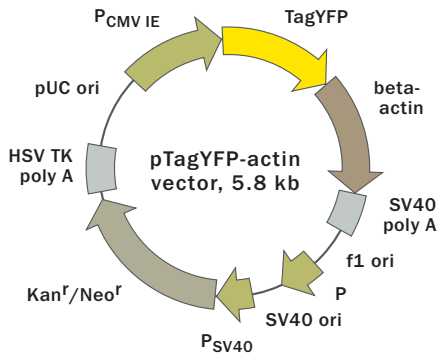


Mammalian expression vector pTagYFP-actin



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

Use

- Expression of TagYFP fusion with beta-actin in mammalian cells under the control of CMV promoter for labeling of actin filaments
- Source of TagYFP-beta-actin fusion coding sequence

| Product | Cat.# | Size |
|---------------|--------------|-------|
| pTagYFP-actin | FP134 | 20 µg |

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

| | |
|----------------------|---|
| Vector type | mammalian expression vector |
| Reporter | TagYFP-actin |
| 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

pTagYFP-actin is a mammalian expression vector encoding TagYFP fusion with human beta-actin. The vector can be used for fluorescent labeling of actin filaments in living cells.

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

pTagYFP-actin is not intended as a cloning vector; however, vector backbone contains unique restriction sites that permit excision of the TagYFP-actin 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

pTagYFP-actin vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the TagYFP-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.

Location of features

P_{CMV IE}: 1-589

Enhancer region: 59-465

TATA box: 554-560

Transcription start point: 583

Kozak consensus translation initiation site 606-616

TagYFP-actin fusion

Start codon (ATG): 613-615

Last amino acid in TagYFP: 1327-1329

Beta-actin 1351-2478

Stop codon: 2476-2478

SV40 early mRNA polyadenylation signals

Polyadenylation signals: 2639-2644 & 2668-2673

mRNA 3' ends: 2677 & 2689

f1 single-strand DNA origin: 2736-3191

Bacterial promoter for expression of Kan^r gene

-35 region: 3253-3258

-10 region: 3276-3281

Transcription start point: 3288

SV40 origin of replication: 3532-3667

SV40 early promoter

Enhancer (72-bp tandem repeats): 3365-3436 & 3437-3508

21-bp repeats: 3512-3532, 3533-3553 & 3555-3575

Early promoter element: 3588-3594

Major transcription start points: 3584, 3622, 3628 & 3633

Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 3716-3718

Stop codon: 4508-4510

G->A mutation to remove Pst I site: 3898

C->A (Arg to Ser) mutation to remove BssH II site: 4244

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

Polyadenylation signals: 4746-4751 & 4759-4764

pUC plasmid replication origin: 5095-5738

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.

Notice to Purchaser:

TagYFP-related products: These 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.