

pNiFty2-IFA-SEAP

Inducible reporter plasmid selectable with Zeocin™

Catalog code: pnf2-ifasp

For research use only

Version 20L03-MM

PRODUCT INFORMATION

Contents:

- 20 µg of pNiFty2-IFA-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 for 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 full-length ORF sequencing.
- Plasmid DNA was purified by ion exchange chromatography.

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 phosphatase (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-IFA-SEAP features the mouse IFNα promoter.

PLASMID FEATURES

- **mIFNα prom** is the mouse interferon alpha 4 minimal promoter¹. Transcription of mIFNα4 is mediated by a virus responsive element (VRE-A4) located in the promoter. VRE-A4 contains four cooperating DNA modules that bind to IRF3 and IRF7². Co-expression of IFNα-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**
- **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.
- **hEF1/HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter³ and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat⁴. 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 Zeocin™ 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 CMV enhancer/promoter in tandem with the bacterial EM7 promoter allowing selection in both mammalian cells and *E. coli*.
- **BGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription⁵.
- **pMB1 Ori** is a minimal *E. coli* origin of replication with the same activity as the longer Ori.

1. Braganca J. et al., 1997. Synergism between multiple virus-induced factor-binding elements involved in the differential expression of interferon A genes. *J Biol Chem.* 272(35):22154-62. **2. Morin P. et al., 2002.** Preferential binding sites for interferon regulatory factors 3 and 7 involved in interferon-A gene transcription. *J Mol Biol.* 316(5):1009-22. **3. 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. **4. Takebe Y. et al., 1988.** SR alphapromoter: 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.* 8(1):466-72. **5. 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

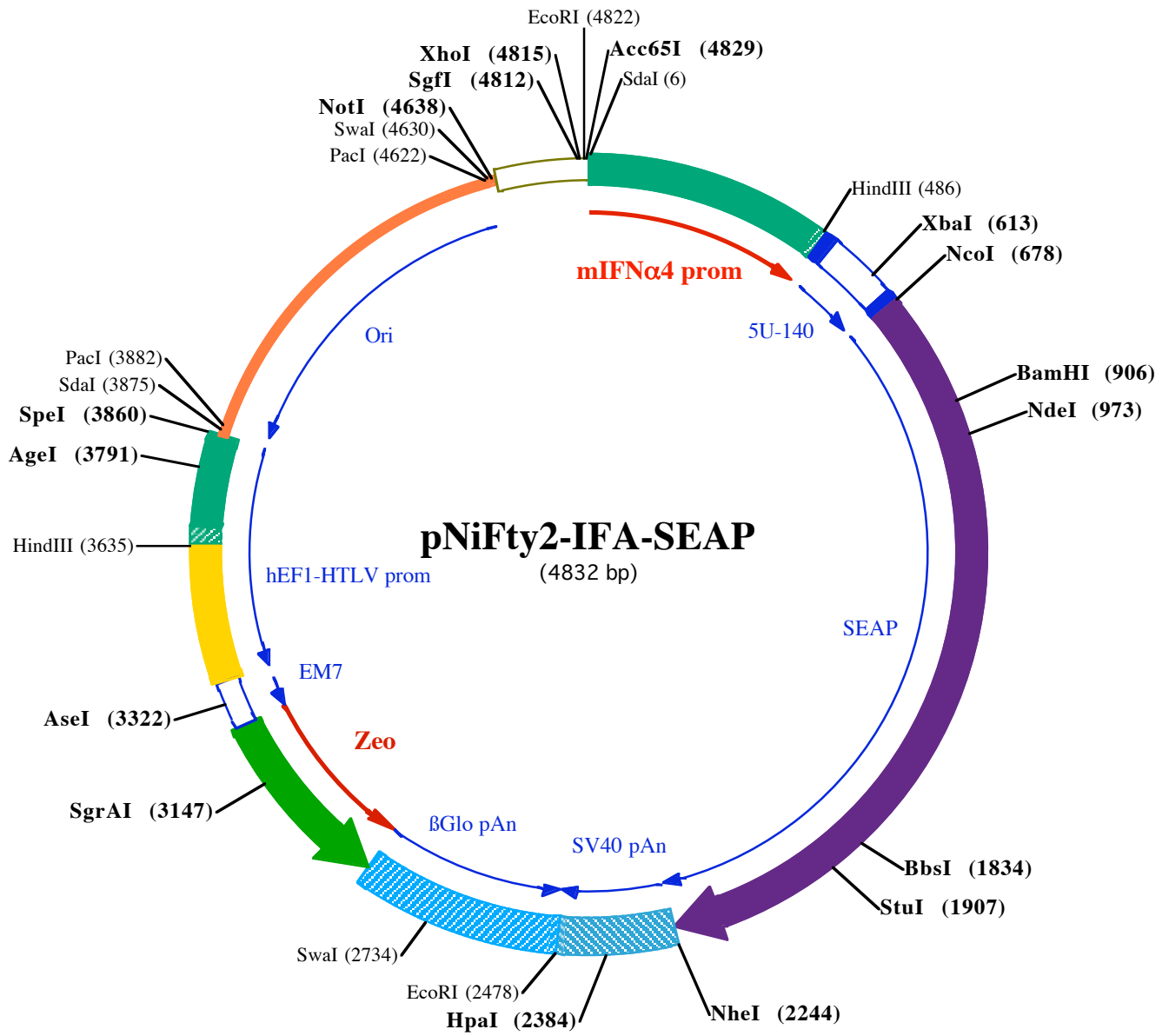
Plasmid amplification and cloning can be performed in *E. coli* GT116 or in other commonly used laboratory *E. coli* strains, such as DH5α.

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.

TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873
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E-mail: info@invivogen.com



SdaI (6)
1 CCTGCAGGGGCAGGTTTCTGTCATCCCTGGACCCTGCTGGTTCCAGTTACCCCCACACTTTACTTTTTTTGACAGAAATATTTATGTAATAGTATAAATA
101 AAACAAAAAGGTTGTAATAAATTACAAAACCTTAAATACAGTCATAGGGGAAATTTGTGTTTGCAGTAGATGTGCAGTTCACACAGAGACTGT
201 ATATCTGTGGAGTAGTGTTAATGACAATTAAGTGAATTTAAAGAGAAACCTGGAGAGTAGCTTCTGAGGGCAGCAGTGAAGTGAAGCAATGAT
301 TGAACCCACATTTCCAGGGGGCGGGGAGGGGAGGACAAAATCCAGACACAAGCAGAGAGTGAAGTAAAGAAAGTAAAAGAGAATTGAAAGCAAG
HindIII (486)
401 GGGAGGGTATTCCGAAAGGAGAACTTGTATTGTCCCTATTTAAGAGAGATGTACACAGCAGGCTCTCAGAGAACCCTGTAGGAGAAGCTTCTGCCCTCT
501 CCCTCTGTGAGTTTgtaagtcaactgactgtctatgctctgggaaagggtggcaggagatgggagcagtgaggaaagtggcactatgaaccTGCAGC
XbaI (613) NcoI (678)
601 CCTAGGAATGCATCTAGAcaattgtactaaccttctctctctctctctctgacagTTGGTGTACAGTAGCTCCACCATGGTTCTGGGGCCCTGCATG
701 CTGCTGCTGCTGCTGCTGCTGGGCTGAGGCTACAGCTCTCCCTGGGCATCATCCAGTTGAGGAGGAGAACCAGGACTTCTGGAACCGCAGGACGGCCG
801 AGGCCCTGGTGGCCCAAGAGCTGCAGCCTGCACAGACAGCCCAAGAACCTCATCTCTCTGGGCGATGGGATGGGGGTGTCTACGGTGACAGC
901 TGCCAGGATCTAAAAGGGCAGAAGAAGGACAAAACCTGGGCTGAGATACCCCTGGCTATGGACCGCTTCCATATGTGGCTGTCCAAGACATACAAT
1001 GTAGACAAACATGTGCCAGACAGTGGAGCCACAGCCAGCCCTACTGTGCGGGGTCAAGGGCAACTCCAGACCATTGGCTTGAGTGCAGCCGCGCCGCT
1101 TTAACCAAGTCAACACGACGACCGCGCAACGAGGTACTCCGCTGATCAATCGGGCCAAAGAGCAGGGAAGTCAAGTGGGAGTGGTAAACCCACACAGAT
1201 GCAGCAGCCCTCGCCAGCCGGCAGCTACGCCACACCGTGAACCGCAACTGGTACTCGGACCGCCAGCTGCCTCGCCCGCCAGGAGGGGTGCCAG
1301 GACATCGCTACGACGCTCATCTCAACATGGACATTGATGTGATCTGGGTGGAGCCGAAAGTACATGTTTCGCATGGGAACCCAGACCTGAGTACC
1401 CAGATGACTACAGCCAAGGTGGGACCAAGCTGGACGGGAAGAATCTGGTGCAGGAATGGCTGGCAAGCGCCAGGGTGGCCCGGTGTGTGGAACCCAGC
1501 TGAGCTCATGCAGGCTTCCCTGGACCGCTGTGACCCATCTATGGGCTCTTTGAGCCTGGAGACATGAAATACGAGATCCACCGAGACTCCACACTG
1601 GACCCCTCCCTGATGGAGATGACAGAGGCTGCCTGCGCCTGCTGAGCAGGAACCCCGCGGCTTCTCTCTTCTGAGGGTGGTGCATCGACCAGC
1701 GTCATACGAAAGCAGGCTTACCGGCACTGACTGAGACGATGTTGACAGACCCATTGAGAGGGCGGGCCAGCTCACCGCCGCGGACACCGCT
1801 GAGCCTCGTCACTGCCACCCTCCACGCTTCTCTTCGGAGCTACCCCTGCGAGGGAGCTCCATCTTGGGCTGGCCCTGGCAAGGCCCGGGAC
1901 AGGAAGGCTACACGGTCTCTATACGAAACCGTCCAGGCTATGTGCTCAAGGACGGCCCGCCGGATGTTACCGAGAGCGAGAGCGGGAGCCCG
2001 AGTATCGGACGAGTACAGCAGTCCCTGGACGAGAGACCACGACGCGGAGGACGCTGGCGGTGTTGCGCGCGCCCGCAGGCGACCTGGTTCACGG
2101 CGTGCAGGAGCAGACTTACGCGCAGCTCATGGCTTCCGCTGCTGAGCCTGAGGACCCCTACCGCCCTGCGACCTGGCGCCCGCCGCGGACACCGG
2201 GCCGCGCACCCGGGGCGGTCCCGGTCCAAGCTGCTGGATTGAAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCAACTAGA
2301 ATGCAGTGAATAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCAATTAAAGTGAATAAACAAGTAAACAACAATTGCA
2401 TTCATTTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGTTTTTTAAAGCAAGTAAACCTCTACAATGTGGTATGGAATTTCAAAAATACAGCATAGCA
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3301 GCCGATATACTATGCCGATGATTAATTGTCAACTACTGTTTGTAGGCGCCGTACAGCTTGATCTGTAACGGCGCAGAACAGAAAACGAACAAAGAC
3401 GTAGAGTTGAGCAAGCAGGTCAGGCAAGCGTGGAGAGCCGGCTGAGTCTAGTGTAGGCTCAAGGGAGCGCCGGACAAAGCCCGGCTCGACCTGAGC
3501 TTTAAACTTACTAGACGCGGACGAGTTCAGGAGGACACAGGCGGGAGGCGGAGAACCGACTCAACCGCGGTGGATGGCGCCCTCAGGTAGGGC

3601 **HindIII (3635)**
GGCGGGCGCGTGAAGGAGAGATGCGAGCCCTCGAAGCTTCAGCTGTGTTCTGGCGGCAAACCCGTTGCGAAAAAGAACGTTACGGCGACTACTGCACT

3701 **AgeI (3791)**
TATATACGGTTCTCCCCACCCTCGGAAAAAGGGGAGCCAGTACACGACATCACTTCCAGTTTACCCCGCGCCACCTTCTCTAGGCACCGGTTCAA

3801 **SpeI (3860)** SdaI (3875) PaeI (3882)
TTGCGACCCCTCCCCCACTTCTCGGGACTGTGGCGATGTGCGCTCTGCCACTGACTAGTGGCCCTGCAGGTTAATTAAGAACATGTGAGCAA

3901 AGGCCAGCAAAGGCCAGGAACCGTAAAAAGGCCGTTGCTGGCGTTTTCCATAGGCTCCGCCCTGACGAGCATCACAAAATCGACGCTCAAGT

4001 CAGAGGTGGCGAAACCCGACAGGACTATAAGATACCAGGCGTTTCCCCTGGAAGCTCCCTCGTGCCTCTCCTGTTCCGACCTGCCGCTTACGGAT

4101 ACCTGTCCGCTTTCTCCTTCGGGAAGCGTGGCGTTTTCTCATAGCTCAGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCAAGCTGGGCTG

4201 TGTGCACGAACCCCGTTTCCAGCCGACCCTGCGCCTTATCCGTAATATCGTCTTGAAGTCCAACCCGTAAGACACGACTTATCGCCACTGGCAGCA

4301 GCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTG

4401 GTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTTTGATCCGGCAAACAACACCGCTGGTAGCGGTGGTTTTTTGTTTG

4501 CAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGGTCTGACGCTCAGTGAACGAAACTCACGTTAAGGG

4601 **NotI (4638)** PaeI (4622) SmaI (4630)
ATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCCAATAAAATATCTTTATTTTCATTACATCTGTGTGGTTTTTTTGTGTAATCGTA

4701 ACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACAACTAGCAAAATAGGCTGTCCCAAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGA

4801 **SgfI (4812)** **XhoI (4815)** **Acc65I (4829)** EcoRI (4822)
AGGATCTGCGATCGCTCGAGTGAATTCGGTA