

pBOOST2-wthIRF1

New DNA vaccine adjuvant of the pVAC plasmids expressing the wild-type IRF1 gene

Catalog # pbst2-wthirf1

For research use only

Version 20K16-MM

PRODUCT INFORMATION

Content:

- 20 µg of lyophilized pBOOST2-wthIRF1 plasmid expressing the human wild type IRF1 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

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-β 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-β 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) response¹. These biases can be further enhanced by the codelivery of IRFs to increase the efficacy of the vaccination^{2,3}.

PLASMID FEATURES

- **wthIRF1** (wild type human interferon regulatory factor 1) IRF-1 primarily increases Th2 antibody responses². Following intramuscular or gene gun injections of DNA vaccines, IRF-1 can increase the titers of antibodies up to 10-fold.
- **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** 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[®] 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[®] 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. 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

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 pBOOST2 solution by mixing 10 μg of pBOOST2-wthIRF1 and 90 μg of the mock plasmid pBOOST2-null in 50 μl saline solution for low dose, or 100 μg of pBOOST2-wthIRF1 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.

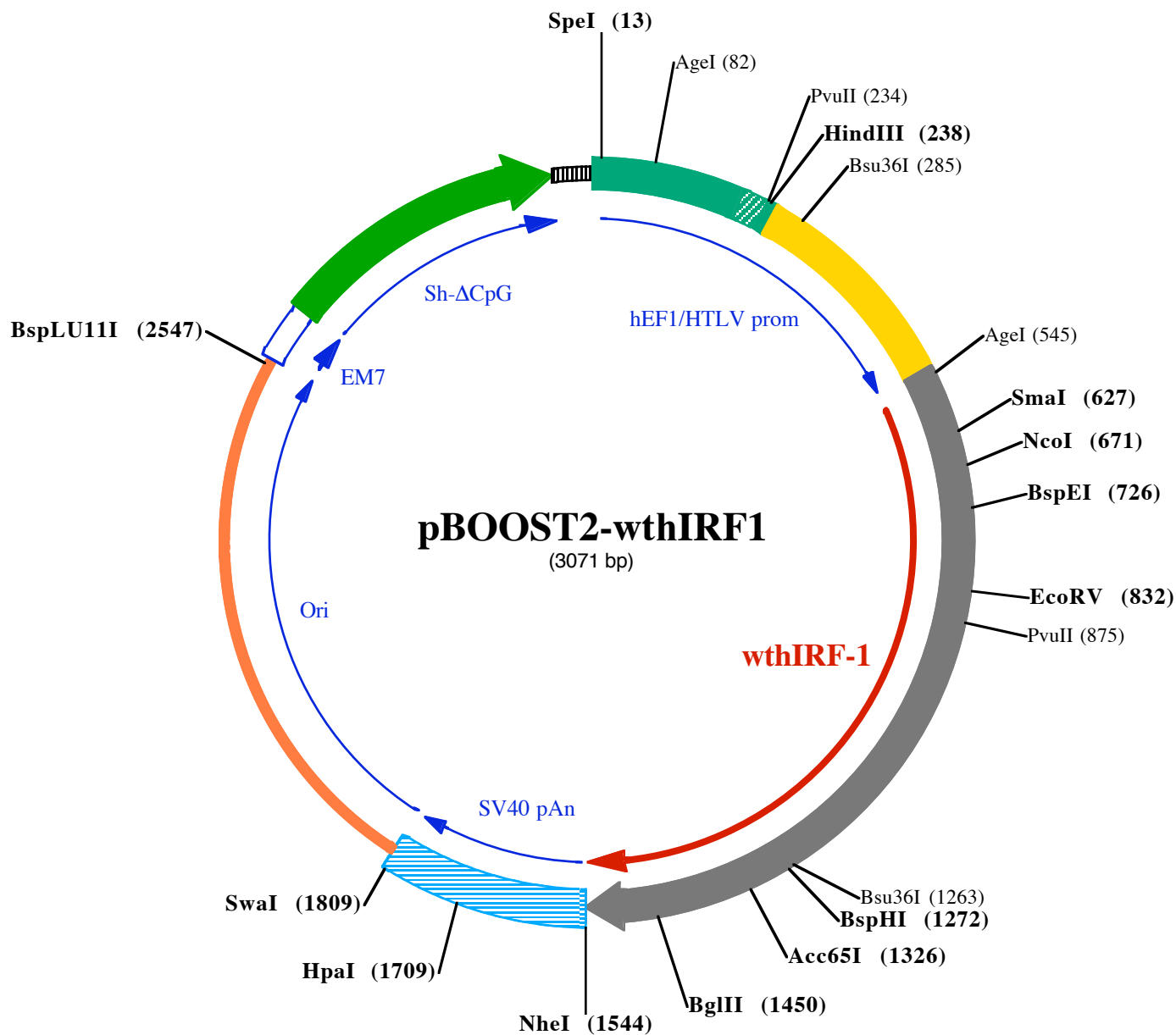
Intramuscular injections

- 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

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



85

1 CCTGCAGGGCCACTAGTCACTGGGCGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGT
AgeI (82)

101 GGCGCGGGTAAACTGGAAAGTGATGTCGTACTGGCTCCGCTTTTTCCGAGGGTGGGGGAAACCGTATATAAGTGCAGTAGTCGCCGTGAACGT

HindIII (238)

201 TCTTTTTGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCCTTACGCGCCCGCCGCTACCTGAGGCCCATCCA
PvuII (234) Bsu36I (285)

301 CGCCGGTTGAGTCGGCTTCTGCCGCTCCCGCTGTGGTGCCTCTGAAGTGCCTCCGCTCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCTTT
401 GTCCGGCTCCCTTGAGCTACCTAGACTCAGCCGGCTCCACGCTTGGCTGACCTGCTTGTCTCAACTCTACGCTTTGTTCTGTTTCTGTTCT

AgeI (545)

501 GCGCGTTACAGATCCAAGCTGTGACCGCGCTACCTGAGATCACCGTAGGAGGCCAGCATGCCATCACTCGGATGCGCATGAGACCTGGCTAGA
1MetProIleThrArgMetArgMetArgProTrpLeuGI

SmaI (627) NcoI (671)

601 GATGCGATTAATCCAACCAATCCCGGGCTCATCTGGATTAATAAGAGGAGATGATCTTCAGATCCATGGAAGCATGTCGCAAGCATGGCTGG
13uMeTGI nIeAsnSerAsnGI nIeP roGI yLeuI eTrpI l eAsnLysGI uGI uMeT l l ePheGI nI l eP roTrpLysHi sAl aAl aLysHi sGI yTrp

BspEI (726)

701 GACATCAACAAGGATGCCTGTTTCCGGAGCTGGCCATTACACAGGCCGATACAAAGCAGGGGAAAAGGAGCCAGATCCAAGACGTGAAAGGCCA
47AspI l eAsnLysAspAl aCysLeuPheArgSer TrpAl a l eHi sThr GI yArgTyrLysAl aGI yGI uLysGI uP roAsp roLysThr TrpLysAl aA

EcoRV (832) PvuII (875)

801 ACTTTCGCTGTGCCATGAACCTCCCTGCCAGATATCGAGGAGTGAAGACCAGAGCAGGAACAAGGCAGCTCAGCTGTGCGAGTGTACCGGATGCTTCC
80snPheArgCysAl aMe tAsnSer LeuProAspI l eGI uGI uVal LysAspGI nSer ArgAsnLysGI ySer Ser Al aVal A rgVal TyrArgMe tLeuP r
901 ACCTCTACCAAGAACCAGAGAAAAGAAAGTCAAGTCCAGCCGAGATGCTAAGAGCAAGGCCAAGGGAAGTTCATGTGGGATTCCAGCCCTGAT
113oP roLeuThr LysAsnGI nArgLysGI uArgLysSer LysSer Ser ArgAspAl aLysSer LysAl aLysArgLysSer CysGI yAspSer Ser P roAsp
1001 ACCTTCTGATGGACTCAGCAGCTCCACTTGCCTGATGACCACAGCAGCTACACAGTTCAGGCTACATGCAGGACTTGGAGGTGGAGCAGGCCCTGA
147Thr PheSerAspGI yLeuSer Ser Ser Thr LeuP roAspAspHi sSer Ser TyrThr Val P roGI yTyrMe tGI nAspLeuGI uVal GI uGI nAl aLeuT
1101 CTCCAGCACTGTCCCATGTGCTGTGACGACACTCTCCCGACTGGCACATCCAGTGAAGTTGTGCCGACAGCAGCTGATCTGTACAACCTCCA
180hr P roAl aLeuSer P roCysAl aVal Ser Ser Thr LeuP roAspTrpHi s l l eP roVal GI uVal Val P roAspSer Thr SerAspLeuTyrAsnPheGI

Bsu36I (1263) BspHI (1272)

1201 GGTGTCACCCATGCCTCCACCTCTGAAGCTACAACAGATGAGGATGAGGAAGGGAAATTACCTGAGGACATCATGAAGCTCTTGGAGCAGTCGGAGTGG
213nVal Ser P roMe tP roSer Thr Ser GI uAl aThr ThrAspGI uAspGI uGI uGI yLysLeuP roGI uAspI l eMe tLysLeuLeuGI uGI nSer GI uTrp

Acc65I (1326)

1301 CAGCAACAACCGTGGATGGAAAGGGTACCTACTCAATGAACCTGGAGTCCAGCCACCTCTGTCTATGGAGACTTTAGCTGTAAGGAGGACAGAAA
247GI nP roThrAsnVal AspGI yLysGI yTyrLeuLeuAsnGI uP roGI yVal GI nP roThr Ser Val TyrGI yAspPheSer CysLysGI uGI uP roGI uI

BglII (1450)

1401 TTGACAGCCAGGGGGATATTGGGCTGAGTCTACAGCGTCTTCCACAGATCTGAAGAACATGGATGCCACCTGGCTGGACAGCTGCTGACCCAGT
280l eAspSer P roGI yGI yAspI l eGI yLeuSer LeuGI nArgVal PheThrAspLeuLysAsnMe tAspAl aThr TrpLeuAspSer LeuLeuThr P roVa

NheI (1544)

1501 CCGTTGCCCTCCATCCAGGCCATTCCCTGTGCACCGTAGCAGGCTAGCTCGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGC
313l A rgLeuP roSer l l eGI nAl a l l eP roCysAl aP ro•••

1601 AGTGAATAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACATTATAAGTGAAT

HpaI (1709)

1701 AAACAAGTTAACAACAACAAATTGCATTCATTTTATGTTTCAGGTTACAGGGGAGGTGGGAGGTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTA

SwaI (1809)

1801 GATCCATTTAATGTTAATTAAGTACCATGACCAAAATCCCTAACGTGAGTTTTCTGTTCCACTGAGCGTCAGACCCGTAAGAAAGATCAAAGGATCT

1901 TCTTGAGATCCTTTTTTCTGCGGTAATCTGCTGCTTGCACAAAAAACCCGCTACCAGCGTGGTTTGTGTTGCCGGATCAAGAGCTACCAACTC

2001 TTTTCCGAAGGTAAGTGGCTTCCAGAGCGCAGATACCAACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCCTCAAGAAGTCTGTAGCACC

2101 GCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCTGTCTTACCAGGTTGGACTCAAGACGATAGTTACCAGGAT

2201 AAGGCGCAGCGGTGGGCTGAACGGGGGTTCTGTGCACACAGCCAGCTTGGAGCGAACGACTACCCGAAGTACCTACAGCGTGAGCTATGAG

2301 AAAGCGCCAGCTTCCGAAAGGAGAAAGCGGACAGGTATCCGGTAAGCGGCAGGGTGGAAAGAGAGCGCACGAGGGAGCTTCCAGGGGAAACGC

2401 CTGGTATCTTTATAGTCTGTGCGGTTTCCGACCTCTGACTTGAGCGTCGATTTTGTGATGCTCGTCAGGGGGCGGAGCTATGAAAAACGCCAGC

BspLU11I (2547)

2501 AACCGGCCTTTTTACGGTCTCGCCCTTTTGTGCGCTTTTGTCTACATGTTCTTAATTAATTTTTCAAAGTAGTTGACAATTAATCATCGGCATAG

2601 TATATCGGCATAGTATAATACGACTCACTATAGGAGGCCATCATGGCCAAGTTGACCAGTGTGTCCAGTGTCCAGTGTCCAGCCAGGGATGTGGCTGGAGCT
1MetAl aLysLeuThr Ser Al aVal P roVal l euThr Al aArgAspVal l Al aGI yAl a

2701 GTTGAAGTCTGGACTGACAGGTTGGGTTTCCAGAGATTTTGTGGAGGATGACTTTGCGAGTGTGGTCAGAGATGATGTCACCTGTTCATCTCAGCAG
20Val l GI uPheTrpThrAspArgLeuGI yPheSer A rgAspPheVal l GI uAspAspPheAl aGI yVal Val A rgAspAspVal l ThrLeuPhe l l eSer Al aV

2801 TCCAGGACCGTGGTGCCTGACAACCCCTGGCTTGGGTGGTGGAGGACTGGATGAGCTGTATGCTGAGTGGAGTGGGTTCCACCAACTT
53al GI nAspGI nVal Val P roAspAsnThr LeuAl aTrpVal l TrpVal l A rgGI yLeuAspGI uLeuTyrAl aGI uTrpSer GI uVal Val Ser ThrAsnPh

2901 CAGGGATGCCAGTGGCCCTGCCATGACAGAGATTGGAGAGCAGCCCTGGGGAGAGAGTTTGGCCTGAGAGACCCAGCAGGCAACTGTGCACTTTGTG
86eArgAspAl aSer GI yP roAl aMe tThr GI u l l eGI yGI uGI nP roTrpGI yA rgGI uPheAl aLeuArgAsp roAl aGI yAsnCysVal l Hi sPheVal

3001 GCAGAGGAGCAGGACTGAGGATAAGAATTTGAACAAAAACCCCGCCGCGGGGTTTTTGTAAATTA

120Al aGI uGI uGI nAsp•••