

pBOOST3-mcs

Negative control plasmid for the pBOOST3 vaccine adjuvant plasmid

Catalog # pbst3-mcs

For research use only

Version 20K16-MM

PRODUCT INFORMATION

Content:

- 20 µg of lyophilized pBOOST3-mcs plasmid
- 1 ml of Zeocin™ (100 mg/ml)

Shipping and storage:

Products are shipped at room temperature.

Lyophilized DNA is stable for 12 months when stored at -20°C. Resuspended DNA is stable for 6 months when stored at -20°C. Avoid repeated freeze-thaw cycles.

Store Zeocin™ at 4 °C or at -20 °C. The expiry date is specified on the product label.

Quality control:

Plasmid construct has been confirmed by restriction analysis and sequencing.

Plasmid DNA was purified by ion exchange chromatography and lyophilized.

GENERAL PRODUCT USE

The pBOOST3-mcs plasmid is a negative control plasmid for use with pBOOST3-mTBK1. The pBOOST3-mTBK1 plasmid was developed as a genetic adjuvant for DNA vaccines to potentiate the immune response to a specific antigen. The pBOOST3-mTBK1 plasmid contains the mouse TANK-binding kinase 1 (mTBK1) gene. TBK1, a non-canonical IκB kinase, was shown to mediate the adjuvant effect of DNA vaccines¹. Administration of DNA vaccines induces the production of type I interferons and inflammatory cytokines in a CpG-independent manner but in TBK1-dependent manner¹.

The method of plasmid DNA vaccine delivery is known to bias the immune response to a specific antigen towards a type 1 (T-cell) response². A DNA vaccine incorporated with genetic adjuvant such as the MyD88 or the TRIF gene has been shown to enhance immune responses³. As TBK1 has been shown to play a crucial role in humoral responses, coadministration of a TBK1-expressing plasmid is expected to further boost DNA vaccine-induced immunogenicity.

PLASMID FEATURES

- **hEF1 / HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter⁴ and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat⁵. The EF-1α promoter exhibits a strong activity and yields long lasting expression of a transgene *in vivo*. The R-U5' has been coupled to the EF-1α core promoter to enhance stability of RNA.
- **SV40 pAn:** The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA.

- **Ori pMB1** is a minimal *E. coli* origin of replication with the same activity as the longer Ori.
- **EM2KC** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- **Sh ble** : The *Sh ble* gene from *Streptoalloteichus hindustanus* encodes a small protein that confers resistance to Zeocin™ by binding to the antibiotic.

References:

1. Ishii KJ. *et al.*, 2008. TANK-binding kinase-1 delineates innate and adaptive immune responses to DNA vaccines. *Nature*. 451:725-729.
2. Robinson HL., 1999. DNA vaccines: basic mechanism and immune responses (Review). *Int J Mol Med*. 4(5):549-55.
3. Takeshita F. *et al.*, 2006. Toll-like receptor adaptor molecules enhance DNA-raised adaptive immune responses against influenza and tumors through activation of innate immunity. *J. Virol*. 80:6218-6224.
4. Kim, D.W. *et al.*, 1990. Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. *Gene* 2: 217-223.
5. Takebe, Y. *et al.*, 1988. R alpha promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. *Mol. Cell Biol*. 1: 466-472.

METHODS

Plasmid resuspension

Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1 µg/µl, resuspend the DNA in 20 µl of sterile H₂O. Store resuspended plasmid at -20 °C.

Plasmid amplification and cloning

Plasmid amplification and cloning can be performed in *E. coli* GT116 or in other commonly used laboratory *E. coli* strains, such as DH5α.

Zeocin™ usage

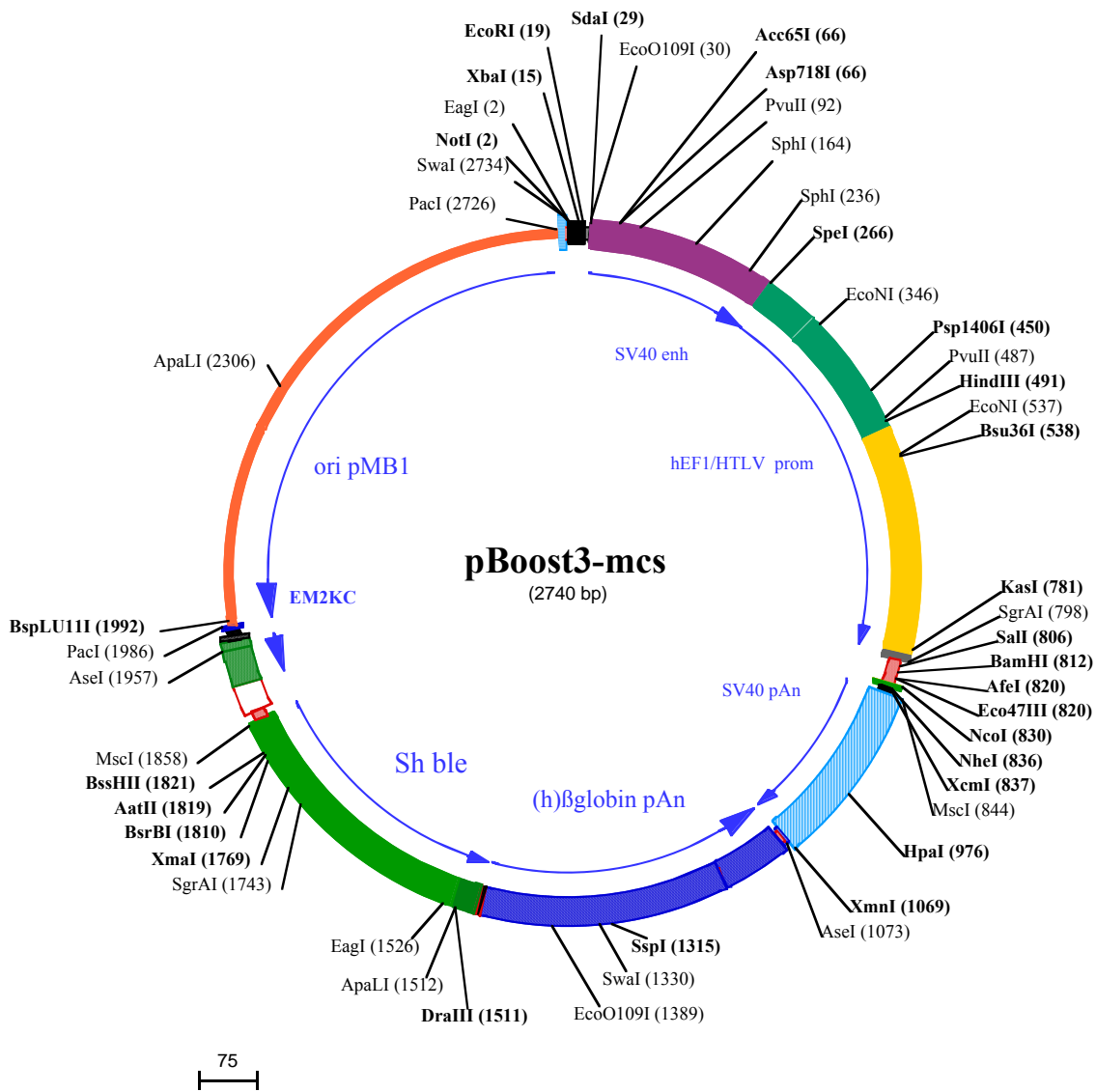
This antibiotic can be used for *E. coli* at 25 µg/ml in liquid or solid media and at 50-200 µg/ml to select Zeocin™-resistant mammalian cells.

TECHNICAL SUPPORT

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EagI (2) EcoRI (19) EcoO109I (30) Asp718I (66) PvuII (92)
 NotI (2) XbaI (15) SdaI (29) Acc65I (66)

1 GCGGCCCTATGCATCTAGAAATTCCTGCAGGGCCTGAAATAACCTCTGAAAGAGGAACTTGGTTAGGTACCTTCTGAGGCGAAAGAAACCAGCTGTGGAA

SphI (164)

101 TGTGTGTCAGTTAGGGTGTGAAAAGTCCCAGGCTCCCAGCAGGCAGAAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGAAAAGTCC

SphI (236) SpeI (266)

201 CCAGGCTCCCAGCAGGCAGAAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGTATGCCACTAGTCAAGTGGGCAGAGCGCACATCGCCACAGT

EcoNI (346)

301 CCCCAGAAAGTTGGGGGGAGGGTTCGGCAATTGAACGGTGCCTAGAGAAGTGGCGCGGGTAAACTGGGAAAAGTGTATGTCGTGTACTGGCTCCGCCTT

Psp1406I (450) PvuII (487) HindIII (491)

401 TTTCCCAGGGTGGGGAGAAACCGTATATAAGTGCAGTAGTCGCGGTGAACGTTCTTTTCGCAACGGTTCGCGCCAGAACACAGCTGAAGCTTCGAG

Bsu36I (538) EcoNI (537)

501 GGGCTCGCATCTCTCCTTCCACGCGCCGCCCTACCCTGAGGCGCCATCCACGCGGTGGAGTCGCGTTCCTGCCGCCCTCCCGCTGTGGTCCCTCCG

601 AACTGCGTCGCGCTCTAGTTAAGTAAAGCTCAGGTCGAGACCGGGCTTTTGTCCGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACG

KasI (781) SgrAI (798)

701 CTTTGCCTGACCTGGCTTGTCTCAACTCTACGTCCTTTTGGTTCGTTCCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCTACCTGAGATCAcc

Eco47III (820) NheI (836) BamHI (812) XcmI (837) SmaI (806) AfeI (820) NcoI (830) MscI (844)

801 ggcgtgtcgacggatccagcgcctctgcagCATGGCTAGCTGGCCAGCATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAAATGCAGTG

HpaI (976)

901 AAAAAATGCTTTATTGTGAAATTTGTGATGCTATTTGCTTTATTGTAACCAATTATAAGCTGCAATAAACAAGTTAACACACAACTGCAATTCATTTT

AseI (1073) XmnI (1069)

1001 ATGTTTACAGGTTACAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAAITTAATCTAAAATACAGCATAGCAAAAC

1101 TTTAACCTCCAAATCAAGCCTCTACTGTAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGTGTGTGCCAATGTGCATTAGCTGTTTGCAGC

1201 CTCACCTCTTTTCATGGAGTTTAAGATATAGTGTATTTTCCCAAGTTTGAAGTACTCTTTTCATTTCTTTATGTTTTAAATGCACTGACCTCCACATTC

SspI (1315) SmaI (1330) EcoO109I (1389)

1301 CCTTTTATGTAATAATTCAGAAATATTAAATACATCATTGCAATGAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAA

1401 TATCCCCAGTTTATAGTGGACTTAGGGAAACAAAGAACCTTAAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCCTGTCTCC

ApaLI (1512) DraIII (1511) EagI (1526)

1501 TCTGCCACAAAGTGCAAGCATGTCCGCGCGGGTTCGCGGAGGGGAACTCCGCCCCACCGCTGCTCGCGATCTCGGTGTCATGGCGCGCGGAGGCGT

120 ▶ E A V F H V C N G A P D R L A F E R G W P Q E G I E T M A P G S A D

1601 CCCGGAAGTTCGTTGACACGACCTCCGACCACTCGCGCTACAGCTCGTCCAGGCGCGCACCCACACCAGGCCAGGGTGTGTCGGGCACCACTGGTC

87 ▶ R F N T S V V E S W E A Y L E D L G R V W V W A L T N D P V V Q D

SgrAI (1743) XmaI (1769)

1701 CTGGACCGCTGATGAACAGGGTCACGTCGTCCCGACCACACCGCGAAGTCGTCCTCCACGAAGTCCCGGAGAACCCGAGCCGGTCCGTCAGAGAAC

54 ▶ Q V A S I F L T V D D R V V G A F D D E V F D R S F G L R D T W F

AatII (1819) BsrBI (1810) BssHIII (1821) MscI (1858)

1801 TCGACCGCTCCGGCGAGCTCGCGCGGGTGTGAGCACCGGAACCGCACTGGTCAACTGGCCATGATGGCTCCTCctgtcaggagaggaagagaagaaggt

20 ▶ E V A G A V D R A T L V P V A S T L K A M

AseI (1957) PacI (1986) BspLU11I (1992)

1901 tagtacaatctgCTATAGTGTGATTATACTATGCAGATATACTATGCAATGATTAAATTGTCAAACTAGGGCTGAGGTAAATTAAGACATGTGAG

2001 CAAAAGGCCAGCAAAGGCCAGGAACCGTAAAAGGCCGGTGTGCGGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCAAAAATCGACGCTC

2101 AAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCTGGAAGCTCCCTCGTGGCTCTCCTGTTCCGACCTCGCGCTTACC

2201 GGATACCTGTCCGCTTTCTCCTCCTCGGGAAGCGTGGCGCTTCTCATAGCTCAGCTGTAGGTATCTCAGTTCGGTGTAGGTGCTTCGCTCCAAGCTGG

ApaLI (2306)

2301 GCTGTGTGACGAACCCCCTTCAGCCCGACCGCTGCGCTTATCCGGTAACTATCGTCTTGAGTCCAACCGGTAAAGACAGCACTTATCGCCACTGGC

2401 AGCAGCCACTGTTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCCTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTA

2501 TTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTG

2601 TTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAAACGAAAATCACGTTA

PacI (2726) SwaI (2734)

2701 AGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCA
