



Section A

Basic Fluorescent Proteins

A variety of colors for *in vivo* labeling applications

- development of cell- and tissue-specific markers
- protein localization and protein interaction studies
- monitoring of promoter activity and gene expression
- cell and subcellular structures labeling
- determination of cell lineage
- selection of transgenic cells
- multicolor labeling
- other cell-live assays

Section content

| | | | |
|---|-------------|--|-------------|
| Overview | A-3 | Other basic fluorescent proteins | A-69 |
| Protein localization tags (TagFPs) | A-5 | Phi-Yellow | |
| TagCFP | | Proteins description | A-71 |
| Protein description | A-7 | Licensing opportunities | A-72 |
| Licensing opportunities | A-8 | Related products | A-73 |
| Related products | A-9 | JRed | |
| TagGFP | | Protein description | A-80 |
| Protein description | A-13 | Licensing opportunities | A-81 |
| Licensing opportunities | A-14 | Related products | A-81 |
| Related products | A-15 | Product list | |
| TagYFP | | Expression/source vectors | A-85 |
| Protein description | A-19 | Vector sets | A-90 |
| Licensing opportunities | A-20 | Recombinant proteins | A-91 |
| Related products | A-21 | Antibodies | A-92 |
| TagRFP | | Stably transfected cell lines | A-93 |
| Protein description | A-25 | Notice to purchaser of Evrogen basic fluorescent protein-related products | A-94 |
| Licensing opportunities | A-26 | | |
| Related products | A-27 | For ordering information, please see “How to order” section | G-3 |
| TagFP635 | | | |
| Protein description | A-31 | | |
| Licensing opportunities | A-32 | | |
| Related products | A-33 | | |
| TurboColors | A-35 | | |
| TurboGFP | | | |
| Protein description | A-37 | | |
| Licensing opportunities | A-39 | | |
| Related products | A-39 | | |
| TurboYFP | | | |
| Protein description | A-46 | | |
| Licensing opportunities | A-47 | | |
| Related products | A-48 | | |
| TurboRFP | | | |
| Protein description | A-53 | | |
| Licensing opportunities | A-54 | | |
| Related products | A-55 | | |
| TurboFP602 | | | |
| Protein description | A-60 | | |
| Licensing opportunities | A-61 | | |
| Related products | A-62 | | |
| TurboFP635 | | | |
| Protein description | A-66 | | |
| Licensing opportunities | A-67 | | |
| Related products | A-67 | | |

Overview

Evrogen offers a wide collection of bright fluorescent proteins for common applications in the field of live-cell assays (labeling of cells, subcellular structures, and proteins; analysis of promoter activity; generation of stably transfected cell lines expressing fluorescent proteins or their fusions; etc).

Ranging in color from cyan to far-red, Evrogen fluorescent proteins can be used for multicolor labeling to observe different cellular events in a particular cell or a cell population.

Advantages

- Wide spectral diversity
- Easy detection by flow cytometry or fluorescence microscopy
- No cofactors, substrate addition, or chemical staining required
- Suitability for stable expression
- Suitability for multicolor labeling
- Special optimization for different applications
- Broad product line: various expression vectors, recombinant proteins, antibodies. Third party stably transfected cell lines are available.
- Free evaluation period during the first six months after purchase for For-Profit entities

Evrogen basic fluorescent proteins are divided into subgroups according to their properties and recommended applications:

TagFPs

Monomeric proteins of different colors, ideal for protein labeling.

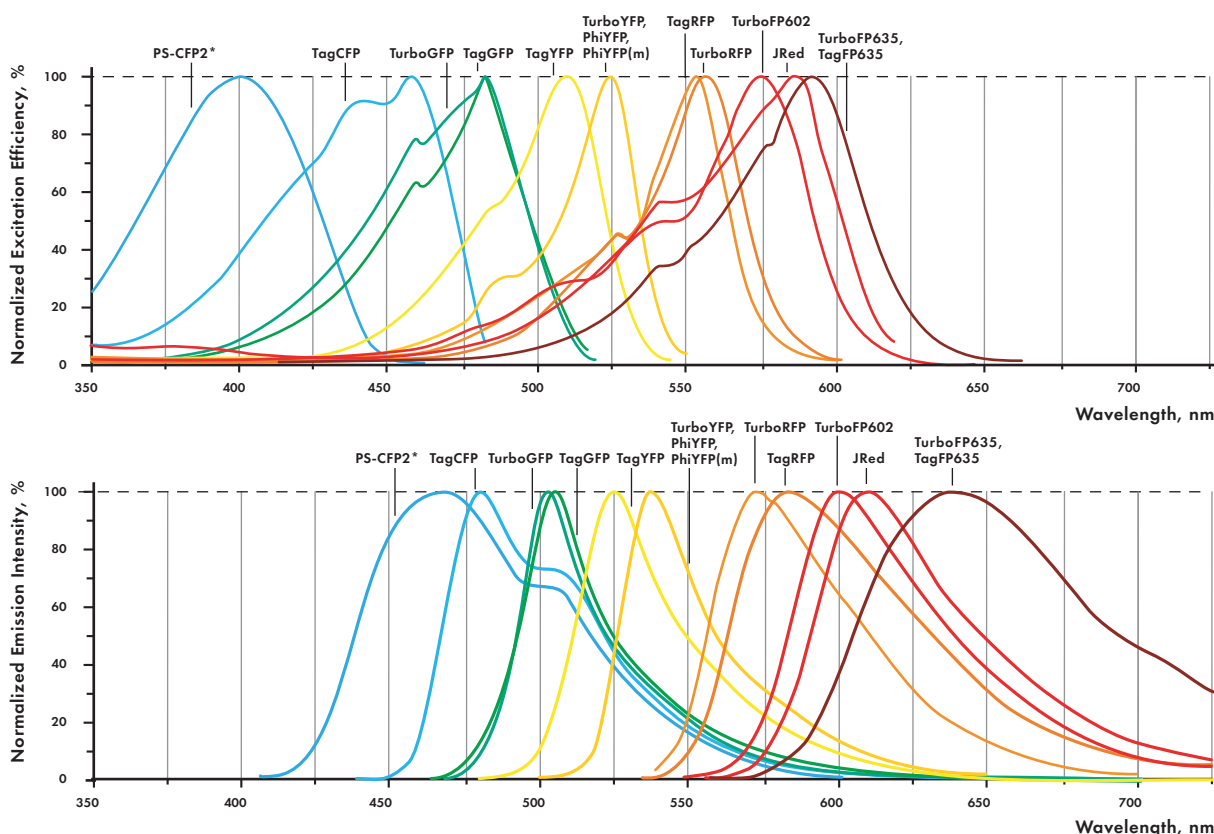
TurboColors

Super-bright and fast-maturing proteins of different colors for cell labeling and monitoring of promoter activity.

Other Basic FPs

Proteins for various common applications.

Basic fluorescent proteins, excitation/emission spectra



* PS-CFP2 is a photoswitchable fluorescent protein (page B-5), however it can be used as a routine cyan fluorescent tag for protein labeling and FRET applications at moderate excitation intensities.

Evrogen basic fluorescent proteins: Main properties and recommendations for use

| Protein | TagCFP | TagGFP | TagYFP | TagRFP | TagFP635 | TurboGFP | TurboYFP | TurboRFP | TurboFP602 | TurboFP635 | PhiYFP | PhiYFP-m | JRed |
|--|------------------------------------|---------|---------|--------------|------------|--|-------------------|--------------|------------|----------------------------------|---------|----------|----------|
| Characteristics | | | | | | | | | | | | | |
| Group | Protein localization tags (TagFPs) | | | | | TurboColors basic fluorescent proteins | | | | Other basic fluorescent proteins | | | |
| Fluorescence color | cyan | green | yellow | red (orange) | far-red | green | yellow | red (orange) | true-red | far-red | yellow | yellow | true-red |
| Excitation max | 458 nm | 482 nm | 508 nm | 555 nm | 588 nm | 482 nm | 525 nm | 553 nm | 574 nm | 588 nm | 525 nm | 525 nm | 584 nm |
| Emission max | 480 nm | 505 nm | 524 nm | 584 nm | 635 nm | 502 nm | 538 nm | 574 nm | 602 nm | 635 nm | 537 nm | 537 nm | 610 nm |
| Quantum yield | 0.57 | 0.59 | 0.62 | 0.48 | 0.33 | 0.53 | 0.53 | 0.67 | 0.35 | 0.34 | 0.40 | 0.39 | 0.20 |
| Extinction coefficient (M ⁻¹ cm ⁻¹) | 37 000 | 58 200 | 64 000 | 100 000 | 45 000 | 70 000 | 105 000 | 92 000 | 74 400 | 65 000 | 130 000 | 124 000 | 44 000 |
| Brightness | 21.1 | 34.3 | 39.7 | 48.0 | 14.9 | 37.1 | 55.7 | 61.6 | 26.0 | 22.1 | 52.2 | 48.4 | 8.8 |
| Brightness, % of EGFP | 64 | 104 | 120 | 145 | 45 | 112 | 169 | 187 | 79 | 67 | 158 | 147 | 26 |
| pKa | 4.7 | 4.7 | 5.5 | 3.8 | 6.0 | 5.2 | 5.9 | 4.4 | 4.7 | 5.5 | 6.0 | 6.0 | 5.0 |
| Structure | monomer | monomer | monomer | monomer | monomer | dimer | dimer | dimer | dimer | dimer | dimer | dimer | dimer |
| Aggregation | no | no | no | no | no | no | at high concentr. | no | no | no | no | no | no |
| Maturation at 37°C | fast | fast | fast | fast | fast | superfast | superfast | superfast | fast | superfast | fast | fast | slow |
| Photostability | high | high | high | medium | high | high | high | high | medium | high | high | high | medium |
| Molecular weight | 27 kDa | 27 kDa | 27 kDa | 27 kDa | 27 kDa | 26 kDa | 26 kDa | 26 kDa | 26 kDa | 26 kDa | 26 kDa | 26 kDa | 27 kDa |
| Recommendations for use | | | | | | | | | | | | | |
| Cell labeling | +++ | +++ | +++ | +++ | +++ | ++++ | ++++ | ++++ | +++ | ++++ | ++++ | ++++ | ++ |
| | | | | | | | | | | | | | |
| Promoter activity testing | +++ | +++ | +++ | +++ | +++ | ++++ | ++++ | ++++ | ++++ | ++++ | +++ | +++ | ++ |
| | | | | | | | | | | | | | |
| Stable transfection | proved | proved | proved | proved | not tested | proved | not tested | not tested | proved | proved | proved | proved | proved |
| In fusions | ++++ | ++++ | ++++ | ++++ | ++++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |

Protein localization tags

Monomeric tags
for protein labeling

TagFPs are monomeric fluorescent proteins specially designed for generation of fusions. Five available colors allow multicolor labeling of different cellular proteins for protein localization and interaction studies.

TagFPs available:

- cyan fluorescent protein TagCFP

source — jellyfish *Aequorea macrodactyla*

excitation max — 458 nm

emission max — 480 nm

- green fluorescent protein TagGFP

source — jellyfish *Aequorea macrodactyla*

excitation max — 482 nm

emission max — 505 nm

- yellow fluorescent protein TagYFP

source — jellyfish *Aequorea macrodactyla*

excitation max — 508 nm

emission max — 524 nm

- red fluorescent protein TagRFP

source — sea anemone *Entacmaea quadricolor*

excitation max — 555 nm

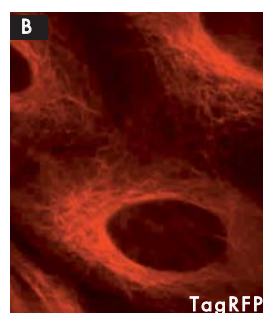
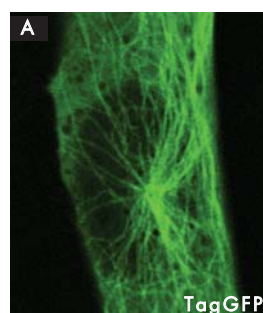
emission max — 584 nm

- far-red fluorescent protein TagFP635

source — sea anemone *Entacmaea quadricolor*

excitation max — 588 nm

emission max — 635 nm



Fluorescent labeling of tubulin filaments in mammalian cells using TagFPs.

(A) — Transiently transfected HeLa cells; (B) — stably transfected U-205 cells.

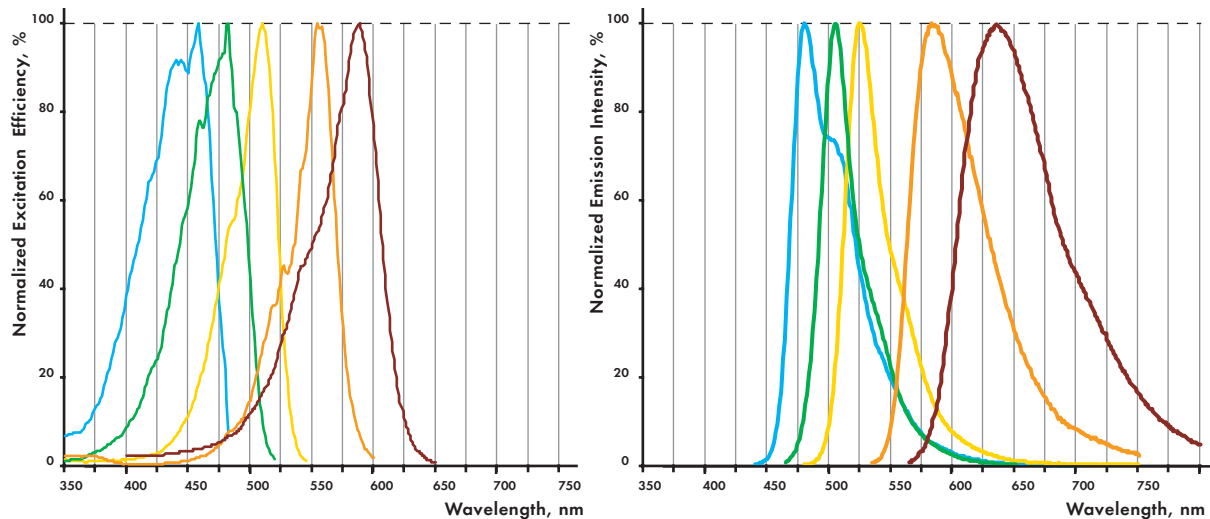
Photograph of stably transfected cell line was provided by Dr. Christian Petzelt (Marinpharm).











Main properties of TagFPs:

| Characteristic | TagCFP | TagGFP | TagYFP | TagRFP | TagFP635 |
|------------------------|---|--|---|--|--|
| Fluorescence color | cyan | green | yellow | red (orange) | far-red |
| Excitation max | 458 nm | 482 nm | 508 nm | 555 nm | 588 nm |
| Emission max | 480 nm | 505 nm | 524 nm | 584 nm | 635 nm |
| Quantum yield | 0.57 | 0.59 | 0.62 | 0.48 | 0.33 |
| Extinction coefficient | 37 000 M ⁻¹ cm ⁻¹ | 58 200 M ⁻¹ cm ⁻¹ | 64 000 M ⁻¹ cm ⁻¹ | 100 000 M ⁻¹ cm ⁻¹ | 45 000 M ⁻¹ cm ⁻¹ |
| Brightness | 21.1 | 34.3 | 39.7 | 48.0 | 14.9 |
| Brightness, % of EGFP | 64 | 104 | 120 | 145 | 45 |
| pKa | 4.7 | 4.7 | 5.5 | 3.8 | 6.0 |
| Structure | monomer | monomer | monomer | monomer | monomer |
| Cell Toxicity | not observed | not observed | not observed | not observed | not observed |
| Aggregation | no | no | no | no | no |
| Maturation at 37°C | fast | fast | fast | fast | fast |
| Photostability | high | high | high | medium | high |
| Molecular weight | 27 kDa | 27 kDa | 27 kDa | 27 kDa | 27 kDa |
| Main advantages | Bright cyan monomeric fluorescent protein | Bright green monomeric fluorescent protein | Bright yellow monomeric fluorescent protein | Bright red monomeric fluorescent protein | Bright far-red monomeric fluorescent protein |
| Possible limitations | no data | no data | no data | medium photostability | no data |

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

TagFPs, excitation/emission spectra



| Characteristic | TagCFP | TagGFP | TagYFP | TagRFP | TagFP635 |
|----------------|---|---|---|--|---|
| Excitation |  |  |  |  |  |
| Emission |  |  |  |  |  |

TagCFP

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

Protein description

TagCFP is a cyan monomeric protein generated from TagGFP mutant of the wild-type GFP-like protein from jellyfish *Aequorea macrodactyla* [1]. It possesses bright fluorescence with excitation/emission maxima at 458 and 480 nm, respectively. TagCFP is significantly brighter than commonly used ECFP.

Main properties of TagCFP

| Characteristic | |
|------------------------|---|
| Molecular weight | 27 kDa |
| Polypeptide length | 239 aa |
| Fluorescence color | cyan |
| Excitation max | 458 nm |
| Emission max | 480 nm |
| Quantum yield | 0.57 |
| Extinction coefficient | 37 000 M ⁻¹ cm ⁻¹ |
| Brightness* | 21.1 |
| Brightness % of EGFP | 64 |
| pKa | 4.7 |
| Structure | monomer |
| Aggregation | no |
| Maturation at 37°C | fast |
| Photostability | high |

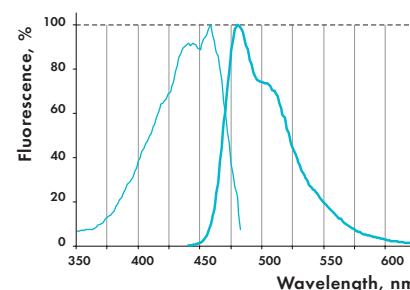
*Brightness is a product of extinction coefficient and quantum yield, divided by 1000.

Performance and use

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

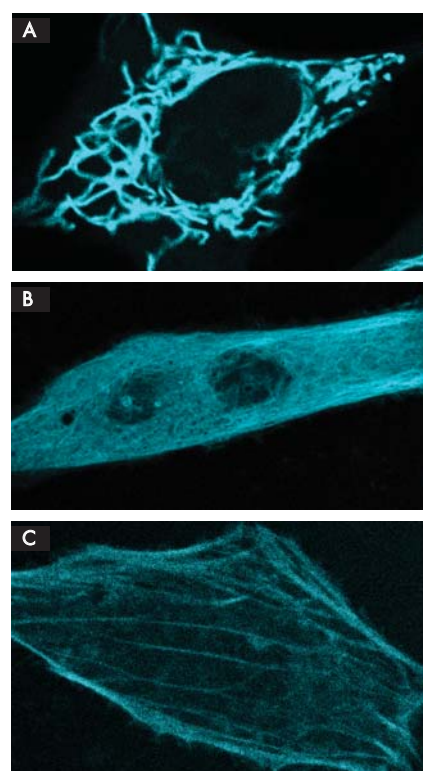
TagCFP can be easily expressed and detected in a wide range of organisms. Mammalian cells transiently transfected with TagCFP expression vectors give bright fluorescent signals within 10-12 hrs after transfection. No cell toxic effects and visible protein aggregation are observed.

TagCFP performance in fusions has been demonstrated in human cytoplasmic beta-actin and alpha-tubulin models. Please visit our Web site at www.evrogen.com/TagCFP.shtml to view 3D video of a cell expressing TagCFP-labeled alpha-tubulin filaments.



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/TagCFP.shtml



TagCFP fusions expression in mammalian cells.

(A) — Confocal microscopy of mitochondria-targeted TagCFP expression in transiently transfected HeLa cells; (B) — confocal microscopy of TagCFP fusion with alpha-tubulin in transiently transfected HeLa cells; (C) — confocal microscopy of TagCFP fusion with cytoplasmic beta-actin in transiently transfected HeLa cells.

| Application | Performance |
|----------------------------------|-------------|
| Cell labeling | |
| mammalian cells | +++ |
| bacterial cells | ++++ |
| Stable transfection | proved |
| Promoter activity testing | +++ |
| In fusions | ++++ |

Compatibility with existing filter sets and antibodies

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

TagCFP can be recognized using Anti-Tag(CGY)FP antibody (Cat.# AB121-AB122, see page D-6 for description) available from Evrogen.

TagCFP licensing opportunities

Evrogen technology embodied in TagCFP 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.

TagCFP comprises the following amino acid substitutions compared with wild-type *A. macrodactyla* GFP (AY013824): K3G, T9A, F64L, S65A, Y66W, F99H, I123V, M128E, D129G, N144S, F145A, N146I, H148D, K162E, V163A, T203C, T205S, T214A, F220L, F223S, C227Y, G228C, K238R. It has 77% amino acid sequence identity with wild-type GFP from *A. victoria*.

References

1. Xia *et al.* (2002) *Mar. Biotechnol.* 4(2):155-162.

TagCFP-related products

TagCFP-related product line includes expression vectors, recombinant protein, and antibodies. For updated product information, please visit the Evrogen Web site (www.evrogen.com/TagCFP.shtml).

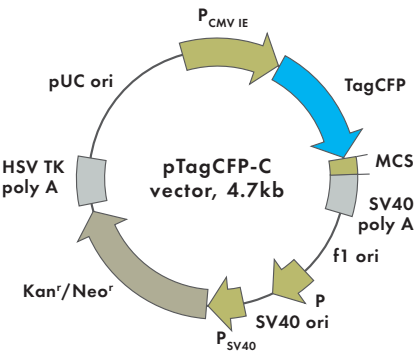
| Product | Cat.# | Description | Size | Page |
|---|-------|---|--------|------|
| TagCFP expression/source vectors | | | | |
| pTagCFP-C | FP111 | Mammalian expression vector encoding humanized TagCFP and allowing TagCFP expression and generation of fusions to the TagCFP C-terminus | 20 µg | A-10 |
| pTagCFP-N | FP112 | Mammalian expression vector encoding humanized TagCFP and allowing TagCFP expression and generation of fusions to the TagCFP N-terminus | 20 µg | A-10 |
| pTagCFP-actin | FP114 | Mammalian expression vector encoding humanized TagCFP fused with human cytoplasmic beta-actin | 20 µg | A-11 |
| pTagCFP-tubulin | FP115 | Mammalian expression vector encoding humanized TagCFP fused with human alpha-tubulin | 20 µg | A-11 |
| pTagCFP-mito | FP117 | Mammalian expression vector encoding humanized TagCFP fused with mitochondria localization signal | 20 µg | A-12 |
| Recombinant protein | | | | |
| rTagCFP | FP151 | Recombinant cyan fluorescent protein | 100 µg | A-12 |
| Antibodies against TagCFP | | | | |
| Anti-Tag(CGY)FP antibody | AB121 | Rabbit polyclonal antibody against TagCFP, TagGFP, TagYFP, and PS-CFP2. | 100 µg | D-6 |
| | AB122 | | 200 µg | |

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

Notice to Purchaser:

TagCFP-related products: These products are intended for research use only and 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 (Appendix A, page G-5).
 CMV Promoter: 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.

Expression/source vectors: pTagCFP-C



For vector sequence, please visit our Web site at www.evrogen.com/pTagCFP-C.shtml

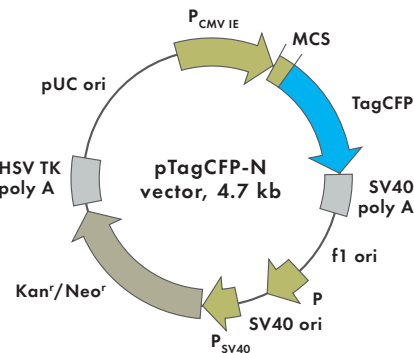
Multiple cloning site (MCS)



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

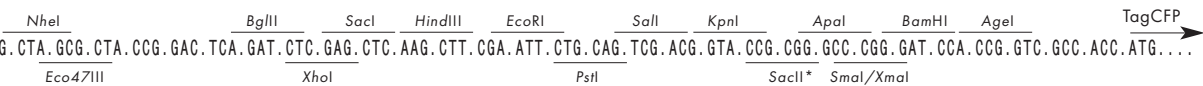
| Product | Cat.# | Size |
|--|--|-------|
| pTagCFP-C | FP111 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | TagCFP | |
| Reporter codon usage | mammalian | |
| Promoter for TagCFP | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | generation of fusions to the TagCFP C-terminus; expression of TagCFP or its fusions in mammalian cells | |

Expression/source vectors: pTagCFP-N



For vector sequence, please visit our Web site at www.evrogen.com/pTagCFP-N.shtml

Multiple cloning site (MCS)

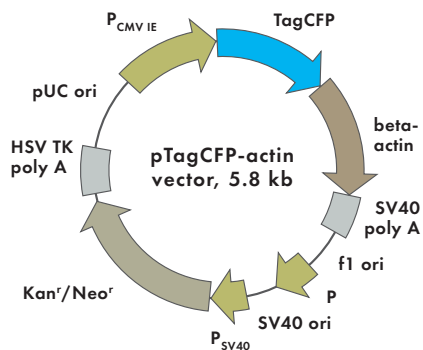


* — not unique sites.

| Product | Cat.# | Size |
|--|--|-------|
| pTagCFP-N | FP112 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | TagCFP | |
| Promoter for TagCFP | P _{CMV IE} | |
| Reporter codon usage | mammalian | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | generation of fusions to the TagCFP N-terminus; expression of TagCFP or its fusions in mammalian cells | |

Notice to Purchaser — please see page A-12

Expression/source vectors: pTagCFP-actin



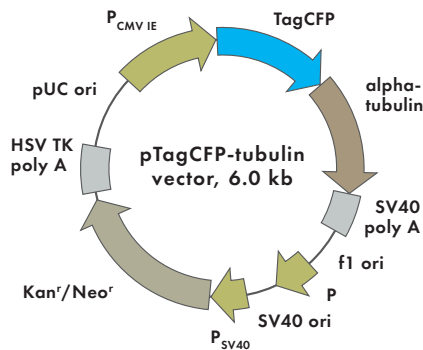
For vector sequence, please visit our Web site at www.evrogen.com/pTagCFP-actin.shtml

| Product | Cat.# | Size |
|---------------|-------|-------|
| pTagCFP-actin | FP114 | 20 µg |

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

| | |
|----------------------|--|
| Vector type | mammalian expression vector |
| Reporter | TagCFP-actin |
| 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 TagCFP fusion with beta-actin in mammalian cells under the control of CMV promoter for labeling of actin filaments; source of TagCFP-beta-actin fusion coding sequence |

Expression/source vectors: pTagCFP-tubulin



For vector sequence, please visit our Web site at www.evrogen.com/pTagCFP-tubulin.shtml

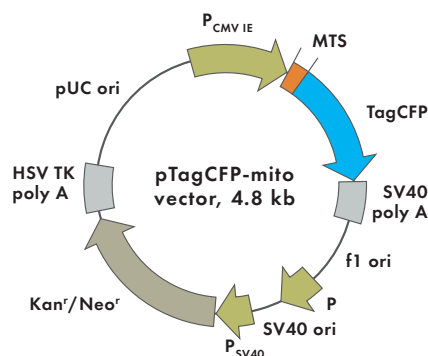
| Product | Cat.# | Size |
|-----------------|-------|-------|
| pTagCFP-tubulin | FP115 | 20 µg |

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

| | |
|----------------------|--|
| Vector type | mammalian expression vector |
| Reporter | TagCFP-tubulin |
| 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 TagCFP fusion with alpha-tubulin in mammalian cells under the control of CMV promoter for labeling of tubulin filaments; source of TagCFP-alpha-tubulin fusion coding sequence |

Notice to Purchaser — please see page A-12

Expression/source vectors: pTagCFP-mito



For vector sequence, please visit our Web site at www.evrogen.com/pTagCFP-mito.shtml

| Product | Cat.# | Size |
|--|---|-------|
| pTagCFP-mito | FP117 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | TagCFP fusion with mitochondrial targeting sequence (MTS) derived from the subunit VIII of human cytochrome C oxidase | |
| Reporter codon usage | mammalian | |
| Promoter for TagCFP | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | expression of mitochondria-targeted TagCFP in mammalian cells under the control of CMV promoter; source of mitochondria-targeted TagCFP coding sequence | |

Recombinant protein rTagCFP

| Product | Cat.# | Size |
|--|-------|--------|
| rTagCFP | FP151 | 100 µg |
| 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

Description

Recombinant TagCFP (rTagCFP) is 27-kDa cyan fluorescent protein. It has excitation and emission spectra identical to those of the expressed TagCFP. rTagCFP is suitable as control reagent for TagCFP expression using the TagCFP expression vectors.

rTagCFP is purified from transformed *E. coli* using organic extraction and hydrophobic chromatography. This method ensures high purity of the recombinant protein and maintenance of fluorescence. The protein concentration is measured by chromophore absorption. rTagCFP contains 6xHis tag at its N-terminus.

Notice to Purchaser:

TagCFP-related products: These products are intended for research use only and 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 (Appendix A, page G-5).
CMV Promoter: 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.

TagGFP

- Bright green fluorescence
- Monomeric protein with successful performance in fusions
- High pH stability and photostability
- Proven suitability to generate stably transfected cell lines
- Recommended for protein labeling

Protein description

TagGFP is an enhanced mutant of wild-type GFP-like protein from jellyfish *Aequorea macrodactyla* [1]. It possesses bright green fluorescence with excitation/emission maxima at 482 and 505 nm, respectively. TagGFP is optimized for expression at 37°C. It is more pH-stable than EGFP.

Main properties of TagGFP

| Characteristic | |
|------------------------|---|
| Molecular weight | 27 kDa |
| Polypeptide length | 238 aa |
| Fluorescence color | green |
| Excitation max | 482 nm |
| Emission max | 505 nm |
| Quantum yield | 0.59 |
| Extinction coefficient | 58 200 M ⁻¹ cm ⁻¹ |
| Brightness* | 34.3 |
| Brightness % of EGFP | 104 |
| pKa | 4.7 |
| Structure | monomer |
| Aggregation | no |
| Maturation at 37°C | fast |
| Photostability | high |

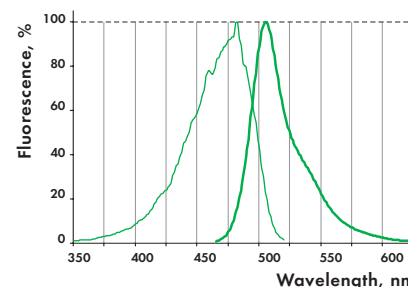
*Brightness is a product of extinction coefficient and quantum yield, divided by 1000.

Performance and use

Because of monomeric nature, TagGFP is mainly intended for protein localization studies and expression in long-term cell cultures. It can also be used for cell labeling and gene expression analysis, although TurboGFP is preferable for such applications because it matures faster and gives brighter fluorescent signal.

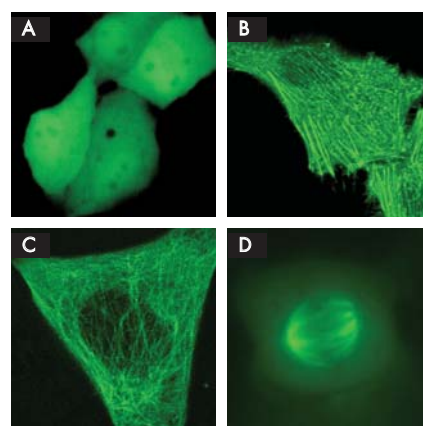
TagGFP can be easily expressed and detected in a wide range of organisms. Being expressed in mammalian cells, TagGFP shows brightness and maturation speed similar to those of EGFP. However, compared with EGFP, TagGFP matures more than two times faster in *E. coli* cells.

Mammalian cells transiently transfected with TagGFP expression vectors give bright fluorescent signals within 10-12 hrs after transfection. No cell



TagGFP normalized excitation (thin line) and emission (thick line) spectra.

Complete TagGFP spectra in Excel format can be downloaded from the Evrogen Web site at www.evrogen.com/TagGFP.shtml



TagGFP use for cell and protein labeling.

(A) — Whole-cell expression in transiently transfected HeLa cells; (B) — expression of TagGFP fusion with beta-actin in transiently transfected HeLa cells; (C) — expression of TagGFP fusion with alpha-tubulin in transiently transfected HeLa cells; (D) — expression of TagGFP fusion with alpha-tubulin in stably transfected MDCK canine kidney epithelial cells.

Photograph of stably transfected cell line was provided by Dr. Christian Petzelt (Marinpharm).

toxic effects and visible protein aggregation are observed. TagGFP performance in fusions has been demonstrated in the beta-actin and alpha-tubulin models. Please visit our Web site at www.evrogen.com/TagGFP.shtml to see 3D video of a cell expressing TagGFP-labeled alpha-tubulin filaments.

In addition, TagGFP suitability to generate stably transfected cells has been proven by Marinpharm company. Cell lines expressing TagGFP fusion with alpha-tubulin are commercially available.

| Application | Performance |
|----------------------------------|-------------|
| Cell labeling | |
| mammalian cells | +++ |
| bacterial cells | ++++ |
| Stable transfection | proved |
| Promoter activity testing | +++ |
| In fusions | ++++ |

Compatibility with existing filter sets and antibodies

TagGFP 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.

The protein can be recognized using Anti-Tag(CGY)FP antibody (Cat.# AB121-AB122, see page D-6 for description) available from Evrogen.

TagGFP licensing opportunities

Evrogen technology embodied in TagGFP 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.

TagGFP comprises the following amino acid substitutions as compared with wild-type *A. macrodactyla* GFP (AY013824): K3G, T9A, F64L, S65C, F99L, M128K, N144S, K162E, I167T, T214A, F220L, F223S, G228C, K238R. It has 79% amino acid sequence identity with wild-type GFP from *A. victoria*.

References
 1. Xia *et al.* (2002) *Mar. Biotechnol.* 4(2):155-162.

TagGFP-related products

TagGFP-related product line includes expression vectors, recombinant protein, and antibodies. For updated product information, please visit the Evrogen Web site (www.evrogen.com/TagGFP.shtml).

| Product | Cat.# | Description | Size | Page |
|---|-------|---|--------|------|
| TagGFP expression/source vectors | | | | |
| pTagGFP-C | FP121 | Mammalian expression vector encoding humanized TagGFP and allowing TagGFP expression and generation of fusions to the TagGFP C-terminus | 20 µg | A-16 |
| pTagGFP-N | FP122 | Mammalian expression vector encoding humanized TagGFP and allowing TagGFP expression and generation of fusions to the TagGFP N-terminus | 20 µg | A-16 |
| pTagGFP-actin | FP124 | Mammalian expression vector encoding humanized TagGFP fused with human cytoplasmic beta-actin | 20 µg | A-17 |
| pTagGFP-tubulin | FP125 | Mammalian expression vector encoding humanized TagGFP fused with human alpha-tubulin | 20 µg | A-17 |
| pTagGFP-mito | FP127 | Mammalian expression vector encoding humanized TagGFP fused with mitochondria localization signal | 20 µg | A-18 |
| Recombinant protein | | | | |
| rTagGFP | FP152 | Recombinant green fluorescent protein | 100 µg | A-18 |
| Antibodies against TagGFP | | | | |
| Anti-Tag(CGY)FP antibody | AB121 | Rabbit polyclonal antibody against | 100 µg | D-6 |
| | AB122 | TagCFP, TagGFP, TagYFP, and PS-CFP2 | 200 µg | |

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

Third party products: stably transfected cell lines expressing TagGFP

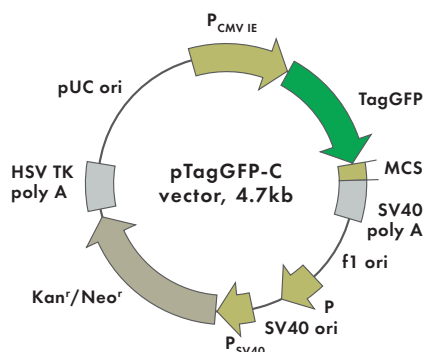
| Cell line | Source | Description |
|---------------|--------|---|
| MDCK-TAG-Tu | canine | MDCK canine kidney epithelial cells expressing TagGFP fusion with alpha-tubulin |
| T24-TG-TAG-Tu | human | T24 human bladder carcinoma expressing TagGFP fusion with alpha-tubulin |

Cell lines are manufactured by Marinpharm GmbH (Berlin, Germany) under the Evrogen license.

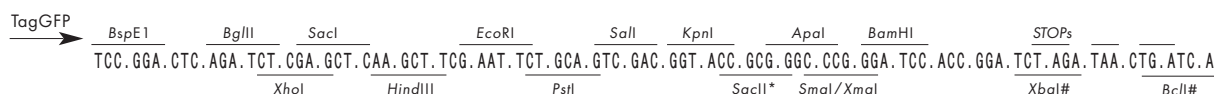
Notice to Purchaser:

TagGFP-related products: These products are intended for research use only and 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 (Appendix A, page G-5).

CMV Promoter: 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.

Expression/source vectors: pTagGFP-C

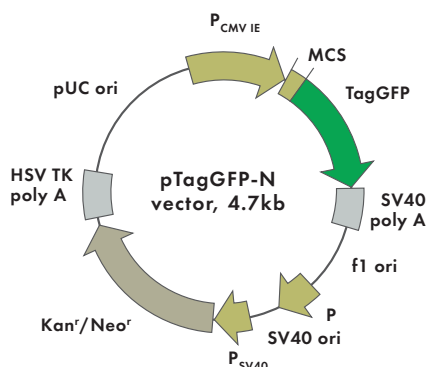
For vector sequence, please visit our Web site at www.evrogen.com/pTagGFP-C.shtml

Multiple cloning site (MCS)

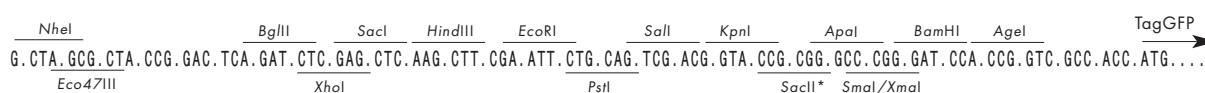
* — not unique sites;

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

| Product | Cat.# | Size |
|--|--|-------|
| pTagGFP-C | FP121 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | TagGFP | |
| Reporter codon usage | mammalian | |
| Promoter for TagGFP | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | generation of fusions to the TagGFP C-terminus; expression of TagGFP or its fusions in mammalian cells | |

Expression/source vectors: pTagGFP-N

For vector sequence, please visit our Web site at www.evrogen.com/pTagGFP-N.shtml

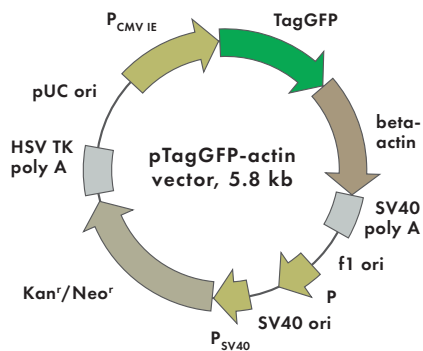
Multiple cloning site (MCS)

* — not unique sites.

| Product | Cat.# | Size |
|--|--|-------|
| pTagGFP-N | FP122 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | TagGFP | |
| Reporter codon usage | mammalian | |
| Promoter for TagGFP | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | generation of fusions to the TagGFP N-terminus; expression of TagGFP or its fusions in mammalian cells | |

Notice to Purchaser — please see page A-18

Expression/source vectors: pTagGFP-actin



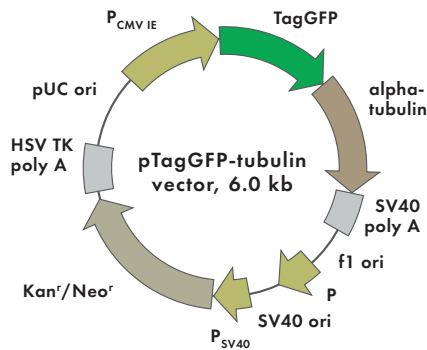
For vector sequence, please visit our Web site at www.evrogen.com/pTagGFP-actin.shtml

| Product | Cat.# | Size |
|---------------|-------|-------|
| pTagGFP-actin | FP124 | 20 µg |

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

| | |
|----------------------|--|
| Vector type | mammalian expression vector |
| Reporter | TagGFP-actin |
| 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 TagGFP fusion with beta-actin in mammalian cells under the control of CMV promoter for labeling of actin filaments; source of TagGFP-beta-actin fusion coding sequence |

Expression/source vectors: pTagGFP-tubulin



For vector sequence, please visit our Web site at www.evrogen.com/pTagGFP-tubulin.shtml

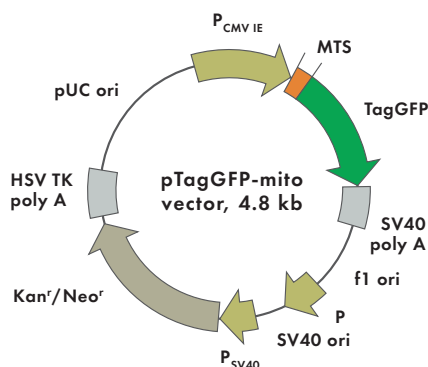
| Product | Cat.# | Size |
|-----------------|-------|-------|
| pTagGFP-tubulin | FP125 | 20 µg |

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

| | |
|----------------------|--|
| Vector type | mammalian expression vector |
| Reporter | TagGFP-tubulin |
| 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 TagGFP fusion with alpha-tubulin in mammalian cells under the control of CMV promoter for labeling of tubulin filaments; source of TagGFP-alpha-tubulin fusion coding sequence |

Notice to Purchaser — please see page A-18

Expression/source vectors: pTagGFP-mito



For vector sequence, please visit our Web site at www.evrogen.com/pTagGFP-mito.shtml

| Product | Cat.# | Size |
|--------------|-------|-------|
| pTagGFP-mito | FP127 | 20 µg |

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

| | |
|-------------------------|---|
| Vector type | mammalian expression vector |
| Reporter | TagGFP fusion with mitochondrial targeting sequence (MTS) derived from the subunit VIII of human cytochrome C oxidase |
| Reporter codon usage | mammalian |
| Promoter for TagGFP-MTS | P _{CMV IE} |
| Host cells | mammalian |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori |
| Use | expression of mitochondria-targeted TagGFP in mammalian cells under the control of CMV promoter; source of mitochondria-targeted TagGFP coding sequence |

Recombinant protein rTagGFP

| Product | Cat.# | Size |
|---------|-------|--------|
| rTagGFP | FP152 | 100 µg |

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

Description

Recombinant TagGFP (rTagGFP) is 27-kDa green fluorescent protein. It has excitation and emission spectra identical to those of the expressed TagGFP. rTagGFP is suitable as control reagent for TagGFP expression using the TagGFP expression vectors.

rTagGFP is purified from transformed *E. coli* using organic extraction and hydrophobic chromatography. This method ensures high purity of the recombinant protein and maintenance of fluorescence. The protein concentration is measured by chromophore absorption. rTagGFP contains 6xHis tag at its N-terminus.

Notice to Purchaser:

TagGFP-related products: These products are intended for research use only and 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 (Appendix A, page G-5).
CMV Promoter: 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.

TagYFP

- Bright yellow fluorescence
- Monomeric protein with successful performance in fusions
- High pH stability and photostability
- Recommended for protein labeling

Protein description

TagYFP is a monomeric yellow fluorescent protein developed from the green fluorescent protein TagGFP. TagYFP possesses single excitation maximum at 508 nm, and emission maximum at 524 nm. TagYFP is more pH stable than EYFP.

Main properties of TagYFP

| Characteristic | |
|------------------------|---|
| Molecular weight | 27 kDa |
| Polypeptide length | 239 aa |
| Fluorescence color | yellow |
| Excitation max | 508 nm |
| Emission max | 524 nm |
| Quantum yield | 0.62 |
| Extinction coefficient | 64 000 M ⁻¹ cm ⁻¹ |
| Brightness* | 39.7 |
| Brightness % of EGFP | 120 |
| pKa | 5.5 |
| Structure | monomer |
| Aggregation | no |
| Maturation at 37°C | fast |
| Photostability | high |

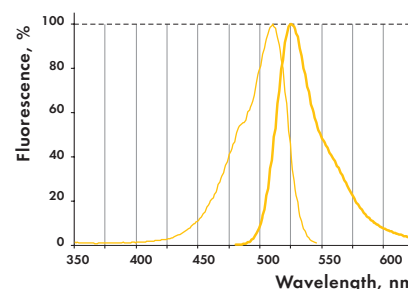
*Brightness is a product of extinction coefficient and quantum yield, divided by 1000.

Performance and use

TagYFP is mainly intended for protein labeling. 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.

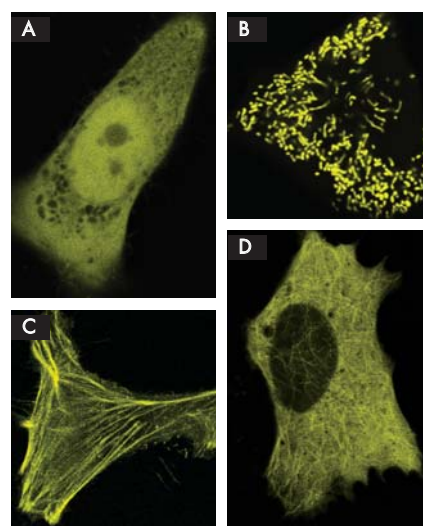
TagYFP can be easily expressed and detected in a wide range of organisms. Mammalian cells transiently transfected with TagYFP expression vectors give bright fluorescent signals within 10-12 hrs after transfection. No cell toxic effects and visible protein aggregation are observed.

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



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/TagYFP.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 beta-actin in transiently transfected 3T3 cells; (D) — confocal microscopy of TagYFP fusion with the alpha-tubulin in transiently transfected 3T3 cells.

| Application | Performance |
|----------------------------------|-------------|
| Cell labeling | |
| mammalian cells | +++ |
| bacterial cells | ++++ |
| Stable transfection | proved |
| Promoter activity testing | +++ |
| In fusions | ++++ |

Compatibility with existing filter sets and antibodies

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

The protein can be recognized using Anti-Tag(CGY)FP antibody (Cat.# AB121-AB122, see page D-6 for description) available from Evrogen.

TagYFP licensing opportunities

Evrogen technology embodied in TagYFP 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.

TagYFP comprises the following amino acid substitutions as compared with wild-type *A. macrodactyla* GFP (AY013824): K3G, T9A, F64L, S65T, I68V, E76K, F99L, M128K, M153T, N144S, K162E, I167T, T203Y, A206D, T214A, F220L, F223S, F224V, G228S, K238R. It has 78% amino acid sequence identity with wild-type GFP from *A. victoria*.

TagYFP-related products

TagYFP-related product line includes expression vectors, recombinant protein, and antibodies. For updated product information, please visit the Evrogen Web site (www.evrogen.com/TagYFP.shtml).

| Product | Cat.# | Description | Size | Page |
|---|-------|---|--------|------|
| TagYFP expression/source vectors | | | | |
| pTagYFP-C | FP131 | Mammalian expression vector encoding humanized TagYFP and allowing TagYFP expression and generation of fusions to the TagYFP C-terminus | 20 µg | A-22 |
| pTagYFP-N | FP132 | Mammalian expression vector encoding humanized TagYFP and allowing TagYFP expression and generation of fusions to the TagYFP N-terminus | 20 µg | A-22 |
| pTagYFP-actin | FP134 | Mammalian expression vector encoding humanized TagYFP fused with human cytoplasmic beta-actin | 20 µg | A-23 |
| pTagYFP-tubulin | FP135 | Mammalian expression vector encoding humanized TagYFP fused with human alpha-tubulin | 20 µg | A-23 |
| pTagYFP-mito | FP137 | Mammalian expression vector encoding humanized TagYFP fused with a mitochondria localization signal | 20 µg | A-24 |
| Recombinant protein | | | | |
| rTagYFP | FP153 | Recombinant yellow fluorescent protein | 100 µg | A-24 |
| Antibodies against TagYFP | | | | |
| Anti-Tag(CGY)FP | AB121 | Rabbit polyclonal antibody against TagCFP, TagGFP, | 100 µg | D-6 |
| antibody | AB122 | TagYFP, and PS-CFP2 | 200 µg | |

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

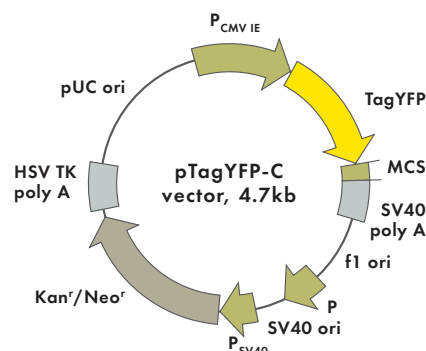
Third party products: stably transfected cell lines expressing TagYFP

| Cell line | Source | Description |
|--------------------|--------|---|
| U205-TAG-YFP-Actin | human | Human osteosarcoma line U205 expressing TagYFP fusion with beta-actin |

Cell lines are manufactured by Marinpharm GmbH (Berlin, Germany) under the Evrogen license.

Notice to Purchaser:

TagYFP-related products: These products are intended for research use only and 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 (Appendix A, page G-5).
CMV Promoter: 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.

Expression/source vectors: pTagYFP-C

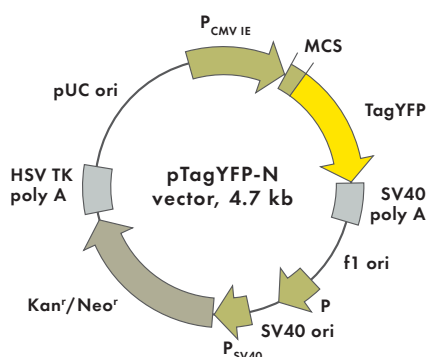
For vector sequence, please visit our Web site at www.evrogen.com/pTagYFP-C.shtml

| Product | Cat.# | Size |
|--|--|-------|
| pTagYFP-C | FP131 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | TagYFP | |
| Reporter codon usage | mammalian | |
| Promoter for TagYFP | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | generation of fusions to the TagYFP C-terminus; expression of TagYFP or its fusions in mammalian cells | |

Multiple cloning site (MCS)

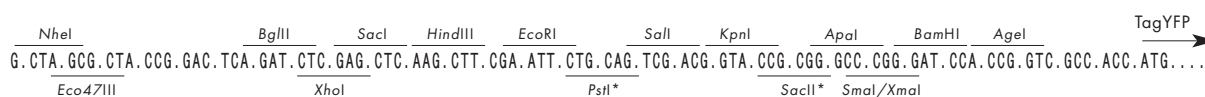
* — not unique sites;

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

Expression/source vectors: pTagYFP-N

For vector sequence, please visit our Web site at www.evrogen.com/pTagYFP-N.shtml

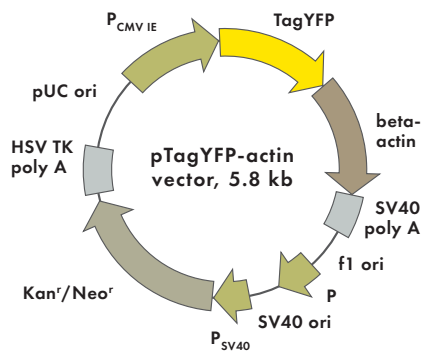
| Product | Cat.# | Size |
|--|--|-------|
| pTagYFP-N | FP132 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | TagYFP | |
| Reporter codon usage | mammalian | |
| Promoter for TagYFP | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | generation of fusions to the TagYFP N-terminus; expression of TagYFP or its fusions in mammalian cells | |

Multiple cloning site (MCS)

* — not unique sites.

Notice to Purchaser — please see page A-24

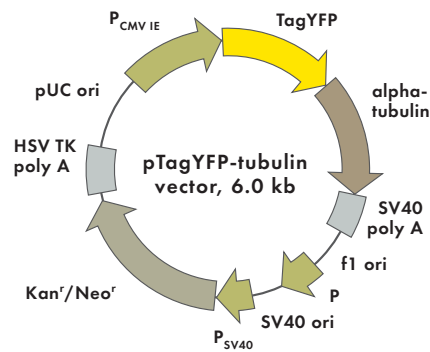
Expression/source vectors: pTagYFP-actin



For vector sequence, please visit our Web site at www.evrogen.com/pTagYFP-actin.shtml

| Product | Cat.# | Size |
|--|--|-------|
| pTagYFP-actin | FP134 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | TagYFP-actin | |
| 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 TagYFP fusion with beta-actin in mammalian cells under the control of CMV promoter for labeling of actin filaments; source of TagYFP-beta-actin fusion coding sequence | |

Expression/source vectors: pTagYFP-tubulin

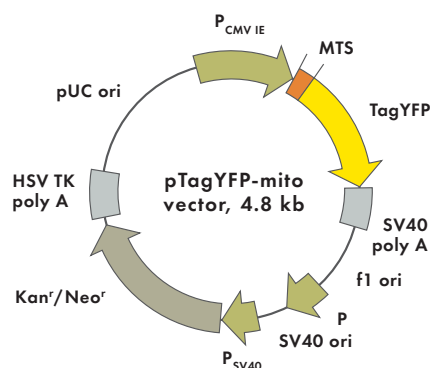


For vector sequence, please visit our Web site at www.evrogen.com/pTagYFP-tubulin.shtml

| Product | Cat.# | Size |
|--|--|-------|
| pTagYFP-tubulin | FP135 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | TagYFP-tubulin | |
| 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 TagYFP fusion with alpha-tubulin in mammalian cells under the control of CMV promoter for labeling of tubulin filaments; source of TagYFP-alpha-tubulin fusion coding sequence | |

Notice to Purchaser — please see page A-24

Expression/source vectors: pTagYFP-mito



For vector sequence, please visit our Web site at www.evrogen.com/pTagYFP-mito.shtml

| Product | Cat.# | Size |
|--------------|-------|-------|
| pTagYFP-mito | FP137 | 20 µg |

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

| | |
|-------------------------|---|
| Vector type | mammalian expression vector |
| Reporter | TagYFP fusion with mitochondrial targeting sequence (MTS) derived from the subunit VIII of human cytochrome C oxidase |
| Reporter codon usage | mammalian |
| Promoter for TagYFP-MTS | P _{CMV IE} |
| Host cells | mammalian |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori |
| Use | expression of mitochondria-targeted TagYFP in mammalian cells under the control of CMV promoter; source of mitochondria-targeted TagYFP coding sequence |

Recombinant protein rTagYFP

| Product | Cat.# | Size |
|---------|-------|--------|
| rTagYFP | FP153 | 100 µg |

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

Description

Recombinant TagYFP (rTagYFP) is 27-kDa yellow fluorescent protein. It has excitation and emission spectra identical to those of the expressed TagYFP. rTagYFP is suitable as control reagent for TagYFP expression using the TagYFP expression vectors.

TagYFP is purified from transformed *E. coli* using organic extraction and hydrophobic chromatography. This method ensures high purity of the recombinant protein and maintenance of fluorescence. The protein concentration is measured by chromophore absorption. rTagYFP contains 6xHis tag at its N-terminus.

Notice to Purchaser:

TagYFP-related products: These products are intended for research use only and 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 (Appendix A, page G-5).
CMV Promoter: 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.

TagRFP

- Bright red (orange) fluorescence
- Monomeric protein with successful performance in fusions
- High pH stability
- Proven suitability to generate stably transfected cell lines
- Recommended for protein labeling

Protein description

TagRFP is a novel monomeric red fluorescent protein generated from the wild-type RFP from sea anemone *Entacmaea quadricolor* [1]. TagRFP is about three times brighter than mCherry protein [2], which makes it the brightest monomeric red fluorescent protein available so far. TagRFP becomes clearly detectable in mammalian cells as early as within 10-12 hrs after transfection.

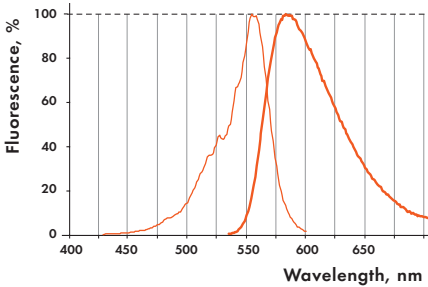
Main properties of TagRFP

| Characteristic | |
|------------------------|--|
| Molecular weight | 27 kDa |
| Polypeptide length | 237 aa |
| Fluorescence color | red (orange) |
| Excitation max | 555 nm |
| Emission max | 584 nm |
| Quantum yield | 0.48 |
| Extinction coefficient | 100 000 M ⁻¹ cm ⁻¹ |
| Brightness* | 48.0 |
| Brightness % of EGFP | 145 |
| pKa | 3.8 |
| Structure | monomer |
| Aggregation | no |
| Maturation at 37°C | fast |
| Photostability | medium |

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

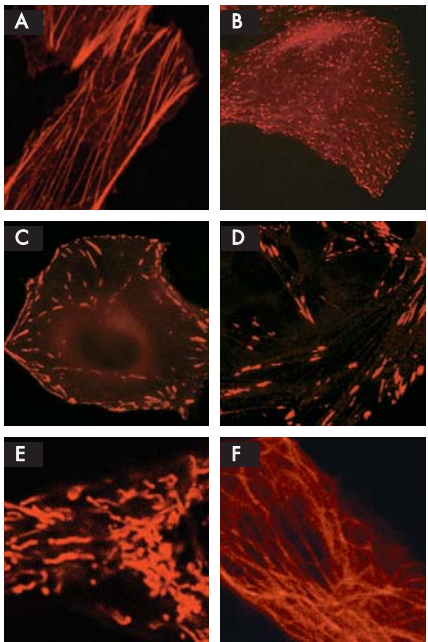
Performance and use

| Application | Performance |
|---------------------------|-------------|
| Cell labeling | |
| mammalian cells | +++ |
| bacterial cells | ++++ |
| Stable transfection | proved |
| Promoter activity testing | +++ |
| In fusions | ++++ |



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/TagRFP.shtml



TagRFP use for cell and protein labeling.

(A) — HeLa cells expressing TagRFP fusion with beta-actin; image from [1]; (B) — HeLa cells expressing TagRFP fusion with end-binding protein 3 (EB3); (C) — HeLa cells expressing TagRFP fusion with vinculin; (D) —HeLa cells expressing TagRFP fusion with zyxin; (E) — HeLa cells expressing TagRFP targeted to mitochondria; (F) — HeLa cells expressing TagRFP fusion with alpha-tubulin. Images B-D were kindly provided by Michael W. Davidson (Florida State University).

TagRFP is mainly intended for protein labeling. It can also be used for cell and organelle labeling and for tracking the promoter activity; however, TurboRFP is preferable for these applications as it is brighter and more photostable than TagRFP.

Successful performance of TagRFP in fusions has been demonstrated in fibrillarin, Bid protein, beta-actin, alpha-tubulin, and other models.

Compatibility with existing filter sets and antibodies

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 can be recognized using Anti-tRFP antibody (Cat.# AB231-AB232, page D-9) available from Evrogen.

TagRFP licensing opportunities

Evrogen technology embodied in TagRFP is available for expanded and commercial use with an adaptable licensing program. Benefits from flexible and market-driven license options are offered for upgrade and novel development of products and applications.

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

References

1. Merzlyak *et al.* (2007) *Nat. Methods.* 4(7): 555-557.
2. Shaner *et al.* (2004) *Nat. Biotechnol.* 12: 1567-1572.

TagRFP-related products

TagRFP-related product line includes expression and source vectors, recombinant protein, and antibodies. For updated product information, please visit the Evrogen Web site (www.evrogen.com/TagRFP.shtml).

| Product | Cat.# | Description | Size | Page |
|---|-------|---|--------|------|
| TagRFP expression/source vectors | | | | |
| pTagRFP-C | FP141 | Mammalian expression vector encoding humanized TagRFP and allowing TagRFP expression and generation of fusions to the TagRFP C-terminus | 20 µg | A-28 |
| pTagRFP-N | FP142 | Mammalian expression vector encoding humanized TagRFP and allowing TagRFP expression and generation of fusions to the TagRFP N-terminus | 20 µg | A-28 |
| pTagRFP-actin | FP144 | Mammalian expression vector encoding humanized TagRFP fused with human beta-actin | 20 µg | A-29 |
| pTagRFP-tubulin | FP145 | Mammalian expression vector encoding humanized TagRFP fused with human alpha-tubulin | 20 µg | A-29 |
| pTagRFP-mito | FP147 | Mammalian expression vector encoding humanized TagRFP fused with mitochondria localization signal | 20 µg | A-30 |
| Recombinant protein | | | | |
| rTagRFP | FP154 | Recombinant red fluorescent protein TagRFP | 100 µg | A-30 |
| Antibodies against TagRFP | | | | |
| Anti-tRFP | AB231 | Rabbit polyclonal antibody against TagRFP, TagFP635, | 100 µg | D-9 |
| antibody | AB232 | TurboRFP, TurboFP602, and TurboFP635 proteins | 200 µg | |

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

Third party products: stably transfected cell lines expressing TagRFP

| Cell line | Source | Description |
|----------------------|--------|--|
| U205-TAG-RFP-Actin | human | Human osteosarcoma line U205 expressing TagRFP fusion with beta-actin |
| U205-TAG-RFP-Tubulin | human | Human osteosarcoma line U205 expressing TagRFP fusion with alpha-tubulin |

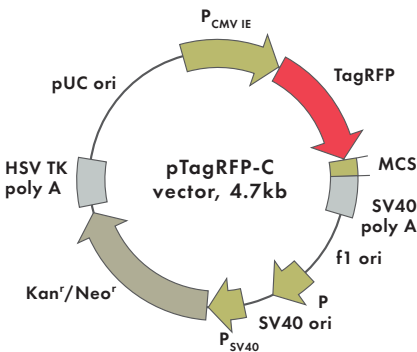
Cell lines are manufactured by Marinpharm GmbH (Berlin, Germany) under the Evrogen license.

Notice to Purchaser:

TagRFP-related products: These products are intended for research use only and 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 (Appendix A, page G-5).

CMV Promoter: 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.

Expression/source vectors: pTagRFP-C



For vector sequence, please visit our Web site at www.evrogen.com/pTagRFP-C.shtml

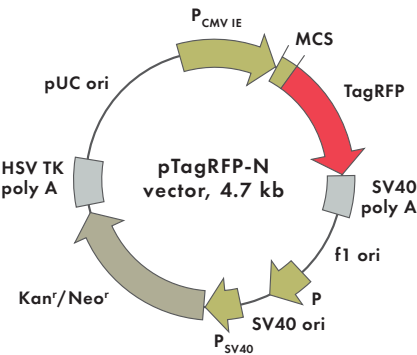
Multiple cloning site (MCS)



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

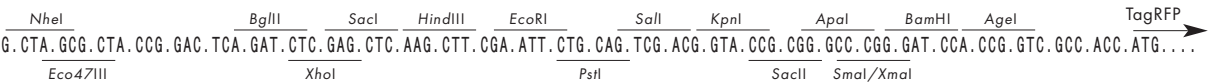
| Product | Cat.# | Size |
|--|--|-------|
| pTagRFP-C | FP141 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | TagRFP | |
| Reporter codon usage | mammalian | |
| Promoter for TagRFP | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | generation of fusions to the TagRFP C-terminus; expression of TagRFP or its fusions in mammalian cells | |

Expression/source vectors: pTagRFP-N



For vector sequence, please visit our Web site at www.evrogen.com/pTagRFP-N.shtml

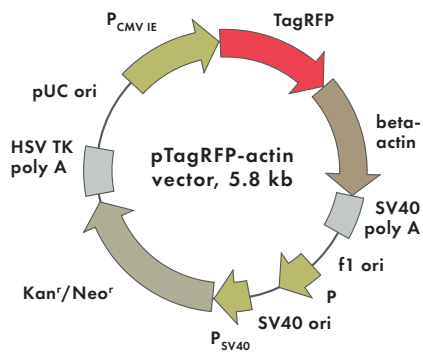
Multiple cloning site (MCS)



| Product | Cat.# | Size |
|--|--|-------|
| pTagRFP-N | FP142 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | TagRFP | |
| Reporter codon usage | mammalian | |
| Promoter for TagRFP | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | generation of fusions to the TagRFP N-terminus; expression of TagRFP or its fusions in mammalian cells | |

Notice to Purchaser — please see page A-30

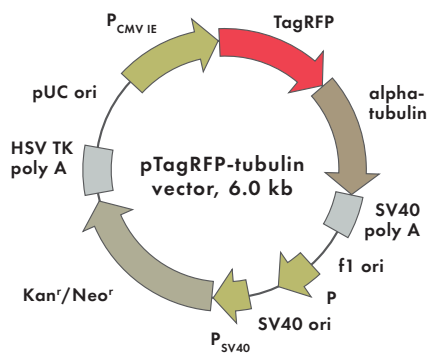
Expression/source vectors: pTagRFP-actin



For vector sequence, please visit our Web site at www.evrogen.com/pTagRFP-actin.shtml

| Product | Cat.# | Size |
|--|--|-------|
| pTagRFP-actin | FP144 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | TagRFP-actin | |
| 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 TagRFP fusion with beta-actin in mammalian cells under the control of CMV promoter for labeling of actin filaments; source of TagRFP-beta-actin fusion coding sequence | |

Expression/source vectors: pTagRFP-tubulin

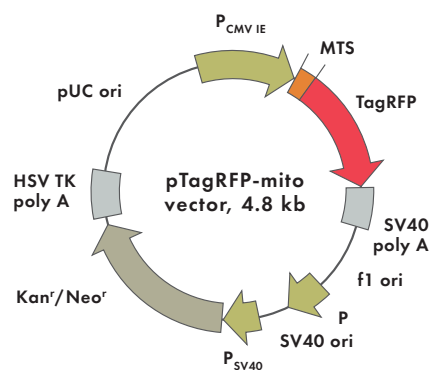


For vector sequence, please visit our Web site at www.evrogen.com/pTagRFP-tubulin.shtml

| Product | Cat.# | Size |
|--|--|-------|
| pTagRFP-tubulin | FP145 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | TagRFP-tubulin | |
| 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 TagRFP fusion with alpha-tubulin in mammalian cells under the control of CMV promoter for labeling of tubulin filaments; source of TagRFP-alpha-tubulin fusion coding sequence | |

Notice to Purchaser — please see page A-30

Expression/source vectors: pTagRFP-mito



For vector sequence, please visit our Web site at www.evrogen.com/pTagRFP-mito.shtml

| Product | Cat.# | Size |
|--|---|-------|
| pTagRFP-mito | FP147 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | TagRFP fusion with mitochondrial targeting sequence (MTS) derived from the subunit VIII of human cytochrome C oxidase | |
| Reporter codon usage | mammalian | |
| Promoter for TagRFP | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | expression of mitochondria-targeted TagRFP in mammalian cells under the control of CMV promoter; source of mitochondria-targeted TagRFP coding sequence | |

Recombinant protein rTagRFP

| Product | Cat.# | Size |
|--|-------|--------|
| rTagRFP | FP154 | 100 µg |
| 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

Description

Recombinant TagRFP (rTagRFP) is a 27-kDa red fluorescent protein. It has excitation and emission spectra identical to those of the expressed TagRFP. rTagRFP is suitable as control reagent for TagRFP expression using the TagRFP expression vectors.

rTagRFP is purified from transformed *E. coli* using organic extraction and hydrophobic chromatography. This method ensures high purity of the recombinant protein and maintenance of fluorescence. The protein concentration is measured by chromophore absorption. rTagRFP contains 6xHis tag at its N-terminus.

Notice to Purchaser:
 TagRFP-related products: These products are intended for research use only and 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 (Appendix A, page G-5).
 CMV Promoter: 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.

TagFP635

- Bright far-red fluorescence
- Monomeric protein with successful performance in fusions
- High photostability
- Recommended for protein labeling
- Fluorescent signal is easily distinguished from background fluorescence

Protein description

TagFP635 (scientific name mKate) is a novel monomeric far-red fluorescent protein generated from the wild-type RFP from sea anemone *Entacmaea quadricolor* [1]. TagFP635 is 6.4-fold brighter than mPlum, 1.25-fold brighter and much more photostable than mRaspberry [1,2], which makes it the best monomeric far-red fluorescent protein available so far. TagFP635 fluorescence allows easy and reliable separation from standard green fluorescent labels in dual-color high-throughput assays. TagFP635 becomes clearly detectable in mammalian cells as early as within 12-14 hrs after transfection.

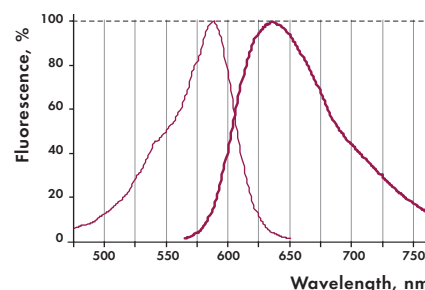
Main properties of TagFP635

| Characteristic | |
|------------------------|---|
| Molecular weight | 27 kDa |
| Polypeptide length | 237 aa |
| Fluorescence color | far-red |
| Excitation max | 588 nm |
| Emission max | 635 nm |
| Quantum yield | 0.33 |
| Extinction coefficient | 45 000 M ⁻¹ cm ⁻¹ |
| Brightness* | 14.9 |
| Brightness % of EGFP | 45 |
| pKa | 6.0 |
| Structure | monomer |
| Aggregation | no |
| Maturation at 37°C | fast |
| Photostability | high |

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

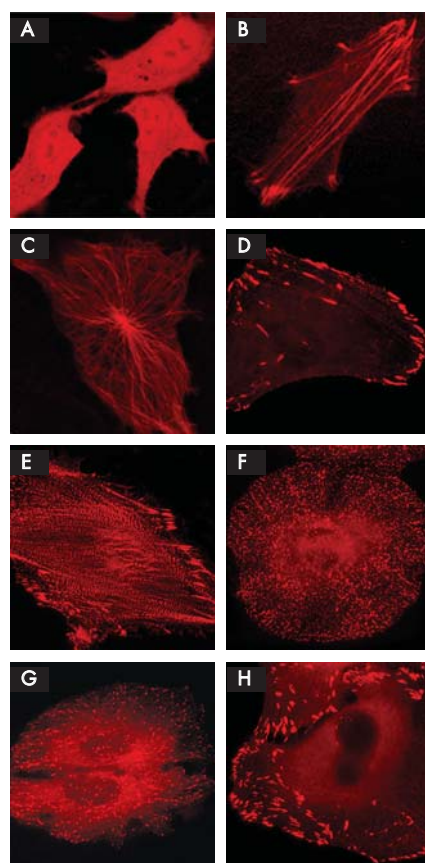
Performance and use

TagFP635 is intended for protein labeling. Successful performance of TagFP635 in fusions has been demonstrated for many proteins. It can also be used for cell and organelle labeling and for tracking the promoter activity; however, TurboFP635 is preferable for these applications as it is brighter and more pH-stable than TagFP635.



TagFP635 normalized excitation (thin line) and emission (thick line) spectra.

Complete TagFP635 spectra in Excel format can be downloaded from the Evrogen Web site at www.evrogen.com/TagFP635.shtml



TagFP635 use for cell and protein labeling.

(A) — Whole-cell expression in transiently transfected Phoenix Eco cells; (B) — fusion with beta-actin in transiently transfected HeLa cells; (C) — fusion with alpha-tubulin in transiently transfected 3T3 cells; (D) — fusion with zyxin in transiently transfected HeLa cells; (E) — fusion with alpha-actinin in transiently transfected HeLa cells; (F) — fusion with clathrin in transiently transfected HeLa cells; (G) — fusion with end-binding protein 3 (EB3) in transiently transfected HeLa cells; (H) — fusion with vinculin in transiently transfected HeLa cells. Images A-C are from ref. 1. Images D-H were kindly provided by Michael W. Davidson (Florida State University).

| Application | Performance |
|----------------------------------|-------------|
| Cell labeling | |
| mammalian cells | +++ |
| bacterial cells | ++++ |
| Stable transfection | not tested |
| Promoter activity testing | +++ |
| In fusions | ++++ |

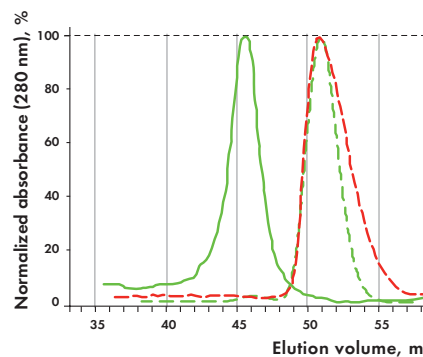
Compatibility with existing filter sets and antibodies

Recommended Omega Optical filter sets are QMAX-Red and XF102-2. TagFP635 can also be detected using Texas Red filter sets or similar. TagFP635 can be recognized using Anti-tRFP antibody (Cat.# AB231-AB232, page D-9) available from Evrogen.

TagFP635 licensing opportunities

Evrogen technology embodied in TagFP635 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.



Gel-filtration of TurboGFP (dimer, solid green line), EGFP (monomer, dashed green line), and TagFP635 (monomer, dashed red line) [1].

References

1. Shcherbo *et al.* (2007) *Nat. Methods* 4(9): 741-746.
2. Shaner *et al.* (2004) *Nat. Biotechnol.* 12: 1567-1572.

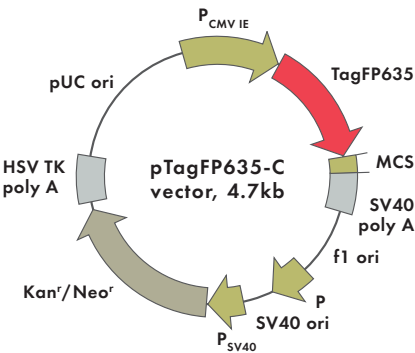
TagFP635-related products

TagFP635-related product line includes expression vectors, and antibodies. For updated product information, please visit the Evrogen Web site (www.evrogen.com/TagFP635.shtml).

| Product | Cat.# | Description | Size | Page |
|---|-------|---|--------|------|
| TagFP635 expression/source vectors | | | | |
| pTagFP635-C | FP161 | Mammalian expression vector encoding humanized TagFP635 and allowing TagFP635 expression and generation of fusions to the TagFP635 C-terminus | 20 µg | A-33 |
| pTagF635P-N | FP162 | Mammalian expression vector encoding humanized TagFP635 and allowing TagFP635 expression and generation of fusions to the TagFP635 N-terminus | 20 µg | A-34 |
| Antibodies against TagFP635 | | | | |
| Anti-IRFP antibody | AB231 | Rabbit polyclonal antibody against TagRFP, TagFP635, | 100 µg | D-9 |
| | AB232 | TurboRFP, TurboFP602, and TurboFP635 proteins | 200 µg | |

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

Expression/source vectors: pTagFP635-C



For vector sequence please visit our website at www.evrogen.com/pTagFP635-C.shtml

| Product | Cat.# | Size |
|--|--|-------|
| pTagFP635-C | FP161 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | TagFP635 | |
| Reporter codon usage | mammalian | |
| Promoter for TagFP635 | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | generation of fusions to the TagFP635 C-terminus; expression of TagFP635 or its fusions in mammalian cells | |

Multiple cloning site (MCS)



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

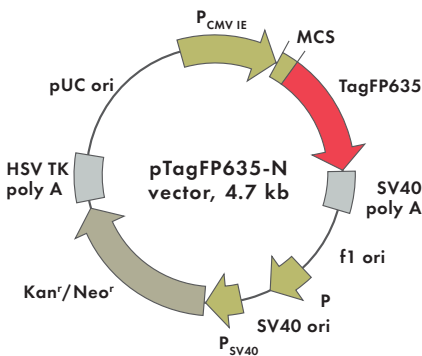
Notice to Purchaser:

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CMV Promoter: 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

ATTENTION: Safety Officer. EVROGEN JSC (Moscow, Russia) hereby confirms that to the best of our knowledge these products do not require a Material Safety Data Sheet. However, all of the properties of these products (and, if applicable, each of its 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.

Expression/source vectors: pTagFP635-N



For vector sequence please visit our website at www.evrogen.com/pTagFP635-N.shtml

| Product | Cat.# | Size |
|--|--|-------|
| pTagFP635-N | FP162 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | TagFP635 | |
| Reporter codon usage | mammalian | |
| Promoter for TagFP635 | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | generation of fusions to the TagFP635 N-terminus; expression of TagFP635 or its fusions in mammalian cells | |

Multiple cloning site (MCS)

NheI

GCTA

CGC

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

TagFP635

Eco47III

XhoI

EcoRI

PstI

SacII

SmaI/XmaI

NcoI*

* — not unique sites.

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basic fluorescent proteins

superbright and
fast-maturing
fluorescent
proteins for cell
labeling and
monitoring gene
expression



TurboColors 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.

TurboColors proteins available:

- green fluorescent protein TurboGFP

source — copepod *Pontellina plumata*

excitation max — 482 nm

emission max — 502 nm

- yellow fluorescent protein TurboYFP

source — jellyfish *Phialidium* sp.

excitation max — 525 nm

emission max — 538 nm

- red fluorescent protein TurboRFP

source — sea anemone *Entacmaea quadricolor*

excitation max — 553 nm

emission max — 574 nm

- true-red fluorescent protein TurboFP602

source — sea anemone *Entacmaea quadricolor*

excitation max — 574 nm

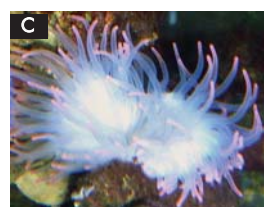
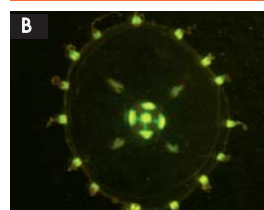
emission max — 602 nm

- far-red fluorescent protein TurboFP635

source — sea anemone *Entacmaea quadricolor*

excitation max — 588 nm

emission max — 635 nm



Marine organisms — sources of TurboColors proteins.

(A) — Planktonic copepod *Pontellina plumata*;

(B) — jellyfish *Phialidium* sp.;

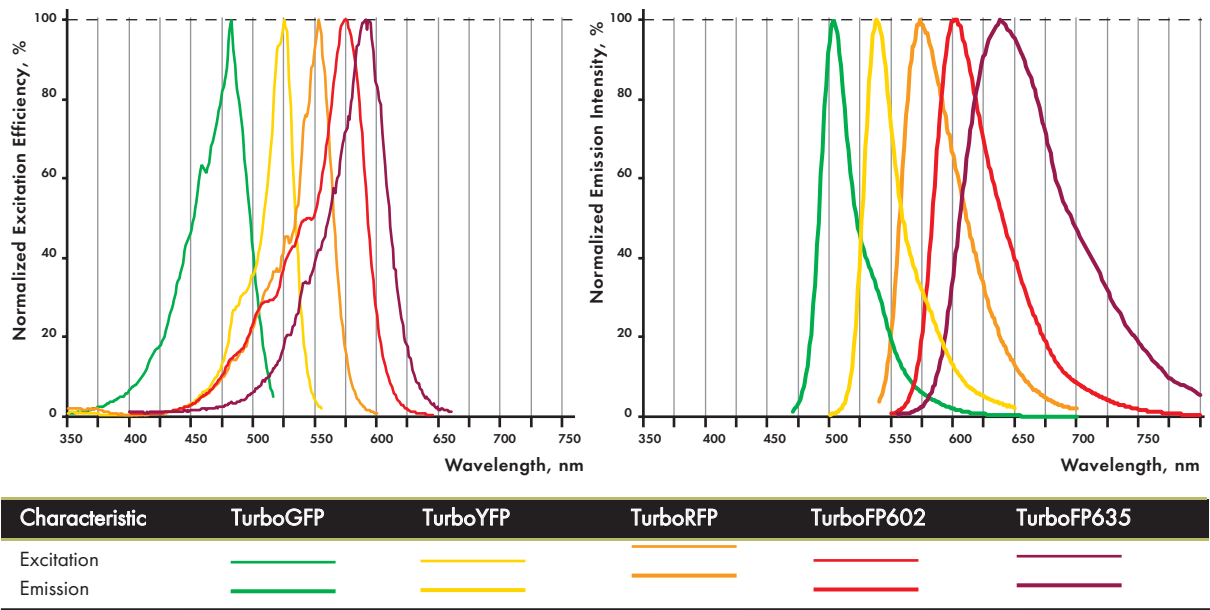
(C) — sea anemone *Entacmaea quadricolor*.

Main properties of TurboColors:

| Characteristic | TurboGFP | TurboYFP | TurboRFP | TurboFP602 | TurboFP635 |
|------------------------|---|--|---|---|---|
| Fluorescence color | green | yellow | red (orange) | true-red | far-red |
| Excitation max | 482 nm | 525 nm | 553 nm | 574 nm | 588 nm |
| Emission max | 502 nm | 538 nm | 574 nm | 602 nm | 635 nm |
| Quantum yield | 0.53 | 0.53 | 0.67 | 0.35 | 0.34 |
| Extinction coefficient | 70 000 M ⁻¹ cm ⁻¹ | 105 000 M ⁻¹ cm ⁻¹ | 92 000 M ⁻¹ cm ⁻¹ | 74 400 M ⁻¹ cm ⁻¹ | 65 000 M ⁻¹ cm ⁻¹ |
| 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 |
| Cell Toxicity | not observed | at high concentrations | not observed | not observed | not observed |
| Aggregation | no | at high concentrations | no | no | no |
| Maturation at 37°C | superfast | superfast | superfast | fast | superfast |
| Photostability | high | high | high | medium | high |
| Molecular weight | 26 kDa | 26 kDa | 26 kDa | 26 kDa | 26 kDa |
| Main advantages | Bright and fast maturing green fluorescent protein | Bright and fast maturing true-yellow fluorescent protein | Bright and fast maturing red fluorescent protein | True-red fluorescent protein, ideal compatibility with popular filter sets | Far-red fluorescent protein, ideal for whole-body imaging |
| Possible limitations | Dimer, limited applicability for fusions generation | Dimer, limited applicability for fusions generation; can form aggregates at very high concentrations | Dimer, limited applicability for fusions generation | Dimer, limited applicability for fusions generation; medium photo-stability | Dimer, limited applicability for fusions generation |

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

TurboColors, excitation/emission spectra



TurboGFP

- Bright green fluorescence
- Extra fast protein maturation
- Proven suitability to generate stably transfected cell lines
- Efficient maturation at a wide range of temperatures
- Destabilized version is available
- Recommended for gene expression analysis, cell and organelle labeling

Protein description

TurboGFP is an improved variant of the green fluorescent protein CopGFP cloned from copepod *Pontellina plumata* (Arthropoda; Crustacea; Maxillopoda; Copepoda) [1]. TurboGFP possesses bright green fluorescence that is visible earlier than fluorescence of other green fluorescent proteins.

Main properties of TurboGFP

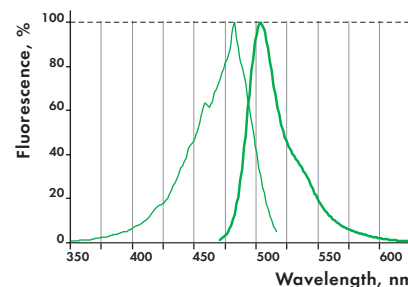
| Characteristic | |
|------------------------|---|
| Molecular weight | 26 kDa |
| Polypeptide length | 232 aa |
| Fluorescence color | green |
| Excitation max | 482 nm |
| Emission max | 502 nm |
| Quantum yield | 0.53 |
| Extinction coefficient | 70 000 M ⁻¹ cm ⁻¹ |
| Brightness* | 37.1 |
| Brightness % of EGFP | 112 |
| pKa | 5.2 |
| Structure | dimer |
| Aggregation | no |
| Maturation at 37°C | superfast |
| Photostability | high |

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

TurboGFP maturation kinetics

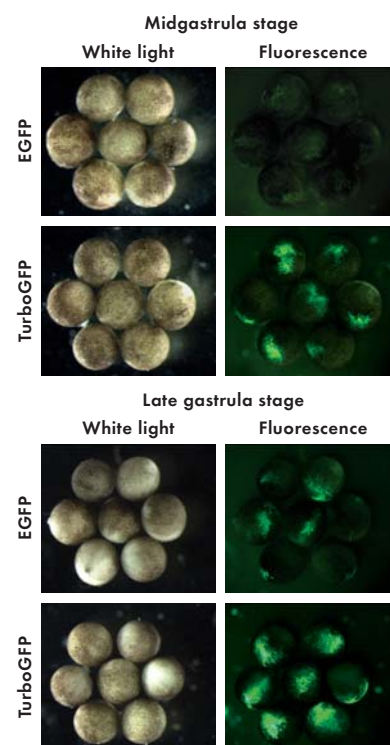
In vivo examination of developing *Xenopus* embryos microinjected with vectors comprising either TurboGFP or EGFP under the control of CMV promoter showed bright fluorescence of TurboGFP immediately after midblastula transition, when gene expression is activated. At the same time, EGFP was practically invisible at this developmental stage. This example clearly demonstrates that TurboGFP is a better tool to study expression in rapidly developing embryos at early stages.

In addition, *in vitro* comparison of TurboGFP refolding and maturation kinetics with that of other fluorescent proteins showed higher TurboGFP maturation rate.



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/TurboGFP.shtml



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. Zairaisky, Institute of Bioorganic Chemistry, RAS (Moscow, Russia).

Refolding and maturation kinetics of TurboGFP and other fluorescent proteins *in vitro*

| Fluorescent protein | Refolding half-time (s) | Maturation half-time (s) | k _{ox} (10 ⁻⁴ s ⁻¹) | Reference |
|---------------------|-------------------------|--------------------------|---|-----------|
| EGFP | 90.6 | 3915 | 1.77 | [2] |
| Venus | 46.2 | 4076 | 1.70 | [3] |
| SYFP2 | 69.3 | 3300 | 2.10 | [3] |
| TurboGFP | 11.0 | 1468 | 4.72 | [2] |

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 MgCl₂, 50 mM Tris, pH 7.5, 1 mM DTT). In maturation assay, 5 mM freshly dissolved dithionite was added to the denaturation solution [4]. Because of the instability of dithionite at high temperatures, to enable complete chromophore reduction, the sample was cooled to 25°C and the addition of 5 mM dithionite followed by heating to 95°C were repeated. Protein refolding and maturation were followed by measuring the recovery of fluorescence using Varian Cary Eclipse Fluorescence Spectrophotometer, with chamber temperature maintained at 25°C. Maturation rate constants (k_{ox}) were determined by computer-fitting the kinetic data to the first-order exponential decay (Origin 6.0).

Destabilized TurboGFP

Destabilized TurboGFP variant (TurboGFP-dest1) is produced by fusing the initial protein with PEST amino acid sequence encoded by region 422-461 of mouse ornithine decarboxylase gene [5]. This sequence targets the protein to degradation and enables a rapid protein turnover. TurboGFP-dest1 retains spectral properties of the initial protein, but has shorter half-life (approximately 2 hrs) as measured by the analysis of fluorescence intensity of cells treated with a protein synthesis inhibitor, cycloheximide. Because of rapid turnover, TurboGFP-dest1 can be used to measure changes in gene expression.

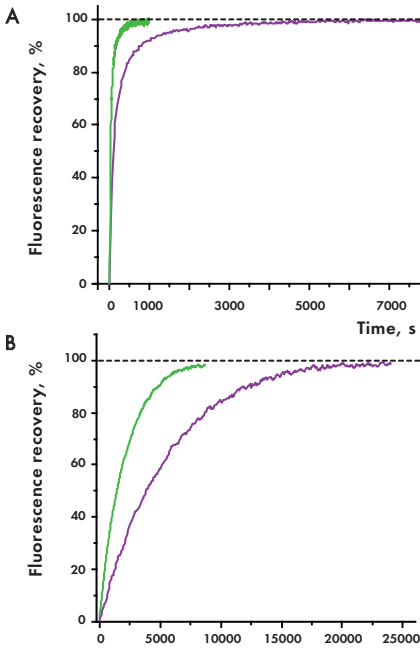
Performance and use

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.

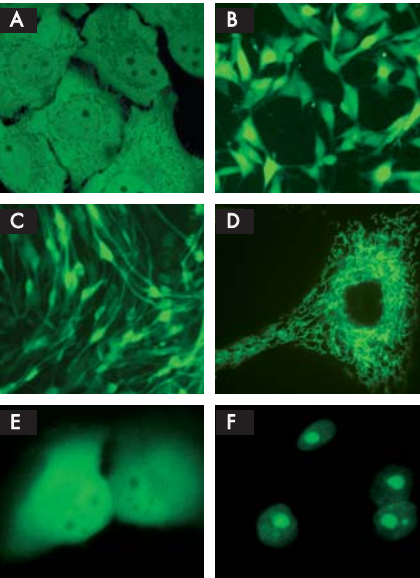
TurboGFP can be expressed and detected in a wide range of organisms. Suitability of the reporter for preparation of stably transfected cell lines expressing TurboGFP alone or in fusions has been shown.

| Application | Performance |
|----------------------------------|-------------|
| Cell labeling | |
| mammalian cells | ++++ |
| bacterial cells | ++++ |
| cold-blooded animals | ++++ |
| Stable transfection | proved |
| Promoter activity testing | ++++ |
| In fusions | ++ |



Comparison of EGFP (violet lines) and TurboGFP (green lines) refolding and maturation speed *in vitro* [2].

Normalized fluorescence recovery plots are shown. (A) — refolding kinetics; (B) — chromophore maturation kinetics.



TurboGFP expression in mammalian cells. (A) — Whole-cell expression in transiently transfected HeLa cells; (B) — in stably transfected M3 mouse melanoma cells; (C) — in stably transfected C2C12 mouse myoblast cells; (D) — mitochondrial TurboGFP expression in stably transfected HeLa cells; (E) — TurboGFP-BID fusion expression in transiently transfected HeLa cells; (F) — TurboGFP-fibrillarin expression in transiently transfected HeLa cells. Photographs of stably transfected cell lines were provided by Dr. Christian Petzelt (Marinpharm).

Despite its dimeric structure, TurboGFP is suitable for generation of fusions; however, we recommend that you use specially optimized monomeric reporters for protein labeling applications. Please see section "Protein Localization Tags" (page A-5) to select a reporter for such purposes.

Compatibility with existing filter sets and antibodies

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.

Antibodies against TurboGFP (Cat.# AB511-AB514, see pages D-3 and D-4 for descriptions) are available from Evrogen.

References

1. Shagin *et al.* (2004) *Mol. Biol. Evol.* 21(5): 841-850.
2. Evdokimov *et al.* (2006) *EMBO Rep.* 7(10): 1006-1012.
3. Kremers *et al.* (2006) *Biochemistry* 45: 6570-6580.
4. Reid and Flynn (1997) *Biochemistry* 36: 6786-6791.
5. Li *et al.* (1998) *J. Biol. Chem.* 273:34970-34975.

TurboGFP licensing opportunities

Evrogen technology embodied in TurboGFP 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.

TurboGFP-related products

TurboGFP-related product line includes expression and source vectors, recombinant protein, and antibodies. For updated product information, please visit the Evrogen Web site (www.evrogen.com/TurboGFP.shtml).

| Product | Cat.# | Description | Size | Page |
|---|-------|---|-------|------|
| 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 | A-41 |
| pTurboGFP-N | FP512 | Mammalian expression vector encoding humanized TurboGFP and allowing its expression and generation of fusions to the TurboGFP N-terminus | 20 µg | A-41 |
| pTurboGFP-B | FP513 | Bacterial expression vector; source of the humanized TurboGFP coding sequence | 20 µg | A-42 |
| pTurboGFP-PRL | FP515 | Promoterless expression vector encoding humanized TurboGFP and designed for monitoring transcription from different promoters and promoter/enhancer combinations | 20 µg | A-42 |
| pTurboGFP-PRL-dest1 | FP518 | Promoterless vector encoding destabilized TurboGFP and designed for monitoring transcription from different promoters and promoter/enhancer combinations | 20 µg | A-43 |
| pTurboGFP-dest1 | FP519 | Mammalian expression vector encoding destabilized TurboGFP for its expression and generation of fusions to the TurboGFP-dest1 N-terminus | 20 µg | A-43 |
| pTurboGFP-mito | FP517 | Mammalian expression vector encoding humanized TurboGFP targeted to mitochondria | 20 µg | A-44 |
| Gateway® TurboGFP-C | FP521 | Gateway® entry clone for generation of fusions to the C-terminus of humanized TurboGFP; transfer of TurboGFP or TurboGFP-tagged fusion into a Gateway® destination vector | 20 µg | A-44 |

| Product | Cat.# | Description | Size | Page |
|------------------------------------|-------|---|--------|------|
| Gateway® TurboGFP-N | FP522 | Gateway® entry clone for generation of fusions to the N-terminus of humanized TurboGFP; transfer of TurboGFP or TurboGFP-tagged fusion into a Gateway® destination vector | 20 µg | A-45 |
| Recombinant protein | | | | |
| rTurboGFP | FP552 | Purified recombinant green fluorescent protein | 100 µg | A-45 |
| Antibodies against TurboGFP | | | | |
| Anti-TurboGFP antibody | AB511 | Rabbit polyclonal antibody against non-denatured | 100 µg | D-3 |
| | AB512 | TurboGFP | 200 µg | |
| Anti-TurboGFP (d) antibody | AB513 | Rabbit polyclonal antibody against denatured | 100 µg | D-4 |
| | AB514 | TurboGFP and CopGFP | 200 µg | |

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

Third party products: stably transfected cell lines expressing TurboGFP

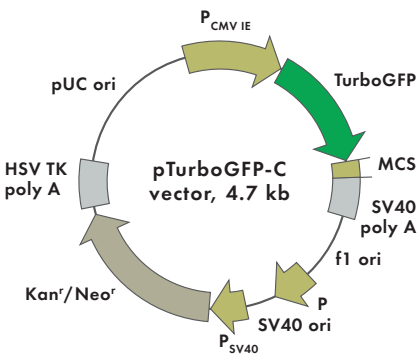
| Cell line | Source | Description |
|-----------------------------------|---------|---|
| M3-TG | mouse | M3 mouse melanoma cells expressing TurboGFP in cytosol |
| PC-TG | rat | PC-12 rat pheochromocytoma expressing TurboGFP in cytosol |
| CHO-K1-TG | hamster | Chinese hamster ovary cells CHO-K1 expressing TurboGFP in cytosol |
| H460-TG | human | H460 human lung carcinoma expressing TurboGFP in cytosol |
| U17-TG | human | UT7 human leukemia cells expressing TurboGFP in cytosol |
| H-TG | human | HeLa human cervical carcinoma expressing TurboGFP in cytosol |
| T24-TG | human | T24 human bladder carcinoma expressing TurboGFP in cytosol |
| C2-TG | mouse | C2C12 mouse myoblast cells expressing TurboGFP in cytosol |
| W-TG | rat | WALKER 256 rat tumor expressing TurboGFP in cytosol |
| 3T3-TG | mouse | 3T3 mouse fibroblasts expressing TurboGFP in cytosol |
| 3T3-TG-D | mouse | T3 mouse fibroblasts expressing destabilized TurboGFP in cytosol |
| M3-TG-Mito | mouse | M3 mouse melanoma cells expressing TurboGFP in mitochondria |
| H-TG-Mito | human | HeLa human cervical carcinoma expressing TurboGFP in mitochondria |
| T24-TG-Mito | human | T24 human bladder carcinoma expressing TurboGFP in mitochondria |
| Fluorescent BID apoptotic protein | human | T24 human carcinoma cells expressing JRed in mitochondria and TurboGFP-BID fusion |
| HeLa-TurboGreen-Actin | human | HeLa human cervical carcinoma expressing TurboGFP fusion with beta-actin |
| Fluorescent fibrillarin | human | HeLa human cervical carcinoma expressing TurboGFP fusion with fibrillarin |

Cell lines are manufactured by Marinpharm GmbH (Berlin, Germany) under the Evrogen license.

Notice to Purchaser:

TurboGFP-related products are intended for research use only and 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 (Appendix A, page G-5). 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. Invitrogen Gateway® Technology: please see Limited Use Label License No. 19: Gateway® Cloning Products, Appendix C, page G-7.

Expression/source vectors: pTurboGFP-C



For vector sequence please visit our Web site at www.evrogen.com/pTurboGFP-C.shtml

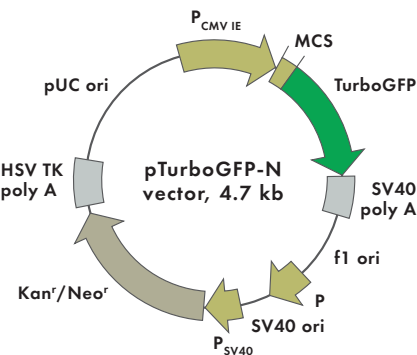
Multiple cloning site (MCS)



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

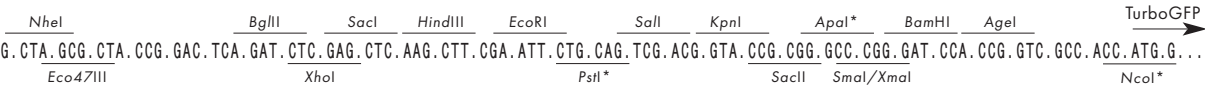
| Product | Cat.# | Size |
|--|--|-------|
| pTurboGFP-C | FP511 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | TurboGFP | |
| Reporter codon usage | mammalian | |
| Promoter for TurboGFP | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | generation of fusions to the TurboGFP C-terminus; expression of TurboGFP or its fusions in mammalian cells | |

Expression/source vectors: pTurboGFP-N



For vector sequence please visit our Web site at www.evrogen.com/pTurboGFP-N.shtml

Multiple cloning site (MCS)

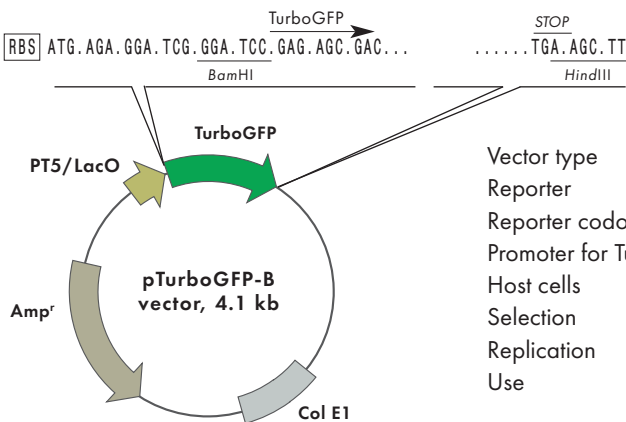


* — not unique sites.

| Product | Cat.# | Size |
|--|--|-------|
| pTurboGFP-N | FP512 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | TurboGFP | |
| Reporter codon usage | mammalian | |
| Promoter for TurboGFP | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | generation of fusions to the TurboGFP N-terminus; expression of TurboGFP or its fusions in mammalian cells | |

Notice to Purchaser — please see page A-45

Expression/source vectors: pTurboGFP-B



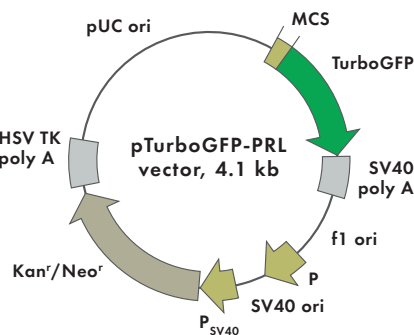
For vector sequence, please visit our Web site at www.evrogen.com/pTurboGFP-B.shtml

| Product | Cat.# | Size |
|-------------|-------|-------|
| pTurboGFP-B | FP513 | 20 µg |

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

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

Expression/source vectors: pTurboGFP-PRL



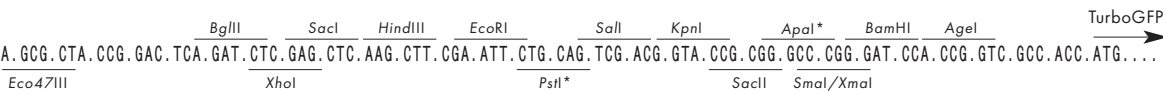
For vector sequence, please visit our Web site at www.evrogen.com/pTurboGFP-PRL.shtml

| Product | Cat.# | Size |
|---------------|-------|-------|
| pTurboGFP-PRL | FP515 | 20 µg |

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

| | |
|-----------------------|---|
| Vector type | promoterless vector |
| Reporter | TurboGFP |
| Reporter codon usage | mammalian |
| Promoter for TurboGFP | NO |
| Host cells | mammalian, bacterial |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori |
| Use | monitoring the activity of promoter or promoter/enhancer combination of interest cloned into vector MCS |

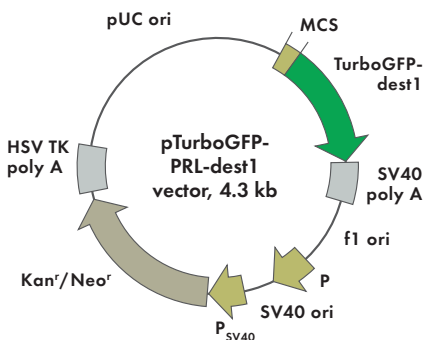
Multiple cloning site (MCS)



* — not unique sites.

Notice to Purchaser — please see page A-45


Expression/source vectors: pTurboGFP-PRL-dest1



For vector sequence, please visit our Web site at www.evrogen.com/pTurboGFP-PRL-dest1.shtml

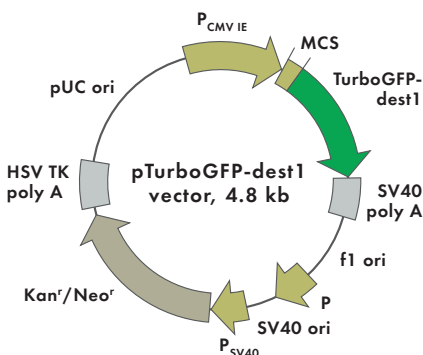
| Product | Cat.# | Size |
|--|--|-------|
| pTurboGFP-PRL-dest1 | FP518 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | promoterless vector | |
| Reporter | destabilized TurboGFP (TurboGFP-dest1) | |
| Reporter codon usage | mammalian | |
| Promoter for TurboGFP-dest1 | NO | |
| Host cells | mammalian, prokaryotic | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | monitoring the activity of promoter or promoter/enhancer combination of interest cloned into vector MCS. Rapid turnover of TurboGFP-dest1 allows exact measuring of changes in gene expression | |

Multiple cloning site (MCS)

A. 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.  TurboGFP-dest1
 Eco47III XhoI* PstI* SacII SmaI/XmaI NcoI*

* — not unique sites.

Expression/source vectors: pTurboGFP-dest1



For vector sequence, please visit our Web site at www.evrogen.com/pTurboGFP-dest1.shtml

| Product | Cat.# | Size |
|--|---|-------|
| pTurboGFP-dest1 | FP519 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | destabilized TurboGFP (TurboGFP-dest1) | |
| Reporter codon usage | mammalian | |
| Promoter for TurboGFP | P _{CMV} IE | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | generation of fusions to the TurboGFP-dest1 N-terminus; expression of TurboGFP-dest1 or its fusions in mammalian cells; positive control for the pTurboGFP-PRL-dest1 vector (Cat.# FP518) | |

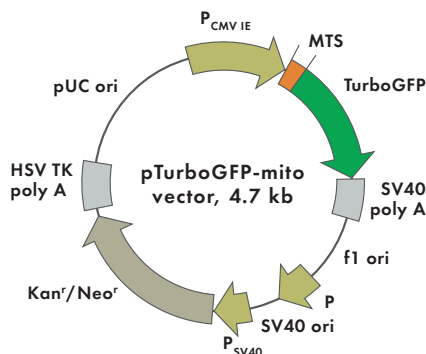
Multiple cloning site (MCS)

NheI BglII* SacI HindIII EcoRI SalI KpnI ApaI* BamHI AgeI TurboGFP-dest1
 G. 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. .
 Eco47III XhoI* PstI* SacII SmaI/XmaI NcoI*

* — not unique sites.

Notice to Purchaser — please see page A-45

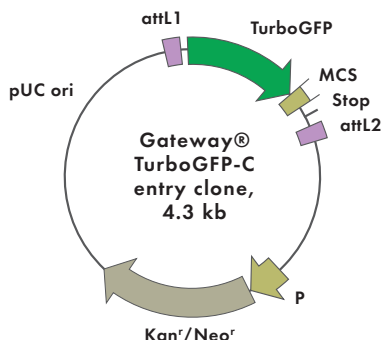
Expression/source vectors: pTurboGFP-mito



For vector sequence, please visit our Web site at www.evrogen.com/pTurboGFP-mito.shtml

| Product | Cat.# | Size |
|--|---|-------|
| pTurboGFP-mito | FP517 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | TurboGFP fusion with mitochondrial targeting sequence (MTS) derived from the subunit VIII of human cytochrome C oxidase | |
| Reporter codon usage | mammalian | |
| Promoter for TurboGFP-MTS | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | expression of mitochondria-targeted TurboGFP in mammalian cells under the control of CMV promoter; source of mitochondria-targeted TurboGFP coding sequence | |

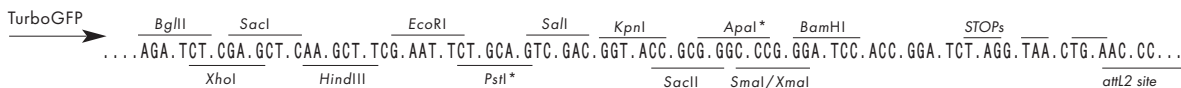
Expression/source vectors: Gateway® TurboGFP-C entry clone



For vector sequence, please visit our Web site at www.evrogen.com/gwTurboGFP-C.shtml

| Product | Cat.# | Size |
|--|---|-------|
| Gateway® TurboGFP-C entry clone | FP521 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | Gateway® entry clone | |
| Reporter | TurboGFP | |
| Reporter codon usage | mammalian | |
| Promoter for TurboGFP | NO | |
| Host cells | prokaryotic | |
| Selection | kanamycin | |
| Replication | prokaryotic — pUC ori | |
| Use | generation of fusions to the N-terminus of TurboGFP using attL1 site; generation of fusions to the C-terminus of TurboGFP using vector MCS; fast cloning into Gateway® expression vectors through site-specific recombination | |

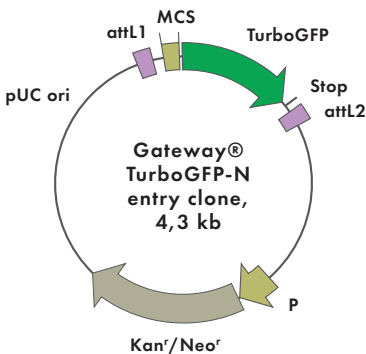
Multiple cloning site (MCS)



* — not unique sites

Notice to Purchaser — please see page A-45

Expression/source vectors: Gateway® TurboGFP-N entry clone



For vector sequence, please visit our Web site at www.evrogen.com/gwTurboGFP-N.shtml

| Product | Cat.# | Size |
|---------------------------------|-------|-------|
| Gateway® TurboGFP-N entry clone | FP522 | 20 µg |

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

| | |
|-----------------------|---|
| Vector type | Gateway® entry clone |
| Reporter | TurboGFP |
| Reporter codon usage | mammalian |
| Promoter for TurboGFP | NO |
| Host cells | prokaryotic |
| Selection | kanamycin |
| Replication | prokaryotic — pUC ori |
| Use | generation of fusions to the N-terminus of TurboGFP using attL1 site or vector MCS; fast cloning into Gateway® expression vectors through site-specific recombination |

Multiple cloning site (MCS)

attL1 site BglII SacI HindIII EcoRI Sall KpnI ApaI* BamHI AgeI 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...
Eco47III XhoI PstI* SacII SmaI/XmaI

* — not unique sites

Recombinant protein rTurboGFP

| Product | Cat.# | Size |
|-----------|-------|--------|
| rTurboGFP | FP552 | 100 µg |

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

Description

Recombinant TurboGFP (rTurboGFP) is 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 (vary in different lots).

Notice to Purchaser:

TurboGFP-related products: These products are intended for research use only and 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 (Appendix A, page G-5). CMV Promoter: 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. Invitrogen Gateway® Technology: please see Limited Use Label License No. 19: Gateway® Cloning Products, Appendix C, page G-7.

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.

TurboYFP

- Superbright true-yellow fluorescence
- Fast maturation
- Emission wavelength is ideally positioned between those of green and red fluorescent proteins
- Destabilized version is available
- Recommended for gene expression analysis and cell labeling

Protein description

TurboYFP is an enhanced variant of the yellow fluorescent protein PhiYFP from jellyfish *Phialidium* sp. [1]. Possessing superbright yellow fluorescence with emission maximum at 538 nm, TurboYFP is ideally positioned between green and red fluorescent proteins, allowing easy separation of these fluorescent markers by flow cytometry using common channels of detection and a single laser excitation line. Compared with PhiYFP, TurboYFP matures faster in mammalian cells.

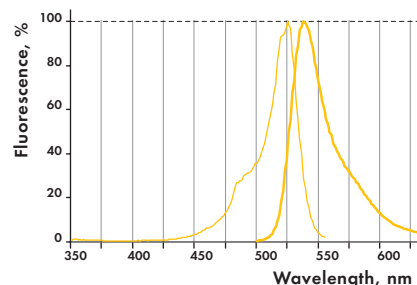
Main properties of TurboYFP

| Characteristic | |
|------------------------|--|
| Molecular weight | 26 kDa |
| Polypeptide length | 234 aa |
| Fluorescence color | yellow |
| Excitation max | 525 nm |
| Emission max | 538 nm |
| Quantum yield | 0.53 |
| Extinction coefficient | 105 000 M ⁻¹ cm ⁻¹ |
| Brightness* | 55.7 |
| Brightness % of EGFP | 169 |
| pKa | 5.9 |
| Structure | dimer |
| Aggregation | at high concentration |
| Maturation at 37°C | superfast |
| Photostability | high |

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

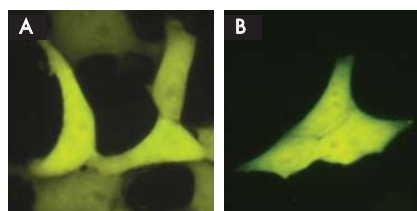
Destabilized TurboYFP

Destabilized TurboYFP variant (TurboYFP-dest1) is produced by fusing the initial protein with PEST amino acid sequence encoded by region 422-461 of mouse ornithine decarboxylase gene [2]. This sequence targets the protein to degradation and enables a rapid protein turnover. TurboYFP-dest1 retains spectral properties of the initial protein, but has shorter half-lives (approximately 1.5-2 hrs) as measured by the analysis of fluorescence intensity of cells treated with a protein synthesis inhibitor, cycloheximide. Because of rapid turnover, TurboYFP-dest1 can be used to measure



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/TurboYFP.shtml



TurboYFP expression in mammalian cells.

(A) — Whole-cell expression in Phoenix cells;
(B) — whole-cell expression in HeLa cells.

Performance and use

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.

| Application | Performance |
|---------------------------|-------------|
| Cell labeling | |
| mammalian cells | ++++ |
| bacterial cells | ++++ |
| Stable transfection | not tested |
| Promoter activity testing | ++++ |
| In fusions | ++ |

TurboYFP can be expressed and detected in a wide range of organisms. Mammalian cells transiently transfected with TurboYFP expression vectors give bright fluorescent signals within 8-10 hrs after transfection.

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 proteins (see description at A-71 page) for stable expression and for organelle labeling.

Despite its dimeric structure, TurboYFP demonstrates successful performance in fusions with subcellular localization signals and many cellular proteins, e.g. with fibrillarin, Bid protein, beta-actin. However, generally we do not recommend using TurboYFP for fusion with oligomerizing cellular proteins (e.g. tubulin). Please see section "Protein Localization Tags" (page A-5) to select a reporter for such purposes.

Compatibility with existing filter sets and antibodies

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

TurboYFP can be recognized using Anti-PhiYFP and Anti-PhiYFP(d) antibodies (Cat.# AB601-AB604, see pages D-7 and D-8 for descriptions) available from Evrogen.

TurboYFP licensing opportunities

Evrogen technology embodied in TurboYFP is available for expanded and commercial use with an adaptable licensing program. Benefits from flexible and market-driven license options are offered for upgrade and novel development of products and applications.

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

References

1. Shagin *et al.* (2004) *Mol. Biol. Evol.* 21(5): 841-850.
2. Li *et al.* (1998) *J. Biol. Chem.* 273:34970-34975.

TurboYFP-related products

TurboYFP-related product line includes expression and source vectors, recombinant protein, and antibodies. For updated product information, please visit the Evrogen Web site (www.evrogen.com/TurboYFP.shtml).

| Product | Cat.# | Description | Size | Page |
|--|-------|--|--------|------|
| <i>TurboYFP expression/source vectors</i> | | | | |
| pTurboYFP-C | FP611 | Mammalian expression vector encoding humanized TurboYFP and allowing TurboYFP expression and generation of fusions to the TurboYFP C-terminus | 20 µg | A-49 |
| pTurboYFP-N | FP612 | Mammalian expression vector encoding humanized TurboYFP and allowing TurboYFP expression and generation of fusions to the TurboYFP N-terminus | 20 µg | A-49 |
| pTurboYFP-B | FP613 | Bacterial expression vector; source of the humanized TurboYFP coding sequence | 20 µg | A-50 |
| pTurboYFP-PRL | FP615 | Promoterless expression vector encoding humanized TurboYFP and designed for monitoring transcription from different promoters and promoter/enhancer combinations | 20 µg | A-50 |
| pTurboYFP-PRL-dest1 | FP618 | Promoterless vector encoding destabilized TurboYFP and designed for monitoring transcription from different promoters and promoter/enhancer combinations | 20 µg | A-51 |
| pTurboYFP-dest1 | FP619 | Mammalian expression vector encoding destabilized TurboYFP for its expression and generation of fusions to the TurboYFP-dest1 N-terminus | 20 µg | A-51 |
| <i>Recombinant protein</i> | | | | |
| rTurboYFP | FP652 | Purified recombinant yellow fluorescent protein | 100 µg | A-52 |
| <i>Antibodies against TurboYFP</i> | | | | |
| Anti-PhiYFP antibody | AB601 | Rabbit polyclonal antibody against non-denatured | 100 µg | D-7 |
| | AB602 | PhiYFP, PhiYFP-m, and TurboYFP | 200 µg | |
| Anti-PhiYFP(d) antibody | AB603 | Rabbit polyclonal antibody against denatured | 100 µg | D-8 |
| | AB604 | PhiYFP, PhiYFP-m, and TurboYFP | 200 µg | |

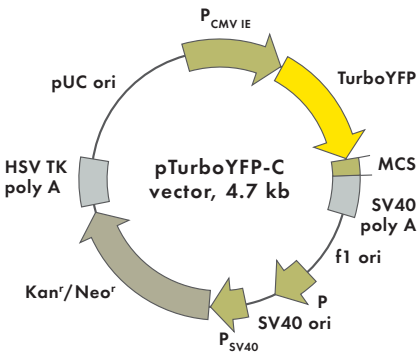
Please contact your local distributor for exact prices and delivery information.

Notice to Purchaser:

TurboYFP-related products: These products are intended for research use only and 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 (Appendix A, page G-5).

CMV Promoter: 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.

Expression/source vectors: pTurboYFP-C



For vector sequence, please visit our Web site at www.evrogen.com/pTurboYFP-C.shtml

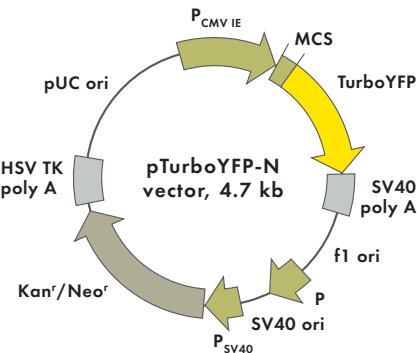
| Product | Cat.# | Size |
|--|--|-------|
| pTurboYFP-C | FP611 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | TurboYFP | |
| Reporter codon usage | mammalian | |
| Promoter for TurboYFP | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | generation of fusions to the TurboYFP C-terminus; expression of TurboYFP or its fusions in mammalian cells | |

Multiple cloning site (MCS)



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

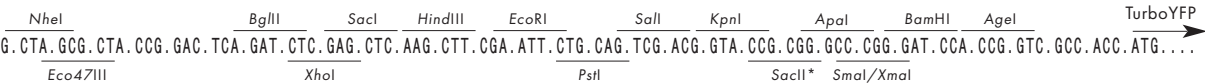
Expression/source vectors: pTurboYFP-N



For vector sequence, please visit our Web site at www.evrogen.com/pTurboYFP-N.shtml

| Product | Cat.# | Size |
|--|--|-------|
| pTurboYFP-N | FP612 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | TurboYFP | |
| Reporter codon usage | mammalian | |
| Promoter for TurboGFP | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | generation of fusions to the TurboYFP N-terminus; expression of TurboYFP or its fusions in mammalian cells | |

Multiple cloning site (MCS)



* — not unique sites.

Notice to Purchaser — please see page A-52

Expression/source vectors: pTurboYFP-B

| Product | Cat.# | Size |
|-------------|-------|-------|
| pTurboYFP-B | FP613 | 20 µg |

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

Vector type
Reporter
Reporter codon usage
Promoter for TurboYFP
Host cells
Selection
Replication
Use

bacterial expression vector
TurboYFP
mammalian
T5 promoter/lac operator
prokaryotic
ampicillin
ColE1 ori
TurboYFP expression in bacterial cells using T5 promoter/lac operator; source of the TurboYFP coding sequence

For vector sequence, please visit our Web site at www.evrogen.com/pTurboYFP-B.shtml

Expression/source vectors: pTurboYFP-PRL

| Product | Cat.# | Size |
|---------------|-------|-------|
| pTurboYFP-PRL | FP615 | 20 µg |

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

Vector type
Reporter
Reporter codon usage
Promoter for TurboGFP
Host cells
Selection
Replication
Use

promoterless vector
TurboYFP
mammalian
NO
mammalian, bacterial
prokaryotic — kanamycin
eukaryotic — neomycin (G418)
prokaryotic — pUC ori
eukaryotic — SV40 ori
monitoring the activity of promoter or promoter/enhancer combination cloned into vector MCS

For vector sequence, please visit our Web site at www.evrogen.com/pTurboYFP-PRL.shtml

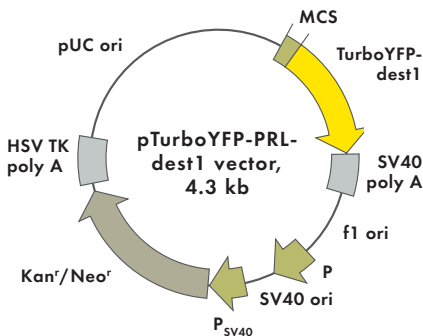
Multiple cloning site (MCS)

A. 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. ...
Eco47III XhoI PstI SacII* SmaI/XmaI

* — not unique sites.

Notice to Purchaser — please see page A-52

Expression/source vectors: pTurboYFP-PRL-dest1



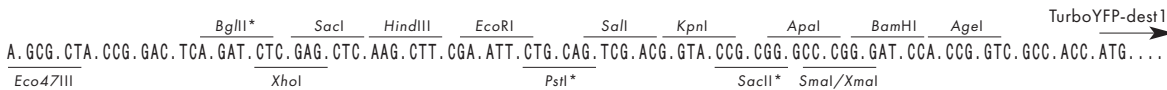
For vector sequence, please visit our Web site at www.evrogen.com/pTurboYFP-PRL-dest1.shtml

| Product | Cat.# | Size |
|---------------------|-------|-------|
| pTurboYFP-PRL-dest1 | FP618 | 20 µg |

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

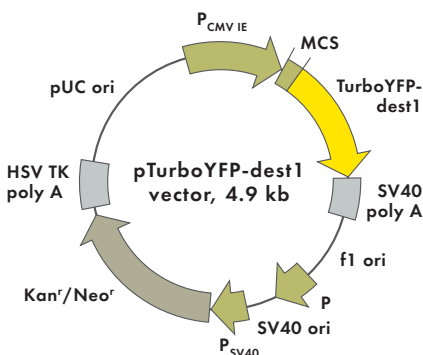
| | |
|-----------------------------|--|
| Vector type | promoterless vector |
| Reporter | destabilized TurboYFP (TurboYFP-dest1) |
| Reporter codon usage | mammalian |
| Promoter for TurboYFP-dest1 | NO |
| Host cells | mammalian, bacterial |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori |
| Use | monitoring the activity of promoter or promoter/enhancer combination cloned into vector MCS. Rapid turnover of TurboYFP-dest1 allows exact measuring of changes in gene expression |

Multiple cloning site (MCS)



* — not unique sites.

Expression/source vectors: pTurboYFP-dest1



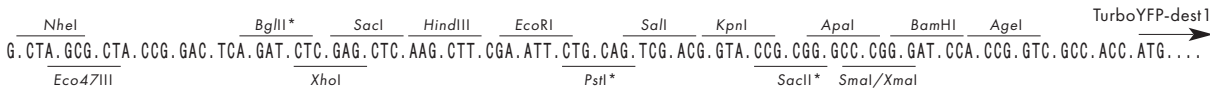
For vector sequence, please visit our Web site at www.evrogen.com/pTurboYFP-dest1.shtml

| Product | Cat.# | Size |
|-----------------|-------|-------|
| pTurboYFP-dest1 | FP619 | 20 µg |

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

| | |
|-----------------------------|---|
| Vector type | mammalian expression vector |
| Reporter | destabilized TurboYFP (TurboYFP-dest1) |
| Reporter codon usage | mammalian |
| Promoter for TurboYFP-dest1 | P _{CMV IE} |
| Host cells | mammalian |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori |
| Use | generation of fusions to the TurboYFP-dest1 N-terminus; expression of TurboYFP-dest1 or its fusions in mammalian cells; positive control for the pTurboYFP-PRL-dest1 vector (Cat.# FP618) |

Multiple cloning site (MCS)



* — not unique sites.

Notice to Purchaser — please see page A-52

Recombinant protein rTurboYFP

| Product | Cat.# | Size |
|-----------|-------|--------|
| rTurboYFP | FP652 | 100 µg |

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

Description

Recombinant TurboYFP (rTurboYFP) is 26-kDa yellow fluorescent protein. It has excitation and emission spectra identical to those of the expressed TurboYFP. rTurboYFP is suitable as control reagent for TurboYFP expression using the TurboYFP expression vectors.

rTurboYFP 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. rTurboYFP may contain 6xHis tag at its N-terminus (vary in different lots).

Notice to Purchaser:

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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.

TurboRFP

- Superbright red (orange) fluorescence
- Fast maturation
- Fluorescent signal is easily distinguished from background fluorescence
- Destabilized version is available
- Recommended for gene expression analysis, cell and organelle labeling

Protein description

TurboRFP is a novel red fluorescent protein (excitation/emission maxima are 553 and 574 nm, respectively) derived from sea anemone *Entacmaea quadricolor* [1]. Possessing high photostability and pH stability, 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.

Main properties of TurboRFP

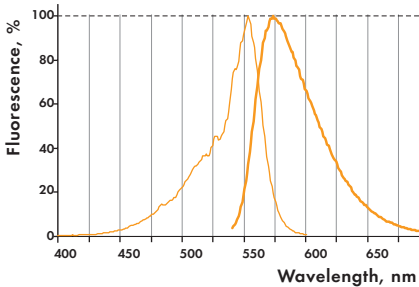
| Characteristic | |
|------------------------|---|
| Molecular weight | 26 kDa |
| Polypeptide length | 231 aa |
| Fluorescence color | red (orange) |
| Excitation max | 553 nm |
| Emission max | 574 nm |
| Quantum yield | 0.67 |
| Extinction coefficient | 92 000 M ⁻¹ cm ⁻¹ |
| Brightness* | 61.6 |
| Brightness % of EGFP | 187 |
| pKa | 4.4 |
| Structure | dimer |
| Aggregation | no |
| Maturation at 37°C | superfast |
| Photostability | high |

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

Comparison of TurboRFP, DsRed2, and DsRed-Express maturation in mammalian cells

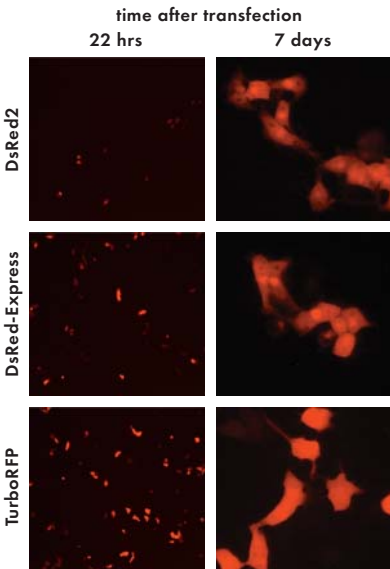
HeLa cells were transiently transfected with mammalian expression vectors comprising TurboRFP, DsRed2, or DsRed-Express fluorescent proteins under the control of CMV promoter. The DNA concentrations were equalized before transfection. Cells were photographed using fluorescent microscope after different periods of cultivation.

Faster appearance of bright fluorescence was detected in the case of TurboRFP. In addition, unlike DsRed-related proteins, no abnormal Golgi-like localization of TurboRFP was observed within 7 days after transfection.



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/TurboRFP.shtml



Fluorescent microscopy of mammalian cells expressing DsRed2, DsRed-Express, and TurboRFP.

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 cytosol.

Destabilized TurboRFP

Destabilized TurboRFP variant (TurboRFP-dest1) is produced by fusing the initial protein with PEST amino acid sequence encoded by region 422-461 of mouse ornithine decarboxylase gene [2]. This sequence targets the protein to degradation and enables a rapid protein turnover. TurboRFP-dest1 retains spectral properties of the initial protein, but has shorter half-life (approximately 1-2 hrs) as measured by the analysis of fluorescence intensity of cells treated with a protein synthesis inhibitor, cycloheximide. Because of rapid turnover, TurboRFP-dest1 can be used to measure changes in gene expression.

Performance and use

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.

| Application | Performance |
|---------------------------|-------------|
| Cell labeling | |
| mammalian cells | ++++ |
| bacterial cells | ++++ |
| Stable transfection | not tested |
| Promoter activity testing | ++++ |
| In fusions | ++ |

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 cell toxic effects are observed

Despite its dimeric structure, TurboRFP demonstrates successful performance in fusions with subcellular localization signals and many cellular proteins. However, we do not recommend using TurboRFP for fusion with oligomerizing cellular proteins (e.g. alpha-tubulin). Please see section "Protein Localization Tags" to select a reporter for such purposes.

Compatibility with existing filter sets and antibodies

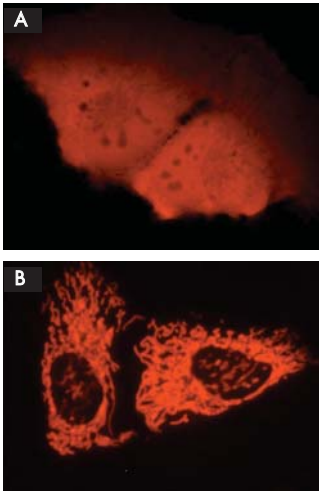
Recommended Omega Optical filter sets are QMAX-Yellow, XF108-2, XF101-2, and XF111-2. TurboRFP can also be detected using TRITC filter set or similar.

TurboRFP can be recognized using Anti-tRFP antibody (Cat.# AB231-AB232, page D-9) available from Evrogen.

TurboRFP licensing opportunities

Evrogen technology embodied in TurboRFP 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.



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.

References
1. Merzlyak *et al.* (2007) *Nat. Methods.* 4(7): 555-557.
2. Li *et al.* (1998) *J. Biol. Chem.* 273:34970-34975.

TurboRFP-related products

TurboRFP-related product line includes expression and source vectors, recombinant protein, and antibodies. For updated product information, please visit the Evrogen Web site (www.evrogen.com/TurboRFP.shtml).

| Product | Cat.# | Description | Size | Page |
|--|-------|--|--------|------|
| <i>TurboRFP expression/source vectors</i> | | | | |
| pTurboRFP-C | FP231 | Mammalian expression vector encoding humanized TurboRFP and allowing TurboRFP expression and generation of fusions to the TurboRFP C-terminus | 20 µg | A-56 |
| pTurboRFP-N | FP232 | Mammalian expression vector encoding humanized TurboRFP and allowing TurboRFP expression and generation of fusions to the TurboRFP N-terminus | 20 µg | A-56 |
| pTurboRFP-B | FP233 | Bacterial expression vector; source of humanized TurboRFP coding sequence | 20 µg | A-57 |
| pTurboRFP-PRL | FP235 | Promoterless mammalian expression vector encoding humanized TurboRFP and designed for monitoring transcription from different promoters and promoter/enhancer combinations | 20 µg | A-57 |
| pTurboRFP-PRL-dest1 | FP238 | Promoterless vector encoding destabilized TurboRFP and designed for monitoring transcription from different promoters and promoter/enhancer combinations | 20 µg | A-58 |
| pTurboRFP-dest1 | FP239 | Mammalian expression vector encoding destabilized TurboRFP for its expression and generation of fusions to the TurboRFP-dest1 N-terminus | 20 µg | A-58 |
| pTurboRFP-mito | FP237 | Mammalian expression vector encoding humanized TurboRFP targeted to mitochondria | 20 µg | A-59 |
| <i>Recombinant protein</i> | | | | |
| rTurboRFP | FP252 | Recombinant red fluorescent protein TurboRFP | 100 µg | A-59 |
| <i>Antibodies against TurboRFP</i> | | | | |
| Anti-tRFP antibody | AB231 | Rabbit polyclonal antibody against TagRFP, TagFP635, | 100 µg | D-9 |
| | AB232 | TurboRFP, TurboFP602, and TurboFP635 proteins | 200 µg | |

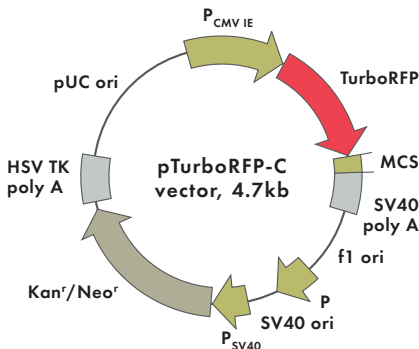
Please contact your local distributor for exact prices and delivery information.

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Expression/source vectors: pTurboRFP-C



For vector sequence, please visit our Web site at www.evrogen.com/pTurboRFP-C.shtml

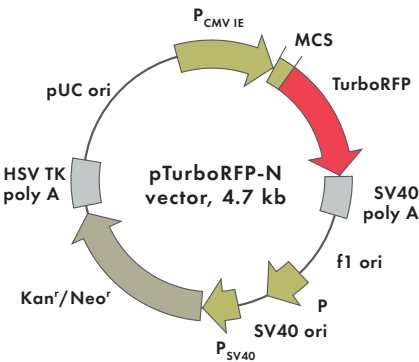
Multiple cloning site (MCS)



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

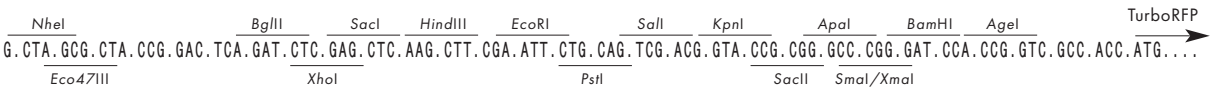
| Product | Cat.# | Size |
|--|--|-------|
| pTurboRFP-C | FP231 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | TurboRFP | |
| Reporter codon usage | mammalian | |
| Promoter for TurboRFP | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin | |
| | eukaryotic — neomycin (G418) | |
| | prokaryotic — pUC ori | |
| Replication | eukaryotic — SV40 ori | |
| | generation of fusions to the TurboRFP C-terminus; expression of TurboRFP or its fusions in mammalian cells | |
| Use | | |

Expression/source vectors: pTurboRFP-N



For vector sequence, please visit our Web site at www.evrogen.com/pTurboRFP-N.shtml

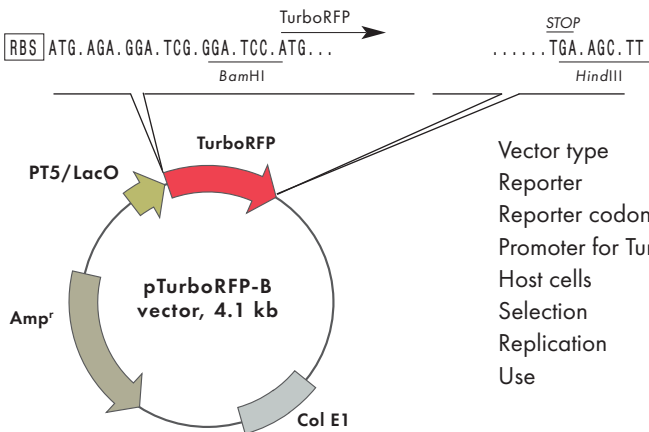
Multiple cloning site (MCS)



| Product | Cat.# | Size |
|--|--|-------|
| pTurboRFP-N | FP232 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | TurboRFP | |
| Reporter codon usage | mammalian | |
| Promoter for TurboRFP | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin | |
| | eukaryotic — neomycin (G418) | |
| | prokaryotic — pUC ori | |
| Replication | eukaryotic — SV40 ori | |
| | generation of fusions to the TurboRFP N-terminus; expression of TurboRFP or its fusions in mammalian cells | |
| Use | | |

Notice to Purchaser — please see page A-59

Expression/source vectors: pTurboRFP-B



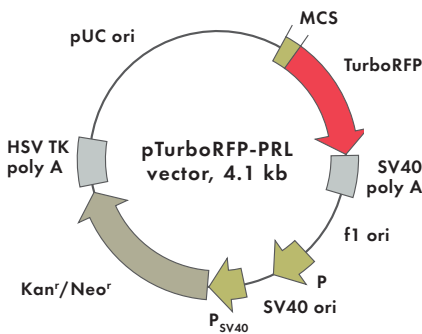
For vector sequence, please visit our Web site at www.evrogen.com/pTurboRFP-B.shtml

| Product | Cat.# | Size |
|-------------|-------|-------|
| pTurboRFP-B | FP233 | 20 µg |

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

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

Expression/source vectors: pTurboRFP-PRL



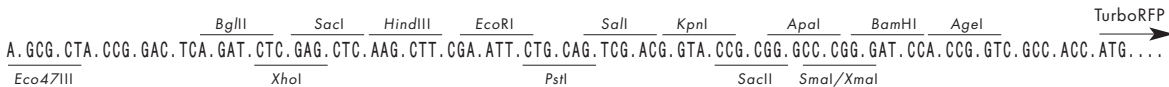
For vector sequence, please visit our Web site at www.evrogen.com/pTurboRFP-PRL.shtml

| Product | Cat.# | Size |
|---------------|-------|-------|
| pTurboRFP-PRL | FP235 | 20 µg |

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

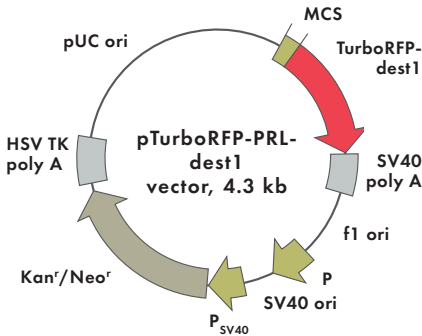
| | |
|-----------------------|---|
| Vector type | promoterless vector |
| Reporter | TurboRFP |
| Reporter codon usage | mammalian |
| Promoter for TurboRFP | NO |
| Host cells | mammalian, bacterial |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori |
| Use | monitoring the activity of promoter or promoter/enhancer combination cloned into vector MCS |

Multiple cloning site (MCS)



Notice to Purchaser — please see page A-59

Expression/source vectors: pTurboRFP-PRL-dest1



For vector sequence, please visit our Web site at www.evrogen.com/pTurboRFP-PRL-dest1.shtml

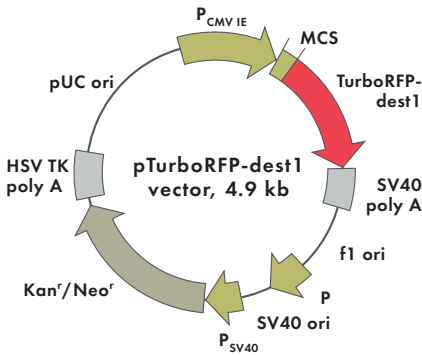
| Product | Cat.# | Size |
|--|--|-------|
| pTurboRFP-PRL-dest1 | FP238 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | promoterless vector | |
| Reporter | destabilized TurboRFP (TurboRFP-dest1) | |
| Reporter codon usage | mammalian | |
| Promoter for TurboRFP-dest1 | NO | |
| Host cells | mammalian, prokaryotic | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | monitoring the activity of promoter or promoter/enhancer combination cloned into vector MCS. Rapid turnover of TurboRFP-dest1 allows exact measuring of changes in gene expression | |

Multiple cloning site (MCS)

A. 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. . . . TurboRFP-dest1
Eco47III BglII* SacI HindIII EcoRI SalI KpnI ApaI BamHI AgeI
XhoI PstI* SacII SmaI/XmaI

* — not unique sites

Expression/source vectors: pTurboRFP-dest1



For vector sequence, please visit our Web site at www.evrogen.com/pTurboRFP-dest1.shtml

| Product | Cat.# | Size |
|--|---|-------|
| pTurboRFP-dest1 | FP239 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | destabilized TurboRFP (TurboRFP-dest1) | |
| Reporter codon usage | mammalian | |
| Promoter for TurboRFP-dest1 | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | generation of fusions to the TurboRFP-dest1 N-terminus; expression of TurboRFP-dest1 or its fusions in mammalian cells; positive control for the pTurboRFP-PRL-dest1 vector (Cat.# FP238) | |

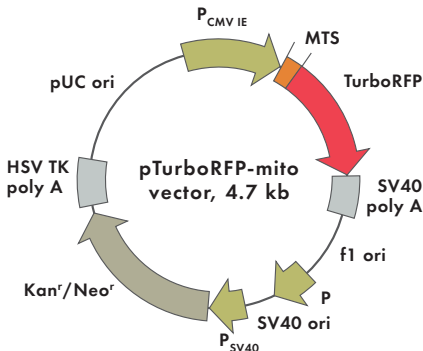
Multiple cloning site (MCS)

G. 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. . . . TurboRFP-dest1
NheI BglII* SacI HindIII EcoRI SalI KpnI ApaI BamHI AgeI
Eco47III XhoI PstI* SacII SmaI/XmaI

* — not unique sites

Notice to Purchaser — please see page A-59

Expression/source vectors: pTurboRFP-mito



For vector sequence, please visit our Web site at www.evrogen.com/pTurboRFP-mito.shtml

| Product | Cat.# | Size |
|--|---|-------|
| pTurboRFP-mito | FP237 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | TurboRFP fusion with mitochondrial targeting sequence (MTS) derived from the subunit VIII of human cytochrome C oxidase | |
| Reporter codon usage | mammalian | |
| Promoter for TurboRFP-MTS | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | expression of mitochondria-targeted TurboRFP in mammalian cells under the control of CMV promoter; source of mitochondria-targeted TurboRFP coding sequence | |

Recombinant protein rTurboRFP

| Product | Cat.# | Size |
|--|-------|--------|
| rTurboRFP | FP252 | 100 µg |
| 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

Description

Recombinant TurboRFP (rTurboRFP) is 26-kDa red fluorescent protein. It has excitation and emission spectra identical to those of the expressed TurboRFP. rTurboRFP is suitable as control reagent for TurboRFP expression using the TurboRFP expression vectors.

rTurboRFP 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. rTurboRFP may contain 6xHis tag at its N-terminus (vary in different lots).

Notice to Purchaser:

TurboRFP-related products: These products are intended for research use only and 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 (Appendix A, page G-5).
CMV Promoter: 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.

TurboFP602

- Bright true-red fluorescence
- Fast maturation
- High pH stability
- Proven suitability to generate stably transfected cell lines
- Fluorescent signal is easily distinguished from background fluorescence
- Optimized for common filter sets
- Recommended for gene expression analysis and cell labeling in autofluorescent environment

Protein description

TurboFP602 is a red-shifted variant of red fluorescent protein TurboRFP. TurboFP602 possesses true-red fluorescence (with excitation/emission maxima at 574/ 602 nm, respectively), optimal for detection via most popular filter sets, and is easily distinguished from background signals. TurboFP602 exhibits fast maturation and high pH stability.

Main properties of TurboFP602

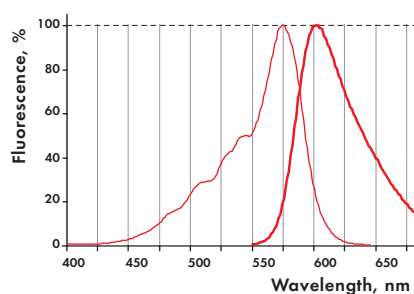
| Characteristic | |
|------------------------|---|
| Molecular weight | 26 kDa |
| Polypeptide length | 231 aa |
| Fluorescence color | true-red |
| Excitation max | 574 nm |
| Emission max | 602 nm |
| Quantum yield | 0.35 |
| Extinction coefficient | 74 400 M ⁻¹ cm ⁻¹ |
| Brightness* | 26.0 |
| Brightness % of EGFP | 79 |
| pKa | 4.7 |
| Structure | dimer |
| Aggregation | no |
| Maturation at 37°C | fast |
| Photostability | medium |

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

Performance and use

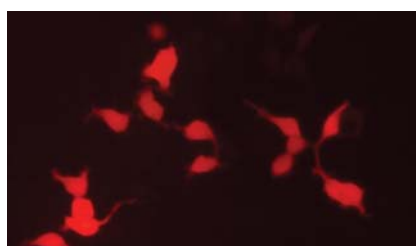
TurboFP602 is mainly intended for applications where fast appearance of true-red fluorescence is crucial. It is specially recommended for cell and organelle labeling and for tracking the promoter activity in autofluorescent tissues.

TurboFP602 can be expressed and detected in a wide range of organisms. Mammalian cells transiently transfected with TurboFP602 expression vec-



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/TurboFP602.shtml

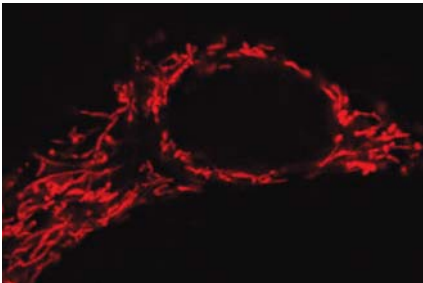


TurboFP602 whole-cell expression in Phoenix cells.

toxic effects and visible protein aggregation are observed.

Despite its dimeric structure, TurboFP602 can be used for fusion construction with cellular proteins. However, we do not recommend that you use TurboFP602 for fusions with oligomerizing cellular proteins (e.g. alpha-tubulin). Please see section "Protein Localization Tags" to select a reporter for such purposes.

| Application | Performance |
|----------------------------------|-------------|
| Cell labeling | |
| mammalian cells | +++ |
| bacterial cells | ++++ |
| Stable transfection | proved |
| Promoter activity testing | ++++ |
| In fusions | ++ |



TurboFP602 mitochondrial expression in HeLa cells.

Compatibility with existing filter sets and antibodies

TurboFP602 can be detected using TRITC filter set or similar. Recommended Omega Optical filter sets are QMAX-Red and XF102-2.

TurboFP602 can be recognized using Anti-tRFP antibody (Cat.# AB231-AB232) available from Evrogen (see description at D-9 page).

TurboFP602 licensing opportunities

Evrogen technology embodied in TurboFP602 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.

TurboFP602-related products

TurboFP602-related product line includes expression and source vectors, recombinant protein, and antibodies. For updated product information, please visit the Evrogen Web site (www.evrogen.com/TurboFP602.shtml).

| Product | Cat.# | Description | Size | Page |
|--|-------|--|--------|------|
| <i>TurboFP602 expression/source vectors</i> | | | | |
| pTurboFP602-C | FP711 | Mammalian expression vector encoding humanized TurboFP602 and allowing TurboFP602 expression and generation of fusions to the TurboFP602 C-terminus | 20 µg | A-63 |
| pTurboFP602-N | FP712 | Mammalian expression vector encoding humanized TurboFP602 and allowing TurboFP602 expression and generation of fusions to the TurboFP602 N-terminus | 20 µg | A-63 |
| pTurboFP602-B | FP713 | Bacterial expression vector; source of humanized TurboFP602 coding sequence | 20 µg | A-64 |
| pTurboFP602-PRL | FP715 | Promoterless mammalian expression vector encoding humanized TurboFP602 and designed for monitoring transcription from different promoters and promoter/enhancer combinations | 20 µg | A-64 |
| pTurboFP602-mito | FP717 | Mammalian expression vector encoding humanized TurboFP602 targeted to mitochondria | 20 µg | A-65 |
| <i>Recombinant protein</i> | | | | |
| rTurboFP602 | FP751 | Recombinant TurboFP602 protein | 100 µg | A-65 |
| <i>Antibodies against TurboFP602</i> | | | | |
| Anti-tRFP antibody | AB231 | Rabbit polyclonal antibody against TagRFP, TagFP635, TurboRFP, TurboFP602, and TurboFP635 proteins | 100 µg | D-9 |
| | AB232 | | 200 µg | |

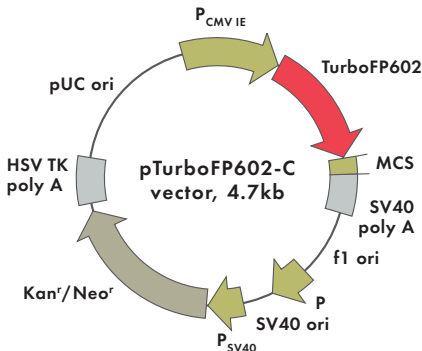
Please contact your local distributor for exact prices and delivery information.

Notice to Purchaser:

TurboFP602-related products: These products are intended for research use only and 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 (Appendix A, page G-5).

CMV Promoter: 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.

Expression/source vectors: pTurboFP602-C



For vector sequence, please visit our Web site at www.evrogen.com/pTurboFP602-C.shtml

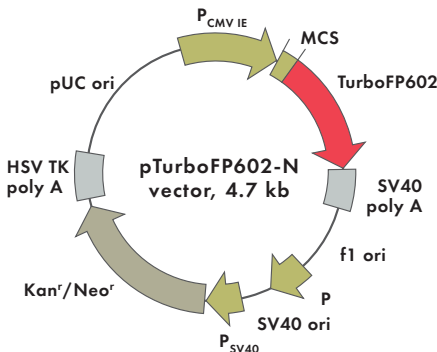
| Product | Cat.# | Size |
|--|--|-------|
| pTurboFP602-C | FP711 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | TurboFP602 | |
| Reporter codon usage | mammalian | |
| Promoter for TurboFP602 | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin | |
| | eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori | |
| | eukaryotic — SV40 ori | |
| Use | generation of fusions to the TurboFP602 C-terminus; expression of TurboFP602 | |
| | or its fusions in mammalian cells | |

Multiple cloning site (MCS)



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

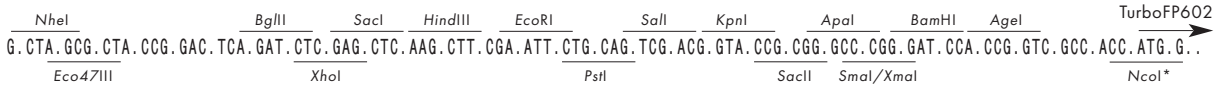
Expression/source vectors: pTurboFP602-N



For vector sequence, please visit our Web site at www.evrogen.com/pTurboFP602-N.shtml

| Product | Cat.# | Size |
|--|--|-------|
| pTurboFP602-N | FP712 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | TurboFP602 | |
| Reporter codon usage | mammalian | |
| Promoter for TurboFP602 | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin | |
| | eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori | |
| | eukaryotic — SV40 ori | |
| Use | generation of fusions to the TurboFP602 N-terminus; expression of TurboFP602 | |
| | or its fusions in mammalian cells | |

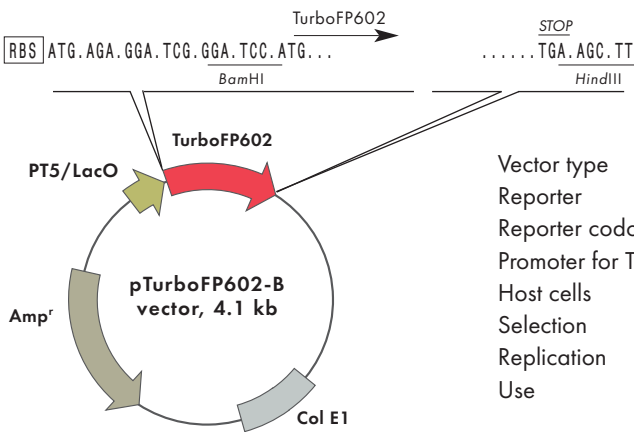
Multiple cloning site (MCS)



* — not unique sites

Notice to Purchaser — please see page A-65

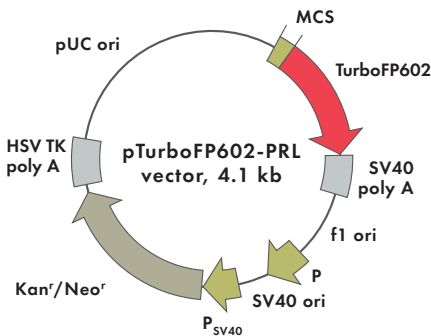
Expression/source vectors: pTurboFP602-B



For vector sequence, please visit our Web site at www.evrogen.com/pTurboFP602-B.shtml

| | |
|-------------------------|---|
| Vector type | bacterial expression vector |
| Reporter | TurboFP602 |
| Reporter codon usage | mammalian |
| Promoter for TurboFP602 | T5 promoter/lac operator |
| Host cells | prokaryotic |
| Selection | ampicillin |
| Replication | ColE1 ori |
| Use | TurboFP602 expression in bacterial cells using T5 promoter/lac operator; source of the TurboFP602 coding sequence |

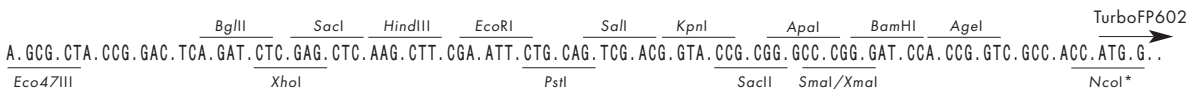
Expression/source vectors: pTurboFP602-PRL



For vector sequence, please visit our Web site at www.evrogen.com/pTurboFP602-PRL.shtml

| | |
|-------------------------|---|
| Vector type | promoterless vector |
| Reporter | TurboFP602 |
| Reporter codon usage | mammalian |
| Promoter for TurboFP602 | NO |
| Host cells | mammalian, bacterial |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori |
| Use | monitoring the activity of promoter or promoter/enhancer combination cloned into vector MCS |

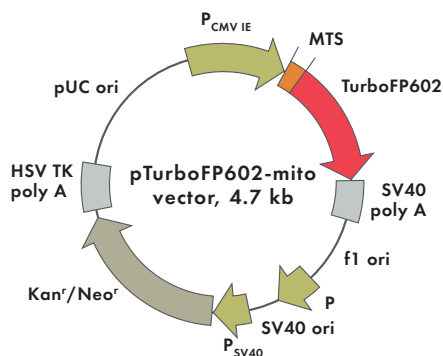
Multiple cloning site (MCS)



* — not unique sites

Notice to Purchaser — please see page A-65

Expression/source vectors: pTurboFP602-mito



For vector sequence, please visit our Web site at www.evrogen.com/pTurboFP602-mito.shtml

| Product | Cat.# | Size |
|--|---|-------|
| pTurboFP602-mito | FP717 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | TurboFP602 fusion with mitochondrial targeting sequence (MTS) derived from the subunit VIII of human cytochrome C oxidase | |
| 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 mitochondria-targeted TurboFP602 in mammalian cells under the control of CMV promoter; source of mitochondria-targeted TurboFP602 coding sequence | |

Recombinant protein rTurboFP602

| Product | Cat.# | Size |
|--|-------|--------|
| rTurboFP602 | FP751 | 100 µg |
| 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

Description

Recombinant TurboFP602 (rTurboFP602) is 26-kDa red fluorescent protein. It has excitation and emission spectra identical to those of the expressed TurboFP602. rTurboFP602 is suitable as control reagent for TurboFP602 expression using the TurboFP602 expression vectors.

rTurboFP602 is purified from transformed *E. coli* using organic extraction and hydrophobic chromatography. This method ensures high purity of the recombinant protein and maintenance of fluorescence. The protein concentration is measured by chromophore absorption. rTurboFP602 contains 6xHis tag at its N-terminus.

Notice to Purchaser:

TurboFP602-related products: These products are intended for research use only and 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 (Appendix A, page G-5).
CMV Promoter: 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.

TurboFP635

- Bright far-red fluorescence
- 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 gene expression analysis, cell labeling inside of tissues

Protein description

TurboFP635 (scientific name Katushka) is a novel far-red mutant of the red fluorescent protein derived from sea anemone *Entacmaea quadricolor* [1]. Possessing excitation/emission maxima at 588/635 nm, TurboFP635 is 7 to 10-fold brighter compared to the spectrally close HcRed [2] or mPlum [3], and is characterized by fast maturation and a high pH- and photo-stability. These unique characteristics make TurboFP635 the protein of choice for visualization within living tissues and dual-color high-throughput assays.

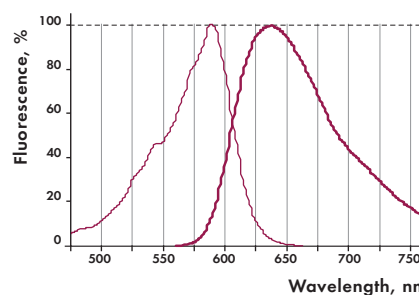
Main properties of TurboFP635

| Characteristic | |
|------------------------|---|
| Molecular weight | 26.3 kDa |
| Polypeptide length | 231 aa |
| Fluorescence color | far-red |
| Excitation max | 588 nm |
| Emission max | 635 nm |
| Quantum yield | 0.34 |
| Extinction coefficient | 65 000 M ⁻¹ cm ⁻¹ |
| Brightness* | 22.1 |
| Brightness % of EGFP | 67 |
| pKa | 5.5 |
| Structure | dimer |
| Aggregation | no |
| Maturation at 37°C | superfast |
| Photostability | high |

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

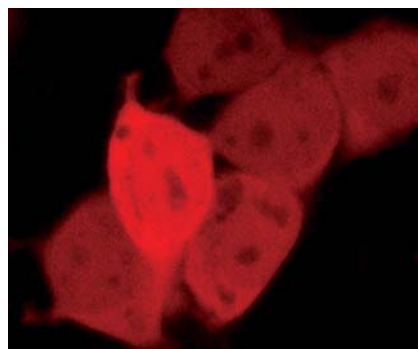
Performance and use

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 expressed and detected in a wide range of organisms. Mammalian cells transiently transfected with TurboFP635 expression vectors give bright fluorescent signals within 10-12 hours after transfection. No cell toxic effects and visible protein aggregation are observed.



TurboFP635 normalized excitation (thin line) and emission (thick line) spectra.

Complete TurboFP635 spectra in Excel format can be downloaded from the Evrogen website at www.evrogen.com/TurboFP635.shtml



TurboFP635 expression in Phoenix cells.



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 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 ref.[1]

Despite its dimeric structure, TurboFP635 can be used for fusion construction with cellular proteins. However we recommend to use TagFPs (see section "Protein Localization Tags") for these purposes.

| Application | Performance |
|----------------------------------|-------------|
| Cell labeling | |
| mammalian cells | ++++ |
| bacterial cells | ++++ |
| Stable transfection | proved |
| Promoter activity testing | ++++ |
| In fusions | ++ |

Compatibility with existing filter sets and antibodies

Recommended Omega Optical filter sets for TurboFP635 are QMAX-Red and XF102-2.

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

TurboFP635 licensing opportunities

Evrogen technology embodied in TurboFP635 is available for expanded and commercial use with an adaptable licensing program. Benefit from flexible and market-driven license options offered for upgrade and novel development of products and applications. For licensing information please contact Evrogen at license@evrogen.com.

References

1. Shcherbo et al. (2007) Nat. Methods 4(9): 741 - 746
2. Gurskaya et al. (2001) FEBS Lett. 507: 16-20.
3. Wang et al. (2004) Proc Natl Acad Sci U S A 101: 16745-16749.

TurboFP635-related products

TurboFP635-related product line include expression vectors and antibodies. Each of these products is described in details in this section below. For updated product information please visit the Evrogen web site (www.evrogen.com/TurboFP635.shtml).

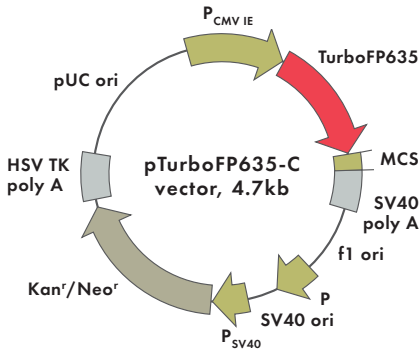
| Product | Cat.# | Description | Size | Page |
|--|-------|--|--------|------|
| <i>TurboFP635 expression/source vectors</i> | | | | |
| pTurboFP635-C | FP721 | C-terminal mammalian expression vector encoding humanized TurboFP635 and allowing TurboFP635 expression and generation of fusions to the TurboFP635 C-terminus | 20 µg | A-68 |
| pTurboFP635-N | FP722 | N-terminal mammalian expression vector encoding humanized TurboFP635 and allowing TurboFP635 expression and generation of fusions to the TurboFP635 N-terminus | 20 µg | A-68 |
| <i>Antibodies against TurboFP635</i> | | | | |
| Anti-tRFP antibody | AB231 | Rabbit polyclonal antibody against TagRFP, TagFP635, | 100 µg | D-9 |
| | AB232 | TurboRFP, TurboFP602 and TurboFP635 proteins | 200 µg | |

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

Notice to Purchaser:

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CMV Promoter: 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.

Expression/source vectors: pTurboFP635-C



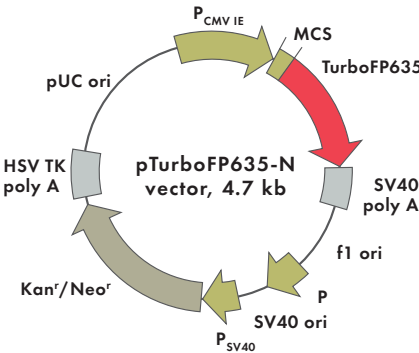
For vector sequence please visit our website at www.evrogen.com/pTurboFP635-C.shtml

Multiple cloning site (MCS)



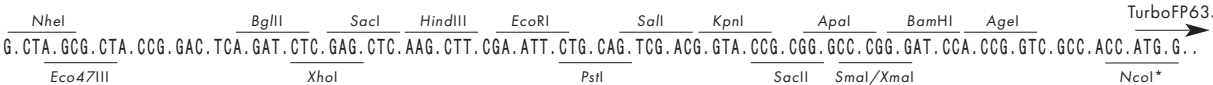
— sites are blocked by methylation. If you wish to digest the vector using these sites, you will need to transform the vector into a dam⁻ host and make fresh DNA; * — not unique sites.

Expression/source vectors: pTurboFP635-N



For vector sequence please visit our website at www.evrogen.com/pTurboFP635-N.shtml

Multiple cloning site (MCS)



* — not unique sites.

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MATERIAL SAFETY DATA SHEET INFORMATION

ATTENTION: Safety Officer. EVROGEN JSC (Moscow, Russia) hereby confirms that to the best of our knowledge these products do not require a Material Safety Data Sheet. However, all of the properties of these products (and, if applicable, each of its 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.

| Product | Cat.# | Size |
|--|--|-------|
| pTurboFP635-C | FP721 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | TurboFP635 | |
| Reporter codon usage | mammalian | |
| Promoter for TurboFP635 | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | generation of fusions to the TurboFP635 C-terminus; expression of TurboFP635 or its fusions in mammalian cells | |

| Product | Cat.# | Size |
|--|--|-------|
| pTurboFP635-N | FP722 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | TurboFP635 | |
| Reporter codon usage | mammalian | |
| Promoter for TurboFP635 | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | generation of fusions to the TurboFP635 N-terminus; expression of TurboFP635 or its fusions in mammalian cells | |

Other fluorescent proteins

Fluorescent proteins
for common use

Proteins available:

- **Phi-Yellow fluorescent proteins**

source — jellyfish *Phialidium* sp.

excitation max — 525 nm

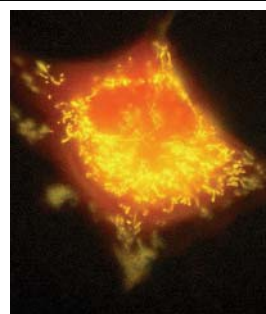
emission max — 537 nm

- **red fluorescent protein JRed**

source — Anthomedusae jellyfish

excitation max — 584 nm

emission max — 610 nm



Stably transfected M3 cells
expressing PhiYFP in mito-
chondria and JRed in cytosol.

Photograph of cells was pro-
vided by Dr. Christian Petzelt
(Marinpharm).

Main properties of Phi-Yellow (PhiYFP and PhiYFP-m) and JRed:

| Characteristic | PhiYFP | PhiYFP-m | JRed |
|------------------------|---|---|---|
| Fluorescence color | yellow | yellow | true-red |
| Excitation max | 525 nm | 525 nm | 584 nm |
| Emission max | 537 nm | 537 nm | 610 nm |
| Quantum yield | 0.40 | 0.39 | 0.20 |
| Extinction coefficient | 130 000 M ⁻¹ cm ⁻¹ | 124 000 M ⁻¹ cm ⁻¹ | 44 000 M ⁻¹ cm ⁻¹ |
| Brightness | 52.0 | 48.4 | 8.8 |
| Brightness, % of EGFP | 158 | 147 | 26 |
| pKa | 6.0 | 6.0 | 5.0 |
| Structure | dimer | dimer | dimer |
| Cell Toxicity | not observed | not observed | at certain excitation wavelengths |
| Aggregation | no | no | no |
| Maturation at 37°C | fast | fast | slow |
| Photostability | high | high | medium |
| Molecular weight | 26 kDa | 26 kDa | 27 kDa |
| Main advantages | Bright and fast-maturing true-yellow fluorescent protein suitable for generation of stably transfected cell lines | Bright and fast-maturing true-yellow fluorescent protein suitable for generation of stably transfected cell lines | True-red fluorescent protein with good compatibility with popular filter sets |
| Possible limitations | Dimer, limited applicability for generation of fusions; unsuitable for generation of fusions to its C-terminus | Dimer, limited applicability for generation of fusions | Dimer, limited applicability for generation of fusions; unsuitable for expression in bacteria |

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

Phi-Yellow proteins

- Bright yellow fluorescence
- Proven availability to generate stably transfected cell lines
- Destabilized variant is available

Protein description

PhiYFP and PhiYFP-m are mutants of a natural yellow fluorescent protein from *Phialidium* sp. (Cnidaria; Hydrozoa; Hydroida; Leptomedusae; Campanulariidae) [1] and previous versions of TurboYFP.

They possess less brightness and maturation rate than TurboYFP, but are more suitable for generation of stably transfected cell lines and for organelle labeling.

The emission wavelength of Phi-Yellow proteins is ideally positioned between those of green and red fluorescent proteins, allowing easy separation of these fluorescent tags by flow cytometry using common channels of detection and a single laser excitation line.

Main properties of Phi-Yellow proteins

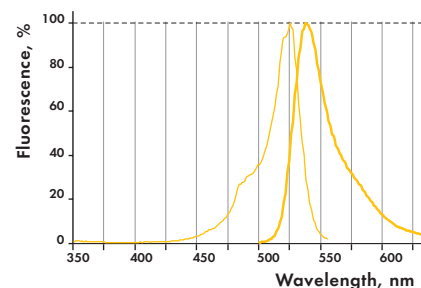
| Characteristic | PhiYFP | PhiYFP-m |
|---|---------|----------|
| Molecular weight, kDa | 26 | 26 |
| Polypeptide length, aa | 234 | 234 |
| Fluorescence color | yellow | yellow |
| Excitation max, nm | 525 | 525 |
| Emission max, nm | 537 | 537 |
| Quantum yield | 0.40 | 0.39 |
| Extinction coefficient, $M^{-1}cm^{-1}$ | 130 000 | 124 000 |
| Brightness* | 52.0 | 48.4 |
| Brightness % of EGFP | 158 | 147 |
| pKa | 6.0 | 6.0 |
| Structure | dimer | dimer |
| Aggregation | no | no |
| Maturation at 37°C | fast | fast |
| Photostability | high | high |

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

Destabilized PhiYFP-m

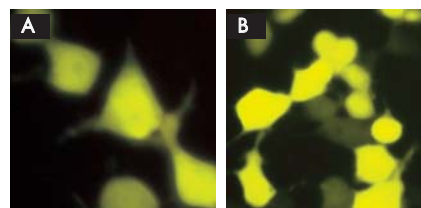
Destabilized PhiYFP-m variant (PhiYFP-m-dest1) is produced by fusing the initial protein with PEST amino acid sequence encoded by region 422-461 of mouse ornithine decarboxylase gene [2]. This sequence targets the protein to degradation and enables a rapid protein turnover.

PhiYFP-m-dest1 retains spectral properties of the initial protein, but has shorter half-life (approximately 2 hrs) as measured by the analysis of fluorescence intensity of cells treated with a protein synthesis inhibitor, cycloheximide. Because of rapid turnover, PhiYFP-m-dest1 can be used to measure changes in gene expression.



PhiYFP and PhiYFP-m normalized excitation (thin line) and emission (thick line) spectra.

Complete Phi-Yellow spectra in Excel format can be downloaded from the Evrogen Web site at www.evrogen.com/PhiYFP.shtml



Expression of Phi-Yellow proteins in transiently transfected mammalian cells: (A) — PhiYFP-m; (B) — PhiYFP.

Performance and use

Phi-Yellow proteins can be easily expressed and detected in a wide range of organisms, from bacteria to mammals. Transient transfection of mammalian cell lines with these proteins results in bright yellow fluorescent signals without visible aggregation. Fluorescence is clearly detected within 12 hrs after transfection.

Despite dimerization capacity, Phi-Yellow proteins demonstrate successful performance in fusions with subcellular localization signals and many cellular proteins. However, we recommend that you use TagFPs for protein labeling applications. Please see section "Protein Localization Tags" to select a reporter for such purposes.

Important note: PhiYFP allows 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.

| Application | Performance |
|----------------------------------|-------------|
| Cell labeling | |
| mammalian cells | ++++ |
| bacterial cells | +++ |
| Stable transfection | proved |
| Promoter activity testing | +++ |
| In fusions | ++ |

Compatibility with existing filter sets and antibodies

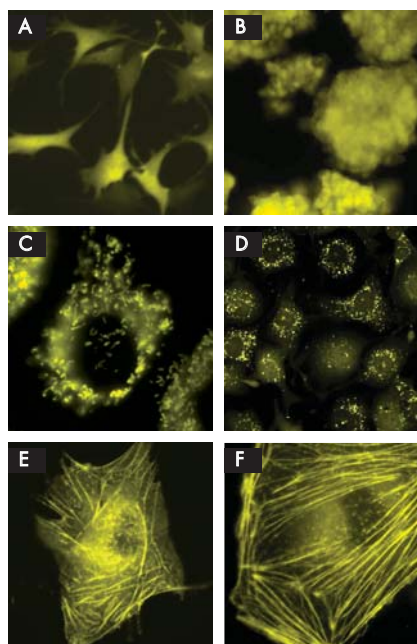
Phi-Yellow proteins can be detected using Omega filter set XF104-3 or Chroma Technology corporation filter set 42003 ("ZsYellow1").

The proteins can be recognized using Anti-PhiYFP and Anti-PhiYFP(d) antibodies (Cat.# AB601-AB604, see pages D-7 and D-8 for descriptions) available from Evrogen.

Phi-Yellow licensing opportunities

Evrogen technology embodied in Phi-Yellow proteins 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.



Phi-Yellow expression in stably transfected mammalian cells.

(A) — PhiYFP whole-cell expression in BC3H1 mouse brain tumor cells; (B) — PhiYFP whole-cell expression in PC-12 pheochromocytoma cells; (C) — mitochondrial expression of PhiYFP in 3T3 mouse fibroblasts; (D) Peroxisomal expression of PhiYFP-m in T-24 human bladder carcinoma cells; (E) — expression of PhiYFP-m fusion with cytoplasmic beta-actin in MADIN-DARBY canine kidney epithelial cells; (F) — expression of PhiYFP-m fusion with cytoplasmic beta-actin in rat kangaroo kidney epithelium cells PIK2.

Photographs of stably transfected cell lines were provided by Dr. Christian Petzelt (Marinpharm).

References

1. Shagin *et al.* (2004) *Mol. Biol. Evol.* 21(5): 841-850.
2. Li *et al.* (1998) *J. Biol. Chem.* 273:34970-34975.

Phi-Yellow-related products

Phi-Yellow-related product line includes expression and source vectors, recombinant protein, and antibodies. For updated product information, please visit the Evrogen Web site (www.evrogen.com/PhiYFP.shtml).

| Product | Cat.# | Description | Size | Page |
|--|-------|--|--------|------|
| <i>Phi-Yellow expression/source vectors</i> | | | | |
| pPhi-Yellow-C | FP601 | Mammalian expression vector encoding humanized PhiYFP-m and allowing PhiYFP-m expression and generation of fusions to the PhiYFP-m C-terminus | 20 µg | A-75 |
| pPhi-Yellow-N | FP602 | Mammalian expression vector encoding humanized PhiYFP and allowing PhiYFP expression and generation of fusions to the PhiYFP N-terminus Note: PhiYFP is not suitable for fusion construction to the reporter C-terminus | 20 µg | A-75 |
| pPhi-Yellow-B | FP603 | Bacterial expression vector; source of humanized PhiYFP coding sequence | 20 µg | A-76 |
| pPhi-Yellow-PRL | FP604 | Promoterless expression vector encoding humanized PhiYFP and designed for monitoring transcription from different promoters and promoter/enhancer combinations | 20 µg | A-76 |
| pPhi-Yellow-PRL-dest1 | FP605 | Promoterless vector encoding destabilized PhiYFP-m and designed for monitoring transcription from different promoters and promoter/enhancer combinations | 20 µg | A-77 |
| pPhi-Yellow-dest1 | FP608 | Mammalian expression vector encoding destabilized PhiYFP-m and allowing PhiYFP-m-dest1 expression and generation of fusions to the N-terminus of PhiYFP-m-dest1 | 20 µg | A-77 |
| pPhi-Yellow-peroxi | FP606 | Mammalian expression vector encoding humanized PhiYFP-m targeted to peroxisomes | 20 µg | A-78 |
| pPhi-Yellow-mito | FP607 | Mammalian expression vector encoding humanized PhiYFP targeted to mitochondria | 20 µg | A-78 |
| <i>Recombinant protein</i> | | | | |
| rPhiYFP | FP651 | Purified recombinant yellow fluorescent protein rPhiYFP | 100 µg | A-79 |
| <i>Antibodies against Phi-Yellow proteins</i> | | | | |
| Anti-PhiYFP antibody | AB601 | Rabbit polyclonal antibody against non-denatured | 100 µg | D-7 |
| | AB602 | TurboYFP, PhiYFP, and PhiYFP-m. | 200 µg | |
| Anti-PhiYFP(d) antibody | AB603 | Rabbit polyclonal antibody against denatured | 100 µg | D-8 |
| | AB604 | TurboYFP, PhiYFP, and PhiYFP-m. | 200 µg | |

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

Third party products: stably transfected cell lines expressing Phi-Yellow proteins

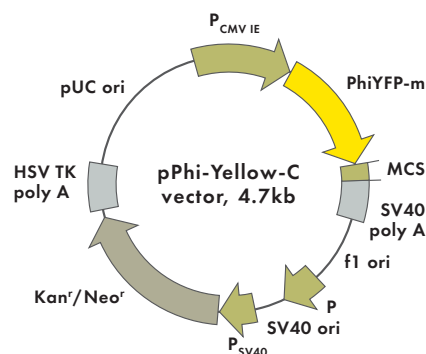
| Cell line | Source | Description |
|---------------|--------|--|
| M3-PY | mouse | M3 mouse melanoma cells expressing PhiYFP in cytosol |
| PC-PY | rat | PC-12 rat pheochromocytoma expressing PhiYFP in cytosol |
| T406-PY | human | T406 human glioma expressing PhiYFP in cytosol |
| BC3-PY | mouse | BC3H1 myoblast cells expressing PhiYFP in cytosol |
| W-PY | rat | WALKER 256 rat tumor expressing PhiYFP in cytosol |
| T24-PY | human | T24 human bladder carcinoma expressing PhiYFP in cytosol |
| T24-PY-dest | rat | Rat kangaroo kidney epithelium PtK2 cells expressing destabilized PhiYFP-m in cytosol |
| T24-PY-P | rat | Rat kangaroo kidney epithelium PtK2 cells expressing PhiYFP-m in peroxisomes |
| C2C12-PY-Mito | mouse | Mouse myoblast cells expressing PhiYFP in mitochondria |
| 3T3-PY-Mito | mouse | Mouse fibroblasts 3T3 expressing PhiYFP in mitochondria |
| P-PY-Mito | rat | Rat kangaroo kidney epithelium PtK2 expressing PhiYFP in mitochondria |
| M3-JR-PY-Mito | mouse | Doubly transfected mouse melanoma M3 cells expressing PhiYFP in mitochondria and JRed in cytosol |
| P-PY-A | rat | Rat kangaroo kidney epithelium PtK2 expressing PhiYFP-m fusion with beta-actin |
| T47-PY-A | human | T47-D human breast cancer cells expressing PhiYFP-m fusion with beta-actin |
| MDCK-PY-A | canine | MADIN-DARBY-canine kidney epithelial cells expressing PhiYFP-m fusion with beta-actin |
| 3T3-PY-A | mouse | Mouse fibroblasts 3T3 expressing PhiYFP-m fusion with beta-actin |
| H-PY-A | human | HeLa human cervical carcinoma expressing PhiYFP-m fusion with beta-actin |

Cell lines are manufactured by Marinpharm GmbH (Berlin, Germany) under the Evrogen license.

Notice to Purchaser:

Phi-Yellow-related products: These products are intended for research use only and 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 (Appendix A, page G-5).
CMV Promoter: 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.

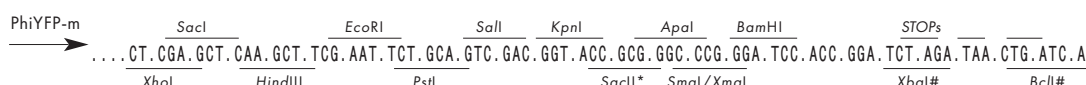
Expression/source vectors: pPhi-Yellow-C



For vector sequences please visit our Web site at
www.evrogen.com/p-phiYFP-C.shtml

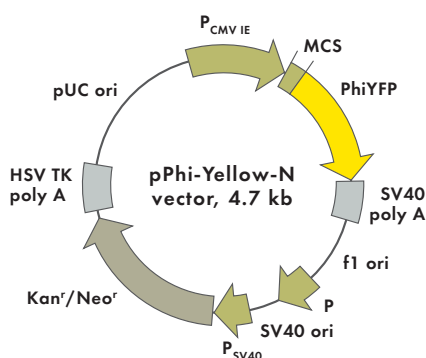
| Product | Cat.# | Size |
|--|--|-------|
| pPhi-Yellow-C | FP601 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | PhiYFP-m | |
| Reporter codon usage | mammalian | |
| Promoter for PhiYFP-m | P _{CMVIE} | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | generation of fusions to the PhiYFP-m C-terminus; expression of PhiYFP-m or its fusions in mammalian cells | |

Multiple cloning site (MCS)



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

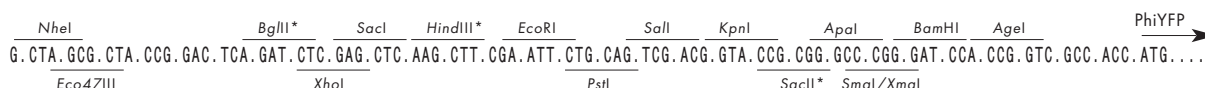
Expression/source vectors: pPhi-Yellow-N



For vector sequence, please visit our Web site at www.evrogen.com/p-phiYFP-N.shtml

| Product | Cat.# | Size |
|--|--|-------|
| pPhi-Yellow-N | FP602 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | PhiYFP | |
| Reporter codon usage | mammalian | |
| Promoter for PhiYFP | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | generation of fusions to the PhiYFP N-terminus; expression of PhiYFP or its fusions in mammalian cells | |

Multiple cloning site (MCS)



* — not unique sites.

Notice to Purchaser — please see page A-79

Expression/source vectors: pPhi-Yellow-B

| Product | Cat.# | Size |
|---------------|-------|-------|
| pPhi-Yellow-B | FP603 | 20 µg |

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

Vector type
Reporter
Reporter codon usage
Promoter for PhiYFP
Host cells
Selection
Replication
Use

bacterial expression vector
PhiYFP
mammalian
T5 promoter/lac operator
prokaryotic
ampicillin
ColE1 ori
PhiYFP expression in bacterial cells using T5 promoter/lac operator; source of the PhiYFP coding sequence

For vector sequence, please visit our Web site at www.evrogen.com/p-phiYFP-B.shtml

Expression/source vectors: pPhi-Yellow-PRL

| Product | Cat.# | Size |
|-----------------|-------|-------|
| pPhi-Yellow-PRL | FP604 | 20 µg |

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

Vector type
Reporter
Reporter codon usage
Promoter for PhiYFP
Host cells
Selection
Replication
Use

promoterless vector
PhiYFP
mammalian
NO
mammalian, bacterial
prokaryotic — kanamycin
eukaryotic — neomycin (G418)
prokaryotic — pUC ori
eukaryotic — SV40 ori
monitoring the activity of promoter or promoter/enhancer combination cloned into vector MCS

For vector sequence, please visit our Web site at www.evrogen.com/p-phiYFP-PRL.shtml

Multiple cloning site (MCS)

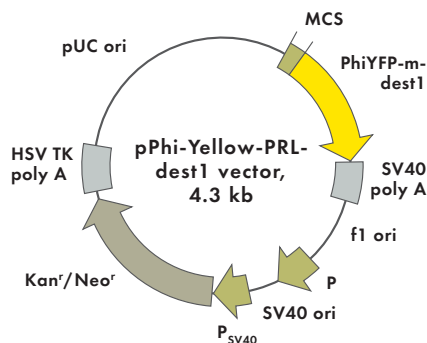
A. GCG. CTA. CCG. GAC. TCA. GAT. CTC. GAG. CTC. AAG. CTT. CGA. ATT. CTG. CAG. TCG. ACG. GTA. CCG. CGG. GCC. CGG. GAT. CCA. CCG. GTC. GCC. ACC. ATG. ...

Eco47III BglII* XhoI SacI* HindIII* EcoRI PstI SalI KpnI SacII* SmaI/XmaI ApaI BamHI AgeI PhiYFP

* — not unique sites.

Notice to Purchaser — please see page A-79

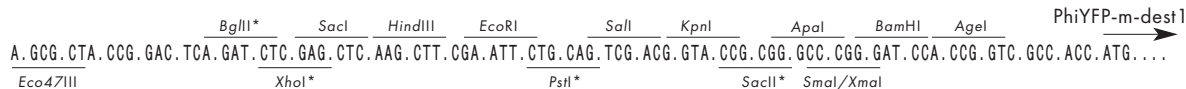
Expression/source vectors: **pPhi-Yellow-PRL-dest1**



For vector sequence, please visit our Web site at www.evrogen.com/p-phiYFP-PRLd1.shtml

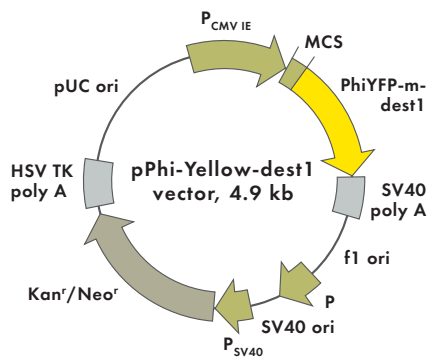
| Product | Cat.# | Size |
|--|--|-------|
| pPhi-Yellow-PRL-dest1 | FP605 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | promoterless vector | |
| Reporter | PhiYFP-m-dest1 | |
| Reporter codon usage | mammalian | |
| Promoter for PhiYFP-m-dest1 | NO | |
| Host cells | mammalian, prokaryotic | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | monitoring the activity of promoter or promoter/enhancer combination cloned into vector MCS. Rapid turnover of PhiYFP-m-dest1 allows exact measuring of changes in gene expression | |

Multiple cloning site (MCS)



* — not unique sites.

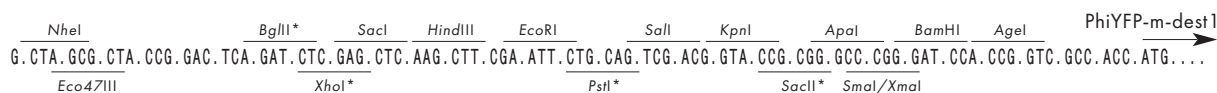
Expression/source vectors: **pPhi-Yellow-dest1**



For vector sequence, please visit our Web site at www.evrogen.com/pPhiYFP-dest1.shtml

| Product | Cat.# | Size |
|--|---|-------|
| pPhi-Yellow-dest1 | FP608 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | PhiYFP-m-dest1 | |
| Reporter codon usage | mammalian | |
| Promoter for PhiYFP-m-dest1 | P_CMV IE | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | generation of fusions to the PhiYFP-m-dest1 N-terminus; expression of PhiYFP-m-dest1 or its fusions in mammalian cells; positive control for the pPhiYFP-m-PRL-dest1 vector (cat.# FP605) | |

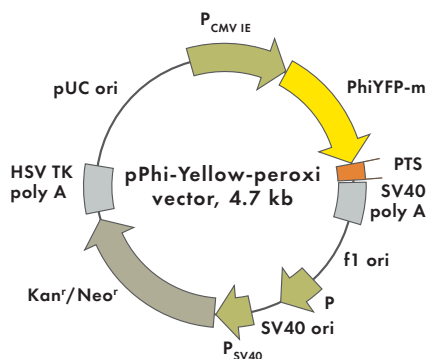
Multiple cloning site (MCS)



* — not unique sites.

Notice to Purchaser — please see page A-79

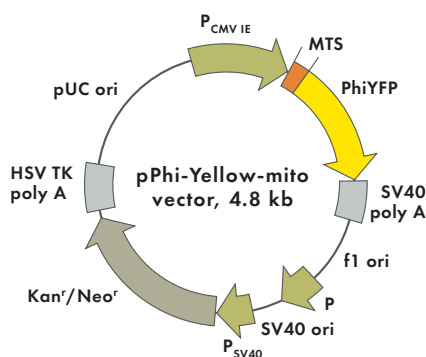
Expression/source vectors: pPhi-Yellow-peroxi



For vector sequence, please visit our Web site at www.evrogen.com/p-phiYFP-peroxi.shtml

| Product | Cat.# | Size |
|--|---|-------|
| pPhi-Yellow-peroxi | FP606 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | PhiYFP-m fusion with the peroxisome targeting signal (PTS) | |
| Reporter codon usage | mammalian | |
| Promoter for PhiYFP-m | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | expression of peroxisome-targeted PhiYFP-m in mammalian cells under the control of CMV promoter; source of peroxisome-targeted PhiYFP-m coding sequence | |

Expression/source vectors: pPhi-Yellow-mito



For vector sequence, please visit our Web site at www.evrogen.com/p-phiYFP-mito.shtml

| Product | Cat.# | Size |
|--|---|-------|
| pPhi-Yellow-mito | FP607 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | PhiYFP fusion with mitochondrial targeting sequence (MTS) derived from the subunit VIII of human cytochrome C oxidase | |
| Reporter codon usage | mammalian | |
| Promoter for PhiYFP | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | expression of mitochondria-targeted PhiYFP in mammalian cells under the control of CMV promoter; source of mitochondria-targeted PhiYFP coding sequence | |

Notice to Purchaser — please see page A-79

Recombinant protein rPhiYFP

| Product | Cat.# | Size |
|---------|-------|--------|
| rPhiYFP | FP651 | 100 µg |

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

Description

Recombinant PhiYFP (rPhiYFP) is 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 (vary in different lots).

Notice to Purchaser:

Phi-Yellow-related products: These products are intended for research use only and 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 (Appendix A, page G-5).

CMV Promoter: 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.

JRed

- True-red fluorescence
- Proven suitability to create stably transfected cell lines

Protein description

JRed is a red fluorescent protein obtained by mutagenesis of Anthomedusae jellyfish chromoprotein [1]. JRed fluorescence can be detected using most popular filter sets.

Main properties of JRed

| Characteristic | |
|------------------------|---|
| Molecular weight | 27 kDa |
| Polypeptide length | 242 aa |
| Fluorescence color | true red |
| Excitation max | 584 nm |
| Emission max | 610 nm |
| Quantum yield | 0.20 |
| Extinction coefficient | 44 000 M ⁻¹ cm ⁻¹ |
| Brightness* | 8.8 |
| Brightness % of EGFP | 26 |
| pKa | 5.0 |
| Structure | dimer |
| Aggregation | no |
| Maturation at 37°C | slow |
| Photostability | medium |

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

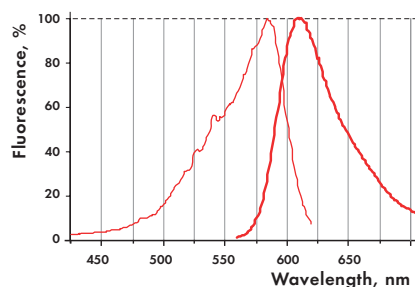
Performance and use

JRed can be expressed in eukaryotic cells; however, it is not appropriate for expression in prokaryotes.

Mammalian cells transiently transfected with JRed vector give red fluorescence without visible aggregation. Fluorescence is clearly detected within 24 hrs after transfection.

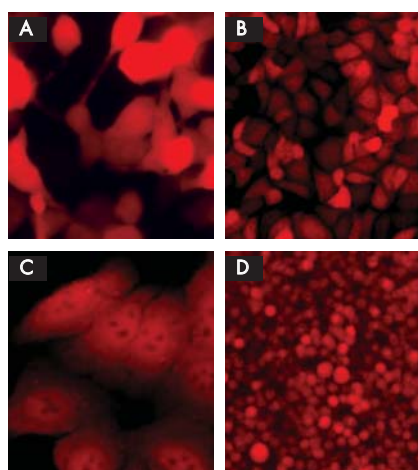
JRed shows successful performance in many fusions including that with cytoplasmic beta-actin, BH3 interacting domain death agonist (BID), nucleolar protein fibrillarin, dopamin transporter (hDAT). In addition, a number of stably transfected cell lines expressing JRed were generated by Marinpharm GmbH.

JRed possesses relatively fast photobleaching rate upon arc lamp irradiation. At the same time, it exhibits high photostability when excited by 543 nm laser line in a confocal microscope, with the photobleaching time several times longer compared with DsRed2. JRed could show phototoxicity when bleached.



JRed normalized excitation (thin line) and emission (thick line) spectra.

Complete JRed spectra in Excel format can be downloaded from the Evrogen Web site at www.evrogen.com/JRED.shtml



Fluorescent microscopy of mammalian cells expressing JRed in cytosol.

(A) — Transiently transfected 293T cells; (B) — stably transfected T24 cells; (C) — stably transfected HeLa cells; (D) — stably transfected WALKER cells. Photographs of stably transfected cell lines were provided by Dr. Christian Petzelt (Marinpharm).

| Application | Performance |
|----------------------------------|-------------|
| Cell labeling | |
| mammalian cells | ++ |
| bacterial cells | - |
| Stable transfection | proved |
| Promoter activity testing | ++ |
| In fusions | ++ |

Compatibility with existing filter sets and antibodies

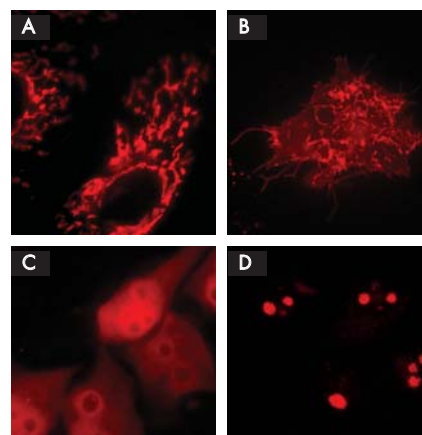
JRed can be detected using TRITC filter set or similar. Recommended Omega filter sets are QMAX-Red and XF174.

JRed can be recognized using Anti-KillerRed antibody (Cat.# AB961-AB962) available from Evrogen (see description on page D-11).

JRed licensing opportunities

Evrogen technology embodied in JRed 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.



Fluorescent microscopy of mammalian cells expressing JRed fusions.

(A) — Mitochondria-targeted JRed in HeLa cells; (B) — JRed-hDAT fusion in PAE cells; (C) — JRed-BID fusion in HeLa cells; (D) — JRed-fibrillarin fusion in HeLa cells

References

1. Shagin *et al.* (2004) *Mol. Biol. Evol.* 21(5): 841-850.

JRed-related products

JRed-related product line includes expression and source vectors for JRed expression. For updated product information, please visit the Evrogen Web site (www.evrogen.com/JRed.shtml).

| Product | Cat.# | Description | Size | Page |
|---------------------------------------|----------------|--|------------------|------|
| JRed expression/source vectors | | | | |
| pJRed-C | FP701 | Mammalian expression vector encoding humanized JRed and allowing JRed expression and generation of fusions to the JRed C-terminus | 20 µg | A-83 |
| pJRed-IN | FP702 | Mammalian expression vector encoding humanized JRed and allowing JRed expression and generation of fusions to the JRed N-terminus | 20 µg | A-83 |
| pJRed-PRL | FP705 | Promoterless expression vector encoding humanized JRed and designed for monitoring transcription from different promoters and promoter/enhancer combinations | 20 µg | A-84 |
| Antibodies against JRed | | | | |
| Anti-KillerRed antibody | AB961 AB962 | Rabbit polyclonal antibody against KillerRed and JRed | 100 µg 200 µg | D-11 |

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

Third party products: stably transfected cell lines expressing JRed

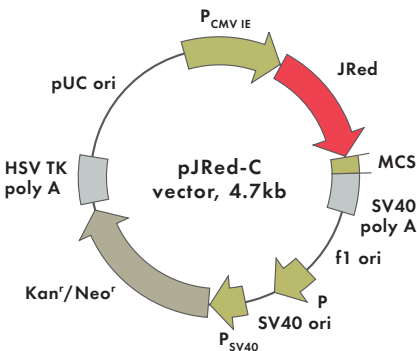
| Cell line | Source | Description |
|-----------------------------------|---------|--|
| W-JR | rat | WALKER 256 rat tumor cells expressing JRed in cytosol |
| PC-JR | rat | PC-12 rat pheochromocytoma expressing JRed in cytosol |
| H-JR | human | HeLa human cervical carcinoma expressing JRed in cytosol |
| T24-JR | human | T24 human bladder carcinoma expressing JRed in cytosol |
| T406-JR | human | T406 human glioma expressing JRed in cytosol |
| ARPE19-JR | human | ARPE19 human retina pigment cells expressing JRed in cytosol |
| CHO-JR | hamster | Chinese hamster ovary cells CHO-K1 expressing JRed in cytosol |
| M3-JR | mouse | M3 mouse melanoma cells expressing JRed in cytosol |
| C2-JR | mouse | C2C12 mouse myoblast cells expressing JRed in cytosol |
| M3-JR-PY-Mito | mouse | Doubly transfected mouse melanoma M3 cells expressing PhiYFP in mitochondria and JRed in cytosol |
| P-JR-Mito | rat | Rat kangaroo kidney epithelium PtK2 expressing JRed in mitochondria |
| ARPE19-JR-Mito | human | ARPE19 human retina pigment cells expressing JRed in mitochondria |
| H-JR-Mito | human | HeLa human cervical carcinoma expressing JRed in mitochondria |
| T24-JR-Mito | human | T24 human bladder carcinoma expressing JRed in mitochondria |
| M3-JR-Mito | mouse | Mouse melanoma M3 cells expressing JRed in mitochondria |
| Fluorescent BID apoptotic protein | human | T24 human carcinoma cells expressing JRed in mitochondria and TurboGFP-BID fusion |

Cell lines are manufactured by Marinpharm GmbH (Berlin, Germany) under the Evrogen license.

Notice to Purchaser:

JRed-related products: These products are intended for research use only and 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 (Appendix A, page G-5).
CMV Promoter: 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.

Expression/source vectors: pJRed-C



For vector sequence, please visit our Web site at www.evrogen.com/pJRed-C.shtml

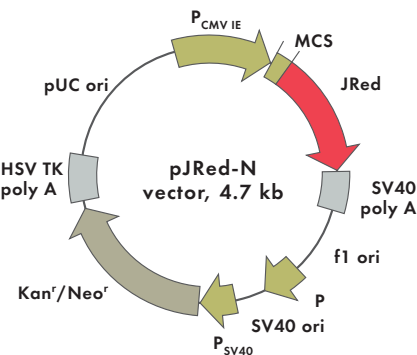
| Product | Cat.# | Size |
|--|--|-------|
| pJRed-C | FP701 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | JRed | |
| Reporter codon usage | mammalian | |
| Promoter for JRed | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | generation of fusions to the JRed C-terminus; expression of JRed or its fusions in mammalian cells | |

Multiple cloning site (MCS)



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

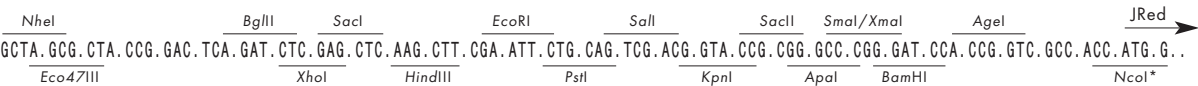
Expression/source vectors: pJRed-N



For vector sequence, please visit our Web site at www.evrogen.com/pJRed-N.shtml

| Product | Cat.# | Size |
|--|--|-------|
| pJRed-N | FP702 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | mammalian expression vector | |
| Reporter | JRed | |
| Reporter codon usage | mammalian | |
| Promoter for JRed | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | generation of fusions to the JRed N-terminus; expression of JRed or its fusions in mammalian cells | |

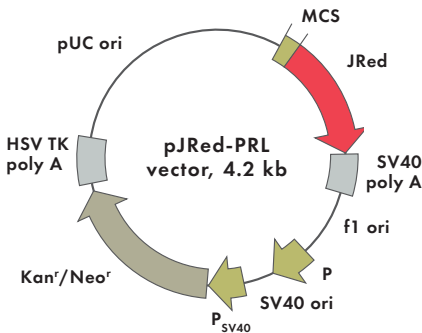
Multiple cloning site (MCS)



* — not unique sites.

Notice to Purchaser — please see page A-84

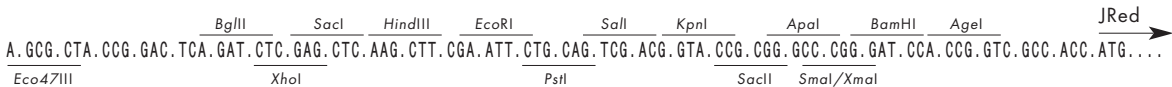
Expression/source vectors: pJRed-PRL



For vector sequence, please visit our Web site at www.evrogen.com/pJRed-PRL.shtml

| Product | Cat.# | Size |
|--|---|-------|
| pJRed-PRL | FP705 | 20 µg |
| Please contact your local distributor for exact prices and delivery information. | | |
| Vector type | promoterless vector | |
| Reporter | JRed | |
| Reporter codon usage | mammalian | |
| Promoter for JRed | NO | |
| Host cells | mammalian, bacterial | |
| Selection | prokaryotic — kanamycin eukaryotic — neomycin (G418) | |
| Replication | prokaryotic — pUC ori eukaryotic — SV40 ori | |
| Use | monitoring the activity of promoter or promoter/enhancer combination cloned into vector MCS | |

Multiple cloning site (MCS)



Notice to Purchaser:

JRed-related products: These products are intended for research use only and 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 (Appendix A, page G-5).
CMV Promoter: 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

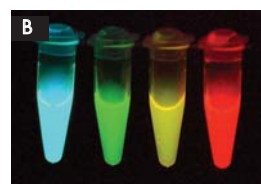
To the best of our knowledge, these products do not require a Material Safety Data Sheet. However, all the properties of these products (and, if applicable, each of their components) have not been thoroughly investigated. Therefore, we recommend that you use gloves and eye protection, and wear a laboratory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

Basic fluorescent proteins: product list

Expression/source vectors

- bacterial expression vectors
- mammalian expression vectors for C- and N- fusion generation
- mammalian expression vectors encoding destabilized basic fluorescent proteins
- promoterless vectors
- promoterless vectors encoding destabilized FPs
- subcellular localization mammalian expression vectors
- Gateway® entry clones

A-85



**Purified fluorescent proteins
of different colors.**

(A) — White light;
(B) — UV light.

Vectors sets

A-90

Recombinant proteins

A-91

Antibodies against Evrogen basic FPs

A-92

Third party products: stably transfected cell lines

A-93

Please see page A-88 for
Notice to purchaser of Evrogen
basic FP-related products

Expression/source vectors

For updated product information, please visit the Evrogen Web site (www.evrogen.com/v-info.shtml).

| Product | Cat.# | Reporter | Use | Group | Size | Page |
|-------------------------------------|-------|--------------------------|---|--------------------|-------|------|
| Bacterial expression vectors | | | | | | |
| pTurboGFP-B | FP513 | TurboGFP (green) | Source of the humanized TurboGFP coding sequence; TurboGFP expression in bacterial cells | Turbo Colors | 20 µg | A-42 |
| pTurboYFP-B | FP613 | TurboYFP (yellow) | Source of the humanized TurboYFP coding sequence; TurboYFP expression in bacterial cells | Turbo Colors | 20 µg | A-50 |
| pTurboRFP-B | FP233 | TurboRFP (red) | Source of the humanized TurboRFP coding sequence; TurboRFP expression in bacterial cells | Turbo Colors | 20 µg | A-57 |
| pTurboFP602-B | FP713 | TurboFP602 (true-red) | Source of the humanized TurboFP602 coding sequence; TurboFP602 expression in bacterial cells | Turbo Colors | 20 µg | A-64 |
| pPhi-Yellow-B | FP603 | PhiYFP (yellow) | Source of the humanized PhiYFP coding sequence; PhiYFP expression in bacterial cells | Other basic FPs | 20 µg | A-76 |

... to be continued

| Product | Cat.# | Reporter | Use | Group | Size | Page |
|--|-------|--------------------------|--|-----------------|-------|------|
| <i>Mammalian expression vectors for fusion construction</i> | | | | | | |
| pTagCFP-C | FP111 | TagCFP (cyan) | TagCFP expression in mammalian cells; generation of fusions to the TagCFP C-terminus | TagFPs | 20 µg | A-10 |
| pTagGFP-C | FP121 | TagGFP (green) | TagGFP expression in mammalian cells; generation of fusions to the TagGFP C-terminus | TagFPs | 20 µg | A-16 |
| pTagYFP-C | FP131 | TagYFP (yellow) | TagYFP expression in mammalian cells; generation of fusions to the TagYFP C-terminus | TagFPs | 20 µg | A-22 |
| pTagRFP-C | FP141 | TagRFP (red) | TagRFP expression in mammalian cells; generation of fusions to the TagRFP C-terminus | TagFPs | 20 µg | A-28 |
| pTagFP635-C | FP161 | TagFP635 (far-red) | TagFP635 expression in mammalian cells; generation of fusions to the TagFP635 C-terminus | TagFPs | 20 µg | A-33 |
| pTurboGFP-C | FP511 | TurboGFP (green) | TurboGFP expression in mammalian cells; generation of fusions to the TurboGFP C-terminus | Turbo Colors | 20 µg | A-41 |
| pTurboYFP-C | FP611 | TurboYFP (yellow) | TurboYFP expression in mammalian cells; generation of fusions to the TurboYFP C-terminus | Turbo Colors | 20 µg | A-49 |
| pTurboRFP-C | FP231 | TurboRFP (red) | TurboRFP expression in mammalian cells; generation of fusions to the TurboRFP C-terminus | Turbo Colors | 20 µg | A-56 |
| pTurboFP602-C | FP711 | TurboFP602 (true-red) | TurboFP602 expression in mammalian cells; generation of fusions to the TurboFP602 C-terminus | Turbo Colors | 20 µg | A-63 |
| pTurboFP635-C | FP721 | TurboFP635 (far-red) | TurboFP635 expression in mammalian cells; generation of fusions to the TurboFP635 C-terminus | Turbo Colors | 20 µg | A-68 |
| pPhi-Yellow-C | FP601 | PhiYFP-m (yellow) | PhiYFP-m expression in mammalian cells; generation of fusions to the PhiYFP-m C-terminus | Other basic FPs | 20 µg | A-75 |
| pJRed-C | FP701 | JRed (red) | JRed expression in mammalian cells; generation of fusions to the JRed C-terminus | Other basic FPs | 20 µg | A-83 |
| pTagCFP-N | FP112 | TagCFP (cyan) | TagCFP expression in mammalian cells; generation of fusions to the TagCFP N-terminus | TagFPs | 20 µg | A-10 |

| Product | Cat.# | Reporter | Use | Group | Size | Page |
|---|-------|------------------------|--|----------------|-------|------|
| pTagGFP-N | FP122 | TagGFP (green) | TagGFP expression in mammalian cells; generation of fusions to the TagGFP N-terminus | TagFPs | 20 µg | A-16 |
| pTagYFP-N | FP132 | TagYFP (yellow) | TagYFP expression in mammalian cells; generation of fusions to the TagYFP N-terminus | TagFPs | 20 µg | A-22 |
| pTagRFP-N | FP142 | TagRFP (red) | TagRFP expression in mammalian cells; generation of fusions to the TagRFP N-terminus | TagFPs | 20 µg | A-28 |
| pTagFP635-N | FP162 | TagFP635 (far-red) | TagFP635 expression in mammalian cells; generation of fusions to the TagFP635 N-terminus | TagFPs | 20 µg | A-34 |
| pTurboGFP-N | FP512 | TurboGFP (green) | TurboGFP expression in mammalian cells; generation of fusions to the TurboGFP N-terminus | Turbo Colors | 20 µg | A-41 |
| pTurboYFP-N | FP612 | TurboYFP (yellow) | TurboYFP expression in mammalian cells; generation of fusions to the TurboYFP N-terminus | Turbo Colors | 20 µg | A-49 |
| pTurboRFP-N | FP232 | TurboRFP (red) | TurboRFP expression in mammalian cells; generation of fusions to the TurboRFP N-terminus | Turbo Colors | 20 µg | A-56 |
| pTurboFP602-N | FP712 | TurboFP602 (true-red) | TurboFP602 expression in mammalian cells; generation of fusions to the TurboFP602 N-terminus | Turbo Colors | 20 µg | A-63 |
| pTurboFP635-N | FP722 | TurboFP635 (far-red) | TurboFP635 expression in mammalian cells; generation of fusions to the TurboFP635 N-terminus | Turbo Colors | 20 µg | A-68 |
| pPhi-Yellow-N | FP602 | PhiYFP (yellow) | PhiYFP expression in mammalian cells; generation of fusions to the PhiYFP N-terminus | Other basic FP | 20 µg | A-75 |
| pJRed-N | FP702 | JRed (red) | JRed expression in mammalian cells; generation of fusions to the JRed N-terminus | Other basic FP | 20 µg | A-83 |
| <i>Mammalian expression vectors encoding destabilized fluorescent proteins</i> | | | | | | |
| pTurboGFP-dest1 | FP519 | TurboGFP-dest1 (green) | TurboGFP-dest1 expression in mammalian cells; generation of fusions to the TurboGFP-dest1 N-terminus | Turbo Colors | 20 µg | A-43 |

... to be continued

| Product | Cat.# | Reporter | Use | Group | Size | Page |
|--|-------|-----------------------------|--|-----------------|-------|------|
| pTurboYFP-dest1 | FP619 | TurboYFP-dest1 (yellow) | TurboYFP-dest1 expression in mammalian cells; generation of fusions to the TurboYFP-dest1 N-terminus | Turbo Colors | 20 µg | A-51 |
| pTurboRFP-dest1 | FP239 | TurboRFP-dest1 (red) | TurboRFP-dest1 expression in mammalian cells; generation of fusions to the TurboRFP-dest1 N-terminus | Turbo Colors | 20 µg | A-58 |
| pPhi-Yellow-dest1 | FP608 | PhiYFP-m-dest1 (yellow) | PhiYFP-m-dest1 expression in mammalian cells; generation of fusions to the reporter | Other basic FPs | 20 µg | A-77 |
| Promoterless vectors | | | | | | |
| pTurboGFP-PRL | FP515 | TurboGFP (green) | Monitoring transcription from different promoters and promoter/enhancer combinations | Turbo Colors | 20 µg | A-42 |
| pTurboYFP-PRL | FP615 | TurboYFP (yellow) | Monitoring transcription from different promoters and promoter/enhancer combinations | Turbo Colors | 20 µg | A-50 |
| pTurboRFP-PRL | FP235 | TurboRFP (red) | Monitoring transcription from different promoters and promoter/enhancer combinations | Turbo Colors | 20 µg | A-57 |
| pTurboFP602-PRL | FP715 | TurboFP602 (true-red) | Monitoring transcription from different promoters and promoter/enhancer combinations | Turbo Colors | 20 µg | A-64 |
| pPhi-Yellow-PRL | FP604 | PhiYFP (yellow) | Monitoring transcription from different promoters and promoter/enhancer combinations | Other Colors | 20 µg | A-76 |
| pJRed-PRL | FP705 | JRed (true-red) | Monitoring transcription from different promoters and promoter/enhancer combinations | Other basic FPs | 20 µg | A-84 |
| Promoterless vectors encoding destabilized fluorescent proteins | | | | | | |
| pTurboGFP-PRL-dest1 | FP518 | TurboGFP-dest1 (green) | Monitoring transcription from different promoters and promoter/enhancer combinations | Turbo Colors | 20 µg | A-43 |
| pTurboYFP-PRL-dest1 | FP618 | TurboYFP-dest1 (yellow) | Monitoring transcription from different promoters and promoter/enhancer combinations | Turbo Colors | 20 µg | A-51 |
| pTurboRFP-PRL-dest1 | FP238 | TurboRFP-dest1 (red) | Monitoring transcription from different promoters and promoter/enhancer combinations | Turbo Colors | 20 µg | A-58 |

| Product | Cat.# | Reporter | Use | Group | Size | Page |
|---|-------|-------------------------------|--|-----------------|-------|------|
| pPhi-Yellow-PRL-dest1 | FP605 | PhiYFP-m dest1 (yellow) | Monitoring transcription from different promoters and promoter/enhancer combinations | Other basic FPs | 20 µg | A-77 |
| Subcellular localization vectors | | | | | | |
| pTagCFP-actin | FP114 | TagCFP (cyan) | Cyan fluorescent labeling of beta-actin filaments | TagFPs | 20 µg | A-11 |
| pTagGFP-actin | FP124 | TagGFP (green) | Green fluorescent labeling of beta-actin filaments | TagFPs | 20 µg | A-17 |
| pTagYFP-actin | FP134 | TagYFP (yellow) | Yellow fluorescent labeling of beta-actin filaments | TagFPs | 20 µg | A-23 |
| pTagRFP-actin | FP144 | TagRFP (red) | Red fluorescent labeling of beta-actin filaments | TagFPs | 20 µg | A-29 |
| pTagCFP-tubulin | FP115 | TagCFP (cyan) | Cyan fluorescent labeling of alpha-tubulin filaments | TagFPs | 20 µg | A-11 |
| pTagGFP-tubulin | FP125 | TagGFP (green) | Green fluorescent labeling of alpha-tubulin filaments | TagFPs | 20 µg | A-17 |
| pTagYFP-tubulin | FP135 | TagYFP (yellow) | Yellow fluorescent labeling of alpha-tubulin filaments | TagFPs | 20 µg | A-23 |
| pTagRFP-tubulin | FP145 | TagRFP (red) | Red fluorescent labeling of alpha-tubulin filaments | TagFPs | 20 µg | A-29 |
| pTagCFP-mito | FP117 | TagCFP (cyan) | Cyan fluorescent labeling of mitochondria | TagFPs | 20 µg | A-12 |
| pTagGFP-mito | FP127 | TagGFP (green) | Green fluorescent labeling of mitochondria | TagFPs | 20 µg | A-18 |
| pTagYFP-mito | FP137 | TagYFP (yellow) | Yellow fluorescent labeling of mitochondria | TagFPs | 20 µg | A-24 |
| pTagRFP-mito | FP147 | TagRFP (red) | Red fluorescent labeling of mitochondria | TagFPs | 20 µg | A-30 |
| pTurboGFP-mito | FP517 | TurboGFP (green) | Green fluorescent labeling of mitochondria | Turbo Colors | 20 µg | A-44 |
| pTurboRFP-mito | FP237 | TurboRFP (red) | Red fluorescent labeling of mitochondria | Turbo Colors | 20 µg | A-59 |
| pTurboFP602-mito | FP717 | TurboFP602 (true-red) | Red fluorescent labeling of mitochondria | Turbo Colors | 20 µg | A-65 |
| pPhi-Yellow-mito | FP607 | PhiYFP (yellow) | Yellow fluorescent labeling of mitochondria | Other basic FPs | 20 µg | A-78 |
| pPhi-Yellow-peroxi | FP606 | PhiYFP-m (yellow) | Yellow fluorescent labeling of peroxisomes | Other basic FPs | 20 µg | A-78 |
| Gateway® entry clones | | | | | | |
| Gateway® TurboGFP-C | FP521 | TurboGFP (green) | Generation of fusions to the C-terminus of humanized TurboGFP; transfer of TurboGFP or its fusion into a Gateway® destination vector | Turbo Colors | 20 µg | A-44 |

... to be continued

| Product | Cat.# | Reporter | Use | Group | Size | Page |
|------------------------|-------|---------------------|--|-----------------|-------|------|
| Gateway® TurboGFP-N | FP522 | TurboGFP (green) | Generation of fusions to the N-terminus of humanized TurboGFP; transfer of TurboGFP or its fusion into a Gateway® destination vector | Turbo Colors | 20 µg | A-45 |

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

Vector sets

| Vector set | Composition | Cat.# | Size | Pages |
|---|----------------------------|--------------|-------|-------|
| Promoter-tracker Green vector set | | FPP03 | | |
| | pTurboGFP-PRL vector | FP515 | 20 µg | A-42 |
| | pTurboGFP-PRL-dest1 vector | FP518 | 20 µg | A-43 |
| | pTurboGFP-dest1 vector | FP519 | 20 µg | A-43 |
| Promoter-tracker Yellow vector set | | FPP14 | | |
| | pTurboYFP-PRL vector | FP615 | 20 µg | A-50 |
| | pTurboYFP-PRL-dest1 vector | FP618 | 20 µg | A-51 |
| | pTurboYFP-dest1 vector | FP619 | 20 µg | A-51 |
| Promoter-tracker 3-colors vector set | | FPP15 | | |
| | pTurboRFP-PRL vector | FP235 | 20 µg | A-57 |
| | pTurboYFP-PRL vector | FP615 | 20 µg | A-50 |
| | pTurboGFP-PRL vector | FP515 | 20 µg | A-42 |
| Mito-tracker vector set | | FPM01 | | |
| | pTurboGFP-mito vector | FP517 | 20 µg | A-44 |
| | pPhi-Yellow-mito vector | FP607 | 20 µg | A-78 |
| | pKindling-Red-mito vector | FP401 | 20 µg | B-27 |
| Fusion Cyan vector set | | FPF11 | | |
| | pTagCFP-C vector | FP111 | 20 µg | A-10 |
| | pTagCFP-N vector | FP112 | 20 µg | A-10 |
| Fusion Green vector set | | FPF12 | | |
| | pTagGFP-C vector | FP121 | 20 µg | A-16 |
| | pTagGFP-N vector | FP122 | 20 µg | A-16 |
| Fusion Yellow vector set | | FPF13 | | |
| | pTagYFP-C vector | FP131 | 20 µg | A-22 |
| | pTagYFP-N vector | FP132 | 20 µg | A-22 |
| Fusion Red vector set | | FPF14 | | |
| | pTagRFP-C vector | FP141 | 20 µg | A-28 |
| | pTagRFP-N vector | FP142 | 20 µg | A-28 |

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

Recombinant proteins

For updated product information, please visit the Evrogen Web site (www.evrogen.com/p1_BFP.shtml).

| Product | Cat.# | Description | Size | Page |
|-------------|-------|--|--------|------|
| rTagCFP | FP151 | Purified recombinant cyan fluorescent protein TagCFP | 100 µg | A-12 |
| rTagGFP | FP152 | Purified recombinant green fluorescent protein TagGFP | 100 µg | A-18 |
| rTagYFP | FP153 | Purified recombinant yellow fluorescent protein TagYFP | 100 µg | A-24 |
| rTagRFP | FP154 | Purified recombinant red fluorescent protein TagRFP | 100 µg | A-30 |
| rTurboGFP | FP552 | Purified recombinant green fluorescent protein TurboGFP | 100 µg | A-45 |
| rTurboYFP | FP652 | Purified recombinant yellow fluorescent protein TurboYFP | 100 µg | A-52 |
| rTurboRFP | FP252 | Purified recombinant red fluorescent protein TurboRFP | 100 µg | A-59 |
| rTurboFP602 | FP751 | Purified recombinant red fluorescent protein TurboFP602 | 100 µg | A-65 |
| rPhiYFP | FP651 | Purified recombinant yellow fluorescent protein PhiYFP | 100 µg | A-79 |

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

Antibodies against Evrogen basic FPs

For updated product information, please visit the Evrogen Web site (www.evrogen.com/AB.shtml).

| Product | Cat.# | Description | Size | Page |
|------------------------------|-------|---|--------|------|
| Anti-TurboGFP antibody | AB511 | Rabbit polyclonal antibody against non-denatured | 100 µg | D-3 |
| | AB512 | TurboGFP; it also recognizes denatured TurboGFP but with lesser affinity than Anti-TurboGFP(d) antibody | 200 µg | |
| Anti-TurboGFP(d) antibody | AB513 | Rabbit polyclonal antibody against denatured TurboGFP | 100 µg | D-4 |
| | AB514 | and CopGFP; it also recognizes non-denatured TurboGFP but with lesser affinity than Anti-TurboGFP antibody | 200 µg | |
| Anti-PhiYFP antibody | AB601 | Rabbit polyclonal antibody against non-denatured | 100 µg | D-7 |
| | AB602 | PhiYFP, PhiYFP-m, and TurboYFP | 200 µg | |
| Anti-PhiYFP(d) antibody | AB603 | Rabbit polyclonal antibody against denatured PhiYFP, | 100 µg | D-8 |
| | AB604 | PhiYFP-m, and TurboYFP; it also recognizes non-denatured TurboYFP and Phi-Yellow proteins, but with lesser affinity than Anti-PhiYFP antibody | 200 µg | |
| Anti-tRFP antibody | AB231 | Rabbit polyclonal antibody against TagRFP, TagFP635 | 100 µg | D-9 |
| | AB232 | TurboRFP, TurboFP602, and TurboFP635 proteins | 200 µg | |
| Anti-Tag(CGY)FP antibody | AB121 | Rabbit polyclonal antibody against TagGFP, TagCFP, | 100 µg | D-6 |
| | AB122 | TagYFP and PS-CFP2 | 200 µg | |
| Anti-KillerRed antibody | AB961 | Rabbit polyclonal antibody against KillerRed and JRed | 100 µg | D-11 |
| | AB962 | | 200 µg | |

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

Third party products: stably transfected cell lines

| Cell line | Reporter | Description |
|------------------------------|----------------|--|
| Whole cell labeling | | |
| M3-TG | green | M3 mouse melanoma cells expressing TurboGFP in cytosol |
| M3-PY | yellow | M3 mouse melanoma cells expressing PhiYFP in cytosol |
| M3-JR | red | M3 mouse melanoma cells expressing JRed in cytosol |
| PC-TG | green | PC-12 rat pheochromocytoma expressing TurboGFP in cytosol |
| PC-PY | yellow | PC-12 rat pheochromocytoma expressing PhiYFP in cytosol |
| PC-JR | red | PC-12 rat pheochromocytoma expressing JRed in cytosol |
| CHO-TG | green | Chinese hamster ovary cells CHO-K1 expressing TurboGFP in cytosol |
| CHO-JR | red | Chinese hamster ovary cells CHO-K1 expressing JRed in cytosol |
| H460-TG | green | H460 human lung carcinoma expressing TurboGFP in cytosol |
| U17-TG | green | UT7 human leukemia cells expressing TurboGFP in cytosol |
| H-TG | green | HeLa human cervical carcinoma expressing TurboGFP in cytosol |
| H-JR | red | HeLa human cervical carcinoma expressing JRed in cytosol |
| C2-TG | green | C2C12 mouse myoblast cells expressing TurboGFP in cytosol |
| C2-JR | red | C2C12 mouse myoblast cells expressing JRed in cytosol |
| W-TG | green | WALKER 256 rat tumor expressing TurboGFP in cytosol |
| W-PY | yellow | WALKER 256 rat tumor expressing PhiYFP in cytosol |
| W-JR | red | WALKER 256 rat tumor cells expressing JRed in cytosol |
| 3T3-TG | green | 3T3 mouse fibroblasts expressing TurboGFP in cytosol |
| 3T3-TG-D | green | T3-mouse fibroblasts expressing destabilized TurboGFP in cytosol |
| T406-PY | yellow | T406 human glioma expressing PhiYFP in cytosol |
| T406-JR | red | T406 human glioma expressing JRed in cytosol |
| BC3-PY | yellow | BC3H1 myoblast cells expressing PhiYFP in cytosol |
| T24-TG | green | T24 human bladder carcinoma expressing TurboGFP in cytosol |
| T24-PY | yellow | T24 human bladder carcinoma expressing PhiYFP in cytosol |
| T24-JR | red | T24 human bladder carcinoma expressing JRed in cytosol |
| T24-PY-dest | yellow | Rat kangaroo kidney epithelium PtK2 cells expressing destabilized PhiYFP-m in cytosol |
| ARPE19-JR | red | ARPE19 human retina pigment cells expressing JRed in cytosol |
| Mitochondria labeling | | |
| H-TG-Mito | green | HeLa human cervical carcinoma expressing TurboGFP in mitochondria |
| H-JR-Mito | red | HeLa human cervical carcinoma expressing JRed in mitochondria |
| T24-TG-Mito | green | T24 human bladder carcinoma expressing TurboGFP in mitochondria |
| T24-JR-Mito | red | T24 human bladder carcinoma expressing JRed in mitochondria |
| M3-TG-Mito | green | M3 mouse melanoma cells expressing TurboGFP in mitochondria |
| M3-JR-Mito | red | Mouse melanoma M3 cells expressing JRed in mitochondria |
| M3-JR-PY-Mito | yellow and red | Doubly transfected mouse melanoma M3 cells expressing PhiYFP in mitochondria and JRed in cytosol |
| C2C12-PY-Mito | yellow | Mouse myoblast cells expressing PhiYFP in mitochondria |
| 3T3-PY-Mito | yellow | Mouse fibroblasts 3T3 expressing PhiYFP in mitochondria |
| P-PY-Mito | yellow | Rat kangaroo kidney epithelium PtK2 expressing PhiYFP in mitochondria |
| P-JR-Mito | red | Rat kangaroo kidney epithelium PtK2 expressing JRed in mitochondria |
| ARPE19-JR-Mito | red | ARPE19 human retina pigment cells expressing JRed in mitochondria |
| Peroxisome labeling | | |
| T24-PY-P | yellow | T24 human bladder carcinoma cells expressing PhiYFP-m in peroxisomes |
| Beta-actin labeling | | |
| HeLa-TurboGreen-Actin | green | HeLa human cervical carcinoma expressing TurboGFP fusion with beta-actin |
| H-PY-A | yellow | HeLa human cervical carcinoma expressing PhiYFP-m fusion with beta-actin |

| Cell line | Reporter | Description |
|-----------------------------------|---------------|--|
| P-PY-A | yellow | Rat kangaroo kidney epithelium PtK2 expressing PhiYFP-m fusion with beta-actin |
| T47-PY-A | yellow | T47-D T47-D human breast cancer cells expressing PhiYFP-m fusion with beta-actin |
| MDCK-PY-A | yellow | MADIN-DARBY-canine kidney epithelial cells expressing PhiYFP-m fusion with beta-actin |
| 3T3-PY-A | yellow | Mouse fibroblasts 3T3 expressing PhiYFP-m fusion with beta-actin |
| U205-TAG-GFP-Actin | green | Human osteosarcoma line U205 expressing TagGFP fusion with beta-actin |
| U205-TAG-YFP-Actin | yellow | Human osteosarcoma line U205 expressing TagYFP fusion with beta-actin |
| U205-TAG-RFP-Actin | red | Human osteosarcoma line U205 expressing TagRFP fusion with beta-actin |
| Alpha-tubulin labeling | | |
| MDCK-TAG-Tu | green | MDCK canine kidney epithelial cells expressing TagGFP fusion with alpha-tubulin |
| T24-TG-TAG-Tu | green | T24 human bladder carcinoma expressing TagGFP fusion with alpha-tubulin |
| U205-TAG-RFP-Tubulin | red | Human osteosarcoma line U205 expressing TagRFP fusion with beta-actin |
| Other constructs | | |
| Fluorescent BID-apoptotic protein | red and green | Doubly transfected T24 human carcinoma cells expressing JRed in mitochondria and TurboGFP-BID fusion |
| Fluorescent fibrillarin | green | HeLa human cervical carcinoma expressing TurboGFP fusion with fibrillarin |

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