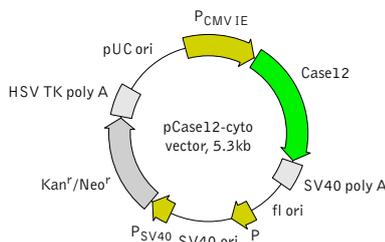


pCase12-cyto vector

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
 Kozak consensus translation initiation site: 672-682
 Start codon (ATG): 679-681; Stop codon: 1924-1926
 Last amino acid in Case12: 1921-1923
 SV40 early mRNA polyadenylation signal
 Polyadenylation signals: 1922-1927, 2080-2085 & 2109-2114
 mRNA 3' ends: 2118 & 2130
 f1 single-strand DNA origin: 2177-2632
 Eukaryotic promoter for expression of Kan^r gene
 -35 region: 2694-2699; -10 region: 2717-2722
 Transcription start point: 2729
 SV40 origin of replication: 2973-3108
 SV40 early promoter
 Enhancer (72-bp tandem repeats): 2806-2877 & 2878-2949
 21-bp repeats: 2953-2973, 2974-2994 & 2996-3016
 Early promoter element: 3029-3035
 Major transcription start points: 3025, 3063, 3069 & 3074
 Kanamycin/neomycin resistance gene
 Neomycin phosphotransferase coding sequences:
 Start codon (ATG): 3157-3159; Stop codon: 3949-3951
 G->A mutation to remove Pst I site: 3339
 C->A (Arg to Ser) mutation to remove BssH II site: 3685
 Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
 Polyadenylation signals: 4187-4192 & 4200-4205
 pUC plasmid replication origin: 4536-5179

Product	Cat.#	Size
pCase12-cyto vector	FP991	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 fluorescent Ca ²⁺ sensor Case12 in mammalian cells under the control of CMV promoter; source of Case12 coding sequence	

Vector description

pCase12-cyto is a mammalian expression vector encoding a fluorescent sensor Case12. To increase mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of the Case12 sequence [Kozak 1987].

The vector can be also used as a source of Case12 coding sequence. Flanking restriction sites are convenient for excision of Case12 sequence and its further insertion into other expression vectors of choice. Alternatively, 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-cyto 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.

Kozak (1987) "An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs." *Nucleic Acids Res*, 15 (20): 8125-8148 / pmid: 3313277

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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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