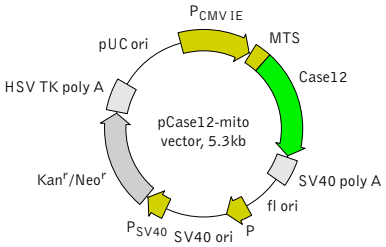
pCase12-mito vector

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
pCase12-mito vector	**FP992**	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 mitochondria-targeted fluorescent Ca^{2+} sensor Case12 in mammalian cells under the control of CMV promoter; source of mitochondria-targeted Case12 coding sequence

Location of features

$P_{CMV\ IE}$: 1-589
Enhancer region: 59-465
TATA box: 554-560
Transcription start point: 583
Case12-Mito fusion
Start codon (ATG): 597-599
Mitochondrial localization signal (MLS): 597-683
Start of Case12 coding sequence (ATG): 705-707
Stop codon: 1950-1952
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1948-1953, 2106-2111 & 2135-2140
mRNA 3' ends: 2144 & 2156
f1 single-strand DNA origin: 2203-2658
Bacterial promoter for expression of Kan^r gene
-35 region: 2720-2725; -10 region: 2743-2748
Transcription start point: 2755
SV40 origin of replication: 2999-3134
SV40 early promoter
Enhancer (72-bp tandem repeats): 2832-2903 & 2904-2975
21-bp repeats: 2979-2999, 3000-3020 & 3022-3042
Early promoter element: 3055-3061
Major transcription start points: 3051, 3089, 3095 & 3100
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 3183-3185; Stop codon: 3975-3977
G->A mutation to remove Pst I site: 3365
C->A (Arg to Ser) mutation to remove BssH II site: 3711
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 4213-4218 & 4226-4231
pUC plasmid replication origin: 4562-5205

Vector description

pCase12-mito is a mammalian expression vector encoding mitochondria-targeted fluorescent Ca^{2+} sensor Case12. Case12 codon usage is optimized for high expression in mammalian cells (humanized) [Haas et al. 1996]. Mitochondrial targeting sequence (MTS) is fused to the Case12 N-terminus. MTS was derived from the subunit VIII of human cytochrome C oxidase [Rizzuto et al. 1989, Rizzuto et al. 1995].

pCase12-mito can be used as a source of MTS-Case12 hybrid sequence. The vector backbone contains unique restriction sites that permit its excision and further insertion into expression vector of choice. Alternatively, MTS-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-mito can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the Case12-MTS 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

Rizzuto et al. (1989) "A gene specifying subunit VIII of human cytochrome c oxidase is localized to chromosome 11 and is expressed in both muscle and non-muscle tissues." J Biol Chem, 264 (18): 10595–10600 / pmid: 2543673

Rizzuto et al. (1995) "Chimeric green fluorescent protein as a tool for visualizing subcellular organelles in living cells." Curr Biol, 5 (6): 635–642 / pmid: 7552174