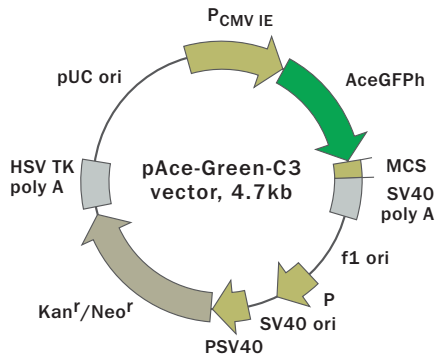


Mammalian expression vector pAce-Green-C3



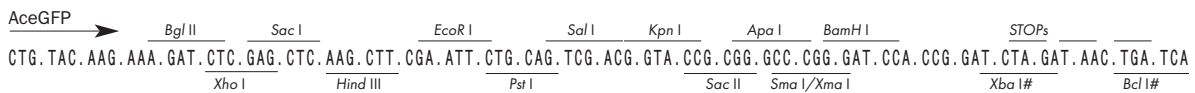
For vector sequence, please visit our Web site at www.evrogen.com/support/vector-info.shtml

Product	Cat.#	Size
pAce-Green-C3	FP103	20 µg

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

Vector type	mammalian expression vector
Reporter	AceGFP
Reporter codon usage	mammalian
Promoter for AceGFP	P _{CMV IE}
Host cells	mammalian
Selection	prokaryotic — kanamycin eukaryotic — neomycin (G418)
Replication	prokaryotic — pUC ori eukaryotic — SV40 ori

Multiple cloning site (MCS)



- sites are blocked by methylation.

Use

- Generation of fusions to the AceGFP C-terminus
- Expression of AceGFP or its fusions in mammalian cells

Vector description

pAce-Green-C3 vector is an eukaryotic (mammalian) expression vector encoding the *Aequorea coerulea* enhanced green fluorescent protein AceGFP. The vector allows generation of fusions to the AceGFP C-terminus and expression AceGFP fusions or AceGFP alone in eukaryotic (mammalian) cells.

AceGFP codon usage is optimized for high expression in mammalian cells (humanized, Haas *et al.*, 1996). To increase AceGFP translation, Kozak consensus translation initiation site is generated upstream of the AceGFP sequence (Kozak, 1987). Multiple cloning site (MCS) is located between AceGFP coding sequence and SV40 polyadenylation signal (SV40 polyA).

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.

Generation of fusions

A localization signal (or a gene of interest) should be cloned into MCS of the vector. It will be expressed as a fusion to AceGFP C-terminus when inserted in the same reading frame as AceGFP and no intervening stop codons are present. AceGFP-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*. Unmodified vector will express AceGFP, when transfected into eukaryotic (mammalian) cells.

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

pAce-Green-C3 can be transfected into mammalian cells by any known transfection method. 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.

Location of features

P_{CMV IE}: 1-589

Enhancer region: 59-465

TATA box: 554-560

Transcription start point: 583

AceGFP

Kozak consensus translation initiation site: 606-616

Start codon (ATG): 613-615; Stop codon: 1392-1394

Last amino acid in AceGFP: 1327-1329

MCS: 1330-1401

SV40 early mRNA polyadenylation signal

Polyadenylation signals: 1534-1539 & 1563-1568

mRNA 3' ends: 1572 & 1579

f1 single-strand DNA origin: 1631-2086

(Packages the noncoding strand of AceGFP)

Bacterial promoter for expression of Kan^r gene

-35 region: 2148-2153; -10 region: 2171-2176

Transcription start point: 2183

SV40 origin of replication: 2427-2562

SV40 early promoter

Enhancer (72-bp tandem repeats): 2260-2331 & 2332-2403

21-bp repeats: 2407-2427, 2428-2448, & 2450-2470

Early promoter element: 2483-2488

Major transcription start points: 2479, 2517, 2523 & 2528

Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 2611-2613; stop codon: 3403-3405

G->A mutation to remove Pst I site: 2788

C->A (Arg to Ser) mutation to remove BssH II site: 3139

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

Polyadenylation signals: 3641-3646 & 3654-3659

pUC plasmid replication origin: 3990-4633

References

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

Haas J. et al. (1996) Curr. Biol. 6: 315-324.

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

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