

pTagFP635-actin vector

Cat# FP390

Vector description

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

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

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

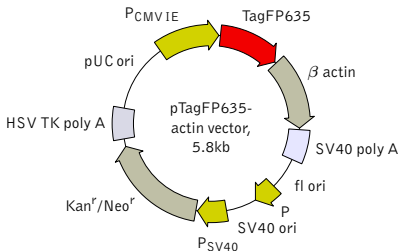
Note: The plasmid DNA was isolated from dam^+ -methylated *E. coli*. Therefore some restriction sites are blocked by methylation. If you wish to digest the vector using such sites you will need to transform the vector into a dam^- host and make fresh DNA.

The vector backbone also contains immediate early promoter of cytomegalovirus ($P_{\text{CMV IE}}$) 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 (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-actin can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the TagFP635-actin 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

Kozak consensus translation initiation site: 606-616

TagFP635

Start codon (ATG): 613-615

Last amino acid in TagFP635: 1321-1323

Beta-Actin: 1345-2472

Stop codon: 2470-2472

SV40 early mRNA polyadenylation signal

Polyadenylation signals: 2633-2638 2662-2667

mRNA 3' ends: 2671 2683

f1 single-strand DNA origin: 2730-3185

Bacterial promoter for expression of Kan^r gene

-35 region: 3247-3252

-10 region: 3270-3275

Transcription start point: 3282

SV40 origin of replication: 3526-3661

SV40 early promoter

Enhancer (72-bp tandem repeats): 3359-3430 3431-3502

21-bp repeats: 3506-3526, 3527-3547 3549-3569

Early promoter element: 3582-3588

Major transcription start points: 3578, 3616, 3622 3627

Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 3710-3712

Stop codon: 4502-4504

G->A mutation to remove Pst I site: 3892

C->A (Arg to Ser) mutation to remove BssH II site: 4238

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

Polyadenylation signals: 4740-4745 4753-4758

pUC plasmid replication origin: 5089-5732

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

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