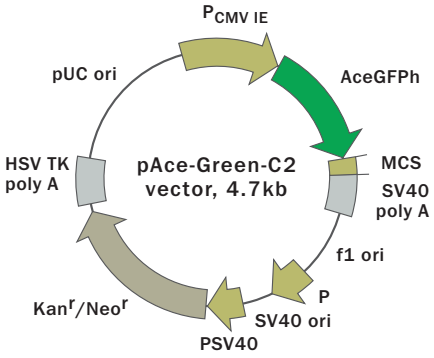
Mammalian expression vector pAce-Green-C2



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

Product	Cat.#	Size
pAce-Green-C2	**FP102**	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)

AceGFP →

Bgl II	Mfe I	EcoR I	Sal I	Kpn I	Apa I	BamH I	STOPs		
CTG.TAC.AAG.AAA.GAT.CTC.GAC.TTA.AGC.TTC.GAA.TTC.TGC.AGT.CGA.CGG.TAC.CGC.GGGCCC.GGG.ATC.CAC.CGG.ATC.TAG.ATA.ACT.GAT.CA									
	Hind III	Pst I		Sac II	Sma I/Xma I		Xba I#	Bcl I#	

- 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-C2 vector is an eukaryotic (mammalian) expression vector encoding the *Aequrea coerulescens* 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 bloked 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-C2 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: 1391-1393
Last amino acid in AceGFP: 1327-1329
MCS: 1330-1400
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1533-1538 & 1562-1567
mRNA 3' ends: 1571 & 1578
f1 single-strand DNA origin: 1630-2085
(Packages the noncoding strand of AceGFP.)
Bacterial promoter for expression of Kan^r gene
-35 region: 2147-2152; -10 region: 2170-2175
Transcription start point: 2182
SV40 origin of replication: 2426-2561
SV40 early promoter
Enhancer (72-bp tandem repeats): 2259-2330 & 2331-2402
21-bp repeats: 2406-2426, 2427-2447, & 2449-2469
Early promoter element: 2482-2487
Major transcription start points: 2478, 2516, 2522 & 2527
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2610-2612; stop codon: 3402-3404
G->A mutation to remove Pst I site: 2787
C->A (Arg to Ser) mutation to remove BssH II site: 3138
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3640-3645 & 3653-3658
pUC plasmid replication origin: 3989-4632

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.