

pBOOST2-wtmIRF1

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

Catalog # pbst2-wtmirf1

For research use only

Version # 05B28-SV

PRODUCT INFORMATION

Content:

- 20 µg of lyophilized pBOOST2-wtmIRF1 plasmid expressing the mouse 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 mouse 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^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. 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

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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-wtmIRF1 and 90 μg of the mock plasmid pBOOST2-null in 50 μl saline solution for low dose, or 100 μg of pBOOST2-wtmIRF1 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.

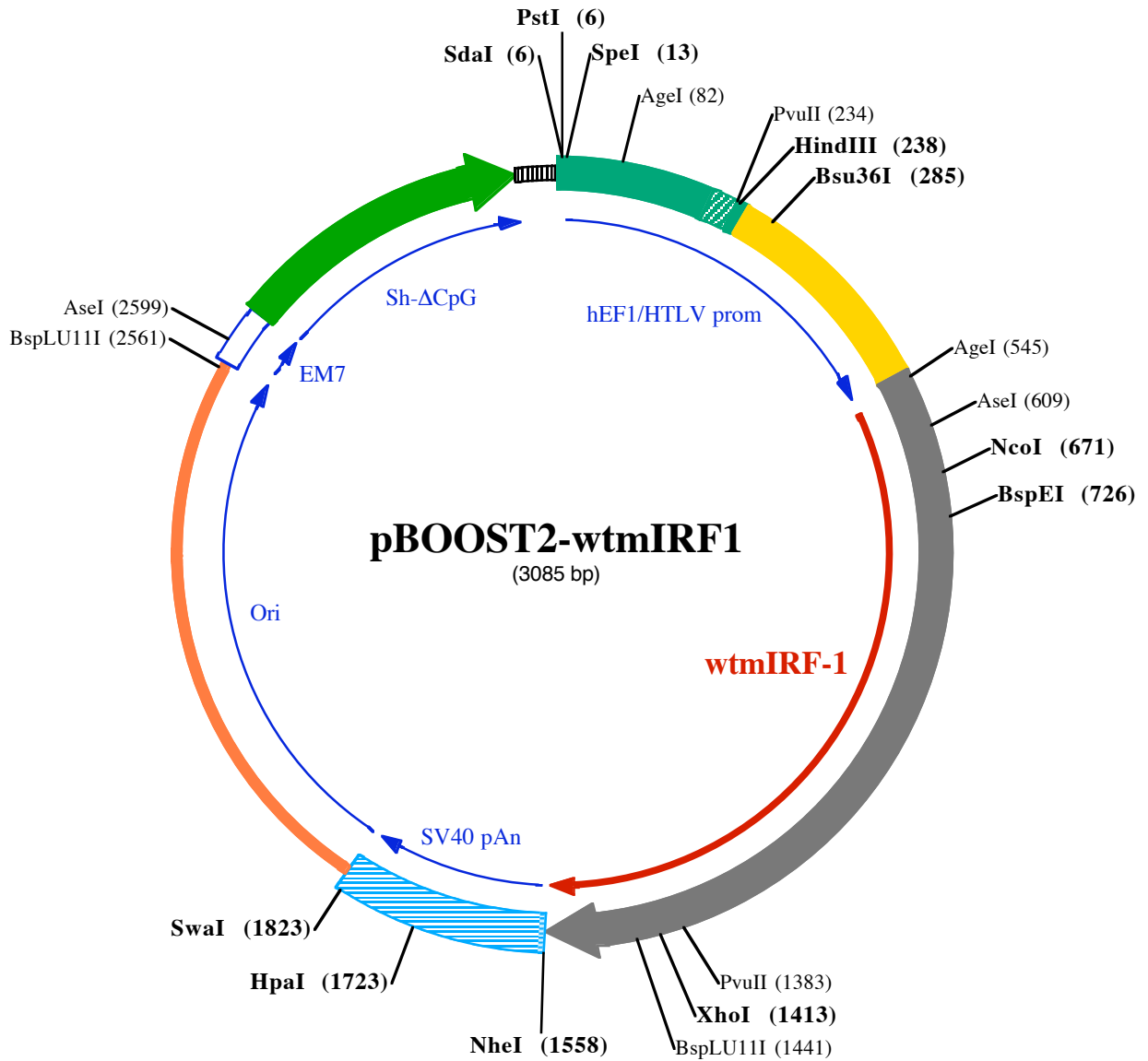
Intramuscular injections

- 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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PstI (6) **SdaI (6)** **SpeI (13)** **AgeI (82)**
1 CCTGCAGGGCCACTAGTCAGTGGGCGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGT
101 GGGCGGGGTAAACTGGAAAGTATGTCGTACTGGCTCCGCTTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTCAGTAGTCGCCGTGAACGT
HindIII (238) **PvuII (234)** **Bsu36I (285)**
201 TCTTTTTCGAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCCTACCTGAGGCCGCCATCCA
301 CGCCGGTTGAGTCGCGTTCTGCCGCTCCCGCTGTGGTGCTCTGAACTCGCTCCGCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTT
401 GTCCGGCGCTCCCTTGAGCCTACCTAGACTCAGCCGGCTCCACGCTTTGCTGACCTGCTTGTCTCAACTCTACGCTTTTGTGTTTTCTGTTCT
AgeI (545)
501 GCGCCGTTACAGATCCAAGCTGTGACCGCGCCCTACCTGAGATCACCGTAGGAGGGCCAGCATGCCAATCACTCGAATGCGGATGAGACCTGGCTAGA
1MetProIleThrArgMetArgMetArgProTrpLeuGI
AseI (609) **NcoI (671)**
601 GATGCAGATTAATTCACAAATCCCAGGGTGATCTGGATCAATAAAGAAGAGATGATCTCCAGATTCCATGGAAGACGCTGCTAAGCACGGCTGG
13MetGI nI eAsnSerAsnGI nI eProGI yLeuI eTrpI eAsnLysGI uGI uMetI ePheGI nI eProTrpLysHisAlaAlaLysHisGI yTrp
BspEI (726)
701 GACATCAACAAGGATGCTGTCTGTTCCGGAGCTGGGCCATTCACACAGGCCGATACAAAGCAGGAGAAAAAGAGCCAGATCCCAAGACATGGAAGGCAA
47AspIleAsnLysAspAlaCysLeuPheArgSerTrpAlaIleHisThrGlyArgTyrLysAlaGI yGI uLysGI uProAspProLysThrTrpLysAlaA
801 ACTTCGGTGTGCCATGAATCCCTGCCAGACATCGAGGAAGTGAAGGATCAGAGTAGGAACAAGGCCAGCTCTGCTGTGCGGGTGTACCGGATGCTGCC
80snPheArgCysAlaMetAsnSerLeuProAspIleGI uGI uValLysAspGI nSerArgAsnLysGI ySerSerAlaValArgValTyrArgMetLeuPr
901 ACCCTCACCAGGAACAGAGAAAGAGAGAAAGTCCAAGTCCAGCCGAGACACTAAGAGCAAAACCAAGAGGAAGCTGTGTGGAGATGTTAGCCCGGAC
113oProLeuThrArgAsnGI nArgLysGI uArgLysSerLysSerArgAspThrLysSerLysThrLysArgLysLeuCysGI yAspValSerProAsp
1001 ACTTCTCTGATGGACTCAGCAGCTCTACCCTACCTGATGACCACAGCAGTTACACCCTCAGGGCTACCTGGGTGAGACTTGGATATGGAAGGGACA
147ThrPheSerAspGI yLeuSerSerSerThrLeuProAspAspHisSerSerTyrThrThrGI nGI yTyrLeuGI yGI nAspLeuAspMetGI uArgAspI
1101 TAATCCAGCACTGTCCCGTGTGTCGTCAGCAGCAGTCTCTGAGTGGCATTATGCAGATGGACATTATACCAGATAGCACCCTGATCTGTATAACCT
180IeThrProAlaLeuSerProCysValValSerSerSerLeuSerGI uTrpHisMetGI nMetAspIleIleProAspSerThrThrAspLeuTyrAsnLe
1201 ACAGGTGTACCATGCCTCCACTCCGAAGCCGACAGACGAGGATGAGGAAGGAAGATAGCCGAAGACCTTATGAAGCTCTTTGAACAGTCTGAG
213uGI nValSerProMetProSerThrSerGI uAlaAlaThrAspGI uAspGI uGI uGI yLysIleAlaGI uAspLeuMetLysLeuPheGI uGI nSerGI u
PvuII (1383)
1301 TGGCAGCCGACACATCGATGGCAAGGGATACTTGTCTCAATGAGCCAGGGACCCAGCTCTCTTGTCTATGGAGACTTCAGCTGCAAAGAGGAACCGAG
247TrpGI nProThrHisIleAspGI yLysGI yTyrLeuLeuAsnGI uProGI yThrGI nLeuSerSerValTyrGI yAspPheSerCysLysGI uGI uProG
XhoI (1413) **BspLU111 (1441)**
1401 AGATTGACAGCCCTCGAGGGGACATTGGGATAGGCATACACATGTCTTCACGGAGATGAAGAATATGGACTCCATCATGTGGATGGACAGCCTGCTGGG
280IuIleAspSerProArgGI yAspIleGI yIleGI yIleGI nHisValPheThrGI uMetLysAsnMetAspSerIleMetTrpMetAspSerLeuLeuGI
NheI (1558)
1501 CAACTCTGTGAGGCTGCCGCCCTTATTAGCCATTCTTGTGACCATAGTTGGGCTAGCTGCAGATGATAAGATACATTGATGAGTTTGGACAAAC
313yAsnSerValArgLeuProProSerIleGI nAlaIleProCysAlaPro●●●
1601 CACAAC TAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCA
HpaI (1723)
1701 TTATAAGCTGCAATAAACAAGTTAAACAACAACAAATGCATTCATTTTATGTTTCAGGTTACAGGGGAGGTGTGGAGGTTTTTAAAGCAAGTAAACCT
SwaI (1823)
1801 CTACAAATGTGGTAGATCCATTTAAATGTTAATTAAGTACGCATGACCAAAATCCCTTAACGTGAGTTTTCTGTTCCACTGAGCGTCAGACCCGTAGAAA
1901 AGATCAAAGGATCTTTCTTGAGATCCTTTTTTCTGCGGTAATCTGCTGCTTGCACAAACAAAAAACACCGCTACCAGCGGTGGTTTTGTTGCGGGATCA
2001 AGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTCCAGCAGAGCGCAGATACCAAACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAG
2101 AACTCTGTAGCACCGCTACATACCTCGCTCTGCTAATCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGCTTACCAGGTTGGACTCAAGAC
2201 GATAGTTACCGGATAAGGCGCAGCGGTGCGGCTGAACGGGGGTTCTGTCACACAGCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACA
2301 GCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGACAGGTCGGAACAGGAGAGCGCACGAGGGAGCTT
2401 CCAGGGGAAACGCCTGATCTTTATAGTCTGTGCGGTTTTGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGCGGAGCCTAT
BspLU111 (2561) **AseI (2599)**
2501 GGAAAAACGCCAGCAACCGGCCCTTTTACGGTTCCTGGCCTTTTGTGGCCTTTTGTCTACATGTTCTTAATTAATTTTTCAAAGTAGTTGACAATT
2601 AATCATCGGCATAGTATATCGGCATAGTATAATACGACTCACTATAGGAGGGCCATCATGGCCAAGTTGACCAGTGTGTCCAGTGTCCACAGCCAGGG
1MetAlaLysLeuThrSerAlaValProValLeuThrAlaArgA
2701 ATGTGGCTGGAGCTGTTGAGTTCTGGACTGACAGGTTGGGGTCTCCAGAGATTTGTGGAGGATGACTTTGCAGGTGTGGTCAGAGATGATGTCCCT
15spValAlaGI yAlaValGI uPheTrpThrAspArgLeuGI yPheSerArgAspPheValGI uAspAspPheAlaGI yValValArgAspAspValThrLe
2801 GTTCATCTCAGCAGTCCAGGACCGAGGTTGCTGCAACACCCTGGCTGGGTGGGTGAGAGGACTGGATGACTGTATGCTGAGTGGAGTGGGTG
48uPheIleSerAlaValGI nAspGI nValValProAspAsnThrLeuAlaTrpValTrpValArgGI yLeuAspGI uLeuTyrAlaGI uTrpSerGI uVal
2901 GTCTCCACCAACTTCAGGGATGCCAGTGGCCCTGCCATGACAGAGATTGGAGAGCAGCCCTGGGGGAGAGATTTGCCCTGAGAGACCCAGCAGGCAACT
82ValSerThrAsnPheArgAspAlaSerGI yProAlaMetThrGI uIleGI yGI uGI nProTrpGI yArgGI uPheAlaLeuArgAspProAlaGI yAsnC
3001 GTGTGACTTTGTGGCAGAGGAGCAGGATGAGGATAAGAATTTAACAAAAACCCCGCCCGGGGTTTTTGTTAATTA
115ysValHisPheValAlaGI uGI uGI nAsp●●●