pBOOST3-mTBK1

New DNA vaccine adjuvant of the pBOOST3 plasmids expressing the mouse TBK1 gene

Catalog # pbst3-mtbk1

For research use only

Version 20K16-MM

PRODUCT INFORMATION

Content:

• 20 µg of lyophilized pBOOST3-mTBK1 plasmid expressing the mouse TBK1 gene

• 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 12 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 plasmid was developed as a genetic adjuvant for DNA vaccines to potentiate the immune response to a specific antigen. The plasmid contains the mouse TANK-binding kinase 1 (mTBK1) gene. TBK1, a non-canonical IkB kinase, was shown to mediate the adjuvant effect of DNA vaccines[1]. Administration of DNA vaccines induces the production of type I interferons and inflammatory cytokines in a CpG-independent manner but in TBK1-dependent manner[1].

The method of plasmid DNA vaccine delivery is known to bias the immune response to a specific antigen towards a type 1 (T-cell) response[2]. A DNA vaccine incorporated with genetic adjuvant such as the MyD88 or the TRIF gene has been shown to enhance immune responses[3]. 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

• **mTBK1**

TBK1 signaling is thought to be critical for optimal humoral response as well as for helper T (Th)1 cytokine production after DNA vaccination[1].

• **hEF1 / HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter[4] and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat[5]. 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 H_2O. 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

InvivoGen USA (Toll-Free): 888-457-5873

InvivoGen USA (International): +1 (858) 457-5873

InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Hong Kong: +852 3622-3480

E-mail: info@invivogen.com

Intramuscular inoculation

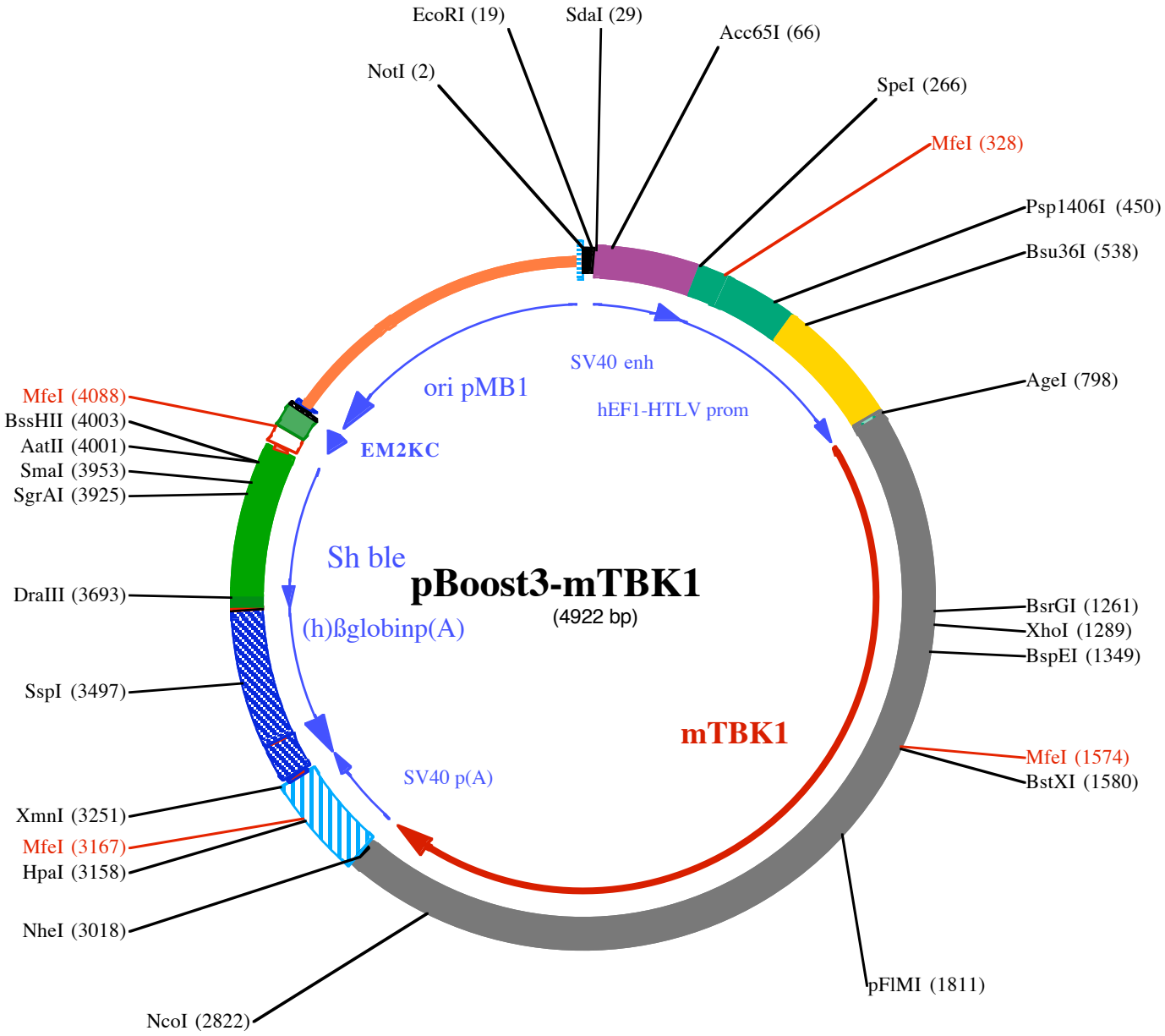
Plasmid DNA solution

- Prepare the vaccine plasmid solution by resuspending 10 µg of the vaccine plasmid DNA in 50 µl saline solution.
- Prepare the pBOOST3 solution by mixing 10 µg of pBOOST3-mTBK1 and 90 µg of the mock plasmid pBOOST3-null in 50 µl saline solution for low dose, or 100 µg of pBOOST3-mTBK1 in 50 µl saline solution for high dose.
- Combine both solutions to obtain a total of 110 µg DNA in 100 µl saline solution.

Note: The quantities are per mouse.

Intramuscular injections

- Inoculate 6 to 8-week old female BALB/c mice with 100 µl plasmid DNA solution (described above) into the quadriceps at 0 and 4 weeks.
- Collect sera and analyze for antibodies at 8 weeks.


EcoRI (19)
SdaI (29)
Acc65I (66)
NotI (2)
SpeI (266)
MfeI (328)
Psp1406I (450)
Bsu36I (538)
AgeI (798)
SV40 enh
ori pMB1
hEF1-HTLV prom
MfeI (4088)
BssHII (4003)
AatII (4001)
SmaI (3953)
SgrAI (3925)
EM2KC
Sh ble
pBoost3-mTBK1
(4922 bp)
DraIII (3693)
(h)ßglobinp(A)
BsrGI (1261)
XhoI (1289)
BspEI (1349)
SspI (3497)
mTBK1
MfeI (1574)
BstXI (1580)
SV40 p(A)
XmnI (3251)
MfeI (3167)
HpaI (3158)
NheI (3018)
pFlMI (1811)
NcoI (2822)

InvivoGen


SdaI (29)
NotI (2)
EcoRI (19)
Acc65I (66)
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SpeI (266)
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Psp1406I (450)
GCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGG
Bsu36I (538)
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AgeI (798)
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GlyMetAsnHisLeuArgGluAsnGlyIleValHisArgAspIleLysProGlyAsnIleMetArgValIleGlyGlu
BsrGI (1261)
XhoI (1289)
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BstXI (1580)
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NcoI (2822)
NheI (3018)
HpaI (3158)
XmnI (3251)
SspI (3497)
DraIII (3693)
SgrAI (3925)
SmaI (3953)
BssHII (4003)
AatII (4001)


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