

pTagFP635-tubulin vector

Cat# FP391

Vector description

pTagFP635-tubulin is a mammalian expression vector encoding TagFP635-tubulin fusion protein. The vector can be used for fluorescent labeling of α -tubulin in living cells.

TagFP635 codon usage is optimized for high expression in mammalian cells, i.e. humanized [Haas *et al.*, 1996]. Human α -tubulin is fused to the TagFP635 C-terminus. To increase mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of TagFP635-tubulin coding sequence [Kozak, 1987].

pTagFP635-tubulin can be used as a source of TagFP635-tubulin hybrid sequence. The vector backbone contains unique restriction sites that permit its excision and further insertion into expression vector of choice.

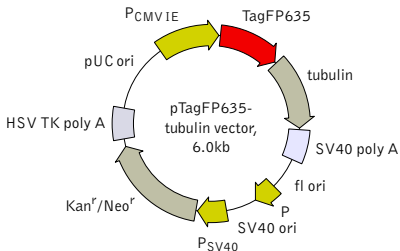
Note: The plasmid DNA was isolated from dam^+ -methylated *E. coli*. Therefore some restriction sites are blocked by methylation. If you wish to digest the vector using such sites you will need to transform the vector into a dam^- host and make fresh DNA.

The vector backbone also contains immediate early promoter of cytomegalovirus (P_{CMVIE}) 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.

Vector map

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



Expression in mammalian cells

pTagFP635-tubulin can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the TagFP635-tubulin fusion in eukaryotic cells. If required, stable transformants can be selected using G418 [Gorman, 1985].

Location of features

PCMV IE: 1-589

Enhancer region: 59-465

TATA box: 554-560

Transcription start point: 583

TagFP635

Kozak consensus translation initiation site: 606-616

Start codon (ATG): 613-615

Stop codon: 2695-2697

Last amino acid in TagFP635: 1321-1323

Tubulin: 1342-2697

SV40 early mRNA polyadenylation signal

Polyadenylation signals: 2858-2863 2887-2892

mRNA 3' ends: 2896 2908

f1 single-strand DNA origin: 2955-3410

Bacterial promoter for expression of Kan^r gene

-35 region: 3472-3477

-10 region: 3495-3500

Transcription start point: 3507

SV40 origin of replication: 3751-3886

SV40 early promoter

Enhancer (72-bp tandem repeats): 3584-3655 3656-3727

21-bp repeats: 3731-3751, 3752-3772 3774-3794

Early promoter element: 3807-3813

Major transcription start points: 3803, 3841, 3847 3852

Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 3935-3937

Stop codon: 4727-4729

G->A mutation to remove Pst I site: 4117

C->A (Arg to Ser) mutation to remove BssH II site: 4463

Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal

Polyadenylation signals: 4965-4970 4978-4983

pUC plasmid replication origin: 5314-5957

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 C. High efficiency gene transfer into mammalian cells. In DNA cloning: A Practical Approach, Vol. II. Ed. D. M. Glover. (IRL Press, Oxford, U.K.). 1985; 143-90.

Haas J, Park EC, Seed B. Codon usage limitation in the expression of HIV-1 envelope glycoprotein. *Curr Biol.* 1996; 6 (3):315-24. / pmid: 8805248

Kozak M. An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs. *Nucleic Acids Res.* 1987; 15 (20):8125-48. / pmid: 3313277

Notice to Purchaser:

Evrogen FP-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 (enclosed).

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.