pBOOST2-sahIRF3

New DNA vaccine adjuvant of the pVAC plasmids expressing a super-activated IRF3 gene

Catalog # pbst2-sahirf3

For research use only

Version 20K16-MM

PRODUCT INFORMATION

Content:

• 20 µg of lyophilized pBOOST2-sahIRF3 plasmid expressing a human super-activated IRF3 gene

• 1 ml of Zeocin™ (100 mg/ml)

Shipping and storage:

Products are shipped at room temperature.

Lyophilized DNA should be resuspended upon receipt and stored at -20°C. Lyophilized DNA is stable 12 months at -20°C. Resuspended DNA is stable more than one year 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

pBOOST2 plasmids were developed as genetic adjuvants for DNA vaccines to potentiate the immune response to a specific antigen. They feature different genes from the interferon regulatory factor family (IRF). IRFs are transcriptional activators for IFN-α, IFN-ß and IFN-stimulated genes. In particular IRF-1, IRF-3 and IRF-7 act as direct transducers of virus-mediated signaling pathways activating IFN-α and IFN-ß in infected cells. Recently, IRF-1, IRF-3 and IRF-7 were shown to be able to bias T cells towards type 1 or type 2 immune responses, leading to the activation of cytotoxic T cells and/or the production of antibodies. The method of plasmid DNA vaccine delivery is known to bias the immune response to a specific antigen towards a type 1 (T-cell) or type 2 (antibody) response[1]. These biases can be further enhanced by the codelivery of IRFs to increase the efficacy of the vaccination[2, 3].

PLASMID FEATURES

• **sahIRF3** (super-activated human interferon regulatory factor 3)
IRF-3 primarily increases Th1 T-cell responses[2]. A constitutively active form of IRF-3 was generated by creating a single point mutation of Ser^{396} to Asp. This super-activated IRF-3 presents a >10-fold enhanced transactivating potential over the wild-type IRF-3 for the IFN-α and IFN-ß promoters[4].

• **hEF1 / HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter[5] 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[6]. 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** is a minimal *E. coli* origin of replication 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-ΔCpG* (Synthetic Zeocin^r gene):** The *Sh ble* gene from *Streptoalloteichus hindustanus* encodes a small protein that confers resistance to Zeocin™ by binding to the antibiotic. To reduce the amount of CpG motifs that may skew the raised antigen-specific immune response, pBOOST2 contains a CpG-free allele of the Zeo^R gene. All CpGs from the wild-type gene (50) were removed by synthesizing a new allele that contains no CpGs but encodes the exact same protein sequence.

References:

1. Robinson HL., 1999. DNA vaccines: basic mechanism and immune responses (Review).Int J Mol Med. 4(5):549-55.
2. Sasaki S. *et al*., 2002. Regulation of DNA-raised immune responses by cotransfected interferon regulatory factors. J Virol.76(13):6652-9.
3. Bramson JL. *et al*., 2003. Super-activated interferon-regulatory factors can enhance plasmid immunization. Vaccine. 21(13-14):1363-70.
4. Servant MJ. *et al*., 2003. Identification of the minimal phosphoacceptor site required for in vivo activation of interferon regulatory factor 3 in response to virus and double-stranded RNA. J Biol Chem. 278(11):9441-7.
5. 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.
6. 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
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Intramuscular inoculation

Plasmid DNA solution

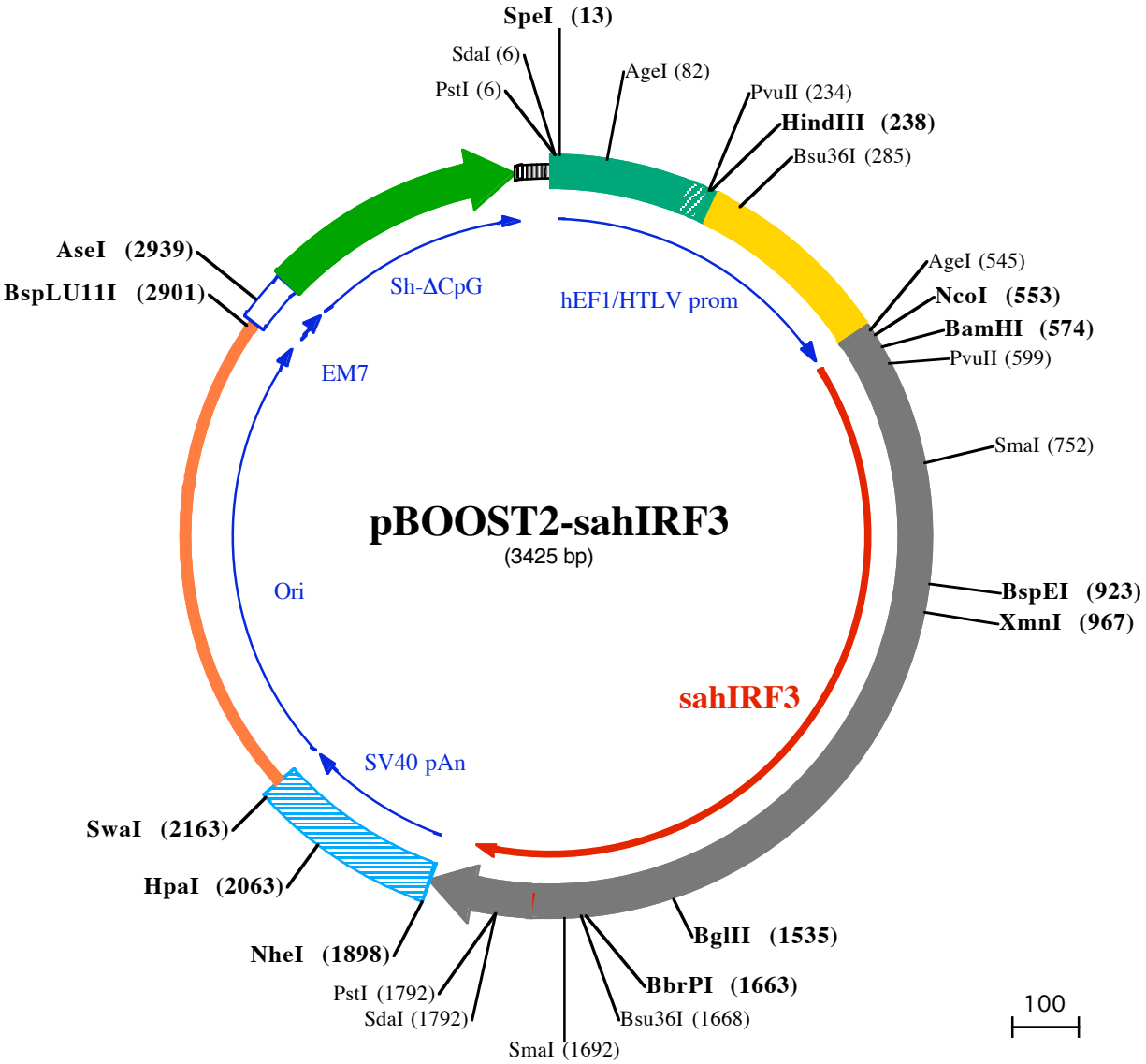
- Prepare the vaccine plasmid solution by resuspending 10 µg of the vaccine plasmid DNA in 50 µl saline solution.
- Prepare the pBOOST2 solution by mixing 10 µg of pBOOST2-sahIRF3 and 90 µg of the mock plasmid pBOOST2-null in 50 µl saline solution for low dose, or 100 µg of pBOOST2-sahIRF3 in 50 µl saline 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.

Note: For more information see the article by Sasaki S. et al.[1]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SdaI (6)
PstI (6)
AgeI (82)
PvuII (234)
HindIII (238)
Bsu36I (285)
AseI (2939)
BspLU11I (2901)
Sh-ΔCpG
hEF1/HTLV prom
AgeI (545)
NcoI (553)
BamHI (574)
PvuII (599)
EM7
SmaI (752)
pBOOST2-sahIRF3
(3425 bp)
Ori
BspEI (923)
XmnI (967)
sahIRF3
SV40 pAn
SwaI (2163)
HpaI (2063)
NheI (1898)
BglII (1535)
PstI (1792)
SdaI (1792)
BbrPI (1663)
Bsu36I (1668)
SmaI (1692)
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InvivoGen


PstI (6)
SdaI (6)
SpeI (13)
AgeI (82)
HindIII (238)
PvuII (234)
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