

# 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- $\alpha$ , IFN- $\beta$  and IFN-stimulated genes. In particular IRF-1, IRF-3 and IRF-7 act as direct transducers of virus-mediated signaling pathways activating IFN- $\alpha$  and IFN- $\beta$  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<sup>1</sup>. These biases can be further enhanced by the codelivery of IRFs to increase the efficacy of the vaccination<sup>2,3</sup>.

## PLASMID FEATURES

- **sahIRF3** (super-activated human interferon regulatory factor 3) IRF-3 primarily increases Th1 T-cell responses<sup>2</sup>. A constitutively active form of IRF-3 was generated by creating a single point mutation of Ser<sup>396</sup> to Asp. This super-activated IRF-3 presents a >10-fold enhanced transactivating potential over the wild-type IRF-3 for the IFN- $\alpha$  and IFN- $\beta$  promoters<sup>4</sup>.
- **hEF1 / HTLV prom** is a composite promoter comprising the Elongation Factor-1 $\alpha$  (EF-1 $\alpha$ ) core promoter<sup>5</sup> 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<sup>6</sup>. The EF-1 $\alpha$  promoter exhibits a strong activity and yields long lasting expression of a transgene *in vivo*. The R-U5' has been coupled to the EF-1 $\alpha$  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- $\Delta$ CpG (Synthetic Zeocin' 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<sup>R</sup> 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<sub>2</sub>O. 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 DH5 $\alpha$ .

### **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

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## **Intramuscular inoculation**

### Plasmid DNA solution

- Prepare the vaccine plasmid solution by resuspending 10  $\mu\text{g}$  of the vaccine plasmid DNA in 50  $\mu\text{l}$  saline solution.
- Prepare the pBOOST2 solution by mixing 10  $\mu\text{g}$  of pBOOST2-sahIRF3 and 90  $\mu\text{g}$  of the mock plasmid pBOOST2-null in 50  $\mu\text{l}$  saline solution for low dose, or 100  $\mu\text{g}$  of pBOOST2-sahIRF3 in 50  $\mu\text{l}$  saline solution for high dose.
- Combine both solutions to obtain a total of 110  $\mu\text{g}$  DNA in 100  $\mu\text{l}$  saline solution.

*Note: The quantities are per mouse.*

### Intramuscular injections

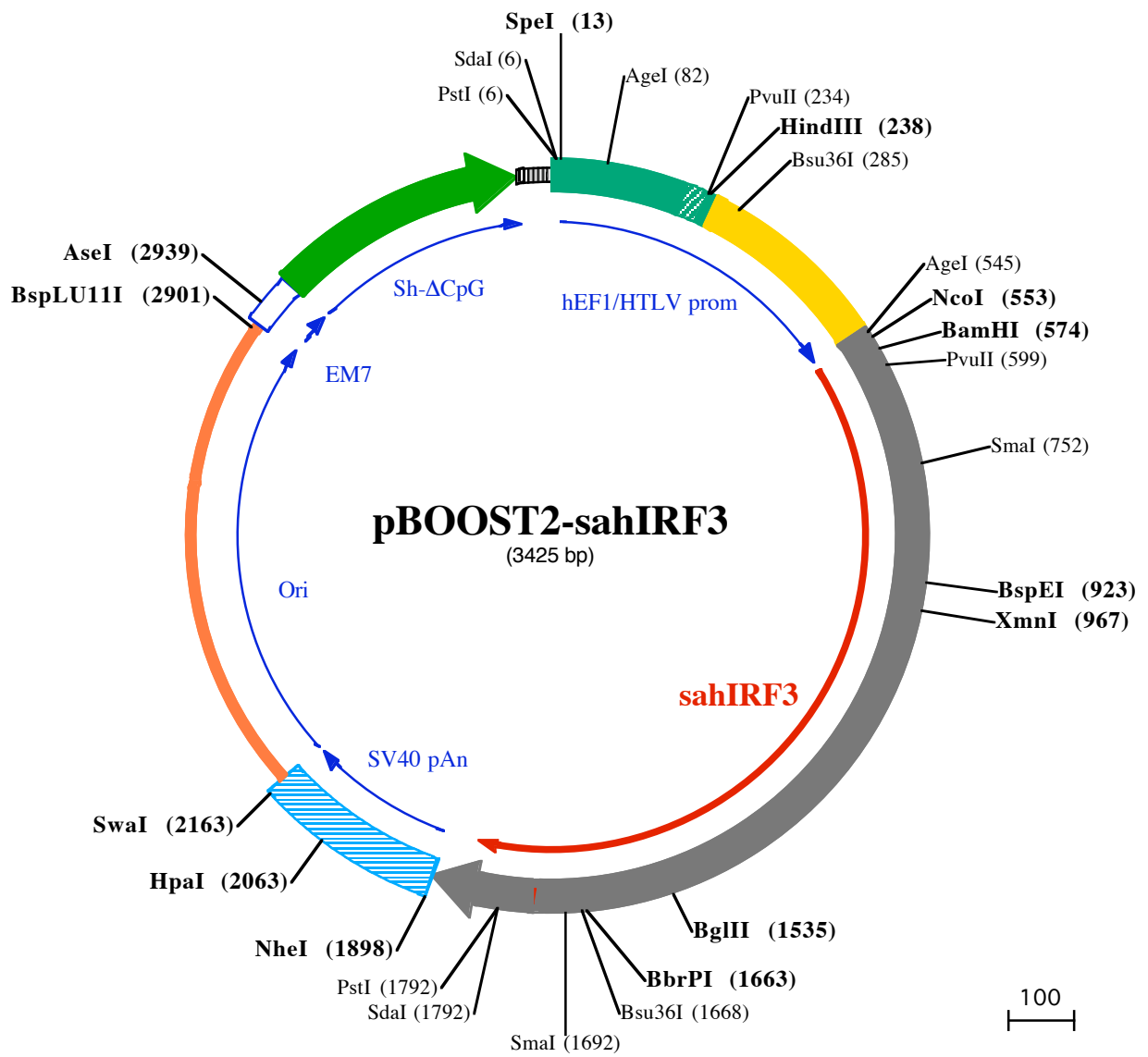
- Inoculate 6 to 8-week old female BALB/c mice with 100  $\mu\text{l}$  plasmid DNA solution (described above) into the quadriceps at 0 and 4 weeks.
- Collect sera and analyze for antibodies at 8 weeks.

*Note: For more information see the article by Sasaki S. et al.<sup>1</sup>*

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PstI (6) SdaI (6) SpeI (13) AgeI (82)  
 1 CCTGCAGGGCCCACTAGTCACTGAGGGGAGGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGT  
 101 GGGCGGGGTAACCTGGGAAAGTGATGTCGTGTACTGGCTCCGCCCTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCCCGTGAACGT

HindIII (238) PvuII (234) Bsu36I (285)  
 201 TCTTTTTCGAACGGGTTTCCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCTTCCACGCGCCGCGCCCTACCTGAGGCCCTACCA  
 301 CGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAAGTGCCTCCGCGCTAGGTAAGTTAAAGCTCAGGTCGAGACCGGGCCTTT  
 401 GTCCGGCGCTCCCTGGAGCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGTCTCAACTCTACGCTTTTGTTCGTTTTCTGTTCT

AgeI (545) NcoI (553) BamHI (574) PvuII (599)  
 501 GCGCCGTTACAGATCAAGCTGTGACCGGCCCTACCTGAGATCACCGGTCCACATGGGAACCCAAAGCCACGGATCTGCCTGGCTGGTGTGCGCAGC  
 601 TGGACCTGGGCAACTGGAGGGCGTGGCTGGGTGAACAAGAGCCGACGCGCTTCCGCATCCCTTGAAGCACGGCTACGGCAGGATGCACAGCAGGA  
 1601 euAspLeuGI yGI nLeuGI uGI yVal AI aTrpVal AsnLysSer ArgThr ArgPheArgI I eP roTrpLysHi sGI yLeuArgGI nAspAI aGI nGI nGI  
 701 GGATTTCCGAATCTCCAGGCTGGGCGGAGGCACTGGTGCATATGTTCCCGGAGGGATAAGCCAGACCTGCCAACCTGGAAGAGGAATTTCCGCTCT  
 490 uAspPheGI y I I ePheGI nAI aTrpAI aGI uAI aThr GI yAI aTyrVal I P roGI yA rgAspLysP roAspLeuP roThr TrpLysArgAsnPheArgSer  
 801 GCCCTCAACCCGAAAGAGGGTTGCGTTTAGCAGAGGACCGGAGCAAGGACCTCACGACCACATAAAATCTACGAGTTTGTGAAGTTCAGGAGTTGGGG  
 830 AI aLeuAsnArgLysGI uGI yLeuArgLeuAI aGI uAspArgSer LysAspP roHi sAspP roHi sLys I I eTyrGI uPheVal I AsnSer GI yVal I GI yA

BspEI (923) XmnI (967)  
 901 ACTTTTCCAGCCAGACACCTCTCCGACACCAATGGTGGAGGCACTTCTGATACCCAGGAAGACATTCTGGATGAGTACTGGGTAACATGGTGT  
 1160 spPheSer GI nP roAspThr Ser P roAspThr AsnGI yGI yGI ySer Thr Ser AspThr GI nGI uAspI I eLeuAspGI uLeuLeuGI yAsnMeT Val I Le  
 1001 GGCCCACTCCAGATCCGGACCCCAAGCCTGGCTGTAGCCCTGAGCCCTGCCCTCAGCCCTCGGGAGCCCAAGCTTGGACAATCCCACTCCCTTC  
 1490 uAI aP roLeuP roAspP roGI yP roP roSer LeuAI aVal AI aP roGI uP roCysP roGI nP roLeuArgSer P roSer LeuAspAsnP roThr P roP he  
 1101 CCAAACTGGGCGCTCTGAGAACCCTGAAGCGGCTGTGGTGGCGGGAAGAGTGGGAGTTCGAGTGACAGCCTCTACCGGGCCGCGCAAGT  
 1830 P roAsnLeuGI yP roSer GI uAsnP roLeuLysArgLeuLeuVal P roGI yGI uGI uTrpGI uPheGI uVal I Thr AI aP heTyrArgGI yA rgGI nVal I P  
 1201 TCCAGCAGACCATCTCTGCCGAGGGCCTGCCGCTGGTGGGGTCCGAAGTGGGAGACAGGACGCTGCCTGGATGGCCAGTCACTGCCAGACCCTGG  
 2160 heGI nGI nThr I I eSer CysP roGI uGI yLeuArgLeuVal I GI ySer GI uVal I GI yAspArgThr LeuP roGI yTrpP roVal I Thr LeuP roAspP roGI  
 1301 CATGTCCCTGACGACAGGGAGTGTAGTACGTGAGGCACTGTGCTGAGCTGGCTGGGTGGGGGACTGGCTCTCTGGCGGGCCGGGCAAGTGGCTGTGG  
 2490 yMeT SerLeuThr AspArgGI yVal I MeT Ser TyrVal I ArgHi sVal I LeuSer CysLeuGI yGI yGI yLeuAI aLeuTrpArgAI aGI yGI nTrpLeuTrp  
 1401 GCCCAGCGGCTGGGCACTGCCACATACTGGCAGTGGCAGGAGCTGCTCCCAACAGCGGGCATGGGCTGATGGCAGGTCCCAAGGACAAGG  
 2830 AI aGI nArgLeuGI yHi sCysHi sThr TyrTrpAI aVal I Ser GI uGI uLeuLeuP roAsnSer GI yHi sGI yP roAspGI yVal I P roLysAspGI yLysG

BglIII (1535) Bsu36I (1668)  
 1501 AAGGAGCGTGTGGTTCAGCTGGGCGCCTTATTGTAGTCTGATTACCTTACGGAAGGAAGCGGACGCTCACCACGCTATGCCCTCTGGTCTGTGTGGG  
 3160 I uGI yGI yVal I P heAspLeuGI yP roP heI I eVal I AspLeuI I eThr P heThr GI uGI ySer GI yA rgSer P roArgTyrAI aLeuTrpP heCysVal I GI

BbrPI (1663) SmaI (1692)  
 1601 GGAGTCATGGCCCAAGGACCGCTGGACCAAGAGGCTCGTGATGGTCAAGGTTGTGCCCAAGTGGCTCAGGGCCTGGTAGAATGGCCCGGGTAGGG  
 3490 yGI uSer TrpP roGI nAspGI nP roTrpThr LysArgLeuVal I MeT Val I LysVal I Val I P roThr CysLeuArgAI aLeuVal I GI uMeT AI aArgVal I GI y

PstI (1792) SdaI (1792)  
 1701 GGTGCTCCTCCCTGGAGAATACTGTGGACCTGCACATTGACCAAGCCACCCACTCTCCCTCACCTCCGACCAAGTACAAGGCTTACCTGCAGGACTTGG  
 3830 GI yAI aSer Ser LeuGI uAsnThr Val I AspLeuHi s I I eAspAsnSerHi sP roLeuSer LeuThr Ser AspGI nTyrLysAI aTyrLeuGI nAspLeuV

NheI (1898)  
 1801 TGGAGGCGATGGATTTCCAGGGCCCTGGGAGACCTGAGCCCTGCCTCATGGTGTCCCAACCCCGTGTCCCAACCACTCAACCAATAAGCT  
 4160 aI GI uGI yMeT AspPheGI nGI yP roGI yGI uThr ●●●  
 1901 AGCTCGACATGATAAGATACTTGTAGTTGGACAAACCACTAGAATGCAAGTGAAGAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTT

HpaI (2063)  
 2001 ATTTGTGAAATTTGTGATGCTATTGCTTTATTGTAACATTATAAGCTGCAATAAACAAGTTAAACAACAACAATTGCATTCTTTATGTTTCAGGTTCT

SmaI (2163)  
 2101 AGGGGAGGTGTGGAGGTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTAGATCCATTTAAATGTTAATTAAGTAACTAGCCATGACCAAAATCCCTTAA  
 2201 CGTGAGTTTTCTTCCACTGAGCGTCAGACCCGTAAGAAAGATCAAAGGATCTTCTTGTAGATCCTTTTTCTGCGCGTAATCTGCTGCTTGCAAAACA  
 2301 AAAAACCACCGCTACCAGCGGTGTTTTGTTGCCGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTCAGCAGAGCGCAGATACCAATAAC  
 2401 TGTTCTTAGTGTAGCCGTAGTTAGGCCACCACTCAAGAACTCTGTAGCACCGCTACATACTCGCTCTGCTAATCTGTTACCAGTGGCTGCTGCC  
 2501 AGTGGCATAAGTCTGTCTTACCAGGTTGGACTCAAGACGATAGTTACCAGGATAAGGCGCAGCGGTCCGGCTGAACGGGGGTTCTGTCACACAGCCCA  
 2601 GCTTGGAGCGAACGACTACCCGAAGTACCTACAGCGTGTAGCTATGAGAAAGCGCCACGCTCCCGAAGGGAGAAAGCGGACAGGTATCCGGT  
 2701 AAGCGGAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGAAACCGCTGGTATCTTTATAGTCTGTGCGGTTTTCCGACCTCTGACTTGAG  
 2801 CGTCGATTTTTGTGATGCTGCTCAGGGGGCGGAGCCTATGAAAAACGCCAGCAACGCGGCTTTTTACGGTCTCGGCTTTTGTGCGCTTTTGTCT

BspLU11I (2901) AseI (2939)  
 2901 ACATGTTCTTAATTAATTTTCAAAGTAGTTGACAATAATCATCGGCATAGTATATCGGCATAGTATAATACGACTCACTATAGGAGGGCCATCATG  
 3001 GCCAAGTTGACCACTGTCTCCAGTGTCCAGGCTCACAGCCAGGATGTGGCTGGAGCTGTTGAGTTCTGGACTGACAGGTTGGGTTCTCCAGAGATTTGTGG  
 200 AI aLysLeuThr Ser AI aVal I P roVal I LeuThr AI aArgAspVal I aGI yAI aVal I GI uP heTrpThr AspArgLeuGI yP heSer ArgAspP heVal I G  
 3101 AGGATGACTTTGACGTTGGTGTGAGGATGATGTACCCTGTTATCTCAGCAGTCCAGGACAGGTTGGTGCCTGACAACCCCTGGCTTGGGTGGTGGT  
 350 I uAspAspP heAI aGI yVal I Val I ArgAspAspVal I Thr LeuP heI I eSer AI aVal I GI nAspGI nVal I Val I P roAspAsnThr LeuAI aTrpVal I TrpVal  
 3201 GAGAGGACTGGATGAGTGTATGCTGAGTGGAGTGGTGTCTCCCAACTTCAGGATGCCAGTGGCCCTGCCATGACAGAGATTGGAGAGCAGCCC  
 680 I A rgGI yLeuAspGI uLeuTyrAI aGI uTrpSer GI uVal I Val I Ser Thr AsnP heArgAspAI aSer GI yP roAI aMeT Thr GI u I I eGI yGI uGI nP ro  
 3301 TGGGGAGAGATTTGCCCTGAGAGACCCAGCAGGCAACTGTGTGCACTTTGTGGCAGAGGAGCAGGACTGAGGATAAGAATTTGTAACAAAAACCCCGC  
 1020 TrpGI yA rgGI uP heAI aLeuArgAspP roAI aGI yAsnCysVal I Hi sP heVal AI aGI uGI uGI nAsp●●●  
 3401 CCCGGCGGGTTTTTTGTAATTA