

# pNiFty3-IAN-SEAP

An inducible reporter plasmid selectable with Zeocin™

Catalog code: pnf3-sp7

For research use only

Version 20L03-MM

## PRODUCT INFORMATION

### Content:

- 20 µg of pNiFty3-IAN-SEAP provided as lyophilized DNA
- 1 ml of Zeocin™ (100 mg/ml)

### Storage and stability:

- Products are shipped at room temperature.
- Store lyophilized DNA at -20 °C.
- 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

Pattern recognition receptor (PRR) activation triggers a complex signaling cascade that leads to the activation of different transcription factors, each playing an important role in the subsequent immune response. To monitor the induction of PRR signaling in response to ligand stimulation in a simple and efficient manner, InvivoGen has designed pNiFty, a family of reporter plasmids expressing a reporter gene under the control of a minimal promoter inducible by these different transcription factors, either individually or in combination. Most pNiFty plasmids are selectable with Zeocin™ in both *E. coli* and mammalian cells, and can be used to generate stable clones.

pNiFty plasmids are composed of three key elements: a proximal promoter, repeated transcription factor binding sites (TFBS) and a reporter gene. The proximal promoters are shorter than 500 bp and contain transcription factor binding sites. Upon stimulation in 293 cells, their expression level remains undetectable. With the addition of repeated TFBS, the proximal promoters become inducible by the appropriate stimulus and drive the expression of the reporter gene.

## PLASMID FEATURES

- **ISRE binding site:** PRRs involved in the antiviral response induce the activation of interferon regulatory factors (IRFs) and the production of type I interferons (IFNs). IFNs trigger the formation of the ISGF3 complex which contains signal transducer and activator of transcription (STAT) 1, STAT2 and IRF9. ISGF3 and IRFs bind to specific nucleotide sequences called interferon-stimulated response elements (ISREs; AGTTTCNNTTCC) in the promoter of IFN-stimulated genes (ISGs) leading to their activation<sup>1</sup>.
- **AP-1 binding site:** Activator protein 1 (AP-1) is a transcription factor activated by most PRRs. AP-1 is a heterodimeric complex composed of members of Fos, Jun and, ATF protein families. AP-1 binds to the TPA responsive element (TRE; ; TGAG/CTCA)<sup>2</sup>. AP-1 activation in TLR signaling is mostly mediated by MAP kinases such as c-Jun N-terminal kinase (JNK), p38 and extracellular signal regulated kinase (ERK).

- **NF-κB binding site:** Nuclear factor (NF)-κB is a “rapid-acting” primary transcription factor activated by a wide variety of PRRs. NF-κB is a protein complex that belongs to the Rel-homology domain-containing protein family. The prototypical NF-κB is composed of the p65(RelA) and p50 subunits<sup>3</sup>. NF-κB binds specific decameric DNA sequences (GGGRNNYYCC, R-purine Y=pyrimidine) and activates genes involved in the regulation of the innate and adaptive immune response.

- **IFN-β promoter:** the mouse IFN-β minimal promoter comprises several positive regulatory domains that bind different cooperating transcription factors such as NF-κB, IRF3 and IRF7<sup>4</sup>.

- **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.

- **EF1/HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) 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-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 EF1-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<sup>7</sup>.

1. **Wesoly J. et al., 2007.** STAT activation and differential complex formation dictate selectivity of interferon responses. *Acta Biochim Pol.* 54(1):27-38. 2. **Hess J. et al., 2004.** AP-1 subunits: quarrel and harmony among siblings. *J Cell Sci.* 117(Pt 25):5965-73. 3. **Kawai T. & Akira S., 2007.** Signaling to NF-κB by Toll-like receptors. *Trends Mol Med.* 13(11):460-9. 4. **Vodjdani G. et al., 1988.** Structure and characterization of a murine chromosomal fragment containing the interferon β gene. *J Mol Biol.* 204(2):221-31. 5. **Kim D. et al., 1990.** Use of the human elongation factor 1α promoter as a versatile and efficient expression system. *Gene* 91(2): 217-23. 6. **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: 466-72. 7. **Yu J & Russell J., 2001.** Structural and functional analysis of an mRNP complex that mediates the high stability of human β-globin mRNA. *Mol Cell Biol.* 21(17):5879-88.

## 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 3-622-34-80

E-mail: [info@invivogen.com](mailto:info@invivogen.com)

## 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 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 |
|-----------------|--------------|
| ChemiComp GT116 | gt116-11     |
| Zeocin™         | ant-zn-1     |

---

### TECHNICAL SUPPORT

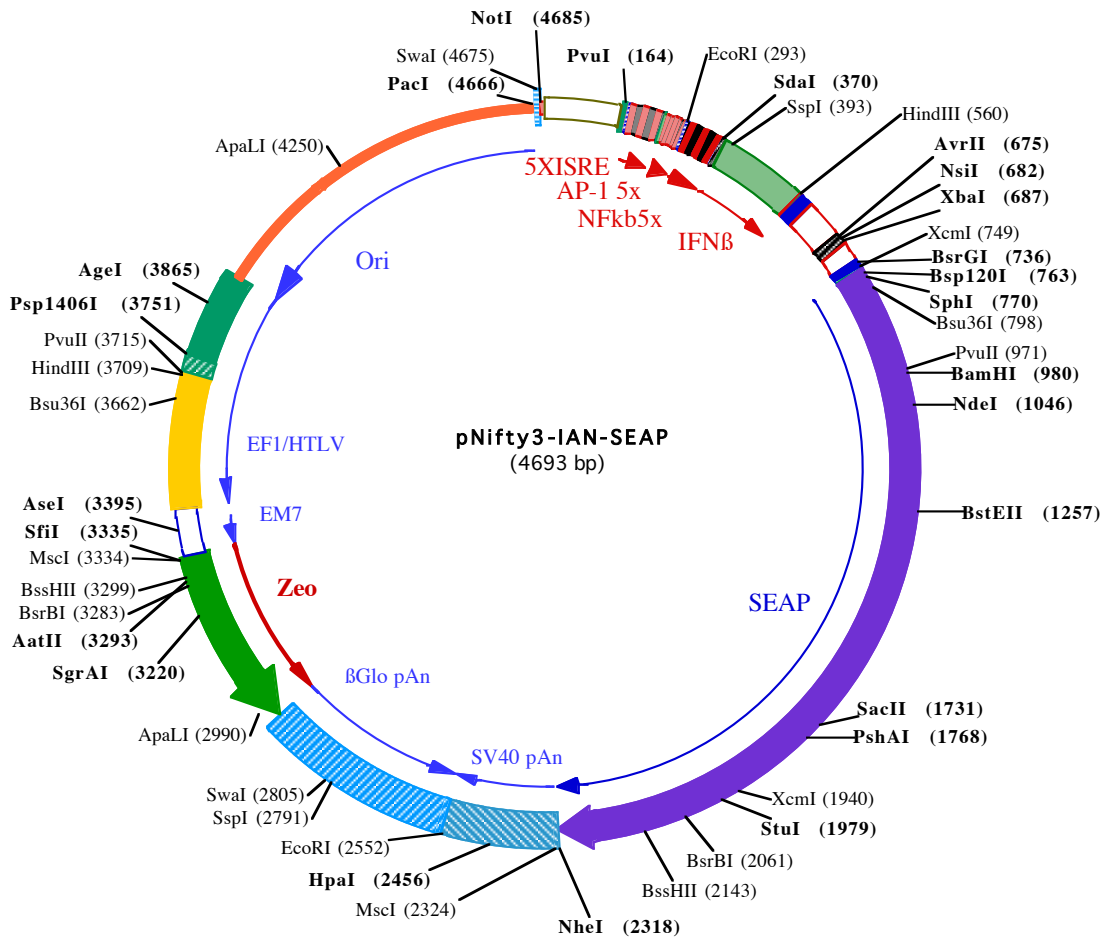
InvivoGen USA (Toll-Free): 888-457-5873

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

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

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

E-mail: [info@invivogen.com](mailto:info@invivogen.com)



1 AATAAAATATCTTTATTTTTCATTACATCTGTGTGGTTTTTGTGTGAATCGTAACATAACGCTCTCCATCAAAAACAAACGAAACAAAACAAAC

101 TAGCAAATAGGCTGCCAGTGAAGTGCAAGTGCAGAGTCCAGAACATTTCTCTACGAAAGGATCTCGCATCGCTGAATTAGTTTCACTTTCCAGTTTCAGTT

201 TCCAGTTTCACTTTCCAGTTTCATTTCCAGTTTCACTTTCTGATCGAGCTCGAGTCACTGACTCAGTGAGTCACTGACTCAGTGAGTAAAGGAATTCT

301 GGGGACTTTCCACTGGGACTTTCCACTGGGACTTTCCACTGGGACTTTCCACTGGGACTTTCCACTCTGCAGGagcttgaataaaatgaatatta

401 gaagctgttagaataagaaaatgacagaggaAAACTGAAAGGgAGAACTGAAAGTGGaaattcctctgaggcagaaggaccatccctTATAAAAt

499 agcacagccatgaaggaagatcattctcactgcagcctttgacagcctttgctcatcttGAGCTTCTGCTTCTCCCTCTGTGAGTTTgtaagtc

599 actgactgtctatgcctgggaagggtgggcaggagatggggcagtgaggaaaagtggcactatgaaccTGCAGCCTAGGAATGCATCTAGACaatt

699 gtactaaccttcttctcttctctctcctgacagGTTGGGTACAGTAGTCTCCACCATGATTCTGGGGCCCTGCATGCTGCTGCTGCTGCTGGG

799 CCTGAGGCTACAGCTCTCCCTGGGCATCATCCAGTTGAGGAGGAGAACCAGGACTTCTGGAACCGCAGGCAGCCGAGGCCCTGGGTGCCCAAGAAG

899 CTGCAGCCTGCACAGACAGCCGCAAGAACCTCATCATCTTCTGGGCGATGGGATGGGGGTGTCTACGGTGACAGCTGCCAGGATCTAAAAGGGCAGA

999 AGAAGGCAAACTGGGGCTGAGATAACCCCTGGCTATGGACCGCTTCCCATATGTGGCTGTCCAAGACATACAATGTAGACAAACATGTGCCAGACAG

1099 TGGAGCCACAGCCAGGCCCTACCTGTGCGGGTCAAGGGCAACTCCAGACCATTGGCTTGAAGTGCAGCCGCGGCTTAAACAGTGCACACGACAGCCG

1199 GGCAACGAGGTCATCTCCGTGATGAATCGGGCCAAAGCAAGGAGTCAAGTGGGAGTGGTAACACCACAGGAGTGCAGCAGCCCTGCCAGCCGGCA

1299 CCTACGCCACAGCTTGAACCGCACTGGTACTCGGACCGCAGCTGCCTCGCCCGCAGGAGGGGTGCCAGGACATCGCTACGCAGCTCATCTC

1399 CAACATGGACATTGATGTATCTGGTGAGGCGCAAGTACATGTTTCGCATGGGAACCCAGACCTGAGTACCCAGATGACTACAGCAAGGTGGG

1499 ACCAGCTGGACGGGAAGAATCTGGTGCAGGAATGGCTGGCGAAGCGCCAGGGTGGCCGCTATGTGGAAACCGCACTGAGCTCATGAGGCTTCCCTGG

1599 ACCCGTCTGTGACCATCTCATGGGTCTCTTGGACCTGGAGACATGAATACGAGATCCACCGAGACTCCACACTGGACCCCTCCCTGATGGAGATGAC

1699 AGAGGCTGCCTGCCTGCTGAGCAGGAACCCCGCGGCTTCTTCTTCTCGTGGAGGGTGGTGCATCGACCAGGTCATCACGAAAGCAGGGCTTAC

1799 CGGGCACTGACTGAGACGATCATGTTGACGAGCCATTGAGAGGGCGGGCCAGCTCACCAGCAGGAGGACACGCTGAGCCTCGTACTGCCGACCACT

1899 CCCACGCTTCTCCTTGGAGGCTACCCCTGGCAGGGAGCTCCATCTTGGGCTGGCCCTGGCAAGGCCGGGACAGGAAGGCTACAGGTCCTCCT

1999 ATACGAAACGGTCCAGGCTATGTCTCAAGGACGGCGCCCGGCGGATGTTACCAGAGCAGGAGCGGGAGCCCGAGTATCGGAGCAGTGCAGCATG

2099 CCCCTGGACGAAAGAGCCACGCAGCGAGGACGTGGCGGTGTTCCGGCGCGCCCGCAGGCGCACCTGGTTCACGGCGTGCAGGAGCAGACCTTCATAG

2199 CGCAGCTCATGGCTTCCGCGCTGCCTGGAGCCCTACCCGCTGCGACCTGGCGCCCGCCCGGCCACCACCGACCCGCGCACCCGGGGCGGTCCCG

2299 GTCCAAGCGTCTGGATTGAAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCAACTAGAATGCAGTGAAAAAATGCTTTAT

2399 TTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTAAGTCAATAAACAAGTTAAACAACAATTGCATTCATTTTTATGTTTCAGTTTCAG

2499 GGGGAGGTGTGGAGGTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAATTCTAAAATACAGCATAGCAAACTTTAACCTCCAATCAAGC

2599 CTCTACTTGAATCCTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTGCTGTTTGCAGCCTCACCTCTTTTCATGGAG

2699 TTTAAGATATAGTGATTTTTCCAAAGTTTGAACGTCTTCTTCTTTATGTTTAAATGCACTGACCTCCACATTCCTTTTTAGTAAAAATATTC

2799 AGAAATAATTTAAATACATCATGCAATGAAAATAAATGTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGTAGT

2899 TGGACTTAGGGAACAAAGGAACCTTAAATAGAAATTTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCTGCTCCTGCCACAAAGTGACGC

2999 AGTTGCCGGCCGGTTCGCGCAGGCGAACTCCCGCCCGCAGGCTGCTCGCCGATCTCGGTGATGGCCGGCCCGAGGCGTCCGGGAAGTTCTGTGGACAC

3099 GACCTCCGACCACTGGCGTACAGCTCGTCCAGGCGCGCACCCACACCCAGGCGAGGGTGTGTCGGCACCACTGGTCTGGACCGCGCTGATGAAC

3199 AGGGTACGTCGCTCCCGACCACACCGGCGAAGTCTGCTTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCCGGAACCTGACCCGCTCCGGCGACGT

3299 CGCGCGGTTGAGCAGCCGGAACGCGCACTGGTCAACTGGCCATGATGGCCCTCTATAGTGAAGTATTACTATGCGGATATACTATGCCGATGATT

3399 CGCGCGGTTGAGCAGCCGGAACGCGCACTGGTCAACTGGCCATGATGGCCCTCTATAGTGAAGTATTACTATGCGGATATACTATGCCGATGATT

3399 AATTGTCAACTACTGTTTGTAGGC GCCGGTCACAGCTTGATCTGTAACGGCGCAGAACAGAAAACGAAACAAAGACGTAGAGTTGAGCAAGCAGGGTCA  
3499 GGCAAAGCGTGGAGAGCCGGCTGAGTCTAGGTAGGCTCCAAGGGAGCGCCGGACA AAGGCCCGTCTCGACCTGAGCTTTAAACTTACCTAGACGGCGGA  
3599 CGCAGTTCAGGAGGCACCCAGGC GGGAGGCGGAGAACGCGACTCAACCGCGTGGATGGCGGCCTCAGGTAGGGCGGCGGCGCTGAAGGAGAGATG  
3699 CGAGCCCTCGAAGCTTCAAGCTGTGTCTGGCGCAAACCCGTTGCGAAAAAGAACGTTACGCGGACTACTGCATTATATACGGTTCCTCCCCACCT  
3799 CGGGAAAAGGGGAGCCAGTACACGACATCACTTCCAGTTTACCCGCGCCACCTTCTAGGCACCGGTTCAATTGCCACCCCTCCCCCACTT  
3899 CTCGGGGACTGTGGCGATGTGCGCTCTGCCACTGACACATGTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAAAGGCCGCTTGTGCGGTTT  
3999 TTCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACAGGCGTTTCCCGC  
4099 CTGGAAGCTCCCTCGTGCCTCTCTGTTCCGACCTGCCGCTTACCGGATACCTGTCCGCTTCTCCCTTCGGGAAGCGTGGCGTTTCTCATAGCTC  
4199 ACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTGCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTACGCCGACCGCTGCGCTTATCCGGTAAC  
4299 TATCGTCTTGAGTCCAACCCGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAG  
4399 AGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAG  
4499 CTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTG  
4599 ATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGC

Bsu36I (3662)  
PvuII (3715)  
HindIII (3709)  
Psp1406I (3751)  
AgeI (3865)  
ApaLI (4250)  
Swal (4675)  
PacI (4666)  
NotI (4685)