

Phi-Yellow fluorescent proteins (PhiYFP and PhiYFP-m)

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

Description

PhiYFP and PhiYFP-m are mutants of a natural yellow fluorescent protein from *Phialidium* sp. (Cnidaria; Hydrozoa; Hydroida; Leptomedusae; Campanulariidae) and previous versions of TurboYFP (Shagin *et al.*, 2002).

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 PhiYFP

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

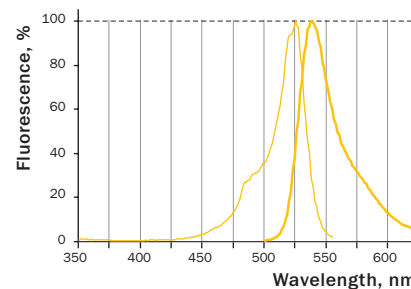
*Brightness is a product of extinction coefficient and quantum yield, divided by 1000.

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.

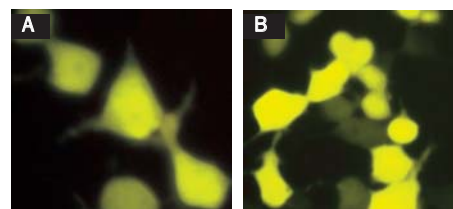
Suitability of Phi-Yellow proteins to generate stably transfected cells has been proven by Marinpharm company (www.marinpharm.com). Various cell lines are commercially available.

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 monomeric TagFPs for



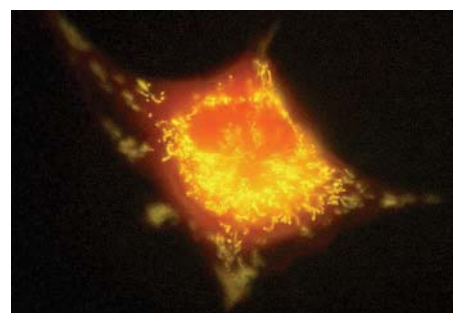
Normalized excitation (thin line) and emission (thick line) spectra of Phi-Yellow proteins.

Complete PhiYFP and PhiYFP-m 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.



Stably transfected M3 cells expressing PhiYFP in mitochondria and JRed in cytosol.

Photograph was provided by Dr. Christian Petzelt (Marinpharm).

protein labeling applications. Please see section "Protein Localization Tags" (available at www.evrogen.com/TagFPs.shtml) 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.

Phi-Yellow proteins can be used in multicolor labeling applications with cyan, green, red, and far-red fluorescent dyes.

Available variants and fusions

PhiYFP

PhiYFP codon usage is optimized for high expression in mammalian cells (Haas *et al.*, 1996), but it can be successfully expressed in many other heterologous systems. PhiYFP allows generation of fusions to its N-terminus but unsuited to generate C-terminal fusions.

PhiYFP-m variant

PhiYFP-m variant is a mutant of PhiYFP. It is suitable for fusion generation to its C-terminus.

Destabilized PhiYFP-m variant

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 (Li *et al.*, 1998). 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-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, PhiYFP-m-dest1 can be used to measure changes in gene expression.

PhiYFP -mito variant

A mitochondrial targeting sequence (MTS) is linked to the PhiYFP N-terminus. MTS was derived from the subunit VIII of human cytochrome C oxidase (Rizzuto *et al.*, 1989; Rizzuto *et al.*, 1995). When expressed in mammalian cells, this variant provides green fluorescent labeling of mitochondria.

PhiYFP-m -peroxi variant

Peroxisomal targeting signal (Gould *et al.*, 1989) encoding tripeptide SKL was fused to the 3' end of PhiYFP-m sequence. This tripeptide targets the fusion protein to the matrix of peroxisomes.

Recommended filter sets and antibodies

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

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

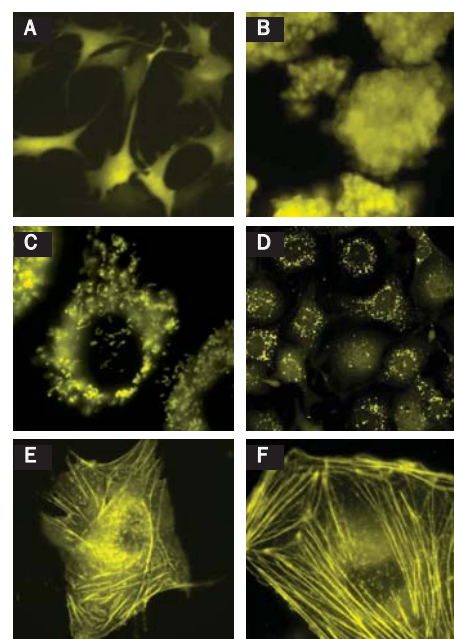
PhiYFP licensing opportunities

Evrogen technology embodied in PhiYFP 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.

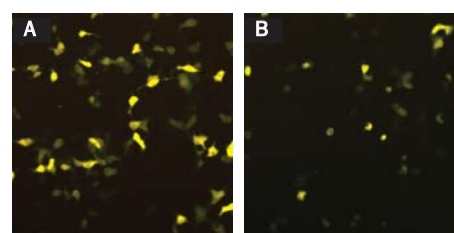
References

- Gould *et al.* (1989) *J. Biol. Chem.* 108:1657-1664.
- Haas *et al.* (1996) *Curr. Biol.* 6: 315-324.
- Li *et al.* (1998) *J. Biol. Chem.* 273:34970-34975.
- Rizzuto *et al.* (1989) *J. Biol. Chem.* 264: 10595-10600.
- Rizzuto *et al.* (1995) *Curr. Biol.* 5: 635-642.
- Shagin *et al.* (2004) *Mol. Biol. Evol.* 21(5): 841-850.



Phi-Yellow expression in stably transfected mammalian cells.

A — PhiYFP in BC3H1 mouse brain tumor cells; B — PhiYFP in PC-12 pheochromocytoma cells; C — mitochondria-targeted PhiYFP in 3T3 mouse fibroblasts; D — peroxisome-targeted PhiYFP-m in T-24 human bladder carcinoma cells; E — PhiYFP-m fusion with beta-actin in MADIN-DARBY canine kidney epithelial cells; F — PhiYFP-m fusion with beta-actin in rat kangaroo kidney epithelium cells PtK2. Photographs were provided by Dr. Christian Petzelt (Marinpharm).



Fluorescence intensities of cells expressing PhiYFP-m-dest1 rapidly decrease in response to cycloheximide (CHX).

Mammalian cells expressing PhiYFP-m-dest1 under the control of CMV promoter were treated with CHX. After 1.5 hours CHX treatment, fluorescence intensity of cells was greatly reduced. A — no CHX treatment; B — after CHX treatment.

PhiYFP-related products

Product	Cat.#	Description	Size
PhiYFP expression/source vectors			
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
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
pPhi-Yellow-B	FP603	Bacterial expression vector; source of humanized PhiYFP coding sequence	20 µg
pPhi-Yellow-PRL	FP604	Promoterless expression vector encoding humanized PhiYFP and designed for monitoring transcription from different promoters and promoter/enhancer combinations	20 µg
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
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
pPhi-Yellow-peroxi	FP606	Mammalian expression vector encoding humanized PhiYFP-m targeted to peroxisomes	20 µg
pPhi-Yellow-mito	FP607	Mammalian expression vector encoding humanized PhiYFP targeted to mitochondria	20 µg
Recombinant protein			
rPhiYFP	FP651	Purified recombinant yellow fluorescent protein	100 µg
Antibodies against PhiYFP			
Anti-PhiYFP antibody	AB601	Rabbit polyclonal antibody against non-denatured PhiYFP, PhiYFP-m, and PhiYFP	100 µg
Anti-PhiYFP(d) antibody	AB603	Rabbit polyclonal antibody against denatured PhiYFP, PhiYFP-m, and TurboYFP	100 µg
	AB604		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

Cell line	Source	Description
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, www.marinpharm.com) under the Evrogen license.

Notice to Purchaser:

PhiYFP-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 (available at www.evrogen.com/Evrogen-FP-license.shtml).

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