

Basic Fluorescent Proteins



Evrogen Collection of Basic Fluorescent Proteins

- 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
- Free evaluation period during the first six months after purchase for For-Profit entities

Overview

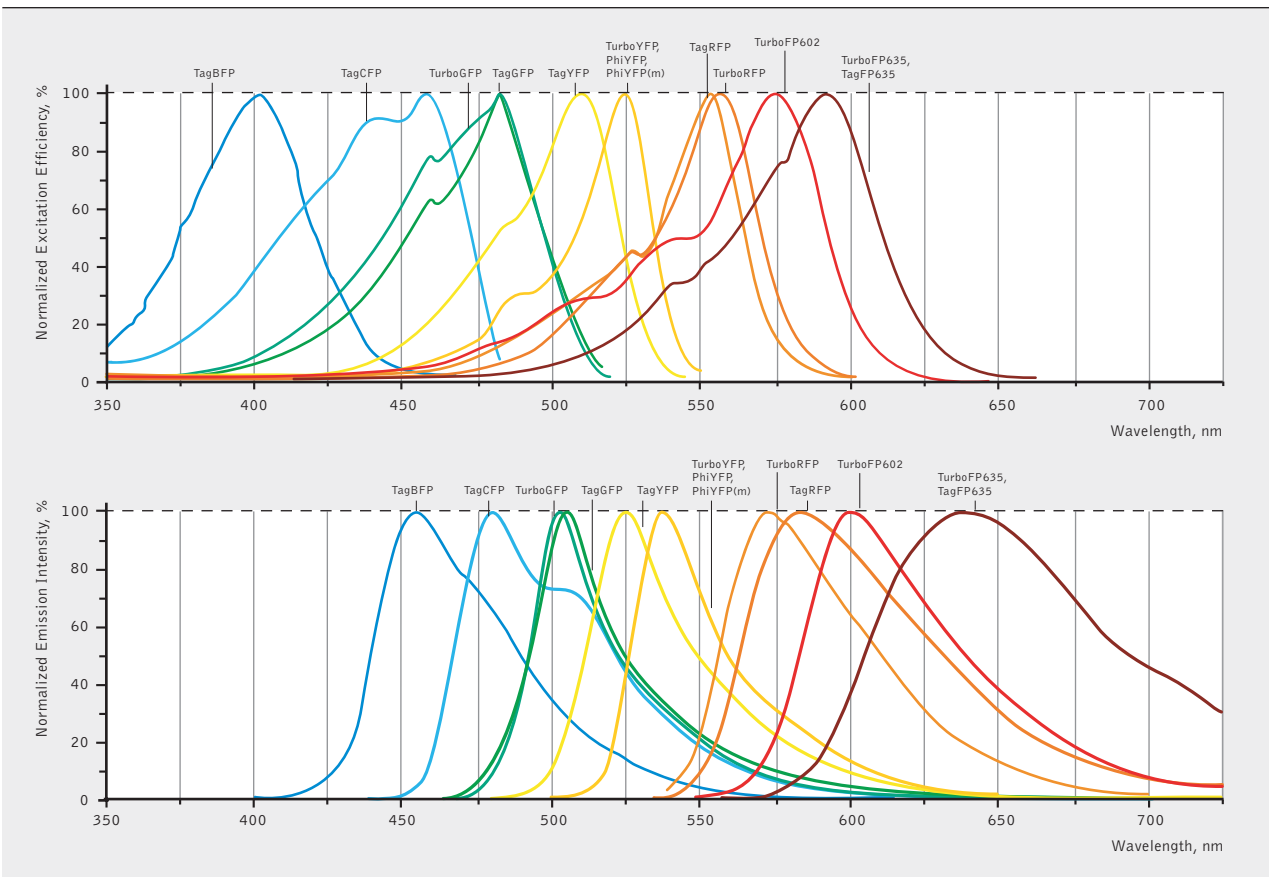
Evrogen offers a wide collection of bright fluorescent proteins for common applications in the field of live-cell assays (labeling of cells, subcellular structures, and proteins; analysis of promoter activity; generation of stably transfected cell lines expressing fluorescent proteins or their fusions;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

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

- **Protein Localization 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.

Spectral diversity

Basic fluorescent proteins, excitation/emission spectra



Main properties and recommendations for use

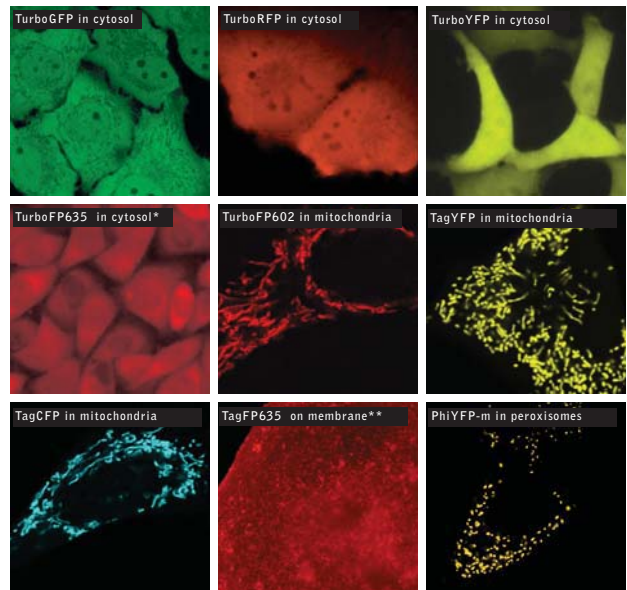
Protein	TagBFP	TagCFP	TagGFP	TagYFP	TagRFP	TagFP635	TurboGFP	TurboYFP	TurboRFP	TurboFP602	TurboFP635	PhiYFP	PhiYFP-m	
Characteristics														
Group	Tag's fluorescent proteins						TurboColors basic fluorescent proteins						Other proteins	
Fluorescence color	blue	cyan	green	yellow	red (orange)	far-red	green	yellow	red (orange)	true-red	far-red	yellow	yellow	
Excitation max	402 nm	458 nm	482 nm	508 nm	555 nm	588 nm	482 nm	525 nm	553 nm	574 nm	588 nm	525 nm	525 nm	
Emission max	457 nm	480 nm	505 nm	524 nm	584 nm	635 nm	502 nm	538 nm	574 nm	602 nm	635 nm	537 nm	537 nm	
Quantum yield	0.63	0.57	0.59	0.62	0.48	0.33	0.53	0.53	0.67	0.35	0.34	0.40	0.39	
Extinction coefficient (M ⁻¹ ·cm ⁻¹)	52 000	37 000	58 200	64 000	100 000	45 000	70 000	105 000	92 000	74 400	65 000	130 000	124 000	
Brightness	32.8	21.1	34.3	39.7	48.0	14.9	37.1	55.7	61.6	26.0	22.1	52.2	48.4	
Brightness, % of EGFP	99	64	104	120	145	45	112	169	187	79	67	158	147	
pKa	2.7	4.7	4.7	5.5	3.8	6.0	5.2	5.9	4.4	4.7	5.5	6.0	6.0	
Structure	monomer	monomer	monomer	monomer	monomer	monomer	dimer	dimer	dimer	dimer	dimer	dimer	dimer	
Aggregation	no	no	no	no	no	no	no	at high concentr.	no	no	no	no	no	
Maturation rate at 37°C	fast	fast	fast	fast	fast	fast	superfast	superfast	superfast	fast	superfast	fast	fast	
Photostability	high	high	high	high	medium	high	high	high	high	medium	high	high	high	
Molecular weight	26 kDa	27 kDa	27 kDa	27 kDa	27 kDa	27 kDa	26 kDa	26 kDa	26 kDa	26 kDa	26 kDa	26 kDa	26 kDa	
Recommendations for use														
Cell labeling - mammalian - bacterial	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	
Promoter activity testing	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	
Stable transfection	not tested	proved	proved	proved	proved	not tested	proved	not tested	not tested	proved	proved	proved	proved	
In fusions	+++	+++	+++	+++	+++	+++	++	++	++	++	++	++	++	

Performance and use

Bright labels of cells and cell organelles

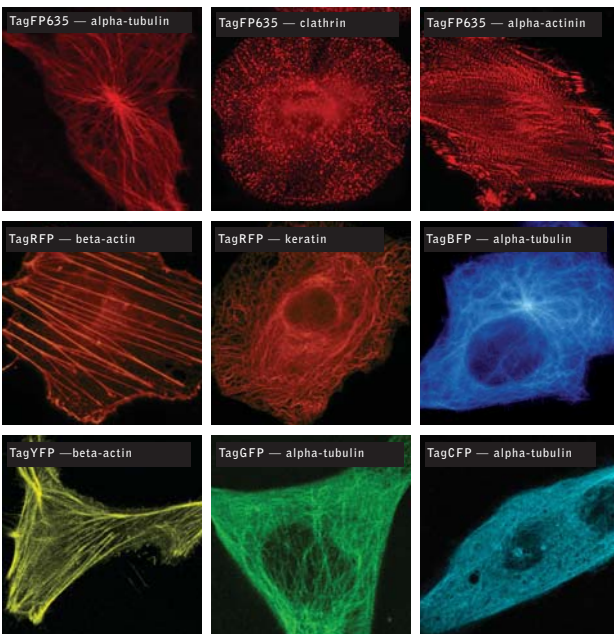
Bright fluorescence of Evrogen fluorescent proteins allows easy labeling of cell and cellular structures. The proteins are stable allowing monitoring fluorescence over extended periods of time. No adding cofactors or substrates is required for protein detection.

Evrogen vectors is designed for labeling of mammalian and bacterial cells; ready-to-use subcellular and protein localization vectors allow easy labeling of cellular organelles (mitochondria, peroxisomes); C- and N- vectors can be used for generation of fluorescent proteins fused with a localization signal of interest.



Labeling of mammalian cells and organelles using Evrogen fluorescent proteins.

* Image was kindly provided by Dr. Christian Petzelt (Marinpharm).
** Image was kindly provided by Michael W. Davidson (Florida State University).



Protein labeling using TagFPs.

Images of TagRFP and TagFP635 fusions were kindly provided by Michael W. Davidson (Florida State University).

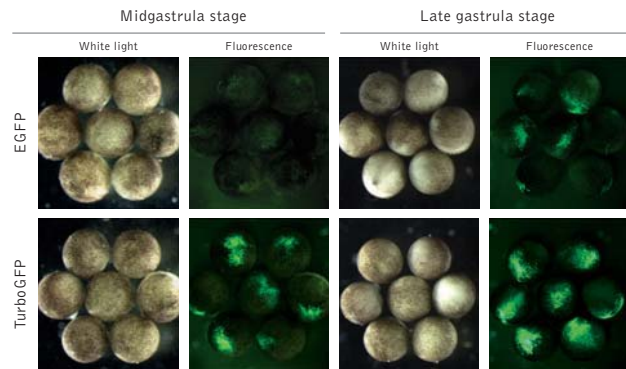
Excellent protein tags

Monomeric TagFPs are the optimal choice for use as fluorescent protein tags. When expressed in mammalian cells, these proteins are homogeneously distributed within the cytosol, without aggregation. Successful performance in protein labeling applications of these proteins was validated in various models including highly oligomerizing cellular proteins like cytoplasmic beta-actin and alpha-tubulin.

Perfect reporters of gene expression

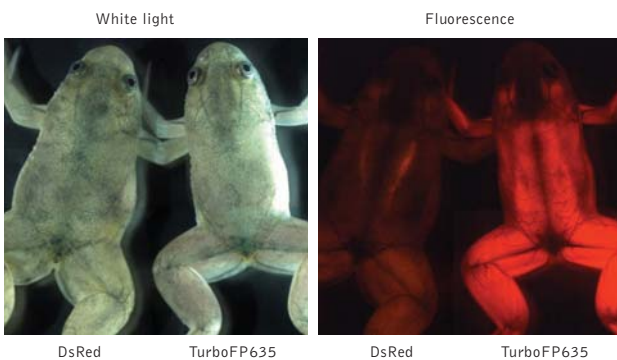
Superbright and fast-maturing TurboColors are protein of choice for applications where fast appearance of bright fluorescence is crucial.

TurboColors mature noticeably faster than other fluorescent proteins and allow monitoring of gene expression from early promoters. Destabilized protein variants allow accurate analysis of rapid and/or transient events in gene regulation.



In vivo comparison of TurboGFP and EGFP maturation in developing *Xenopus* embryos.

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. EGFP was practically invisible at this developmental stage. Living embryos were then photographed from the animal pole at the middle and late gastrula stages. Experimental data were provided by Dr. A. Zaraisky (Moscow, Russia).



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 Shcherbo *et al.*, Nat. Methods 2007.

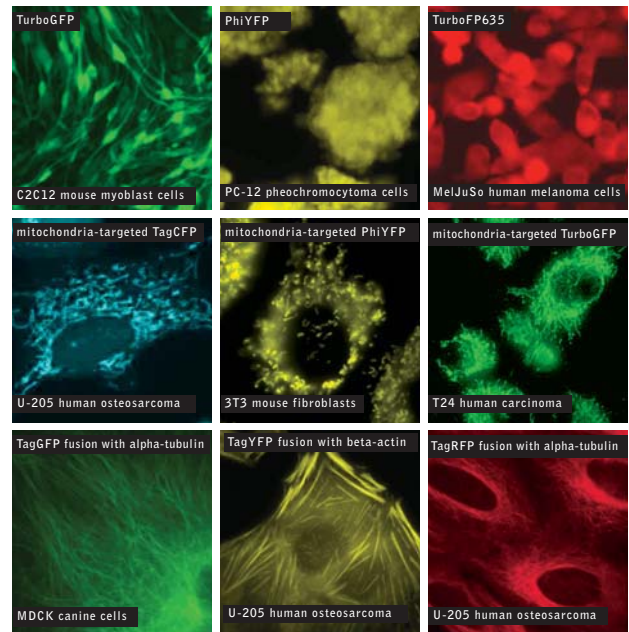
Suitable markers for whole body imaging

For deep imaging of animal tissues, the optical window favorable for light penetration is in near-infrared wavelengths, which requires proteins with emission spectra in the far-red wavelengths. Evrogen TurboFP635 and TagFP635 have emission maxima at 635 nm and are more brighter, photostable and pH-stable than other cloned far-red fluorescent proteins.

Superiority of TurboFP635 for whole-body imaging has been demonstrated by direct comparison with other red and far-red fluorescent proteins.

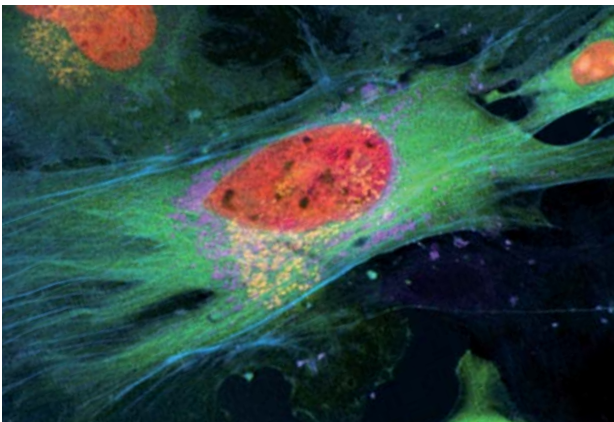
Proven suitability for generation of stably transfected cell lines

Evrogen proteins suitability to generate stably transfected cells has been proven by Marinpharm company (www.marinpharm.com). Various cell lines expressing Evrogen fluorescent proteins are commercially available.



Stably transfected cell lines expressing Evrogen fluorescent proteins.

Images were kindly provided by Dr. Christian Petzelt (Marinpharm).



Multicolor labeling of mammalian cells.

TagCFP-actin fusion (cyan), TagYFP-tubulin fusion (green), TagFP635-H2B fusion (red), Golgi-targeted TagRFP (yellow) and mitochondria-targeted TagBFP (purple). Image was kindly provided by Michael W. Davidson (Florida State University).

Ideal tool for multicolor labeling applications

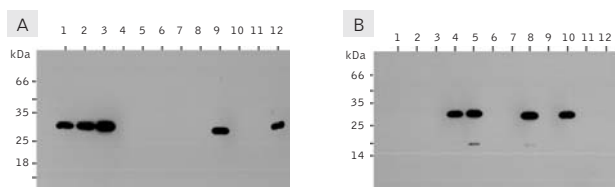
Ranging in color from blue to far-red, Evrogen fluorescent proteins can be used for multicolor labeling and fluorescence resonance energy transfer (FRET) applications for visualization of protein translocation against other subcellular structures, investigation of protein-protein co-localization, detection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

Visualization parameters

Color	Protein	Ex/em max, nm	Suitable Omega filter set #	Component type	Part #	Also suitable are
BLUE	TagBFP	402/457	XF119-2	Exciter Dichroic Emitter	XF1076 XF2040 XF3078	Semrock filter set DAPI-5060B
			QMAX-Blue	Exciter Dichroic Emitter	QMAX_EX355-405 QMAX_DI410LP QMAX_EM420-480	Chroma Tech. filter set 31016 (Hydroxycoumarin)
CYAN	TagCFP	458/480	XF114-2	Exciter Dichroic Emitter	XF1071 XF2034 XF3075	Common fluorescence filter sets for ECFP
			XF130-2	Exciter Dichroic Emitter	XF1071 XF2034 XF3087	
GREEN	TagGFP TurboGFP	482/505 482/502	QMAX-Green	Exciter Dichroic Emitter	QMAX/ EX 450-490 QMAX/ DI 500LP QMAX/EM/510-560	Common fluorescence filter sets for EGFP, FITC, and other green dyes
			XF100-2	Exciter Dichroic Emitter	XF1073 XF2010 XF3084	
			XF100-3	Exciter Dichroic Emitter	XF1087 XF2077 XF3105	
			XF115-2	Exciter Dichroic Emitter	XF1073 XF2010 XF3086	
			XF116-2	Exciter Dichroic Emitter	XF1072 XF2037 XF3080	
YELLOW	TagYFP	508/524	XF104-3	Exciter Dichroic Emitter	XF1068 XF2030 XF3106	Chroma Tech. filter set 41028 Yellow GFP BP (10C/Topaz) or the similar
			XF105-2	Exciter Dichroic Emitter	XF1068 XF2030 XF3082	
	TurboYFP PhiYFP	525/538 525/537	XF104-3	Exciter Dichroic Emitter	XF1068 XF2030 XF3106	Chroma Tech. filter set 42003 (ZsYellow1)
RED (ORANGE)	TagRFP TurboRFP	555/584 553/574	QMAX-Yellow	Exciter Dichroic Emitter	QMAX/EX 510-550 QMAX/DI 560LP QMAX/EM 570-600	TRITC filter set or similar
			XF108-2	Exciter Dichroic Emitter	XF1074 XF2017 XF3083	
			XF101-2	Exciter Dichroic Emitter	XF1074 XF2017 XF3085	
			XF111-2	Exciter Dichroic Emitter	XF1077 XF2015 XF3089	
TRUE-RED	TurboFP602 JRed	574/602 584/610	QMAX-Red	Exciter Dichroic Emitter	QMAX/ EX 530-570 QMAX/DI 580LP QMAX/EM 600-650	TRITC filter set or similar
FAR-RED	TagFP635 TurboFP635	588/635 588/635	QMAX-Red	Exciter Dichroic Emitter	QMAX/ EX 530-570 QMAX/DI 580LP QMAX/EM 600-650	Texas Red filter set or similar
			XF102-2	Exciter Dichroic Emitter	XF1067 XF2029 XF3081	

Immunological detection

Rabbit polyclonal antibodies are available from Evrogen for immunological detection of fluorescent proteins. The antibodies were purified by affinity chromatography and can be used for Western blot, immunoblotting, immunohistochemistry, ELISA, in-cell-Western, and immunoprecipitation.



Western blot detection of fluorescent proteins using Anti-Tag(CGY)FP (A) and Anti-tRFP (B) antibodies.

1 — TagCFP; 2 — TagGFP; 3 — TagYFP; 4 — TagRFP; 5 — TurboFP602; 6 — TurboGFP; 7 — TurboYFP; 8 — TurboRFP; 9 — PS-CFP2; 10 — Dendra2; 11 — KillerRed; 12 — EGFP.

Recombinant proteins were purified from transformed *E. coli*. 25 ng of each protein were separated by SDS PAGE (14% acrylamide). The samples were boiled before loading. Antibody was used at a 1/10000 dilution. Secondary antibody: Goat anti-Rabbit HRP-conjugated IgG.

Available vectors

Evrogen vector collection includes vectors optimized for various research needs: bacterial expression vectors; mammalian expression vectors allowing fusion generation; promoterless vectors; vectors encoding destabilized basic fluorescent proteins; subcellular and protein localization vectors; and Gateway® entry clones.

For complete list of available vectors, please visit Evrogen Web site at www.evrogen.com/products/product_list/basicFPs_vectors.shtml

Licensing opportunities

Evrogen technologies embodied in fluorescent proteins are 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.

Notice to Purchaser:

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Protein	Antibody	Cat.#
Protein localization TagFPs		
TagBFP	Anti-tRFP antibody	AB231, AB232
TagCFP	Anti-Tag(CGY)FP antibody	AB121, AB122
TagGFP	Anti-Tag(CGY)FP antibody	AB121, AB122
TagYFP	Anti-Tag(CGY)FP antibody	AB121, AB122
TagRFP	Anti-tRFP antibody	AB231, AB232
TagFP635	Anti-tRFP antibody	AB231, AB232
TurboColors		
TurboGFP	Anti-TurboGFP antibody	AB511, AB512
	Anti-TurboGFP(d) antibody	AB513, AB514
TurboYFP	Anti-PhiYFP antibody	AB601, AB602
	Anti-PhiYFP(d) antibody	AB603, AB604
TurboRFP	Anti-tRFP antibody	AB231, AB232
TurboFP602	Anti-tRFP antibody	AB231, AB232
TurboFP635	Anti-tRFP antibody	AB231, AB232
Others		
PhiYFP	Anti-PhiYFP antibody	AB601, AB602
	Anti-PhiYFP(d) antibody	AB603, AB604
PhiYFP-m	Anti-PhiYFP antibody	AB601, AB602
	Anti-PhiYFP(d) antibody	AB603, AB604

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