

pNiFty3-AN-SEAP

An inducible reporter plasmid selectable with Zeocin™

Catalog code: pnf3-sp6

For research use only

Version 20L03-MM

PRODUCT INFORMATION

Content:

- 20 µg of pNiFty3-AN-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

- **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)¹. 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 subunits². 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 IRF7³.
- **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 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.
- **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 transcription⁶.

References

1. Hess J, et al., 2004. AP-1 subunits: quarrel and harmony among siblings. J Cell Sci. 117(Pt 25):5965-73.
2. Kawai T. & Akira S., 2007. Signaling to NF-kappaB by Toll-like receptors. Trends Mol Med. 13(11):460-9.
3. Vodjdani G. et al., 1988. Structure and characterization of a murine chromosomal fragment containing the interferon beta gene. J Mol Biol. 204(2):221-31.
4. 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.
5. 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.
6. 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



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

TECHNICAL SUPPORT

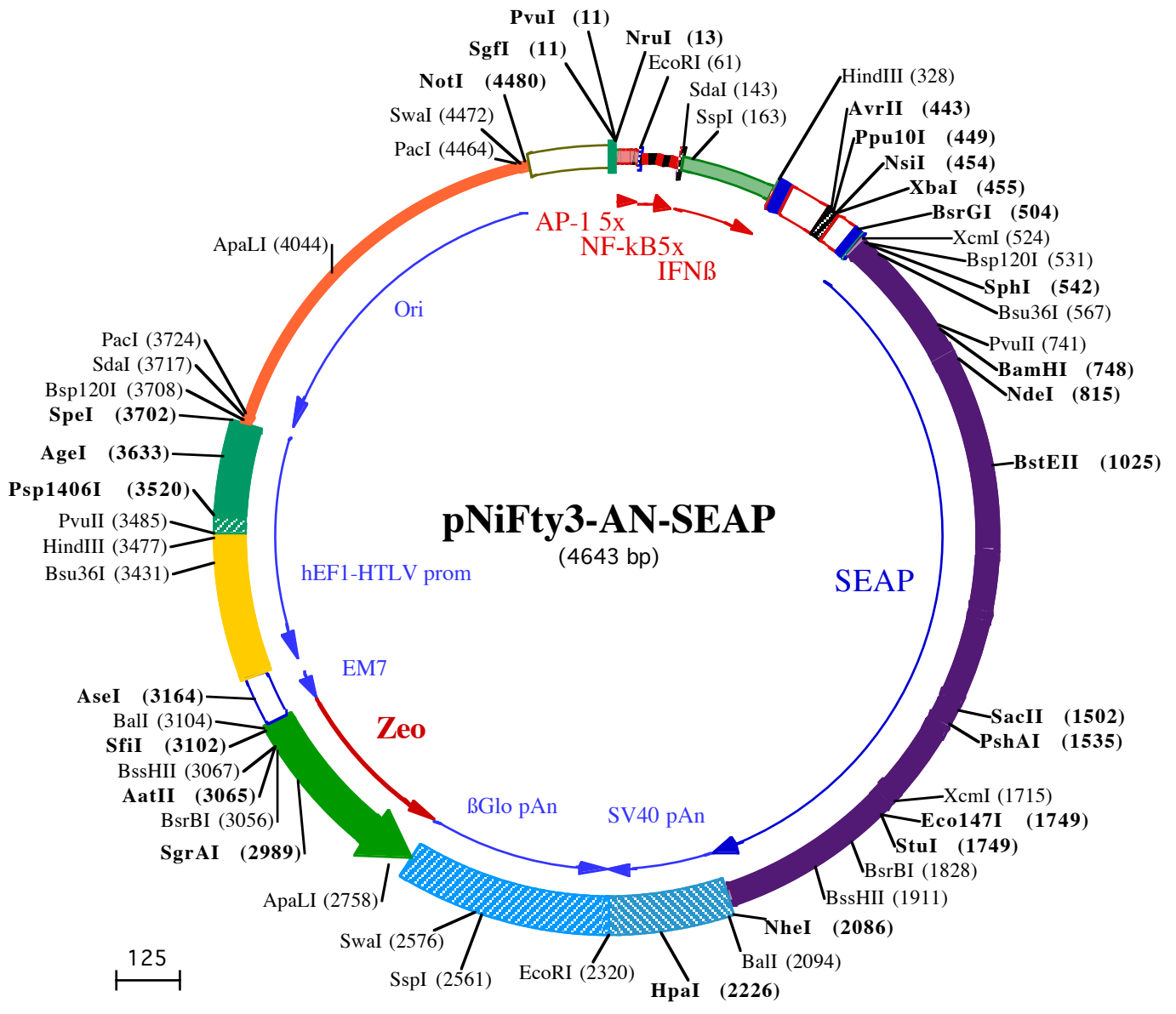
InvivoGen USA (Toll-Free): 888-457-5873

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



NruI (13)
 PvuI (11)
 SgfI (11)
 EcoRI (61)

1 GGATCTGCGATCGGAGCTCTGAGTCACTGACTCAGTGAGTCACTGACTCAGTGAGTAAAGGAATTCGGGGACTTTCCACTGGGGACTTTCCACTGGGA

SdaI (143) SspI (163)
 101 CTTTCCACTGGGGACTTTCCACTGGGGACTTTCCACTCTGCAGGagcttgaaataaatgaaatattagaagctgttagaataagagaaaatgacagagga

201 AAAGTAAAGGgAGAAGTAAAGTggaattctctgaggcagaagaccatccctTATAAAtagcacagccatgaaggaagatcattctcactg

HindIII (328)
 299 cagcctttgacagcctttgcctcatctgAAGCTTCTGCCTTCTCCCTCCTGTGAGTTTGtaagtcactgactgtctatgcctgggaaggggggag

Ppu10I (449)
 NsiI (454)
 AvrII (443) XbaI (455)
 399 gagatggggcagtgaggaaagtggcactatgaaccctGCAGCCCTAGGAATGCATCTAGACcaattgtactaaccttcttctcttctcctctcctgacag

SphI (542)
 BsrGI (504) XcmI (524) Bsp120I (531) Bsu36I (567)
 499 GTTGGTGTACAGTAGCTTCCACCATGATTCTGGGGCCCTGCATGCTGCTGCTGCTGCTGCTGGGGCTGAGGCTACAGCTCTCCCTGGGCATCATCCC

599 AGTTGAGGAGGAGAAACCCGACTTCTGGAACCGGAGGACGCGGAGCCCTGGGTGCCGCAAGAAGCTGCAGCCTGCACAGACAGCCGCAAGAACCTC

26 V E E E N P D F W N R E A A E A L G A A K K L Q P A Q T A A K N L

699 ATCATCTTCTGGGCGATGGGATGGGGGTGTCTACGGTGCAGCTGCCAGGATCTAAAAGGGCAGAAGAAGGACAAACTGGGGCCTGAGATACCCCTGG

60 I I F L G D G M G V S T V T A A R I L K G Q K K D K L G P E I P L

NdeI (815)
 799 CTATGACCCTTCCCATATGTGGCTCTGTCCAAGACATACAATGTAGACAACATGTGCCAGACAGTGGAGCCACAGCCACGGCCTACCTGTGCGGGGT

93 A M D R F P Y V A L S K T Y N V D K H V P D S G A T A T A Y L C G V

899 CAAGGGCAACTTCCAGACCACTGGCTGAGTGCAGCCGCGCTTAAACCAAGTGCACACGACAGCAGGCACGCGGCAACGAGGTCATCTCCGTGATGAATCGGGC

126 K G N F Q T I G L S A A A R F N Q C N T R G N E V I S V M N R A

BstEII (1025)
 999 AAGAAAGCAGGGAAGTCAGTGGGAGTGGTAACACCCACACAGAGTGCAGCAGCCCTCGCCAGCCGACCTACGCCACAGCCTGCAACCGGCAACTGGTACT

160 K K A G K S V G V V T T T R V Q H A S P A G T Y A H T V N R N W Y

1099 CGGACGCCGAGCCTCGCTCGCCCGGAGGGGTGCCAGGACATGCTACGACAGTCACTCAACATGGACATTGATGTCCTGGGTGGAGG

193 S D A D V P A S A R Q E G C Q D I A T Q L I S N M D I D V I L G G G

1199 CCGAAAGTACATGTTTCGATGGGAACCCAGACCCTGAGTACCCAGATGACTACAGCCAAGGTGGGACCAGGCTGGACGGGAAGAATCTGGTGCAGGAA

226 R K Y M F R M G T P D P E Y P D D Y S Q G G T R L D G K N L V Q E

1299 TGGCTGGCGAAGCGCCAGGGTGGCCGATGTTGTAACCGCATGAGCTCATGACGAGCTTCCCTGGACCCGCTGTGACCATCTCATGGGTCTCTTTG

260 W L A K R Q G A R Y V W N R T E L M Q A S L D P S V T H L M G L F

1399 AGCCTGGAGACATGAAATACGAGATCCACCGAGACTCCACACTGGACCCCTCCCTGATGGAGATGACAGAGGCTGCCCTGCGCTGCTGAGCAGGAACCC

293 E P G D M K Y E I H R D S T L D P S L M E M T E A A A L R L L S R N P

SacII (1502) PshAI (1535)
 1499 CCGCGCTTCTTCTTCTCGTGGAGGGTGGTCGATCGACCAGGTCATCACGAAAGCAGGGCTTACCGGGCACTGACTGAGACGATCATGTTGCGACGAC

326 R G F F L F V E G G R I D H G H H E S R A Y R A L T E T I M F D D

1599 GCCATTGAGAGGGCGGGCAGCTCACCAGCAGGAGGACAGCCTGAGCCTCTCTACTGCCAGCCTCCACGTCTTCTCTTCCGAGGCTACCCCTGCC

360 A I E R A G Q L T S E E D T L S L V T A D H S H V F S F G G Y P L

StuI (1749)
 Eco147I (1749)
 1699 GAGGGAGCTCCATCTTCGGGCTGGCCCCTGGCAAGGCCGGGACAGGAAGGCCCTACAGGTCCTCTATACGGAAACGGTCCAGGCTATGTCTCAAGGA

393 R G S S I F G L A P G K A R D R K A Y T V L L Y G N G P G Y V L K D

BsrBI (1828)
 1799 CGGCGCCGCGCGGATGTTACCGAGAGCGAGAGCCGCGGAGTATCGGCGAGCAGTCAAGTCCGCTGGACGAAGAGACCCACGAGCGGAGGAC

426 G A R P D V T E S E S G S P E Y R Q Q S A V P L D E E T H A G E D

BssHIII (1911)
 1899 GTGGCGGTGTTTCGCGCGCGGCCGAGGCCACCTGGTTCACGGCGTGCAGGAGCAGACCTTCTAGCAGCAGCTATGGCCCTCGCCGCTGCTGGAGC

460 V A V F A R G P Q A H L V H G V Q E Q T F I A H V M A F A A C L E

Ball (2094)
 NheI (2086)
 1999 CCTACACCCTGCGACCTGGCCGCCCCCGGGCACCCGACCCGGGCGGTCCCGGTCCAAGCGTCTGATTGAAGCTAGCTGGCCAG

493 P Y T A C D L A P P A G T T D A A H P G R S R S K R L D

2099 ACATGATAAGATACATTGATGAGTTTGACAAAACCACAAC TAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTG

HpaI (2226)
 2199 AACCAATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGTTTTTTAAAGCAAGTAA

EcoRI (2320)
 2299 AACCTCTACAAATGTGGTATGGAATCTAAAATACAGCATAGCAAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCTTTTTCTGAGGGATGAATA

2399 GGCATAGGCATCAGGGGCTGTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTTAAAGATATAGTGATTTTTCCCAAGGTTTGAA

SspI (2561) SnaI (2576)
 2499 CTAGCTCTTCATTTCTTATGTTTTAAATGCACTGACCTCCACATTCCCTTTTTTAGTAAAAATTCAGAAATAATTTAAATACATCATTGCAATGAAAA

2599 TAAATGTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTTAGTAGTTGGACTTAGGGAACAAGGAACCTTTAATAGAA

2699 ATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGTCCGCGAGGGCGAACTCCC
1274 • G • D Q E E A V F H V C N G A P D R L A F E R

2799 GCCCCACGGCTGCTCGCCGATCTCGGTATGGCCGGCCGGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAG
1034 G W P Q E G I E T M A P G S A D R F N T S V V E S W E A Y L E D L

2899 GCCGCGCACCCACCCAGGCCAGGGTGTGTCCGGCACCACCTGGTCTGGACCGCGTGTGAACAGGGTACGTCGTCCCGACCACACCGCGGAAG
704 G R V W V W A L T N D P V V Q D Q V A S I F L T V D D R V V G A F

2999 TCGTCTCCACGAAGTCCCAGGAGAACCCGAGCCGGTCCGATCCAGAACTCGACCGTCCGGCGACGTCGCGCGCGGTGAGCACCGGAACGGCACTGGTCA
364 D D E V F D R S F G L R D T W F E V A G A V D R A T L V P V A S T L

3099 ACTTGGCCATGATGGCCCTCCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCACTACTGTTTGTAGGCCCGGTCA
3 K A M

3199 AGCTTGATCTGTAACGGCGCAGAACAGAAAACGAAACAAAGACGTAGAGTTGAGCAAGCAGGGTACGGCAAAGCGTGGAGAGCCGGCTGAGTCTAGGTA

3299 GGCTCCAAGGGAGCGCCGACAAAGGCCCGGTCTCGACCTGAGCTTTAAACTTACCTAGACGGCGGACGCAGTTGAGGAGCACACAGCGGGGAGCGCG

3399 CAGAACCGGACTCAACCGCGTGGATGGCGGCTCAGGTAGGGCGCGGGCGGTGAAGGAGAGATGCGAGCCCTCGAAGCTTCAGCTGTGTTCTGGCG
Bsu36I (3431) PvuII (3485)
HindIII (3477)

3499 GCAAACCCGTTGCGAAAAAGAACGTTACGGCGACTACTGCACTTATATACGGTTCTCCCCACCCCTCGGAAAAAGGCGGAGCCAGTACACGACATCAC
Psp1406I (3520)

3599 TTTCCCAGTTTACCCGCGCCACCTTCTTAGGCACCGGTTCAATTGCCGACCCCTCCCCCAACTTCTCGGGACTGTGGGCGATGTGCGCTCTGCCA
AgeI (3633)

3699 CTGACTAGTGGCCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCTTGTGGCGTTTTCCATA
Bsp120I (3708) SpeI (3702) SdaI (3717) PacI (3724)

3799 GGCTCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGCGCTTCCCCCTGGAAG

3899 CTCCCTCGTGCCTCTCCTGTTCCGACCCCTGCCGTTACCGGATACTGTCCGCCTTCTCCCTTCCGGAAGCGTGGCGCTTCTCATAGCTCACGCTGT

3999 AGGTATCTCAGTTCGGTGTAGGTGTTGCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTCAGCCCAGCCGCTGCGCTTATCCGGTAACTATCGTC
ApaLI (4044)

4099 TTGAGTCCAACCCGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTT

4199 GAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGA

4299 TCCGCAACAAACCACCGTGGTAGCGGTGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCTTTGATCTTTT

4399 CTACGGGGTCTGACGCTCAGTGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGCCGCAATAAAATATCT
PacI (4464) SmaI (4472) NotI (4480)

4499 TTATTTTTCATTACATCTGTGTGTTGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAAACAAAACGAAAACAAAACAACTAGCAAAATAGG

4599 CTGTCGCCAGTGCAAGTGCAAGTGCCAGAACATTTCTCTATCGAA