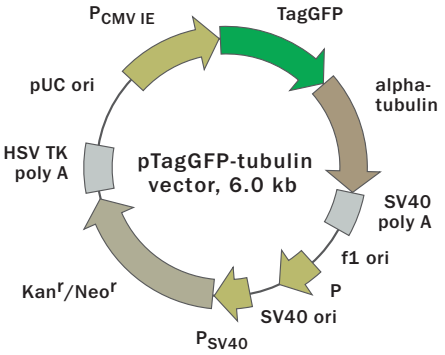
Mammalian expression vector pTagGFP-tubulin



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

Product	Cat.#	Size
pTagGFP-tubulin	**FP125**	20 µg

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

Vector type	mammalian expression vector
Reporter	TagGFP-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

Use

- Expression of TagGFP fusion with alpha-tubulin in mammalian cells under the control of CMV promoter for labeling of tubulin filaments

- Source of TagGFP-alpha-tubulin fusion coding sequence

Vector description

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

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

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

Note: The plasmid DNA was isolated from dam$^+$-methylated *E.coli*. Therefore some restriction sites are bloked 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

pTagGFP-tubulin vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the TagGFP-tubulin 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.

Location of features

$P_{CMV\ IE}$: 1-589
Enhancer region: 59-465
TATA box: 554-560
Transcription start point: 583
TagGFP-tubulin fusion
Kozak consensus translation initiation site: 606-616
Start codon (ATG): 613-615
Last amino acid in TagGFP: 1324-1326
Alpha-tubulin: 1345-2700
Stop codon: 2698-2700
SV40 early mRNA polyadenylation signals
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.