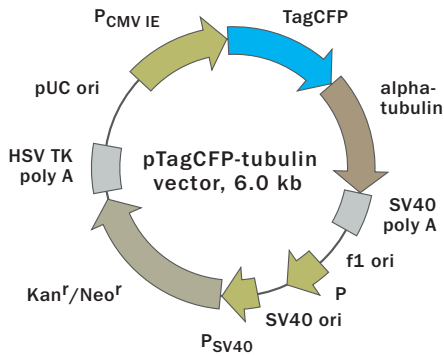


Mammalian expression vector pTagCFP-tubulin



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

Use

- Expression of TagCFP fusion with alpha-tubulin in mammalian cells under the control of CMV promoter for labeling of tubulin filaments
- Source of TagCFP-alpha-tubulin fusion coding sequence

Product	Cat.#	Size
pTagCFP-tubulin	FP115	20 µg

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

Vector type	mammalian expression vector
Reporter	TagCFP-tubulin
Reporter codon usage	mammalian
Promoter	P _{CMV IE}
Host cells	mammalian
Selection	prokaryotic — kanamycin eukaryotic — neomycin (G418)
Replication	prokaryotic — pUC ori eukaryotic — SV40 ori

Vector description

pTagCFP-tubulin is a mammalian expression vector encoding TagCFP fusion with human alpha-tubulin. The vector can be used for fluorescent labeling of tubulin filaments in living cells.

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

pTagCFP-tubulin is not intended as a cloning vector; however, vector backbone contains unique restriction sites that permit excision of the TagCFP-tubulin hybrid sequence.

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_{CMV 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 provides neomycin resistance gene expression to select stably transfected eukaryotic cells using G418.

Bacterial promoter (P) provides kanamycin resistance gene expression 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-tubulin vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the TagCFP-tubulin fusion in eukaryotic cells. If required, stable transformants can be selected using G418 (Gorman, 1985).

TagCFP-tagged fusions retain fluorescent properties of the native fluorescent protein allowing its localization *in vivo*.

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.

Location of features

P_{CMV IE}: 1-589

Enhancer region: 59-465

TATA box: 554-560

Transcription start point: 583

TagCFP-tubulin fusion

Kozak consensus translation initiation site: 606-616

Start codon (ATG): 613-615

Last amino acid in TagCFP: 1324-1326

Alpha-tubulin: 1345-2700

Stop codon: 2698-2700

SV40 early mRNA polyadenylation signal

Polyadenylation signals: 2861-2866 & 2890-2895

mRNA 3' ends: 2899 & 2911

f1 single-strand DNA origin: 2958-3413

Bacterial promoter for expression of Kan^r gene

-35 region: 3475-3480; -10 region: 3498-3503

Transcription start point: 3510

SV40 origin of replication: 3754-3889

SV40 early promoter

Enhancer (72-bp tandem repeats): 3587-3658 & 3659-3730

21-bp repeats: 3734-3754, 3755-3775 & 3777-3797

Early promoter element: 3810-3816

Major transcription start points: 3806, 3844, 3850 & 3855

Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 3938-3940; Stop codon: 4730-4732

G->A mutation to remove Pst I site: 4120

C->A (Arg to Ser) mutation to remove BssH II site: 4466

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

Polyadenylation signals: 4968-4973 & 4981-4986

pUC plasmid replication origin: 5317-5960

References

Gorman C. (1985) In DNA cloning: A Practical Approach, Vol. II. Ed. D. M. Glover. (IRL Press, Oxford, U.K.), pp. 143-190.

Haas J. *et al.* (1996) *Curr. Biol.* 6: 315-324.

Kozak M. (1987) *Nucleic Acids Res.* 15:8125-8148.

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