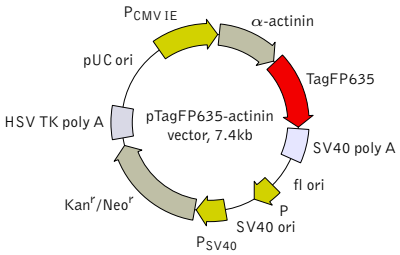
pTagFP635-actinin vector

The vector sequence has been compiled using the information from sequence databases, published literature, and other sources, together with partial sequences obtained by Evrogen. This vector has not been completely sequenced.



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

Product	Cat.#	Size
pTagFP635-actinin vector	**FP380**	20 μg

The price does not include delivery. The price varies in different countries. Please contact your local distributor for exact prices and delivery information.

Vector type	mammalian expression vector
Reporter	TagFP635
Reporter codon usage	mammalian
Promoter for TagFP635	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori
Use	far-red fluorescent labeling of α-actinin

Location of features

$P_{CMV\ IE}$: 1-589
Enhancer region: 59-465
TATA box: 554-560
Transcription start point: 583
Alpha-actinin: 637-3312
TagFP635: 3370-4083
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 4236-4241 & 4265-4270
mRNA 3' ends: 4274 & 4286
f1 single-strand DNA origin: 4333-4788
Bacterial promoter for expression of Kan^r gene
-35 region: 4850-4855; -10 region: 4873-4878
Transcription start point: 4885
SV40 origin of replication: 5129-5264
SV40 early promoter
Enhancer (72-bp tandem repeats): 4962-5033 & 5034-5105
21-bp repeats: 5109-5129, 5130-5150 & 5152-5172
Early promoter element: 5185-5191
Major transcription start points: 5181, 5219, 5225 & 5230
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 5313-5315; Stop codon: 6105-6107
G->A mutation to remove Pst I site: 5495
C->A (Arg to Ser) mutation to remove BssH II site: 5841
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 6343-6348 & 6356-6361
pUC plasmid replication origin: 6692-7335

Vector description

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

TagFP635 codon usage is optimized for high expression in mammalian cells, i.e. humanized (Haas et al. 1996). Human α-actinin is fused to the TagFP635 N-terminus.

pTagFP635-actinin can be used as a source of TagFP635-actinin 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 ($P_{CMV\ IE}$) 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.

Expression in mammalian cells

pTagFP635-actinin can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the TagFP635-actinin 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.

References

Gorman (1985). "High efficiency gene transfer into mammalian cells." In: *DNA cloning: A Practical Approach, Vol. II*. Ed. by Glover. (IRL Press, Oxford, U.K.) Pp. 143–190.

Haas et al. (1996) "Codon usage limitation in the expression of HIV-1 envelope glycoprotein." Curr Biol, 6 (3): 315–324 / pmid: 8805248