

pVAC2-mcs

A plasmid designed for the production of neutralizing antibodies

Catalog # pvac2

For research use only

Version # 09G08-MM

PRODUCT INFORMATION

Content:

- 20 µg of lyophilized pVAC2-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

pVAC2-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 that possesses a natural signal sequence upstream of 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}.

pVAC2-mcs may be used to:

Clone a gene encoding an antigenic protein of your choice. pVAC2-mcs is designed for the cloning of an antigenic gene that already possesses a signal sequence. The MCS is located upstream of the GPI anchoring domain of PLAP. 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 secreted protein is anchored to cell membrane since the MCS is located upstream of the glycosylphosphatidylinositol (GPI) anchoring domain of human PLAP.

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.
- **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.

• **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.

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

• **Sh-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-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-ΔCpG* gene.

• **Term:** The *E. coli rpmB/G* terminator allows efficient transcription termination of the *Sh-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 H₂O. 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:

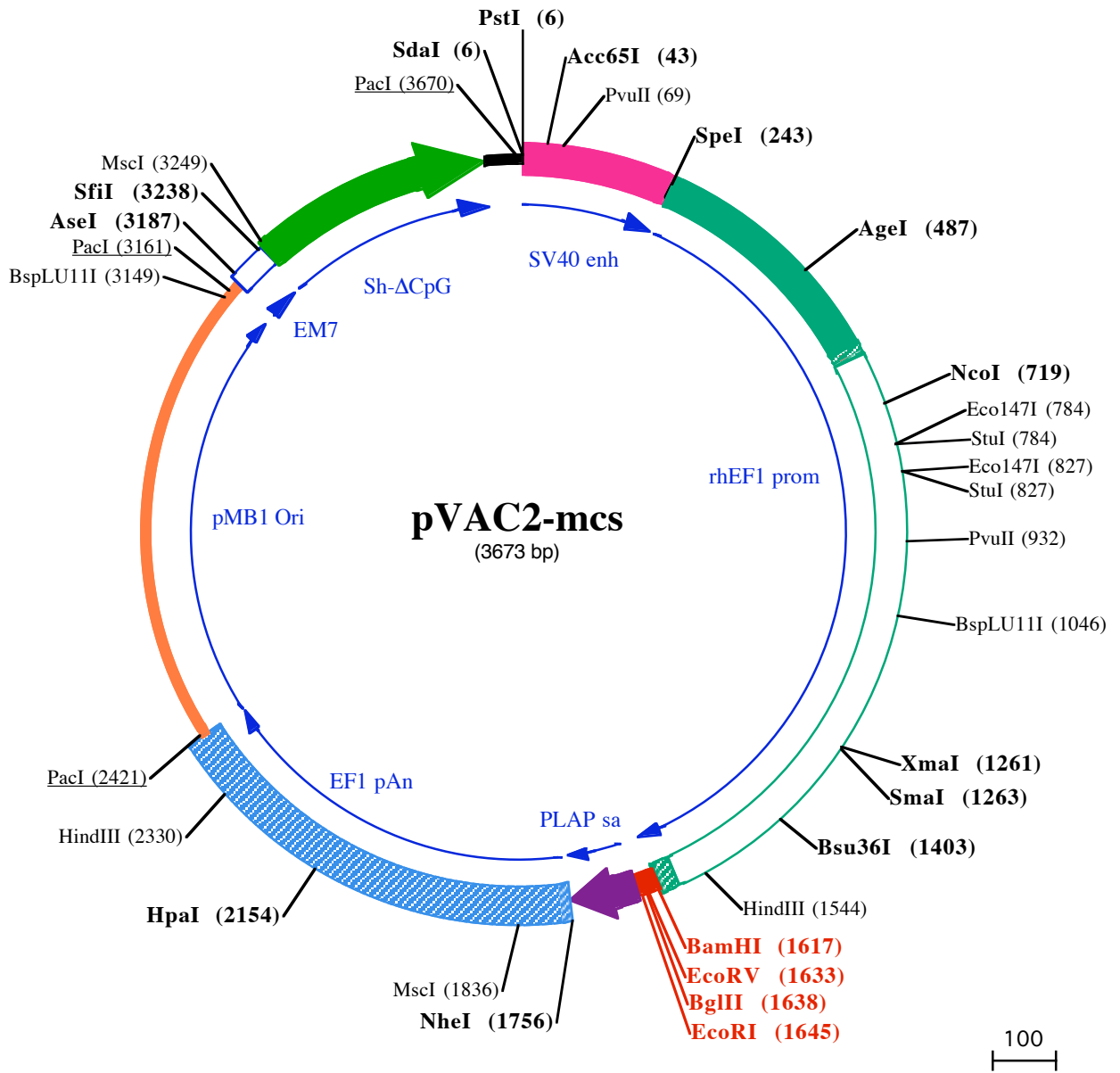
- 1- Corr, M. *et al.* 1999. J. Immunol. 163:4721-7
- 2- Forns, X. *et al.* 1999. Vaccine 17:1992-2002
- 3- McCluskie, M.J. *et al.* 1999. Mol. Med. 5:287-300

TECHNICAL SUPPORT

Toll free (US): 888-457-5873
Outside US: (+1) 858-457-5873
Europe: +33 562-71-69-39
E-mail: info@invivogen.com
Website: www.invivogen.com



3950 Sorrento Valley Blvd. Suite 100
San Diego, CA 92121 - USA



PstI (6)
SdaI (6)

Acc65I (43)

PvuII (69)

1 CCTGCAGGGCCCTGAAATAACCTCTGAAAGAGGAACCTGGTTAGGTACCTTCTGAGCGGAAAGAACACAGCTGTGGAATGTGTGTCAGTTAGGGTGTGGAA

101 AGTCCCAGGCTCCCAGCAGGAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGAAAGTCCCAGGCTCCCAGCAGGAGAAG

SpeI (243)

201 TATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCACTAGTGGAGCGGAGAGTAATTCATACAAAAGGACTGCCCCTGCCTTGGGGAATCCC

301 AGGGACCGCTGTTAAACTCCCACTAACCTAGAACCCAGAGATCGTGCCTCCGCCCTCACACGCCGCTCTCGTCATACCAAGGTGGAGAAGAGC

AgeI (487)

401 ATGCGTGAGGCTCCGGTGCCTGCAGTGGCAGAGCGACATCGCCACAGTCCCGAGAAGTTGGGGGAGGGGTGGCAATTGAACCGTGCCTAGAG

501 AAGGTGGCGCGGGTAAACTGGGAAAGTATGTCGTGACTGGCTCGCCCTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGTGTG

601 AACGTTCTTTTCGCAACGGGTTTCCCGCAGAACACAGGtaagtactgtgtgtggtcctcgcgggcctggcctctttacgggctatggccctcgcgtgc

NeoI (719)

StuI (784)
Eco147I (784)

701 cttttattacttacacgccatggcgcgtgtactgttcttgatcccagcctcgggttggagtggtgggagaggtcgagcccttgaccttaaggag

StuI (827)
Eco147I (827)

801 tcccttcgcctcgtgcttgagtcgagcctggcctgggctcgggctgcccgcgtgcaatctggtagcaccttcgcgcctgccccgctgctttcactaa

PvuII (932)

901 gtttctagccatttaaaatTTTTgatgaccagctgcaacgcctTTTTcttgccgagataatcttataaatgcgagaccaggatctgcacactgatattggg

BspLU11I (1046)

1001 gttttggggccgcccggctcgcacgggctcgtgctcccagcgcacatgttcggcgagggcctgagcgcggccaccgagagtcggagcggggggg

1101 agtctcaagctggccctcctgctggtgcccggcctcgcgcgggtgtgctgcccgcctggctggcaagcctggcccggctggcaccagttgctgtg

XmaI (1261)
SmaI (1263)

1201 agcggaaagatggccgcttcccggcctgcccagggagctcaaaatggaggaagcggcgcccgggagagcgggagggtagtcacccacacaaaggaaa

1301 agggcctttccctcctcgtgctgcttcatgtgacccacggagtagccggcgcctcaggcacctcgattagttctccgagcttttggagtagctct

Bsu36I (1403)

1401 tccttaggtttgggggaggggtttgtgctggtggagtttccccacacttggtgggtggagactgaagagtttagccagcttggcgtcgtatgaattctc

HindIII (1544)

1501 cttggaatttgccttttccaatttggatcctggcctattctcaagcttcagacagtggttcaaaagtttttttctcccatttcagGTGTCGTGAAACT

BglII (1638)

BamHI (1617) **EcoRV (1633)** **EcoRI (1645)**

1601 ACCCTAAAAGCCATcGGATCCAGAGCTCAGATATCCAGATCTTGAATTCACCACTGATGCTGCCATCCTGGAAGGTCTGTGGTGCCTGCCTTGTGCC

1701 TCTGCTGGCTGGCACTCTGCTGCTGCTGGAGACTGCCACTGCTCCCTAAACTGAGCTAGCATTATCCCTAATACCTGCCACCCCACTCTTAATCAGTGG

NheI (1756)

1701 17> oLeuLeuAl aGl yThr LeuLeuLeuLeuGl uThr Al aThr Al aP ro•••

MscI (1836)

1801 TGGAAGAAGCGTCTCAGAACTGTTTGTTCATTGCCATTAAAGTTTAGTGTAGTAAAAGACTGGTAAATGATAACAATGCATCGTAAAACCTCAGAAGG

1901 AAAGGAGAATGTTTTGTGGACCACTTTGGTTTTCTTTTTGCGTGTGGCAGTTTTAAGTTATTAGTTTTTAAATCAGTACTTTTTAATGAAACAACCTT

2001 GACCAAAAATTTGTACAGAATTTGAGACCCATTA AAAAAGTAAATGAGAAACCTGTGTGTTCTTTGGTCAACACCGAGACATTTAGGTGAAAGACA

HpaI (2154)

2101 TCTAATTCTGGTTTACGAATCTGAAACTTCTTGAAATGTAATTCTTGAGTTAACACTTCTGGGTGGAGAATAGGGTTGTTTTCCCCCACATAATTG

2201 GAAGGGGAAGGAATATCATTAAAGCTATGGGAGGTTCTTTGATTACAACACTGGAGAGAAATGCAGCATGTTGCTGATTGCCTGCTACTAAAACAGG

HindIII (2330)

2301 CCAAAAACCTGAGTCTTGGTTGCATAGAAAGCTTCATGTTGCTAAACCAATGTTAAGTGAATCTTTGAAACAAAATGTTTCAAATTACTGGGATGTG

PaeI (2421)

2401 CATGTTGAAACGTGGTTAATTAACTAGCCATGACCAAAATCCCTTAACGTGAGTTTTCTGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGAT

2501 CTTCTTGAGATCCTTTTTTCTGCGGTAATCTGCTGCTTGC AAAACAAAAAACACCGCTACCAGCGGTGGTTTGTGTCGGATCAAGAGCTACCAAC

2601 TCTTTTTCCGAAGGTAAGTGGCTT CAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCA

2701 CCGCTACATACCTCGCTCTGCTAATCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCTGTCTTACC GGTTGGACTCAAGACGATAGTTACC GG

2801 ATAAGGCGCAGCGGTGCGGCTGAACGGGGGTTCTGTGCACACAGCCAGCTTGAGCGAACGACCTACACGAACTGAGATACCTACAGCGTGAGCTATG

2901 AGAAAGCGCCACGCTTCCCGAAGGGAGAAAGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAC

3001 GCCTGGTATCTTTATAGCTGTGCGGTTTCCGCCACTCTGACTTGAGCGTCGATTTTTGTGATGCTGCTCAGGGGGCGGAGCTATGAAAAACGCCA

PacI (3161)

BspLU11I (3149)

AseI (3187)

3101 GCAACGCGCCCTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTAATTAATTTTCAAAGTAGTTGACAATTAATCATCGGCAT

MscI (3249)

SfiI (3238)

3201 AGTATATCGGCATAGTATAATACGACTCACTATAGGAGGCCATCATGGCCAAGTTGACCAGTGTCTCCAGTGTCCAGTGTCCAGCCAGGGATGTGGCTGGAG

1▶MetAl aLysLeuThr SerAl aVal ProVal LeuThrAl aArgAspValAl aGlyA

3301 CTGTTGAGTTCTGGACTGACAGGTTGGGGTTCTCCAGAGATTTGTGGAGGATGACTTTGCAGGTGTGGTCAGAGATGATGCACCTGTTTCATCTCAGC

19▶I aVal Gl uPheTrpThrAspArgLeuGlyPheSerArgAspPheVal Gl uAspAspPheAl aGlyVal Val ArgAspAspVal ThrLeuPheI leSerAl

3401 AGTCCAGGACCAGTGGTGCCTGACAACCCCTGGCTTGGGTGGGTGAGAGGACTGGATGAGCTGTATGCTGAGTGGAGTGGGTGGTCTCCACCAAC

52▶aVal Gl nAspGlnVal Val ProAspAsnThrLeuAl aTrpVal TrpVal ArgGlyLeuAspGluLeuTyrAl aGluTrpSer Gl uVal Val Ser ThrAsn

3501 TTCAGGGATGCCAGTGGCCCTGCCATGACAGAGATTGGAGAGCAGCCCTGGGGGAGAGAGTTTGCCCTGAGAGACCAGCAGGCAACTGTGTGCACTTTG

86▶PheArgAspAl aSer Gl yProAl aMetThr Gl ul leGlyGluGlnProTrpGlyArgGluPheAl aLeuArgAspProAl aGlyAsnCysVal HisPheV

PacI (3670)

3601 TGGCAGAGGAGCAGGACTGAGGATAAGAATTGTAACAAAAACCCCGCCCGGGGTTTTTTGTTAATTA

119▶aAl aGluGluGlnAsp•••