Evrogen products. A complete list of distributors is available on our Web site (www.evrogen.com/distributor.shtml).
For countries not listed in the distributor list, you can order Evrogen products via Axxora Platform Web site (www.axxora.com). For any questions related to ordering, please contact order@evrogen.com

Ordering Evrogen services

Please contact us by e-mail at service@evrogen.com to specify the service you desire; service level (if any); order size, brief description of samples including nucleic acids you plan to provide, nucleic acid amount(s) and source(s); other materials you plan to provide; output materials you desire; and special instructions.
Also, you can fill out a Service Pricing Request Form on-line (please see www.evrogen.com/p-order.shtml page to select an appropriate form). Service manager of Evrogen will contact you within a few days. After clearing up all details of the service you desire, we will send you a Price Quote via e-mail in accordance with your order details.
Order will be initiated upon receiving the corresponding Service Order Form and Purchase Order by fax +7(495) 988 4085. For any questions related to ordering, please contact service@evrogen.com

Special offers

Feedback-based discount program

Evrogen is a rapidly developing innovative company. Our product line is constantly widening, and we aim to provide the best quality services and products to the market. Only scientists can assess the applicability of different products for their applications and can provide feedback, which we value. Evrogen introduces a discount on products granted for your expertise:

1. Send us your article, quoting the use of Evrogen service, and get a 10% discount for next service order.
2. Send us your article, quoting the use of Evrogen products or provide us with data showing successful use of Evrogen products*, and get a 20% discount for next purchase.
3. Send us your data quoting unsuccessful use of Evrogen products*. If problems occurred because of intrinsic imperfections rather than incorrect use of the product, we will do our best to develop an alternative product devoid of these drawbacks and will provide it for you free of charge.

* The data should contain detailed description of experiment and sample photos. The data will not be shared with third party without prior permission of the customer.

Customer Support

Please send your questions and comments related to Evrogen products to customer-support@evrogen.com
We will make all efforts to provide you prompt reply.

How to send samples to Evrogen

Nucleic acid sample preparation

We accept RNA or genomic DNA isolated by all common methods.
Important note: We are not permitted to accept any starting material, which is infectious for humans, animals, or plants. In case starting material for the service of your interest is not infectious but is derived from or related to a pathogen, please contact us via e-mail service@evrogen.com to obtain additional declaration form requested in such cases by local legislation.

Nucleic acid sample transportation

Please ship your nucleic acid samples in ethanol at room temperature via express mail. We strongly recommend the following procedure for DNA or RNA sample preparation:
1. Prepare DNA or RNA water solution with total volume of not less than 30 μl.
2. Add 0.1 volume of 3 M sodium acetate and 3 volumes of 96% ethanol and mix.
Following this, your samples will be ready to ship at room temperature. Please ensure that the total volume of your sample solution is not less than 120 μl.
Please do not send the samples on dry ice or blue ice, as such packages require special customs handling and otherwise will not be delivered!

Send your samples to:

Please send the materials via express mail (TNT, UPS, DHL, the like EXCEPT FedEx*) to:
EVROGEN
Miklukho-Maklaya 16/10
Moscow, 117997, Russia
Phone: +7(495) 988 4084
Fax: + 7(495) 988 4085
Attn: Dmitry Shagin
For any questions please contact service@evrogen.com

Important for Safe and Fast Sample Delivery

Please prepare the following two documents:
1. Proforma-invoice (www.evrogen/support/customs_form.doc). Please complete all items marked YELLOW in the form, and enclose one copy of the printed form inside the package and the other copy to accompanying documents.
2. Description (www.evrogen/support/description.doc). Please print the document on a letterhead of your organization, put any signature and a stamp (if available), and enclose one printed copy of the document INTO the package (please email us a scanned copy of this document prior to sending samples).
Description of samples in the shipping documents may vary depending on country requirements. It is essential to mention "scientific research samples" and "have no commercial value". Please do not mention "medical", "biological" or "ethanol" anywhere in the shipping documents.
Please DO NOT enclose any other documents into the package except for the two above-mentioned forms!
* Please DO NOT send the materials via FedEx due to limitations on delivery of any chemical substances via this carrier to Russian Federation.

Appendix A

Non-exclusive license agreement for Evrogen Fluorescent Protein products

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Appendix B

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The purchase of this product or obtaining the product from Evrogen for evaluation conveys the non-transferable right to the recipient to use the obtained amount of the product and its components ("Product") for Research as long as it takes the recipient to use the Product.

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Appendix C

Limited Use Label License No. 19: Gateway® Cloning Products (Invitrogen)

This product and its use is the subject of one or more of U.S. Patent Nos. 5,888,732, 6,143,557, 6,171,861, 6,270,969, 6,277,608, and 6,720,140 and/or other pending U.S. and foreign patent applications owned by Invitrogen Corporation. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for profit entity). The purchase of this product does not convey a license under any method claims in the foregoing patents or patent applications, or to use this product with any recombination sites other than those purchased from Invitrogen Corporation or its authorized distributor. The right to use methods claimed in the foregoing patents or patent applications with this product for research purposes can only be acquired by the use of Clonase™ purchased from Invitrogen Corporation or its authorized distributors. The buyer cannot modify the recombination sequence(s) contained in this product for any purpose. The buyer cannot sell or otherwise transfer (a) this product, (b) its components, or (c) materials made by the employment of this product or its components to a third party or otherwise use this product or its components or materials made by the employment of this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the employment of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Notwithstanding the preceding, any buyer who is employed in an academic or government institution may transfer materials made with this product to a third party who has a license from Invitrogen under the patents identified above to distribute such materials. Transfer of such materials and/or information to collaborators does not convey rights to practice any methods claimed in the foregoing patents or patent applications. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of the above patents based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that none of (i) this product, (ii) any of its components, or (iii) a method claim of the foregoing patents, was used in the manufacture of such product. Invitrogen Corporation will not assert a claim against the buyer of infringement of the above patents based upon the use of this product to manufacture a protein for sale, provided that no method claim in the above patents was used in the manufacture of such protein. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to use this product for purposes other than those permitted above, contact Licensing Department, Invitrogen Corporation, 1600 Faraday Avenue, Carlsbad, California 92008. Phone (760) 603-7200.

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