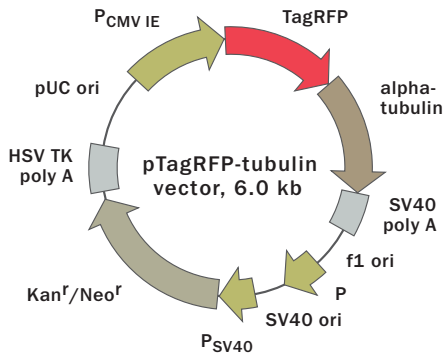


Mammalian expression vector pTagRFP-tubulin



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

Use

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

Product	Cat.#	Size
pTagRFP-tubulin	FP145	20 µg
Please contact your local distributor for exact prices and delivery information.		
Vector type	mammalian expression vector	
Reporter	TagRFP-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

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

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

pTagRFP-tubulin is not intended as a cloning vector; however, vector backbone contains unique restriction sites that permit excision of the TagRFP-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

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

TagRFP-tubulin fusion

Kozak consensus translation initiation site: 606-616

Start codon (ATG): 613-615

Stop codon: 2695-2697

Last amino acid in TagRFP: 1321-1323

Alpha-tubulin: 1342-2697

SV40 early mRNA polyadenylation signals

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 PstI site: 4117

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

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

Polyadenylation signals: 4965-4970 & 4978-4983

pUC plasmid replication origin: 5314-5957

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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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.