Cyan fluorescent protein TagCFP

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

Description

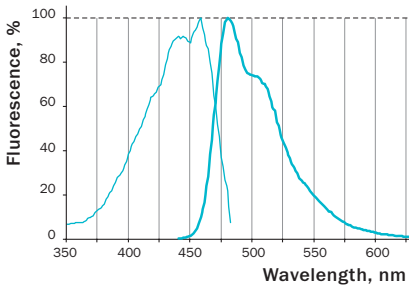
TagCFP is a cyan monomeric protein generated on the basis of GFP-like protein from jellyfish *Aequorea macrodactyla* (Xia *et al.*, 2002). It possesses bright fluorescence with excitation/emission maxima at 458 and 480 nm, respectively. TagCFP is significantly brighter than commonly used ECFP.

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

Main properties of TagCFP

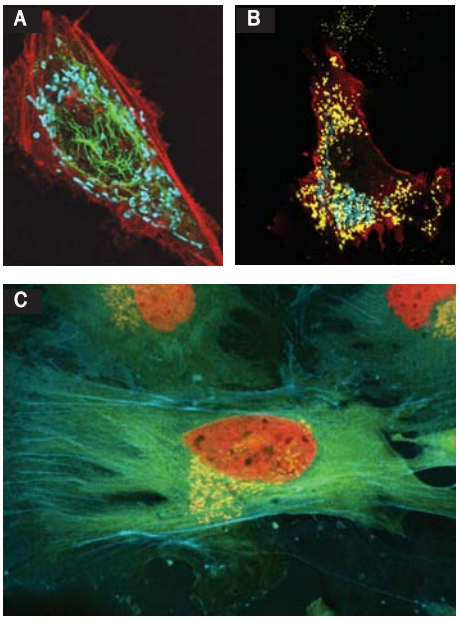
Characteristic	
Molecular weight	27 kDa
Polypeptide length	239 aa
Fluorescence color	cyan
Excitation max	458 nm
Emission max	480 nm
Quantum yield	0.57
Extinction coefficient	37 000 $M^{-1}cm^{-1}$
Brightness*	21.1
Brightness % of EGFP	64
pKa	4.7
Structure	monomer
Aggregation	no
Maturation rate at 37°C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

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



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

Complete TagCFP spectra in Excel format can be downloaded from the Evrogen Web site at www.evrogen.com/support/FP-tech.shtml



Multicolor labeling of mammalian cells.

A — mitochondria-targeted TagCFP (cyan), Dendra2-vimentin fusion (green), and TagRFP-actin fusion (red); B — mitochondria-targeted TagCFP (cyan), peroxisome-targeted PhiYFP-m (yellow), and membrane-targeted KillerRed (red); C — TagCFP-actin fusion (blue), TagYFP-tubulin fusion (green), TTagFP635-H2B fusion (red), and Golgi-targeted TagRFP (orange).

Performance and use

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.

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

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

Available variants and fusions

TagCFP codon usage is optimized for high expression in mammalian cells (Haas *et al.*, 1996), but it can be successfully expressed in many other heterological systems.

TagCFP-mito fusion

A mitochondrial targeting sequence (MTS) is linked to the TagCFP 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 cyan fluorescent labeling of mitochondria.

TagCFP-actin fusion

Human beta-actin is linked to the TagCFP C-terminus. When expressed in mammalian cells, this fusion provides cyan fluorescent labeling of actin filaments.

TagCFP-tubulin fusion

Human alpha-tubulin is linked to the TagCFP C-terminus. When expressed in mammalian cells, this fusion provides cyan fluorescent labeling of tubulin filaments.

Recommended filter sets and antibodies

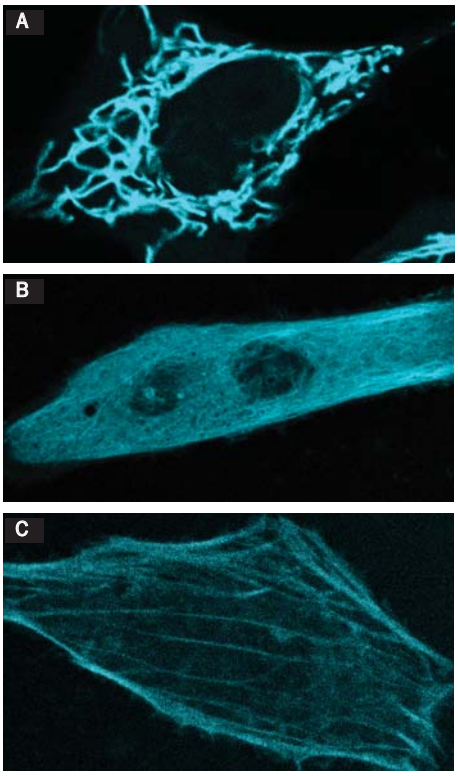
TagCFP can be detected using fluorescence filter sets for ECFP and the similar. Recommended Omega Optical filter sets are XF114-2 and XF130-2.

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

TagCFP licensing opportunities

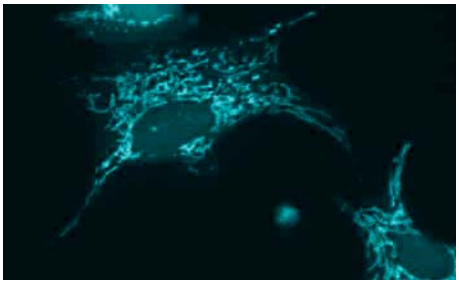
Evrogen technology embodied in TagCFP is available for expanded and commercial use with an adaptable licensing program. Benefits from flexible and market-driven license options are offered for upgrade and novel development of products and applications. For licensing information, please contact Evrogen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



Expression of TagCFP fusions in transiently transfected mammalian cells (HeLa).

Confocal microscopy: A — mitochondria-targeted TagCFP; B — TagCFP fusion with alpha-tubulin; C — TagCFP fusion with cytoplasmic beta-actin.



Expression of mitochondria-targeted TagCFP in stably transfected U-205 human osteosarcoma cells.

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

References

Haas *et al*. (1996) Curr. Biol. 6: 315–324.
Rizzuto *et al*. (1989) J. Biol. Chem. 264: 10595–10600.
Rizzuto *et al.* (1995) Curr. Biol. 5: 635–642.
Xia *et al*. (2002) Mar. Biotechnol. 4(2): 155-162.

TagCFP-related products

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

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

Third party products: stably transfected cell lines expressing TagCFP

Cell line	Source	Description
U-205-TAG-CFP-mitochondria	human	U-205 human osteosarcoma cells expressing mitochondria-targeted TagCFP

Cell lines are manufactured by Marinpharm GmbH (Berlin, Germany) under the Evrogen license. Please visit Marinpharm Web site at www.marinpharm.com for prices and delivery information.