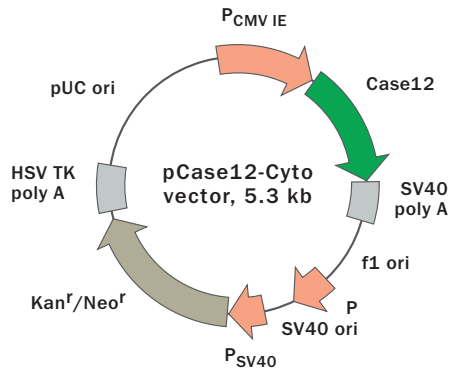


Mammalian expression vector pCase12-Cyto



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

Use

- Case12 expression in mammalian cells under the control of CMV promoter
- Source of Case12 coding sequence

Product	Cat.#	Size
pCase12-Cyto	FP991	20 µg

Please contact your local distributor for exact prices and delivery information.

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

Vector description

pCase12-Cyto is an eukaryotic (mammalian) expression vector encoding a fluorescent sensor for calcium ions, Case12. The vector allows Case12 expression in cell cytosol for monitoring concentration changes of intracellular calcium ions. To increase Case12 translation, Kozak consensus translation initiation site is generated upstream of the Case12 sequence (Kozak, 1987).

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

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 polyA direct proper processing of the 3' end of the reporter mRNA.

SV40 early promoter provides neomycin resistance gene expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression in *E. coli*. Kan^r/Neo^r gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals.

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.

Expression in mammalian cells

pCase12-Cyto vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of Case12 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 mg/ml) to *E. coli* hosts. Copy number in *E. coli* is about 500.

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

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

Bacterial 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 PstI site: 3339

C->A (Arg to Ser) mutation to remove BssHII site: 3685

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

Polyadenylation signals: 4187-4192, 4200-4205

pUC plasmid replication origin: 4536-5179

References

Gorman C. (1985) In DNA cloning: A Practical Approach, Vol. II. Ed. D. M. Glover. (IRL Press, Oxford, U.K.), pp. 143-190.

Kozak M. (1987) Nucleic Acids Res. 15:8125-8148.

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MATERIAL SAFETY DATA SHEET INFORMATION

To the best of our knowledge, these products do not require a Material Safety Data Sheet. However, all the properties of these products (and, if applicable, each of their components) have not been thoroughly investigated. Therefore, we recommend that you use gloves and eye protection, and wear a laboratory coat when working with these products.