pVAC1-mcs

A plasmid designed for the production of neutralizing antibodies

Catalog # pvac1

For research use only

Version # 09G08-MM

PRODUCT INFORMATION

Contents:

- 20 µg of lyophilized pVAC1-mcs plasmid DNA.
- 4 pouches of *E. coli* Fast-Media® Zeo (2 TB and 2 Agar)

Storage and Stability:

- Products are shipped at room temperature.
- Lyophilized DNA is stable 12 months when stored at -20˚C.
- Resuspended DNA is stable 12 months when stored at -20˚C. Avoid repeated freeze-thaw cycles.
- Store *E. coli* Fast-Media® Zeo at room temperature. Fast-Media® pouches are stable 18 months when stored properly.

Quality control:

- Plasmid DNA was prepared using affinity column and lyophilized.
- Plasmid construct has been confirmed by restriction analysis sequencing.

GENERAL PRODUCT USE

pVAC1-mcs is a DNA vaccine vector specifically designed to stimulate a humoral immune response by intramuscular injection. Antigenic proteins are targeted and anchored to the cell surface by cloning the gene of interest in frame between the IL2 signal sequence and the C-terminal transmembrane anchoring domain of the placental alkaline phosphatase (PLAP). The antigenic peptide produced on the surface of muscle cells is thought to be taken up by antigen presenting cells (APCs) and processed through the major histocompatibility complex (MHC) class II pathway[1, 2, 3].

pVAC1-mcs may be used to:

Clone a gene encoding an antigenic protein of your choice. pVAC1-mcs is designed for the cloning of an antigenic gene that is not naturally secreted. A multiple cloning site (MCS) is located downstream of the promoter for convenient cloning of an antigenic gene. The MCS contains several restriction sites that are compatible with many other enzymes, thus facilitating cloning.

Express an antigenic protein directly within transfected cells. The expression of the antigenic protein is driven by the strong rhesus monkey EF1 promoter. The expressed protein is secreted and anchored to cell membrane since the MCS is inserted between the IL2 signal sequence and the glycosylphosphatidylinositol (GPI) anchoring domain of human PLAP.

Note: *Make sure the open reading frame of the antigenic gene is cloned in phase with the IL2 signal sequence.*

PLASMID FEATURES

• **SV40 enhancer** which is comprised of a 72-base-pair repeat allows the enhancement of gene expression in a large host range. The enhancement varies from 2-fold in non-permissive cells to 20-fold in permissive cells. Furthermore, the SV40 enhancer is able to direct nuclear localization of plasmids.

• **rhEF1 prom:** The elongation factor-1 alpha (EF-1α) is one of the most abundant proteins in eukaryotic cells and is expressed in almost all kinds of mammalian cells. The promoter of this 'housekeeping' gene exhibits a strong activity, higher than viral promoters such as SV40 and RSV promoters and, on the contrary to the CMV promoter, yields persistent expression of the transgene *in vivo*. The rhesus monkey EF-1α promoter shares 92.9% homology with its human counterpart and displays an activity similar to the human EF-1α promoter.

• **IL2 ss:** The IL2 signal sequence contains 21 amino acids and share common characteristics with signal peptides of other secretory proteins with respect to abundance and positions of hydrophobic amino acids. The intracellular cleavage of the IL2 signal peptide occurs after Ser20 and leads to the secretion of the antigenic protein

• **MCS** contains the following restriction sites:

Bam HI, *Eco* RV, *Bgl* II, and *Eco* RI

- *Bam* HII is compatible with *Bgl* I, *Bst* YI and *Bcl* I.
- *Eco* RV is compatible with any other blunt-end restriction enzymes.
- *Bam* HII is compatible with *Bgl* I, *Bst* YI and *Bcl* I.
- *Eco*RI is compatible with *Apo* I, *Mfe* I and *Tsp* 509I.

• **PLAP sa** is a hydrophobic COOH-terminal sequence of 32 residues which is eliminated during processing of the preprotein. The proteolytic cleavage of the C-terminal propeptide after Asp3 is catalyzed by a transaminase which simultaneously adds a GPI tail to the Asp residue.

• **EF1 pAn** is a strong polyadenylation signal. InvivoGen uses a sequence starting after the stop codon of the EF1 cDNA and finishing after a bent structure rich in GT.

• **pMB1 ori:** a minimal *E. coli* origin of replication to limit vector size but with the same activity as the longer Ori.

• **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli.*

• **Sh ble-ΔCpG** is a new allele of the *Sh ble* gene conferring resistance to Zeocin™. In order to reduce the immunogenicity of this bacterial gene all CpG motifs have been removed by chemically synthesizing the gene. The *Sh ble-ΔCpG* gene allows the selection of *E. coli* clones transformed with the pVAC plasmid.

Note: *Stable transfection of clones cannot be performed due to the absence of an eukaryotic promoter upstream of the Sh ble-ΔCpG gene.*

• **Term:** The *E. coli rpmB/G* terminator allows efficient transcription termination of the *Sh ble-ΔCpG* gene.

METHODS

Plasmid resuspension:

Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1µg/µl, resuspend the DNA in 20µl of sterile H_2O. Store resuspended plasmid at -20°C.

Selection of bacteria with *E. coli* Fast-Media:

E. coli Fast-Media® Zeo is a **new, fast and convenient** way to prepare liquid and solid media for bacterial culture by using only a microwave. *E. coli* Fast-Media® Zeo is a TB (liquid) or LB (solid) based medium with Zeocin™, and contains stabilizers. *E. coli* Fast-Media® Zeo can be ordered separately (catalog code # fas-zn-l, fas-zn-s).

Method:

1- Pour the contents of a pouch into a clean borosilicate glass bottle or flask.

2- Add 200 ml of distilled water to the flask

3- Heat in a microwave on MEDIUM power setting (about 400Watts), until bubbles start appearing (approximately 3 minutes). **Do not heat a closed container. Do not autoclave Fast-Media®.**

4- Swirl gently to mix the preparation. **Be careful, the bottle and media are hot, use heatproof pads or gloves and care when handling.**

5- Reheat the media for 30 seconds and gently swirl again. Repeat as necessary to completely dissolve the powder into solution. But be careful to avoid overboiling and volume loss.

6- Let agar medium cool to 45˚C before pouring plates. Let liquid media cool to 37˚C before seeding bacteria.

Note: *Do not reheat solidified Fast-Media® as the antibiotic will be permanently destroyed by the procedure.*

References:

1- Corr, M. *et al.* 1999. J. Immunol. 163:4721-7
2- Forns, X. *et al.* 1999. Vaccine 17:1992-2002
3- McCluskie, M.J. *et al.* 1999. Mol. Med. 5:287-300

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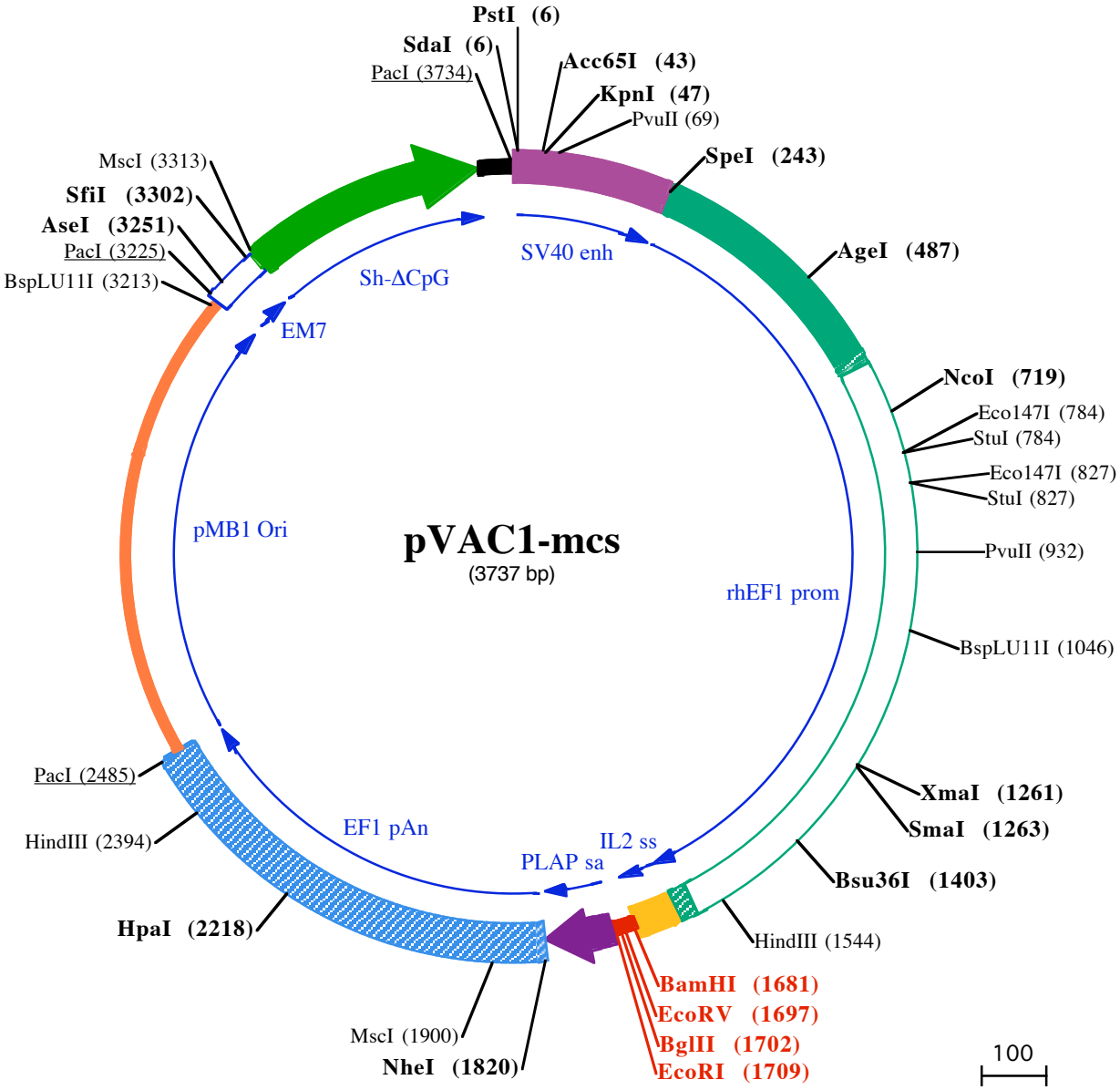
PstI (6)
SdaI (6)
PacI (3734)
Acc65I (43)
KpnI (47)
PvuII (69)
SpeI (243)
MscI (3313)
SfiI (3302)
AseI (3251)
PacI (3225)
BspLU11I (3213)
AgeI (487)
SV40 enh
Sh-ΔCpG
EM7
NcoI (719)
Eco147I (784)
StuI (784)
Eco147I (827)
StuI (827)
pMB1 Ori
pVAC1-mcs
(3737 bp)
PvuII (932)
rhEF1 prom
BspLU11I (1046)
PacI (2485)
XmaI (1261)
SmaI (1263)
HindIII (2394)
EF1 pAn
IL2 ss
PLAP sa
Bsu36I (1403)
HpaI (2218)
HindIII (1544)
BamHI (1681)
EcoRV (1697)
BglII (1702)
EcoRI (1709)
MscI (1900)
NheI (1820)
100


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3101 CTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCT
PacI (3225)
BspLU11I (3213)
AseI (3251)
3201 GGCCTTTTGCTCACATGTTCTTAATTAAATTTTTCAAAAGTAGTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACTCACTATAGG
MscI (3313)
SfiI (3302)
3301 AGGGCCATCATGGCCAAGTTGACCAGTGCTGTCCCAGTGCTCACAGCCAGGGATGTGGCTGGAGCTGTTGAGTTCTGGACTGACAGGTTGGGGTTCTCCA
1 MetAlaLysLeuThrSerAlaValProValLeuThrAlaArgAspValAlaGlyAlaValGluPheTrpThrAspArgLeuGlyPheSerA
3401 GAGATTTTGTGGAGGATGACTTTGCAGGTGTGGTCAGAGATGATGTCACCCTGTTCATCTCAGCAGTCCAGGACCAGGTGGTGCCTGACAACACCCTGGC
31 rgAspPheValGluAspAspPheAlaGlyValValArgAspAspValThrLeuPheIleSerAlaValGlnAspGlnValValProAspAsnThrLeuAl
3501 TTGGGTGTGGGTGAGAGGACTGGATGAGCTGTATGCTGAGTGGAGTGAGGTGGTCTCCACCAACTTCAGGGATGCCAGTGGCCCTGCCATGACAGAGATT
64 aTrpValTrpValArgGlyLeuAspGluLeuTyrAlaGluTrpSerGluValValSerThrAsnPheArgAspAlaSerGlyProAlaMetThrGlulle
3601 GGAGAGCAGCCCTGGGGGAGAGAGTTTGCCCTGAGAGACCCAGCAGGCAACTGTGTGCACTTTGTGGCAGAGGAGCAGGACTGAGGATAAGAATTGTAAC
98 GlyGluGlnProTrpGlyArgGluPheAlaLeuArgAspProAlaGlyAsnCysValHisPheValAlaGluGluGlnAsp•••
PacI (3734)
3701 AAAAAACCCCGCCCCGGCGGGGTTTTTTGTTAATTAA