pNiFty2-IFB-SEAP

Inducible reporter plasmid feature mouse IFNβ promoter

Catalog code: pnf2-ifbsp

For research use only

Version 20L03-MM

PRODUCT INFORMATION

Content:

• 20 µg of pNiFty2-IFB-SEAP provided as lyophilized DNA.

• 1 ml of Zeocin™ (100 mg/ml)

Storage and stability:

• Product is shipped at room temperature.

• Lyophilized DNA should be stored at -20˚C.

• Resuspended DNA should be stored at -20˚C and is stable up to 1 year.

• 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.

GENERAL PRODUCT USE

Interferons are key modulators of the immune response. Their pleiotropic activities are mediated by the induction of many IFN-stimulated genes (ISGs). To help study the transcriptional regulation and signal transduction of type I IFNs, InvivoGen provides several reporter systems, called **pNiFty2**, based on the inducible expression of the secreted embryonic alkaline phophatase (SEAP) gene. The SEAP gene is cloned under the control of three different promoters that are activated by various transcription factors, such as IRF3, IRF5, IRF7 and NF-κB.

pNiFty2-IFB-SEAP features the mouse IFNβ promoter.

PLASMID FEATURES

• **mIFNβ prom:** The mouse interferon beta minimal promoter comprises several positive regulatory domains (PRDs) that bind different cooperating transcription factors such as NF-κB, IRF3 and IRF7[1]. Co-expression of IFNβ-SEAP with constitutively activated IRF3 (saIRF3) or IRF7 (saIRF7) in HEK293 cells led to a strong increase in SEAP expression.

• **5U-140** is a synthetic 5'UTR containing an intron.

• **SEAP** is a secreted form of human embryonic alkaline phosphatase. Unlike endogenous alkaline phosphatases, SEAP is extremely heat stable and resistant to the inhibitor L-homoarginine. It catalyses the hydrolysis of pNitrophenyl phosphate (pNpp) producing a yellow end product. SEAP expression can be readily quantified by collecting samples of culture medium and measuring the hydrolysis of pNpp with a spectrophotometer at 405 nm.

• **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

• **hEF1/HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter[2] 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[3]. 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.

• **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.

• **Zeo:** Resistance to the antibiotic Zeocin™ is conferred by the *Sh ble* gene from *Streptoalloteichus hindustanus*. The *Sh ble* gene is driven by the hEF1-HTLV promoter in tandem with the bacterial EM7 promoter allowing selection in both mammalian cells and *E. coli*.

• **βGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription[4].

1. Vodjdani G. *et al*. 1998. Structure and characterization of a murine chromosomal fragment containing the interferon beta gene. J Mol Biol. 204(2):221-31. **2. Kim DW. *et al.*, 1990.** Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. Gene 91(2):217-23. **3. Takebe Y. *et al.*, 1988.** SR 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. **4. Yu J & Russell JE. 2001.** Structural and functional analysis of an mRNP complex that mediates the high stability of human beta-globin mRNA. Mol Cell Biol, 21(17):5879-88.

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.

RELATED PRODUCTS

Product	Catalog Code
pUNO-sahIRF3	puno-sahirf3
pUNO-sahIRF7Δ	puno-sahirf7d
pNiFty2-IFA-SEAP	pnf2-ifasp
pNiFty2-56K-SEAP	pnf2-56ksp
Zeocin™	ant-zn

TECHNICAL SUPPORT

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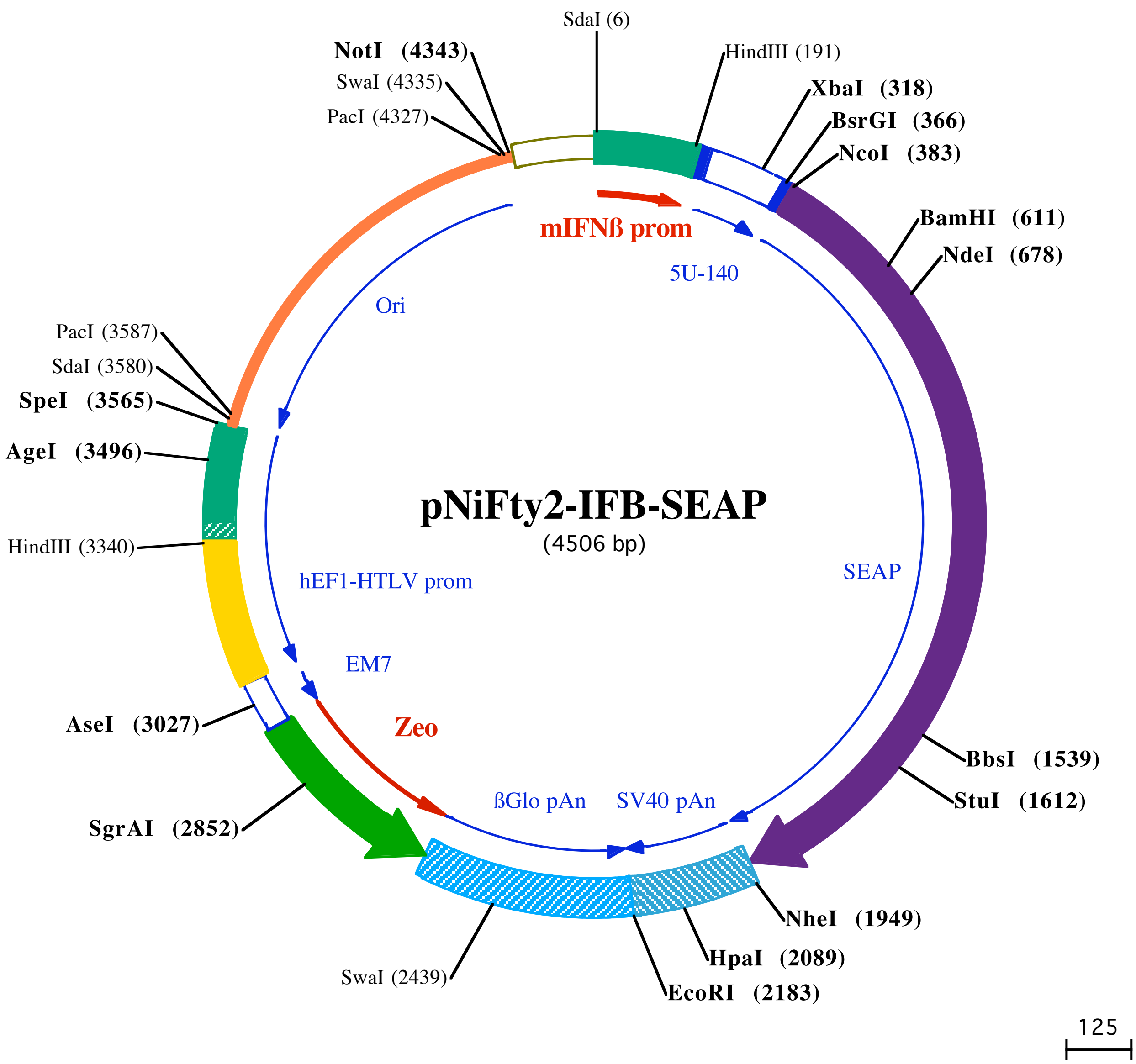
InvivoGen USA (International): +1 (858) 457-5873

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

InvivoGen Hong Kong : +852 3-622-34-80

E-mail: info@invivogen.com



pNiFty2-IFB-SEAP
(4506 bp)
SdaI (6)
HindIII (191)
XbaI (318)
BsrGI (366)
NcoI (383)
BamHI (611)
NdeI (678)
BbsI (1539)
StuI (1612)
NheI (1949)
HpaI (2089)
EcoRI (2183)
SwaI (2439)
SgrAI (2852)
AseI (3027)
HindIII (3340)
AgeI (3496)
SpeI (3565)
SdaI (3580)
PacI (3587)
PacI (4327)
SwaI (4335)
NotI (4343)
mIFNß prom
5U-140
SEAP
SV40 pAn
ßGlo pAn
Zeo
EM7
hEF1-HTLV prom
Ori
125

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     SdaI (6)
   1 CCTGCAGGAGCTTGAATAAAATGAATATTAGAAGCTGTTAGAATAAGAGAAAATGACAGAGGAAAACTGAAAGGGAGAACTGAAAGTGGGAAATTCCTCT

                                                                                          HindIII (191)
 101 GAGGCAGAAAGGACCATCCCTTATAAATAGCACAGGCCATGAAGGAAGATCATTCTCACTGCAGCCTTTGACAGCCTTTGCCTCATCTTGAAGCTTCTGC

 201 CTTCTCCCTCCTGTGAGTTTGgtaagtcactgactgtctatgcctgggaaagggtgggcaggagatggggcagtgcaggaaaagtggcactatgaacccT

                  XbaI (318)                                               BsrGI (366)       NcoI (383)
 301 GCAGCCCTAGGAATGCATCTAGAcaattgtactaaccttcttctctttcctctcctgacagGTTGGTGTACAGTAGCTTCCACCATGGTTCTGGGGCCCT
                                                                                             1▶MetValLeuGlyProC
 401 GCATGCTGCTGCTGCTGCTGCTGCTGGGCCTGAGGCTACAGCTCTCCCTGGGCATCATCCCAGTTGAGGAGGAGAACCCGGACTTCTGGAACCGCGAGGC
   6▶ysMetLeuLeuLeuLeuLeuLeuLeuGlyLeuArgLeuGlnLeuSerLeuGlyIleIleProValGluGluGluAsnProAspPheTrpAsnArgGluAl
 501 AGCCGAGGCCCTGGGTGCCGCCAAGAAGCTGCAGCCTGCACAGACAGCCGCCAAGAACCTCATCATCTTCCTGGGCGATGGGATGGGGGTGTCTACGGTG
  39▶aAlaGluAlaLeuGlyAlaAlaLysLysLeuGlnProAlaGlnThrAlaAlaLysAsnLeuIleIlePheLeuGlyAspGlyMetGlyValSerThrVal
              BamHI (611)                                                          NdeI (678)
 601 ACAGCTGCCAGGATCCTAAAAGGGCAGAAGAAGGACAAACTGGGGCCTGAGATACCCCTGGCTATGGACCGCTTCCCATATGTGGCTCTGTCCAAGACAT
  73▶ThrAlaAlaArgIleLeuLysGlyGlnLysLysAspLysLeuGlyProGluIleProLeuAlaMetAspArgPheProTyrValAlaLeuSerLysThrT
 701 ACAATGTAGACAAACATGTGCCAGACAGTGGAGCCACAGCCACGGCCTACCTGTGCGGGGTCAAGGGCAACTTCCAGACCATTGGCTTGAGTGCAGCCGC
 106▶yrAsnValAspLysHisValProAspSerGlyAlaThrAlaThrAlaTyrLeuCysGlyValLysGlyAsnPheGlnThrIleGlyLeuSerAlaAlaAl
 801 CCGCTTTAACCAGTGCAACACGACACGCGGCAACGAGGTCATCTCCGTGATGAATCGGGCCAAGAAAGCAGGGAAGTCAGTGGGAGTGGTAACCACCACA
 139▶aArgPheAsnGlnCysAsnThrThrArgGlyAsnGluValIleSerValMetAsnArgAlaLysLysAlaGlyLysSerValGlyValValThrThrThr
 901 CGAGTGCAGCACGCCTCGCCAGCCGGCACCTACGCCCACACGGTGAACCGCAACTGGTACTCGGACGCCGACGTGCCTGCCTCGGCCCGCCAGGAGGGGT
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1101 GTACCCAGATGACTACAGCCAAGGTGGGACCAGGCTGGACGGGAAGAATCTGGTGCAGGAATGGCTGGCGAAGCGCCAGGGTGCCCGGTATGTGTGGAAC
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1301 CACTGGACCCCTCCCTGATGGAGATGACAGAGGCTGCCCTGCGCCTGCTGAGCAGGAACCCCCGCGGCTTCTTCCTCTTCGTGGAGGGTGGTCGCATCGA
 306▶hrLeuAspProSerLeuMetGluMetThrGluAlaAlaLeuArgLeuLeuSerArgAsnProArgGlyPhePheLeuPheValGluGlyGlyArgIleAs
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                                      BbsI (1539)
1501 ACGCTGAGCCTCGTCACTGCCGACCACTCCCACGTCTTCTCCTTCGGAGGCTACCCCCTGCGAGGGAGCTCCATCTTCGGGCTGGCCCCTGGCAAGGCCC
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             StuI (1612)
1601 GGGACAGGAAGGCCTACACGGTCCTCCTATACGGAAACGGTCCAGGCTATGTGCTCAAGGACGGCGCCCGGCCGGATGTTACCGAGAGCGAGAGCGGGAG
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 473▶HisGlyValGlnGluGlnThrPheIleAlaHisValMetAlaPheAlaAlaCysLeuGluProTyrThrAlaCysAspLeuAlaProProAlaGlyThrT
                                                       NheI (1949)
1901 CCGACGCCGCGCACCCGGGGCGGTCCCGGTCCAAGCGTCTGGATTGAAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAA
 506▶hrAspAlaAlaHisProGlyArgSerArgSerLysArgLeuAsp•••
                                                                                           HpaI (2089)
2001 CTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAA

                                                                                       EcoRI (2183)
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2201 TAGCAAAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCT

2301 GTTTGCAGCCTCACCTTCTTTCATGGAGTTTAAGATATAGTGTATTTTCCCAAGGTTTGAACTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCT

                                        SwaI (2439)
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2501 CCTTCATAATATCCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAG
                                                                                           127◀•••Gly•••A
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2701 CGGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACACCCAGGCCAGGGTGTTGTCCGGCAC
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                                                             SgrAI (2852)
2801 CACCTGGTCCTGGACCGCGCTGATGAACAGGGTCACGTCGTCCCGGACCACACCGGCGAAGTCGTCCTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCG
  57◀ValGlnAspGlnValAlaSerIlePheLeuThrValAspAspArgValValGlyAlaPheAspGluValPheAspArgSerPheGlyLeuArgAspT
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  23◀hrTrpPheGluValAlaGlyAlaValAspArgAlaThrLeuValProValAlaSerThrLeuLysAlaMet
                         AseI (3027)
3001 ACTATGCCGATATACTATGCCGATGATTAATTGTCAACTACTGTTTGTAGGCGCCGGTCACAGCTTGGATCTGTAACGGCGCAGAACAGAAAACGAAACA

3101 AAGACGTAGAGTTGAGCAAGCAGGGTCAGGCAAAGCGTGGAGAGCCGGCTGAGTCTAGGTAGGCTCCAAGGGAGCGCCGGACAAAGGCCCGGTCTCGACC

3201 TGAGCTTTAAACTTACCTAGACGGCGGACGCAGTTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGGCGGCCTCAGGT

                                           HindIII (3340)
3301 AGGGCGGCGGGCGCGTGAAGGAGAGATGCGAGCCCCTCGAAGCTTCAGCTGTGTTCTGGCGGCAAACCCGTTGCGAAAAAGAACGTTCACGGCGACTACT
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AgeI (3496)

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SpeI (3565) SdaI (3580) PacI (3587)

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3801 CGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTG

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4001 CAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGT

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4201 GTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTT

PacI (4327) SwaI (4335) **NotI (4343)**

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4401 TCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACAAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCT
4501 ATCGAA