pAcVE.02 Baculovirus Transfer Vector

Cat. No.: 20020 Quantity: 10µg Storage: -20°C

This baculovirus transfer vector integrates the co-expression of a vankyrin gene for increased protein production, a honey bee melittin signal sequence for direct secretion and an N-terminal 8x His-tag for ease of purification.

Description

pAcVE.02 is a baculovirus transfer vector designed for high levels of recombinant gene expression under the control of the *Autographa californica* nucleopolyhedro virus (AcMNPV) polyhedrin promoter. pAcVE.02 is derived from pUC57 and contains the pUC origin of replication and the ampicillin resistance gene for propagation and selection in bacteria. pAcVE.02 is designed for the direct secretion of recombinant proteins utilizing the honeybee melittin signal sequence (Tessier *et al.*, 1991). An N-terminal 8xHis-tag is provided for ease of purification. Cloning into pAcVE.02 requires the DNA insert to be in frame with the N-terminal 8xHis tag. Foreign genes may be cloned into the multiple cloning site utilizing the following restriction sites: NotI; SbfI and NheI. PmeI can be used if a C-terminal 8xHis-tag instead of the N-terminal His-tag is required or if the His-tag is not desired. Increased recombinant protein production over conventional baculovirus transfer vectors is achieved by the co-expression of the gene of interest with a vankyrin expression cassette (Fath-Goodin *et al.*, 2006). pAcVE.02 contains ORF 603 and ORF1629 sequences and allows recombination with the viral DNA for insertion into the polyhedrin locus. The baculovirus transfer vector is compatible with any baculovirus system that utilizes homologous recombination in insect cells, including *flash*BAC (Oxford Expression Technologies) and Bac-N-Blue (Invitrogen).

Contents

The plasmid DNA is dissolved in TE buffer (10mM Tris-HCl, 1 mM EDTA pH7.5). pAcVE.02 baculovirus transfer vector is provided at 10 µg in 50 µl.

Storage

The transfer vector should be placed at -20° C for long-term storage.

Handling

For expression of recombinant protein under the polyhedrin promoter, the gene of interest should be ligated into an appropriate restriction site in the multiple cloning site of the pAcVE.02 vector and should be in frame with the N-terminal His-tag (see diagram). Transform the recombinant pAcVE.02 transfer vector in *E. coli* cells (DH5α, Top10 or any other suitable strain), propagate the cells under ampicillin selection and purify the recombinant plasmid using standard plasmid purification protocols. For construction of recombinant AcMNPV perform a co-transfection of the purified recombinant pAcVE.02 vector with linearized baculovirus DNA suitable for homologous recombination in insect cells.

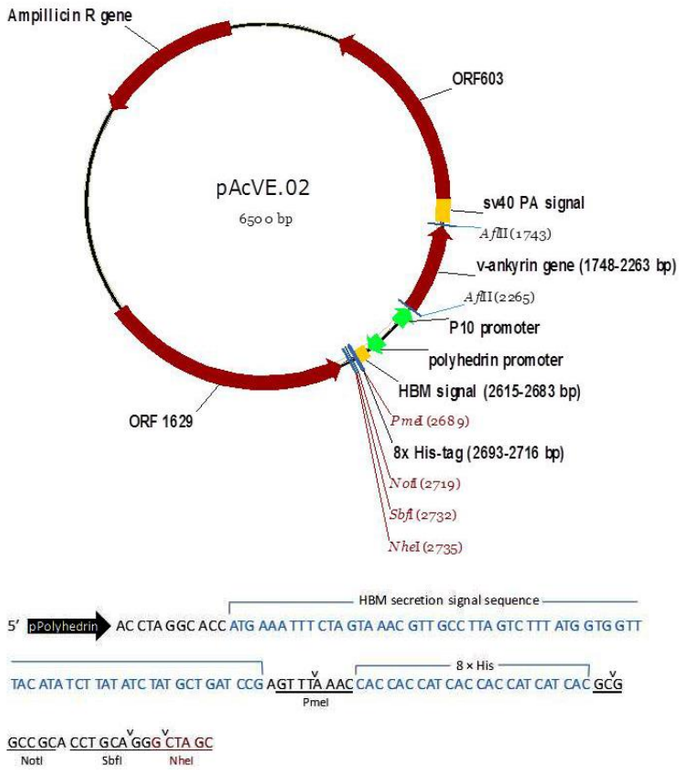
Unique Restriction Sites

AgeI AleI AlwNI ApaI AvaI AvrII BamHI BbvCI BclI BmgBI BmtI BplI BpmI Bpu10I BsaAI BsaBI BseRI BseYI BspMI BstAPI BstBI BstXI EagI Eco53kI EcoNI EcoRI HindIII HpaI KpnI NaeI NdeI NgoMIV NheI NotI NruI PciI PfoI PmeI PpuMI PspOMI SacI SapI SbfI SgrAI SmaI SnaBI SpeI SphI StuI StyI SwaI XbaI XmaI

Absent Restriction Sites

AscI AsiSI BglII BlpI BsiWI BspEI BssHII BstEII BstZ17I Bsu36I BtgI DraIII FseI FspAI MscI NcoI PflMI PmlI PshAI RsrII SacII SanDI SexAI SfiI Tth111I XcmI XhoI

pAcVE.02 Vector Map and Multiple Cloning Site



ParaTechs' Related Products

Product Name	Catalog No.
pAcVE.01	20010
pAcVE.03	20030
VE Insect Cell Line 01	10010
VE Insect Cell Line 02	10020
VE Insect Cell Line 03	10030

Contract service for recombinant virus construction is available upon request. For further information on other ParaTechs products contact our Technical Services at info@paratechs.com or call (859) 317-9213. See also our web-site at www.paratechs.com.

References

Fath-Goodin, A., Kroemer, J.A., Martin, S.B., Reeves, K., and Webb, B.A. (2006). Polydnavirus genes that enhance the Baculovirus Expression Vector System. Advances in Virus Research, vol. 68, pp. 75-90.

Tessier, D.C., Thomas, D.Y., Khouri, H.E., Laliberté, F., and Vernet, T. (1991). Enhanced secretion from insect cells of a foreign protein fused to the honeybee melittin signal peptide. Gene, 98: 177-183.

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Product Use Limitation

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Patent information: United States Patent 7,629,160

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