evrogen

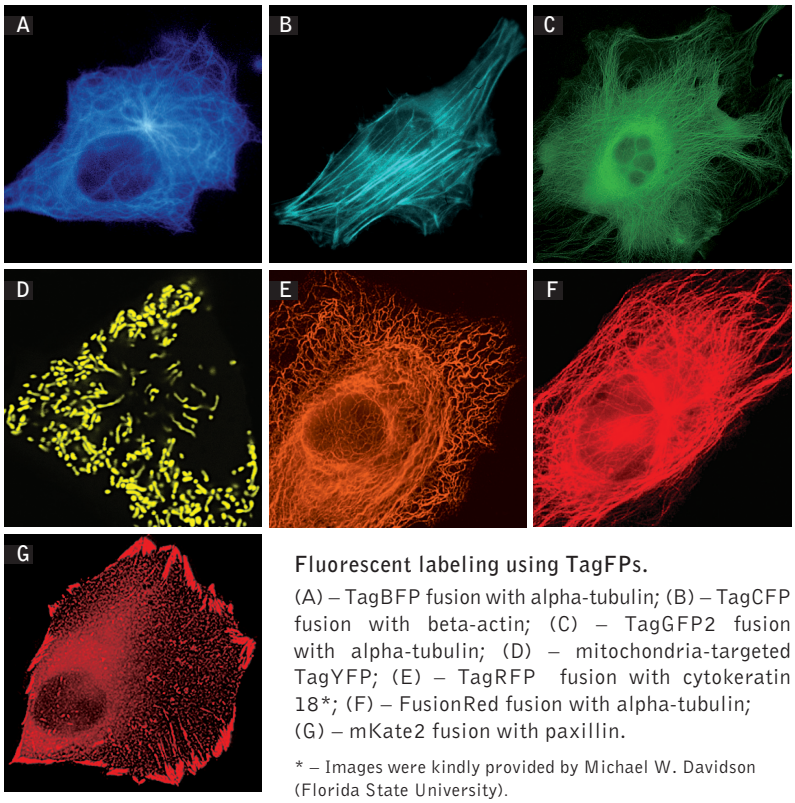
Fluorescent tags for *in vivo* protein labeling

Evrogen TagFPs are monomeric fluorescent proteins specially optimized for protein localization/interaction studies. Successful performance of TagFPs in protein labeling applications was validated in various models including highly oligomerizing cellular proteins like beta-actin and alpha-tubulin.

Protein	TagBFP	TagCFP	TagGFP2	TagYFP	TagRFP	FusionRed	mKate2
Fluorescence color	blue	cyan	green	yellow	red (orange)	red	far-red
Excitation max	402 nm	458 nm	483 nm	508 nm	555 nm	580 nm	588 nm
Emission max	457 nm	480 nm	506 nm	524 nm	584 nm	608 nm	633 nm
Quantum yield	0.63	0.57	0.60	0.62	0.48	0.19	0.40
Extinction coeff. ($M^{-1}cm^{-1}$)	52 000	37 000	56 500	50 000	100 000	94 500	62 500
Brightness*	32.8	21.1	33.9	31.0	48.0	18.0	25.0
Brightness, % of EGFP	99	64	105	94	148	53	74
pKa	2.7	4.7	5.0	5.5	3.8	4.6	5.4

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

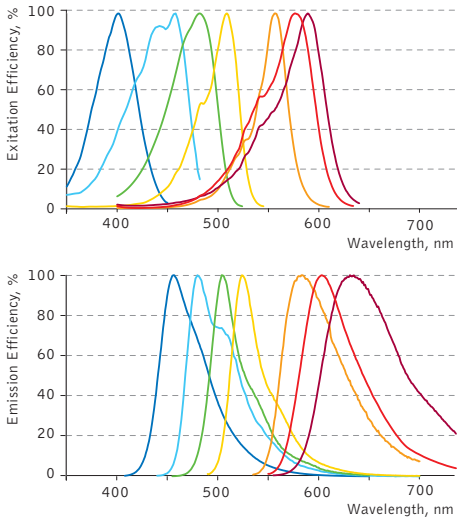
Expression of TagFP-tagged fusions in mammalian cells



Fluorescent labeling using TagFPs.

(A) – TagBFP fusion with alpha-tubulin; (B) – TagCFP fusion with beta-actin; (C) – TagGFP2 fusion with alpha-tubulin; (D) – mitochondria-targeted TagYFP; (E) – TagRFP fusion with cytokeratin 18*; (F) – FusionRed fusion with alpha-tubulin; (G) – mKate2 fusion with paxillin.

* – Images were kindly provided by Michael W. Davidson (Florida State University).



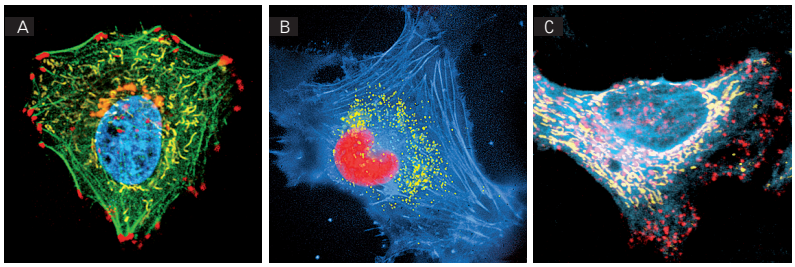
TagFPs normalized excitation/emission spectra.

TagBFP
TagCFP
TagGFP2
TagYFP
TagRFP
FusionRed
mKate2

P.T.O. ⟶

Ideal tool for multicolor labeling and FRET applications

Ranging in color from blue to far-red, Evrogen fluorescent proteins can be used for multicolor labeling and fluorescence resonance energy transfer (FRET) applications for visualization of protein translocation against other subcellular structures, investigation of protein-protein co-localization, detection of the onset of gene expression from distinct promoters, and separation of mixed cell populations.



Multicolor labeling of mammalian cells.
(A) – TagBFP-H2B fusion (blue), TagGFP-actin fusion (green), mitochondria-targeted PhiYFP (yellow); Golgi-targeted TagRFP (orange), mKate2-zyxin fusion (red);
(B) – TagBFP-actin fusion (blue), peroxisomes-targeted PhiYFP (yellow), TagRFP-H2B fusion (red);
(C) – TagCFP-tubulin fusion (cyan), mitochondria-targeted TagYFP (yellow), TagFP635*-clathrin fusion (red).

* TagFP635 is a parental variant of mKate2

TagFPs licensing opportunities

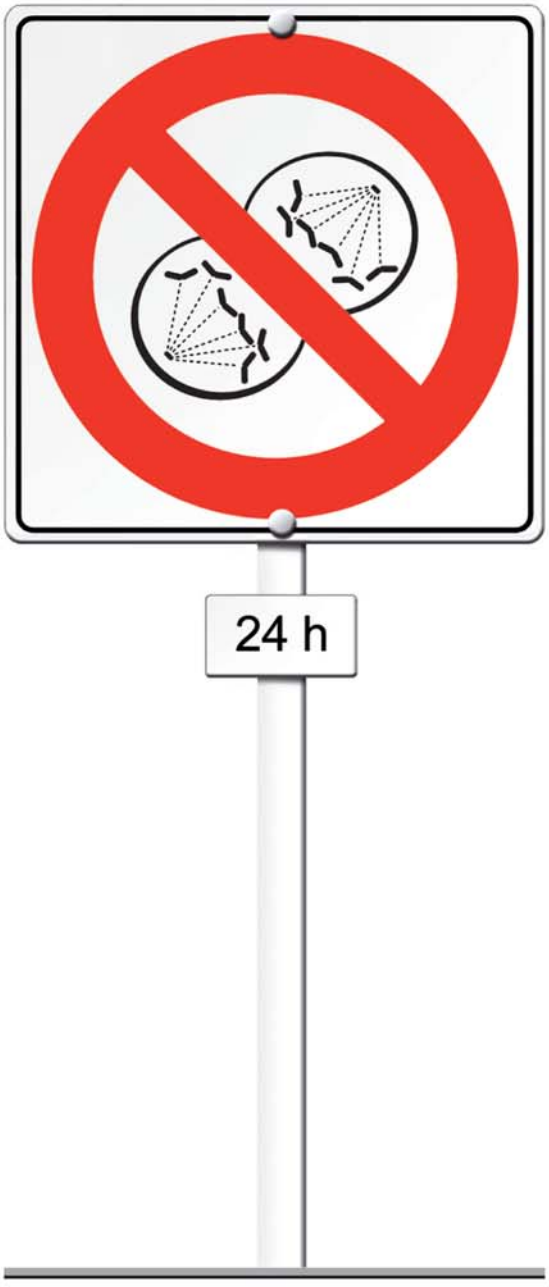
Evrogen technology embodied in TagFPs is available for expanded and commercial use with an adaptable licensing program. Benefits from flexible and market-driven license options are offered for upgrade and novel development of products and applications.
For licensing information, please contact Evrogen at license@evrogen.com

For more information, please visit our web-site:

www.evrogen.com

Protein localization tags: available vectors

Vector	Cat.#
Vectors for TagFPs expression and fusion construction	
pTagBFP-C	FP171
pTagCFP-C	FP111
pTagGFP2-C	FP191
pTagYFP-C	FP131
pTagRFP-C	FP141
pFusionRed-C	FP411
pmKate2-C	FP181
pTagBFP-N	FP172
pTagCFP-N	FP112
pTagGFP2-N	FP192
pTagYFP-N	FP132
pTagRFP-N	FP142
pFusionRed-N	FP412
pmKate2-N	FP182
Ready-to-use subcellular localization vectors	
pTagBFP-actin	FP174
pTagCFP-actin	FP114
pTagGFP2-actin	FP194
pTagRFP-actin	FP144
pmKate2-actin	FP184
pTagRFP-actinin	FP360
pmKate2-annexin	FP321
pFusionRed-cadherin	FP434
pmKate2-clathrin	FP322
pTagRFP-Cx43	FP364
pmKate2-EB3	FP316
pFusionRed-ER	FP420
pTagBFP-H2B	FP176
pTagRFP-H2B	FP368
pFusionRed-H2B	FP421
pmKate2-H2B	FP311
pmKate2-paxillin	FP323
pFusionRed-PDHA1	FP430
pFusionRed-talin	FP432
pTagBFP-tubulin	FP175
pTagGFP2-tubulin	FP195
pTagYFP-tubulin	FP135
pTagRFP-tubulin	FP145
pFusionRed-tubulin	FP433
pFusionRed-endo	FP427
pmKate2-endo	FP314
pTagRFP-Golgi	FP367
pFusionRed-f-mem	FP418
pmKate2-f-mem	FP186
pmKate2-lyso	FP312
pFusionRed-MAP4	FP428
pTagCFP-mito	FP117
pTagGFP2-mito	FP197
pTagYFP-mito	FP137
pTagRFP-mito	FP147
pmKate2-mito	FP187
pmKate2-peroxi	FP313



Photoinducible cell cycle inhibitor ArrestRed

Reversible inhibition of cell cycle progression
Activation by green light irradiation
Easy visualization in cell nuclei
No exogenous chemical compounds required

Genetically-encoded photoinducible cell cycle inhibitor ArrestRed

ArrestRed is a modified red fluorescent protein that can be easily expressed in different systems. The illumination of the ArrestRed expressing cells by green light leads to blockage of cell proliferation for about 24 hours, after that approximately 90% of ArrestRed expressing cells resume division. Repeated light illuminations allow to maintain cells in the non-dividing state for longer periods.

The ability to transiently block cell cycle progression makes ArrestRed a powerful optogenetic tool to study the roles of specific cell populations in development, regeneration, and carcinogenesis.

Performance and use

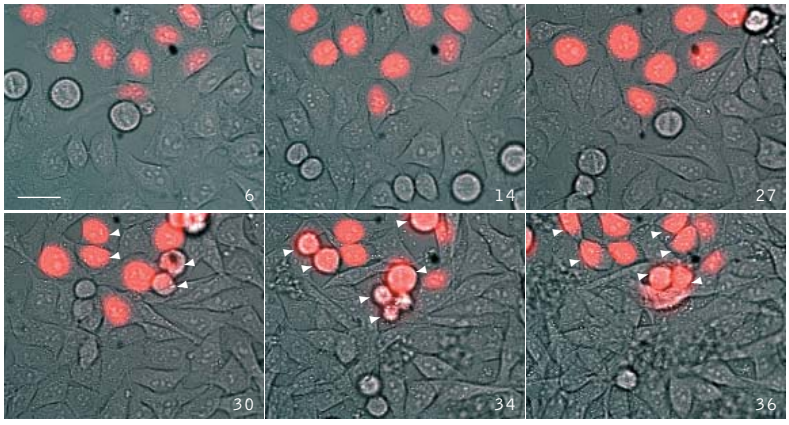
ArrestRed can be used for selective inhibition of cell cycle progression in various experimental systems. Before light activation, ArrestRed enables correct chromatin labeling and does not interfere with cellular division.

Effects of light-activated ArrestRed *in vivo*: The effects of light-induced activation of ArrestRed in the whole organism *in vivo* were demonstrated in transgenic *Xenopus laevis* embryos [Serebrovskaya et al. 2011].

In one set of experiments, the Xag2 promoter was used to specifically direct the ArrestRed expression in the cement gland, a provisory organ located at the rostral end of the embryonic head. Activation of ArrestRed (green light illumination with LED array, 525 nm, 45 mW/cm^2, 1 hour) in transgenic embryos at the early neurula stage leads to clear retardation of the cement gland differentiation observed at the tadpole stage.

In another set of experiments, the tissue-specific promoter of the homebox gene Xanf1 was used to specifically induce the ArrestRed expression in the cells of the anterior neural fold between the middle gastrula and the late neurula stages of the development. Activation of ArrestRed (green light illumination with LED array, 525 nm, 45 mW/cm^2, 1 hour) in transgenic embryos at the early-midneurula stages leads to various degrees of forebrain reduction accompanied by prominent optic stalk dysplasia, which in extreme cases resulted in a complete cyclopic phenotype observed at the tadpole stage.

Effects of light-activated ArrestRed on cell division *in vitro*: Activation of ArrestRed in either transiently or stably transfected HeLa cells results in complete blockage of cell division for about 24 h. During this time, cell nuclei have interphase morphology, and no cells undergo division. At the same time, most cells remain viable with no membrane blebbing, loss of attachments, cell shrinkage, or other signs of cell death. Over a second 24-h period (24-48 h after activation of ArrestRed), approximately 90% of the ArrestRed-transfected cells undergo mitosis.



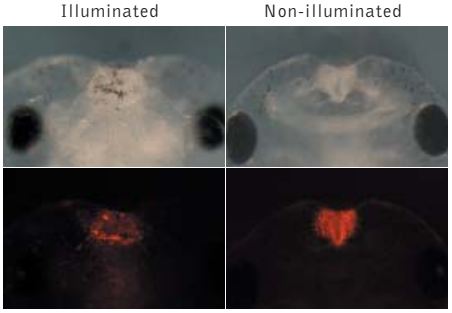
Time-lapse images of representative HeLa cells after activation of ArrestRed by green light illumination. Overlay of red fluorescence and transmitted light are shown (numbers indicate time in hours). Note that in contrast to non-transfected cells, ArrestRed expressing cells do not divide for 27 h, and then undergo mitosis normally (arrows point mitotic or newly appeared daughter cells).

ArrestRed-related products

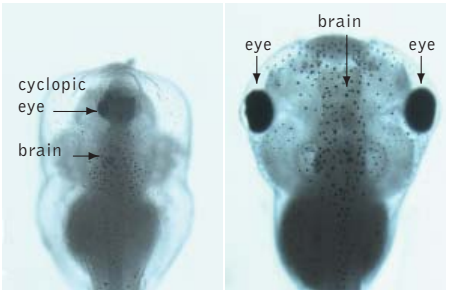
Product	Cat.#
pArrestRed expression/source vector	FP980

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

A The cement gland-specific Xag2 promoter



B The forebrain-specific Xanf1 promoter



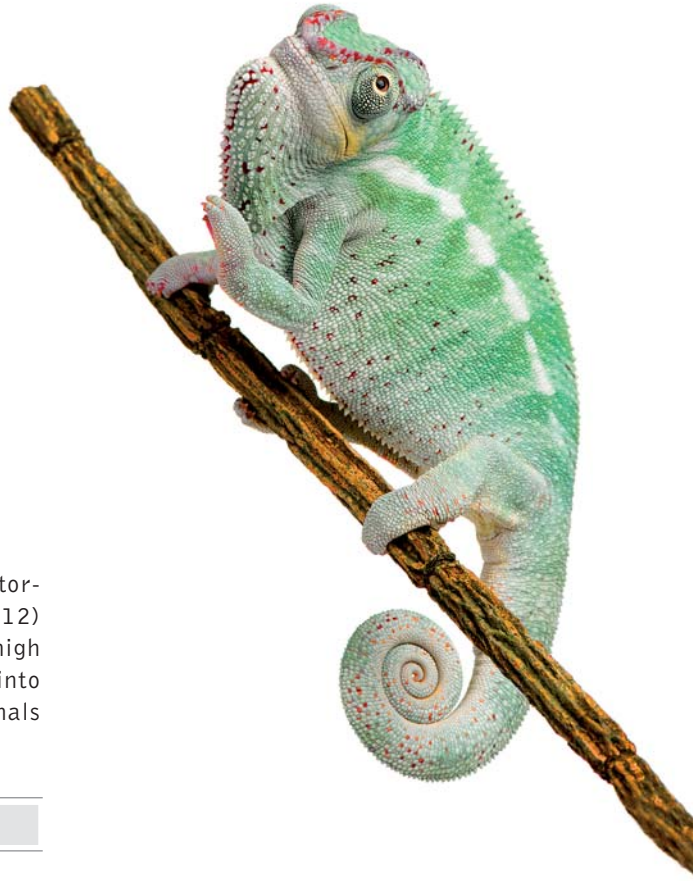
Transgenic tadpoles expressing ArrestRed under the control of certain brain-specific promoters.
(A) ArrestRed was activated (left panels) or non-activated (right panels) at the early neurula stage (top panels – transmitted light, bottom panels – red fluorescence). (B) The left panel shows cyclopic tadpole developed after the ArrestRed activation at the early-midneurula stages, the right panel shows normal embryo developed in the dark. *Data courtesy of Dr. A. Zaraisky, Institute of Bioorganic Chemistry, RAS (Moscow, Russia).*

REFERENCES

Serebrovskaya et al. (2011). Biochem J, 435 (1): 65–71 / pmid: 21214518

Evrogen JSC
16/10 Miklukho-Maklaya str. Moscow 117997, Russia
Tel: +7(495) 988 4084, fax: +7(495) 988 4085
www.evrogen.com

evrogen



Fluorescent sensors for detection of intracellular Ca^{2+} and H_2O_2

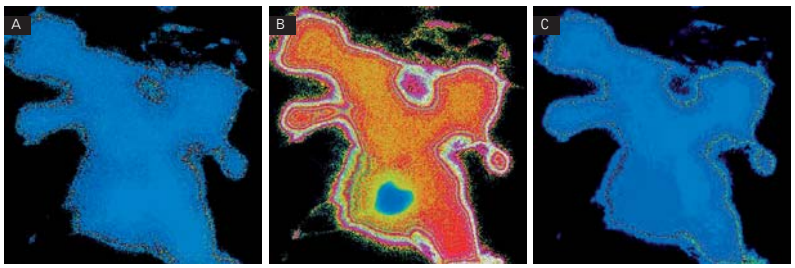
Evrogen offers genetically-encoded fluorescent sensors for monitoring changes in intracellular concentration of calcium ions (Case12) and hydrogen peroxide (HyPer). Both indicators demonstrate high selectivity and sensitivity of detection, allow precise targeting into various subcellular compartments and real time measuring of signals in natural intracellular surroundings.

Protein	HyPer	Case12
Fluorescence color	green	green
Excitation max, nm	420 and 500	491
Emission max, nm	516	516
Specificity	H_2O_2	Ca^{2+}
Aggregation	not observed	not observed

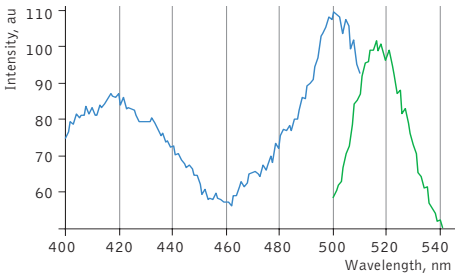
HyPer

Ratiometric sensor for hydrogen peroxide, HyPer, demonstrates submicromolar affinity to H_2O_2 and is insensitive to other oxidants tested, like superoxide, oxidized glutathione, nitric oxide, and peroxinitrite. Unlike many chemical sensors, 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.

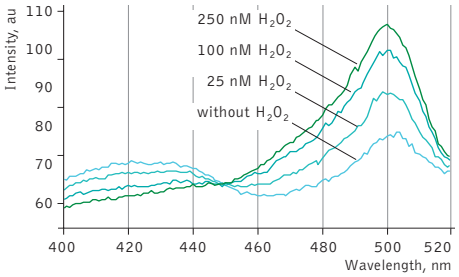
Without H_2O_2, HyPer has two excitation peaks with maxima at 420 nm and 500 nm, and one emission peak with maximum at 516 nm. Upon exposure to H_2O_2, the excitation peak at 420 nm decreases proportionally to the increase in the peak at 500 nm, allowing ratiometric measurement of H_2O_2. Oxidized HyPer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



Ratiometric images of the group of HeLa cells before (A), 20 sec after (B), and 600 sec after (C) addition of 180 ul of H_2O_2. Images were pseudocolored using "ratio" lookup table of NIH ImageJ software: blue-green-red-white colors represent lowest-intermediate-high-highest level of H_2O_2.



HyPer excitation (blue line) and emission (green line) spectra.

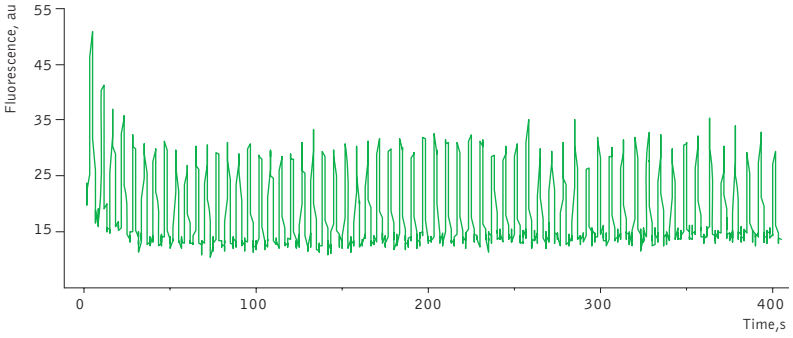


Changes in the excitation spectrum of isolated HyPer in response to H_2O_2 addition. Emission was measured at 530 nm.

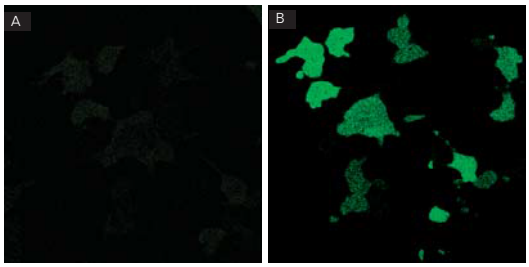
Case12

High dynamic range sensor for calcium ions, Case12, allows measurement of changes of calcium concentration in a physiological range from hundred nanomoles to micromoles with a high signal-to-noise ratio. Binding of Ca^{2+} is fast and reversible, allowing monitoring 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, easily detectable using standard GFP filter sets.

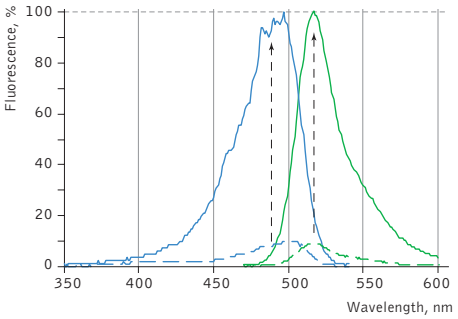
In contrast to conventional calcium sensors, Case12 is stable under physiological pH (pKa 7.2 in the presence of 10 μM Ca^{2+}). This makes it well suitable for *in vivo* use.



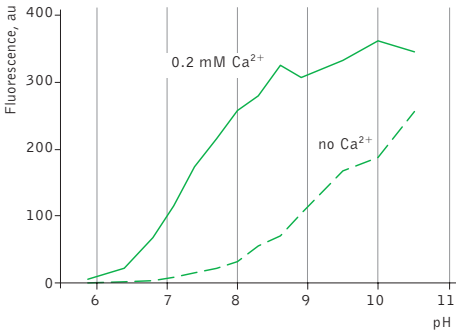
Fluorescence changes of human melanoma-derived M21 cells expressing Case12 in response to 100 μM ATP.



Testing Case12 in living cells. HeLa cells expressing Case12 shown before (A) and after (B) ionophore addition.



Case12 normalized excitation (blue) and emission (green) spectra without Ca^{2+} (dotted line) and in the presence of 1 mM of Ca^{2+} (solid line). Case12 shows multi-fold brightness increase of fluorescence in the response to 1 mM Ca^{2+}.



Dependence of Case12 fluorescence on pH in the presence (solid line) and in the absence (dashed line) of Ca^{2+}.

Available vectors

Vector	Cat#
pCase12-cyto	FP991
pCase12-mito	FP992
pHyPer-cyto	FP941
pHyPer-dMito	FP942
pHyPer-nuc	FP944

For licensing information, please contact Evrogen at license@evrogen.com

For more information, please visit our web-site:
www.evrogen.com

evrogen

High effective FRET pairs

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.

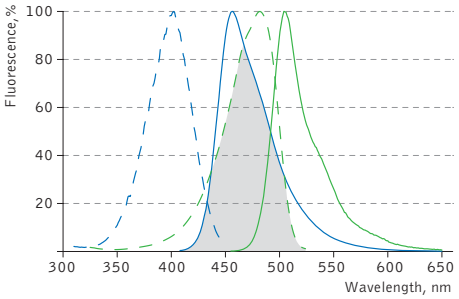
Characteristics	TagBFP-TagGFP2	TagGFP2-TagRFP
FRET efficiency (E)	0.57	0.50
Calc. Forster distance (R_0)	5.25	5.70
FRET donor	TagBFP	TagGFP2
Fluorescence color	blue	green
Excitation maximum, nm	402	483
Emission maximum, nm	457	506
Brightness, % of EGFP	99	105
pKa	2.7	5.0
FRET acceptor	TagGFP2	TagRFP
Fluorescence color	green	red
Excitation maximum, nm	483	555
Emission maximum, nm	506	584
Brightness, % of EGFP	105	148
pKa	5.0	3.8

TagBFP-TagGFP2 pair

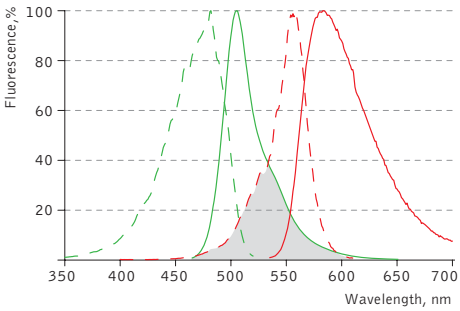
The calculated Forster distance (R_0 = 5.25 nm) for TagBFP-TagGFP2 pair is larger than those reported for the standard ECFP-EYFP and mCyPet-mYPet pairs (R_0 = 4.86 nm and 4.93 nm, respectively). Calculation of FRET efficiency (E) based on the increase of donor emission upon cleavage of the fusion protein in vitro produced E=0.57 for TagBFP-TagGFP2. For comparison, E=0.38 for EBFP2-TagGFP2 pair; E=0.42 for ECFP-EYFP pair; and E=0.51 for mCyPet-mYPet pair.

When TagBFP and TagGFP2 free proteins are coexpressed in HeLa cells, the cross-bleed-corrected FRET normalized to fluorescence of donor is 0.85%. Under the same experimental conditions, the cross-bleed-corrected FRET between ECFP and EYFP free proteins coexpressed in HeLa cells is 6.2%, confirming their weak dimerization. In other words, TagBFP and TagGFP2 proteins derived from the different marine sources and, as a result, lacking ability to form heterodimers, provide more than 6-fold lower background for FRET analysis than the weakly dimerizing FRET pairs, such as the ECFP-EYFP.

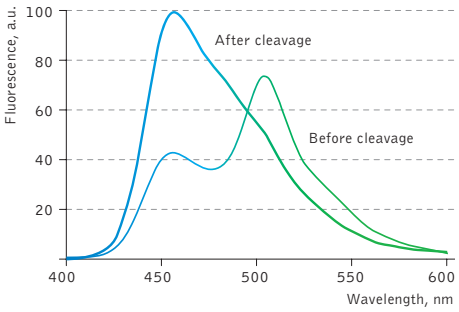
Thus, TagBFP-TagGFP2 pair is not only superior to other BFP-GFP pairs, but is one of the best among available FRET pairs of the true monomeric fluorescent proteins [Subach *et al.*, 2008].



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



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



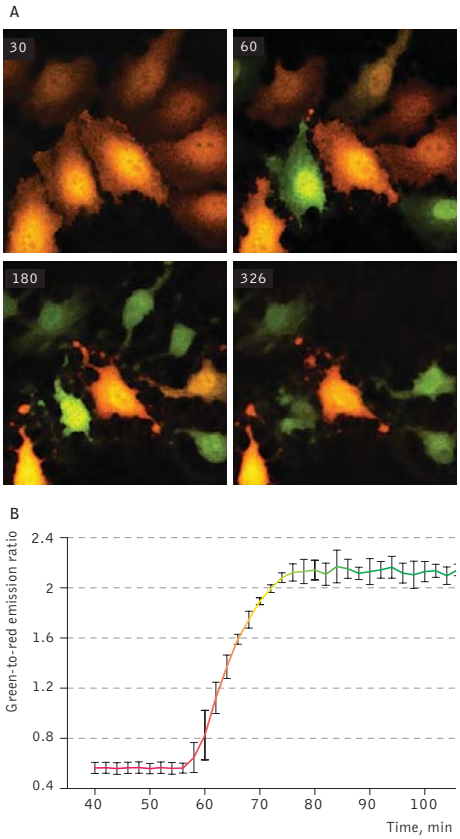
Change in excitation/emission spectra of TagBFP-TagGFP2 FRET pair upon the cleavage of the linker connecting two fluorescent proteins.

TagGFP2-TagRFP pair

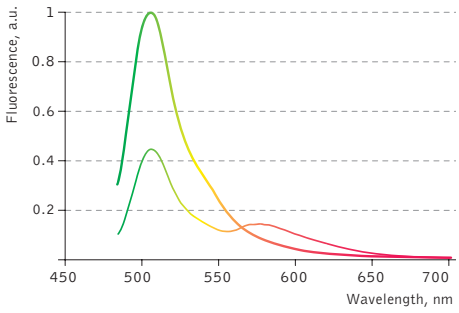
The high fluorescence quantum yield of TagGFP2 along with the high molar extinction coefficient of TagRFP and excellent overlap of donor emission and acceptor excitation spectra result in highly effective FRET between these fluorescent proteins. The calculated Forster distance R_0 = 5.7 nm for TagGFP2 - TagRFP pair is one of the largest 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.

Shifting the wavelengths toward the red part of the spectrum (comparing to traditional cyan and yellow FRET partners) reduces input of cellular autofluorescence. The significantly increased quantum yield of TagRFP is highly beneficial for acceptor-based ratiometric FRET studies. High pH-stability of the both proteins allows using this pair for imaging in acidic organelles. The combined advantages suggest that the TagGFP2-TagRFP is one of the most efficient green/red FRET couple available to date for ratiometric FRET analyses. 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. We believe that advantageous characteristics make TagGFP2-TagRFP one of the preferable FRET pairs to monitor interaction of proteins of interest in living cells and to generate FRET-based sensors of various specificity (see demonstration of FRET-based caspase-3 apoptosis sensor Casper3-GR performance and [Shcherbo *et al.*, 2009]).

The excitation wavelength required to visualize FRET changes of the TagGFP2-TagRFP pair 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.



Green-to-red emission ratio change of Casper3-GR upon staurosporine-induced apoptosis. (A) Two channel fluorescence imaging of Casper3-GR. Time (in min.) is shown after staurosporine infusion. (B) 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.



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

References:

1. Shcherbo D, Souslova EA, Goedhart J, Chepurnykh TV, Gaintzeva A, Shemiakina II, Gadella TW, Lukyanov S, Chudakov DM. Practical and reliable FRET/FLIM pair of fluorescent proteins. BMC Biotechnol. 2009; 9:24. / pmid: 19321010

2. Subach OM, Gundorov IS, Yoshimura M, Subach FV, Zhang J, Gruenwald D, Souslova EA, Chudakov DM, Verkhusha VV. Conversion of Red Fluorescent Protein into a Bright Blue Probe. Chem Biol. 2008; 15 (10):1116-24. / pmid: 18940671

For more information, please visit our web-site:
www.evrogen.com

evrogen

Fluorescent reporters for *in vivo* cell labeling and monitoring of promoter activity

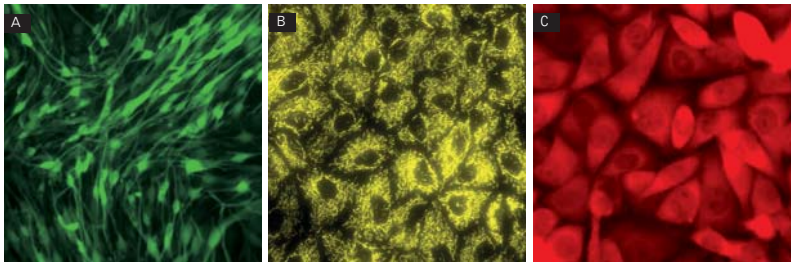
Evrogen TurboColors are superbright and fast maturing fluorescent proteins, specially recommended for applications requiring fast appearance of bright fluorescence, including cell and organelle labeling or tracking promoter activity. Far-red marker TurboFP635 is ideal for whole body imaging applications.

Protein/Characteristics	TurboGFP	TurboYFP	TurboRFP	TurboFP602	TurboFP635	TurboFP650
Fluorescence color	green	yellow	red (orange)	true-red	far-red	near-infrared
Excitation max	482 nm	525 nm	553 nm	574 nm	588 nm	592 nm
Emission max	502 nm	538 nm	574 nm	602 nm	635 nm	650 nm
Quantum yield	0.53	0.53	0.67	0.35	0.34	0.24
Extinction coefficient ($M^{-1}cm^{-1}$)	70 000	105 000	92 000	74 400	65 000	65 000
Brightness*	37.1	55.7	61.6	26.0	22.1	15.6
Brightness, % of EGFP	112	169	187	79	67	47
pKa	5.2	5.9	4.4	4.7	5.5	5.7

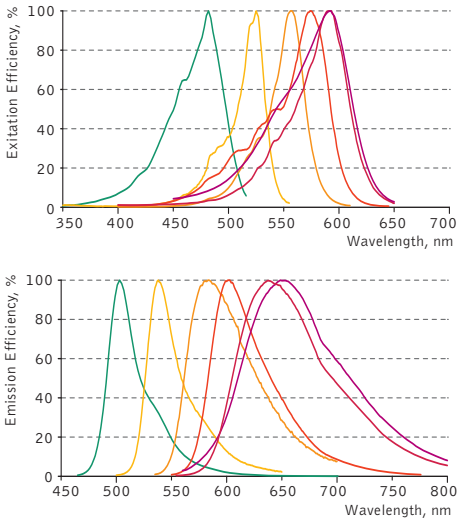
* Brightness is a product of extinction coefficient and quantum yield, divided by 1000.

Bright labels of cells and cell organelles

TurboFPs possess bright stable fluorescence allowing monitoring of cells over extended periods of time. Despite their dimeric structure, TurboFPs are suitable for generation of fusions with subcellular localization signals targeting the reporters to desired cell compartments. Stable cell lines expressing TurboFPs are available.



Expression of TurboFPs in stably transfected mammalian cell lines. (A) - TurboGFP, C2C12 myoblast cells, (B) - mitochondria-targeted PhiYFP*, PtK2 cells, (C) - TurboFP635, T24 cells.
* PhiYFP is a variant of TurboYFP optimized for stable expression.
Images of stably transfected cell lines were kindly provided by Dr. Christian Petzelt (Marinpharm).



TurboFPs normalized exitation/emission spectra

Perfect reporters of gene expression

TurboFPs mature noticeably faster than many other fluorescent proteins, allowing monitoring of gene expression from early promoters. The example below shows *in vivo* examination of the developing *Xenopus* embrios expressing either TurboGFP or EGFP. Destabilized protein variants (*-dest1) allow accurate analysis of rapid and/or transient events in gene regulation.

***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).

Suitable markers for whole body imaging

For deep imaging of animal tissues, the optical window favorable for light penetration is in near-infrared wavelengths, which requires proteins with emission spectra in the far-red wavelengths. TurboFP635 (scientific name Katushka) has emission maxima at 635 nm and is more bright, photostable and pH-stable than other cloned far-red fluorescent proteins. Superiority of TurboFP635 for whole-body imaging has been demonstrated by direct comparison with other red and far-red fluorescent proteins (Shcherbo *at al.* Nat Methods. (2007) 4:741-746).

DsRed-Express and TurboFP635 expression in *Xenopus laevis*.
Transgenic 2.5 months intact animals expressing TurboFP635 and DsRed-Express under the control of cardiac actin promoter are shown from the dorsal side. TurboFP635 (on the right) is clearly visible in the whole body, while DsRed-Express (on the left) is not. 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.

Available vectors

Vector	Cat#
Bacterial expression vectors	
pTurboGFP-B	FP513
pTurboYFP-B	FP613
pTurboRFP-B	FP233
Mammalian expression vectors	
pTurboGFP-C	FP511
pTurboRFP-C	FP231
pTurboFP635-C	FP721
pTurboFP650-C	FP731
pTurboGFP-N	FP512
pTurboYFP-N	FP612
pTurboRFP-N	FP232
pTurboFP602-N	FP712
pTurboFP635-N	FP722
pTurboFP650-N	FP732
Vectors for labeling of mitochondria	
pTurboGFP-mito	FP517
pTurboRFP-mito	FP237
pTurboFP602-mito	FP717
Promoterless vectors	
pTurboGFP-PRL	FP515
pTurboRFP-PRL	FP235
pTurboFP602-PRL	FP715

For licensing information, please contact Evrogen at license@evrogen.com

For more information, please visit our web-site:
www.evrogen.com

Evrogen JSC | 16/10 Miklukho-Maklaya str. Moscow 117997, Russia | Tel: +7(495) 988 4084, fax: +7(495) 988 4085 | www.evrogen.com

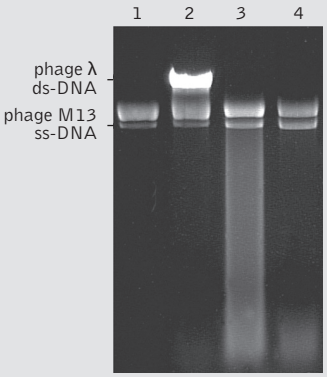
evrogen

Duplex-specific nuclease

Duplex-specific nuclease (DSN) is an enzyme purified from hepatopancreas of Red King (Kamchatka) crab [Shagin *et al.*, 2002].

Applications

- Full-length enriched cDNA normalization
- Construction of normalized RNA-seq libraries for NGS
- Normalization of genomic DNA
- cDNA depletion and ribosomal cDNA depletion
- cDNA subtraction
- SNP detection
- Construction of repeat-free FISH probes
- Multiplexed fluorescence detection of miRNAs
- Quantitative telomeric overhang determination



Effect of DSN on ss- (phage M13) and ds- (phage λ) DNA substrates.
1, 2 – negative control without DSN: M13 DNA (1); M13 and λ DNA mix (2). 3, 4 – M13 and λ DNA mix after incubation with DSN at 70°C for 1.5 min (3) and 5 min (4).

DSN features

- Selectively cleaves ds DNA and DNA in DNA-RNA hybrid duplexes
- Discriminates between perfectly and nonperfectly matched short duplexes
- Inactive towards ss-DNA and RNA
- Thermostable
- Reaction can be stopped with EDTA
- Tolerant to Proteinase K

Available products

Product	Cat.#	Size
Duplex-specific nuclease, lyophilized	EA003	10 U
	EA001	50 U
	EA002	100 U
	EA008	1000 U

Storage: -20°C.

Products are intended for research purposes only.

References

Shagin DA, Rebrikov DV, Kozhemyako VB, Altshuler IM, Shcheglov AS, Zhulidov PA, Bogdanova EA, Staroverov DB, Rasskazov VA, Lukyanov S. A novel method for SNP detection using a new duplex-specific nuclease from crab hepatopancreas. Genome Res. 2002; 12 (12):1935-42. / pmid: 12466298

For more information, please visit our web-site:

www.evrogen.com

Evrogen JSC | 16/10 Miklukho-Maklaya str. Moscow 117997, Russia | Tel: +7(495) 988 4084, fax: +7(495) 988 4085 | www.evrogen.com

evrogen

Trimmer-2
cDNA normalization kit

In an eukaryotic cell, the mRNA population constitutes approximately 1% of total RNA with the number of transcripts varying from several thousand to several tens of thousands. Normally, the high abundance transcripts (several thousand mRNA copies per cell) of as few as 5-10 genes account for 20% of the cellular mRNA. The intermediate abundance transcripts (several hundred copies per cell) of 500-2000 genes constitute about 40-60% of the cellular mRNA. The remaining 20-40% of mRNA is represented by rare transcripts (from one to several dozen mRNA copies per cell) [Alberts *et al.*, 1994] . Such an enormous difference in abundance complicates large-scale transcriptome analysis, which results in recurrent sequencing of more abundant cDNAs.

cDNA normalization decreases the prevalence of high abundance transcripts and equalizes transcript concentrations in a cDNA sample, thereby dramatically increasing the efficiency of sequencing and rare gene discovery.

Trimmer-2 kit is designed to normalize amplified full-length-enriched cDNA prepared using Evrogen Mint-2 cDNA synthesis kit (cat. # SK005). The resulting cDNA contains equalized abundance of different transcripts and can be used for construction of cDNA libraries and for direct sequencing, including high-throughput sequencing on the next generation sequencing platforms (e.g. Illumina).

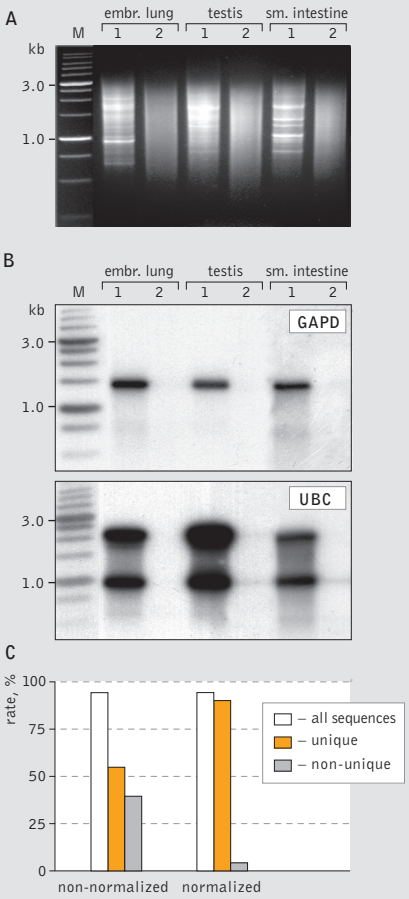
Features

- 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
- Fully compatible with NGS sequencing protocols

Available products

Product	Cat.#	Size
Trimmer-2 kit	NK003	10 rxn

Storage: -20°C.
Kit is intended for research purposes only.



Typical cDNA normalization result.
(A) Agarose/EtBr gel-electrophoresis of non-normalized (1) and normalized (2) human cDNA samples.
(B) Concentration of abundant transcripts in these samples revealed by Virtual Northern blot. GAPD – glyceraldehyde-3-phosphate dehydrogenase; UBC – ubiquitin C; M – 1 kb DNA size markers.
(C) Normalization significantly increases gene discovery rate of cDNA library. Transcript distribution in standard and normalized cDNA libraries from *Aplysia* neurons: white – all sequences, orange – unique sequences; grey – non-unique sequences.

For more information, please visit our web-site:
www.evrogen.com

References

Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD. Molecular biology of the cell. 3rd ed., Garland Publishing, New York. 1994.

Evrogen JSC | 16/10 Miklukho-Maklaya str. Moscow 117997, Russia | Tel: +7(495) 988 4084, fax: +7(495) 988 4085 | www.evrogen.com

evrogen

Mint-2
cDNA synthesis kit

Mint-2 cDNA synthesis kit is designed to synthesize full-length-enriched double stranded (ds) cDNA from total or poly(A)+ RNA.

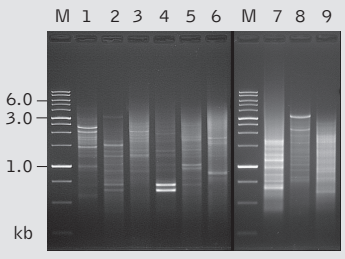
cDNA can be prepared from limited amounts of starting RNA (as little as 100 ng of poly(A)+ or 250 ng of total RNA is required). The synthesis procedure is fast and lasts only 6 hours.

Mint-2 cDNA synthesis kit allows preparation of cDNA flanked with different adapters optimized for various research applications, including construction of cDNA libraries, subtractive hybridization (SSH), high throughput sequencing on the next generation sequencing platforms and other applications.

cDNA prepared with Mint-2 kit can be normalized using Trimmer-2 kit (cat. # NK003) to decrease the prevalence of highly abundant transcripts.

Key features

- High yields of full-length cDNA up to 7.5 kb
- Optimal reaction temp 42-45°C
- No DNase treatment and adaptor-ligation step
- Optimized for various downstream applications including NGS



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 Ladder.

Available products

Product	Cat.#	Size (10 µl rxn)
Mint-2 kit	SK005	20

Storage: -20°C.
Kit is intended for research purposes only.

For more information, please visit our web-site:

www.evrogen.com

Evrogen JSC | 16/10 Miklukho-Maklaya str. Moscow 117997, Russia | Tel: +7(495) 988 4084, fax: +7(495) 988 4085 | www.evrogen.com

evrogen

Polymerases and PCR kits

Polymerases provide an antibody-mediated hot-start ensuring highly specific and sensitive PCR amplification.

Both enzymes are completed with 10X general purpose PCR buffer.

PCR kits includes all components necessary for PCR: polymerase, a mix of high purity deoxyribonucleotides, sterile PCR water and three reaction buffers (general purpose buffer, GC buffer for amplification of GC-rich templates and Red buffer for direct loading on agarose gels).

	Common features	Key features	Applications
Encyclo polymerase	• Proofreading 3'→5' exonuclease activity • Lack of 5'→3' exonuclease activity • Hot start • TA cloning compatibility	High processivity	• cDNA amplification • Long PCR (up to 20 Kb) • Amplification of low-copy-number targets
Tersus polymerase		High fidelity High specificity	• Amplification of DNA for subsequent cloning or sequencing • Site-specific mutagenesis • High specific PCR from complex templates

Available products

Product	Cat.#	Size (25 µl rxn)
Encyclo polymerase	PK002S	200
	PK002L	1000
Encyclo Plus PCR kit	PK101	200
Tersus polymerase	PK123S	200
	PK123L	1000
Tersus Plus PCR kit	PK121	200

Storage: -20°C.
Products are intended for research purposes only.

For more information, please visit our web-site:

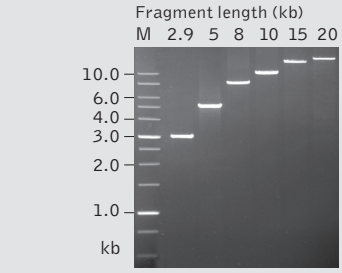
www.evrogen.com

P.T.O. →

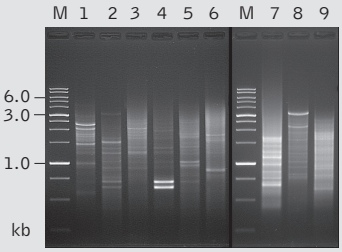
Encyclo polymerase and Encyclo Plus PCR kit

For robust routine PCR, amplification of low-copy-number targets, long PCR, cDNA amplification.

Encyclo polymerase produces high yields of PCR products from a wide variety of templates and is suitable for most PCR and primer extension applications, including the amplification of difficult templates and long PCR.



PCR amplification of phage λ DNA fragments using Encyclo Plus PCR kit.
Fragment length is indicated at the top.
M – 1 kb DNA size Ladder.

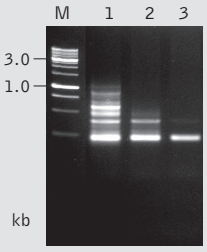


PCR amplification of first-strand cDNA samples (prepared from total RNA from different tissues) using Encyclo Plus PCR kit.
1 – mouse liver; 2 – mouse skeletal muscle; 3 – mouse brain; 4 – human leucocytes; 5 – human lung; 6 – human skeletal muscle; 7 – mosquito grub; 8 – copepod *Pontella sp.*; 9 – tomato *Lycopersicon esculentum*. M – 1 kb DNA size Ladder.

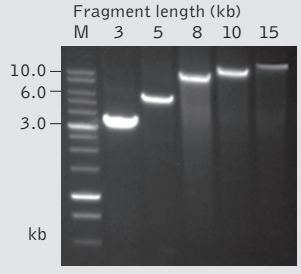
Tersus polymerase and Tersus Plus PCR kit

For high-fidelity PCR.

Tersus polymerase is a specially developed mix of proofreading and highly processive PCR enzymes. Tersus polymerase has about 4 times lower error rate than Encyclo polymerase that makes it an ideal choice for cloning and other applications requiring high-fidelity amplification. High specificity of Tersus polymerase ensures its excellent performance in amplification of difficult templates, such as highly homologous repeats, genomic DNA or cDNA libraries.



Tersus polymerase provides highly specific amplification of difficult templates.
A 230 bp fragment of L1Hs repeat was amplified from 5 ng of human genomic DNA using different polymerases.
M – 1 kb DNA ladder; 1 – Taq polymerase; 2 – Encyclo polymerase;
3 – Tersus polymerase, 28 cycles.



PCR amplification of phage λ DNA fragments using Tersus polymerase.
DNA fragments were amplified from 25 ng of template in 15 cycles (wells 1 and 2) and 19 cycles (wells 3-5).
Fragment length is indicated at the top.
M – 1 kb DNA ladder.