

pTagRFP-FAK vector

Cat# FP366

Vector description

pTagRFP-FAK is a mammalian expression vector encoding TagRFP-FAK fusion protein. The vector can be used for fluorescent labeling of focal adhesion kinase in living cells.

TagRFP codon usage is optimized for high expression in mammalian cells, i.e. humanized [Haas *et al.*, 1996]. Chicken focal adhesion kinase is fused to the TagRFP C-terminus. To increase mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of TagRFP-FAK coding sequence [Kozak, 1987].

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

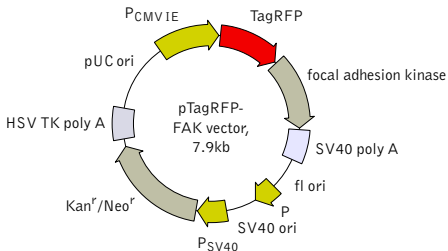
Note: The plasmid DNA was isolated from *dam*⁺-methylated *E. coli*. Therefore some restriction sites are blocked by methylation. If you wish to digest the vector using such sites you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

The vector backbone also contains immediate early promoter of cytomegalovirus (P_{CMVIE}) 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

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

TagRFP

Start codon (ATG): 613-615

Last amino acid in TagRFP: 1321-1323

Focal Adhesion Kinase: 1339-4509

Stop codon: 4507-4509

SV40 early mRNA polyadenylation signal

Polyadenylation signals: 4686-4691 4715-4720

mRNA 3' ends: 4724 4736

f1 single-strand DNA origin: 4783-5238

Bacterial promoter for expression of Kan^r gene

-35 region: 5300-5305

-10 region: 5323-5328

Transcription start point: 5335

SV40 origin of replication: 5579-5714

SV40 early promoter

Enhancer (72-bp tandem repeats): 5412-5483 5484-5555

21-bp repeats: 5559-5579, 5580-5600 5602-5622

Early promoter element: 5635-5641

Major transcription start points: 5631, 5669, 5675 5680

Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 5763-5765

Stop codon: 6555-6557

G->A mutation to remove Pst I site: 5945

C->A (Arg to Ser) mutation to remove BssH II site: 6291

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

Polyadenylation signals: 6793-6798 6806-6811

pUC plasmid replication origin: 7142-7785

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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