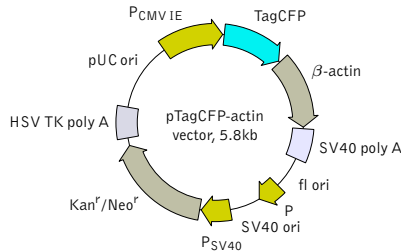


pTagCFP-actin vector

The vector sequence has been compiled using the information from sequence databases, published literature, and other sources, together with partial sequences obtained by Evrogen. This vector has not been completely sequenced.



For vector sequence, please visit our Web site at <http://www.evrogen.com/support/vector-info.shtml>

Location of features

PCMV IE: 1-589
 Enhancer region: 59-465
 TATA box: 554-560
 Transcription start point: 583
 Kozak consensus translation initiation site: 606-616
 TagCFP
 Start codon (ATG): 613-615
 Last amino acid in TagCFP: 1324-1326
 Beta-Actin: 1348-2475
 Stop codon: 2473-2475
 SV40 early mRNA polyadenylation signal
 Polyadenylation signals: 2636-2641 & 2665-2670
 mRNA 3' ends: 2674 & 2686
 f1 single-strand DNA origin: 2733-3188
 Eukaryotic promoter for expression of Kan^r gene
 -35 region: 3250-3255; -10 region: 3273-3278
 Transcription start point: 3285
 SV40 origin of replication: 3529-3664
 SV40 early promoter
 Enhancer (72-bp tandem repeats): 3362-3433 & 3434-3505
 21-bp repeats: 3509-3529, 3530-3550 & 3552-3572
 Early promoter element: 3585-3591
 Major transcription start points: 3581, 3619, 3625 & 3630
 Kanamycin/neomycin resistance gene
 Neomycin phosphotransferase coding sequences:
 Start codon (ATG): 3713-3715; Stop codon: 4505-4507
 G->A mutation to remove Pst I site: 3895
 C->A (Arg to Ser) mutation to remove BssH II site: 4241
 Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
 Polyadenylation signals: 4743-4748 & 4756-4761
 pUC plasmid replication origin: 5092-5735

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

The price does not include delivery. The price varies in different countries. 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	PCMV IE
Host cells	mammalian
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori
Use	cyan fluorescent labeling of β -actin filaments

Vector description

pTagCFP-actin is a mammalian expression vector encoding TagCFP-actin fusion protein. The vector can be used for fluorescent labeling of β -actin in living cells.

TagCFP codon usage is optimized for high expression in mammalian cells, i.e. humanized [Haas et al. 1996]. Human cytoplasmic β -actin is fused to the TagCFP C-terminus. To increase mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of TagCFP-actin coding sequence [Kozak 1987].

pTagCFP-actin can be used as a source of TagCFP-actin hybrid sequence. The vector backbone contains unique restriction sites that permit its excision and further insertion into expression vector of choice.

Note: The plasmid DNA was isolated from dam⁺-methylated *E. coli*. Therefore some restriction sites are blocked by methylation. If you wish to digest the vector using such sites you will need to transform the vector into a dam⁻ host and make fresh DNA.

The vector backbone also contains immediate early promoter of cytomegalovirus (PCMV IE) for protein expression, SV40 origin for replication in mammalian cells expressing SV40 T-antigen, pUC origin of replication for propagation in *E. coli*, and f1 origin for single-stranded DNA production. SV40 polyadenylation signals (SV40 poly A) direct proper processing of the 3' end of the reporter mRNA.

SV40 early promoter (P_{SV40}) provides neomycin resistance gene (Neo^r) expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression (Kan^r) in *E. coli*. Kan^r/Neo^r gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals.

Expression in mammalian cells

pTagCFP-actin can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the TagCFP-actin fusion in eukaryotic cells. If required, stable transformants can be selected using G418 [Gorman 1985].

Propagation in *E. coli*

Suitable host strains for propagation in *E. coli* include DH5alpha, HB101, XL1-Blue, and other general purpose strains. Plasmid incompatibility group is pMB1/ColE1. The vector confers resistance to kanamycin (30 µg/ml) to *E. coli* hosts. Copy number in *E. coli* is about 500.

References

- Gorman (1985). "High efficiency gene transfer into mammalian cells." In: *DNA cloning: A Practical Approach, Vol. II*. Ed. by Glover. (IRL Press, Oxford, U.K.) Pp. 143-190.
- Haas et al. (1996) "Codon usage limitation in the expression of HIV-1 envelope glycoprotein." *Curr Biol*, 6 (3): 315-324 / pmid: 8805248
- Kozak (1987) "An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs." *Nucleic Acids Res*, 15 (20): 8125-8148 / pmid: 3313277

Notice to Purchaser:

Evrogen Fluorescent Protein Products (the Products) are intended for research use only. The Products are covered by U.S. Pat. 7,417,131 and other 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.

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.