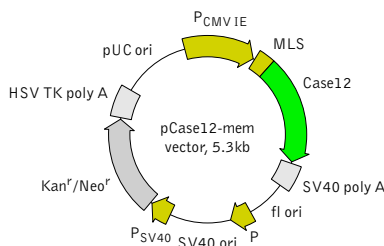


pCase12-mem vector

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>

Location of features

P_{CMV IE}: 1-589
 Enhancer region: 59-465
 TATA box: 554-560
 Transcription start point: 583
 Case12-mem fusion
 Start codon (ATG): 679-681
 Neuromodulin N-terminal sequence (mem): 679-738
 Start of Case12 coding sequence (ATG): 739-741
 Stop codon: 1978-1980
 SV40 early mRNA polyadenylation signal
 Polyadenylation signals: 1976-1981, 2134-2139 & 2163-2168
 mRNA 3' ends: 2172 & 2184
 f1 single-strand DNA origin: 2231-2686
 Bacterial promoter for expression of Kan^r gene
 -35 region: 2748-2753; -10 region: 2771-2776
 Transcription start point: 2783
 SV40 origin of replication: 3027-3162
 SV40 early promoter
 Enhancer (72-bp tandem repeats): 2860-2931 & 2932-3003
 21-bp repeats: 3007-3027, 3028-3048 & 3050-3070
 Early promoter element: 3083-3089
 Major transcription start points: 3079, 3117, 3123 & 3128
 Kanamycin/neomycin resistance gene
 Neomycin phosphotransferase coding sequences:
 Start codon (ATG): 3211-3213; Stop codon: 4003-4005
 G->A mutation to remove Pst I site: 3393
 C->A (Arg to Ser) mutation to remove BssH II site: 3739
 Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
 Polyadenylation signals: 4241-4246 & 4254-4259
 pUC plasmid replication origin: 4590-5233

Product	Cat.#	Size
pCase12-mem vector	FP993	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	Case12	
Reporter codon usage	mammalian	
Promoter for Case12	P _{CMV IE}	
Host cells	mammalian	
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)	
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori	
Use	Expression of membrane-targeted fluorescent Ca ²⁺ sensor Case12 in mammalian cells under the control of CMV promoter; source of membrane-targeted Case12 coding sequence	

Vector description

pCase12-mem is a mammalian expression vector encoding membrane-targeted fluorescent Ca²⁺ sensor Case12. Case12 codon usage is optimized for high expression in mammalian cells (humanized) [Haas et al. 1996]. Membrane localization signal (MLS) of neuromodulin is linked to the Case12 N-terminus. The MLS (N-terminal 20 amino acid residues of neuromodulin) contains a signal for posttranslational palmitoylation of cysteines 3 and 4 that targets Case12 to cellular membranes [Skene and Virág 1989].

pCase12-mem can be used as a source of MLS-Case12 hybrid sequence. The vector backbone contains unique restriction sites that permit its excision and further insertion into expression vector of choice. Alternatively, MLS-Case12 coding sequence can be amplified by PCR.

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 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

pCase12-mem vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive Case12 expression in many cell types. 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
- Skene and Virág (1989) "Posttranslational membrane attachment and dynamic fatty acylation of a neuronal growth cone protein, GAP-43." *J Cell Biol*, 108 (2): 613-624 / pmid: 2918027

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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.

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