

pNiFty3-TAN-SEAP

An inducible reporter plasmid selectable with Zeocin™

Catalog code: pnf3-sp8

For research use only

Version 20L03-MM

PRODUCT INFORMATION

Content:

- 20 µg of pNiFty3-TAN-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

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

• **NFAT binding site:** Nuclear factor of activated T-cell (NFAT) is a family of transcription factors expressed in T cells, but also in other classes of immune and non-immune cells¹. NFAT is activated by stimulation of receptors coupled to calcium mobilization, such as the PRRs Dectin-1 and Mincle^{2,3}. Calcium mobilization induces the calmodulin-dependent phosphatase calcineurin leading to NFAT activation. NFAT binds to a 9 bp element, with the consensus sequence (A/T)GGAAA(A/N)(A/T/C)N.

• **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)⁴. 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 subunits⁵. NF-κB binds specific decameric DNA sequences (GGGRNNYYCC, R-purine Y=pyrimidine) and activates genes involved in the regulation of the innate and adaptative 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 IRF7⁶.

• **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 promoter⁷ and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat⁸. 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 transcription⁹.

TECHNICAL SUPPORT

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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

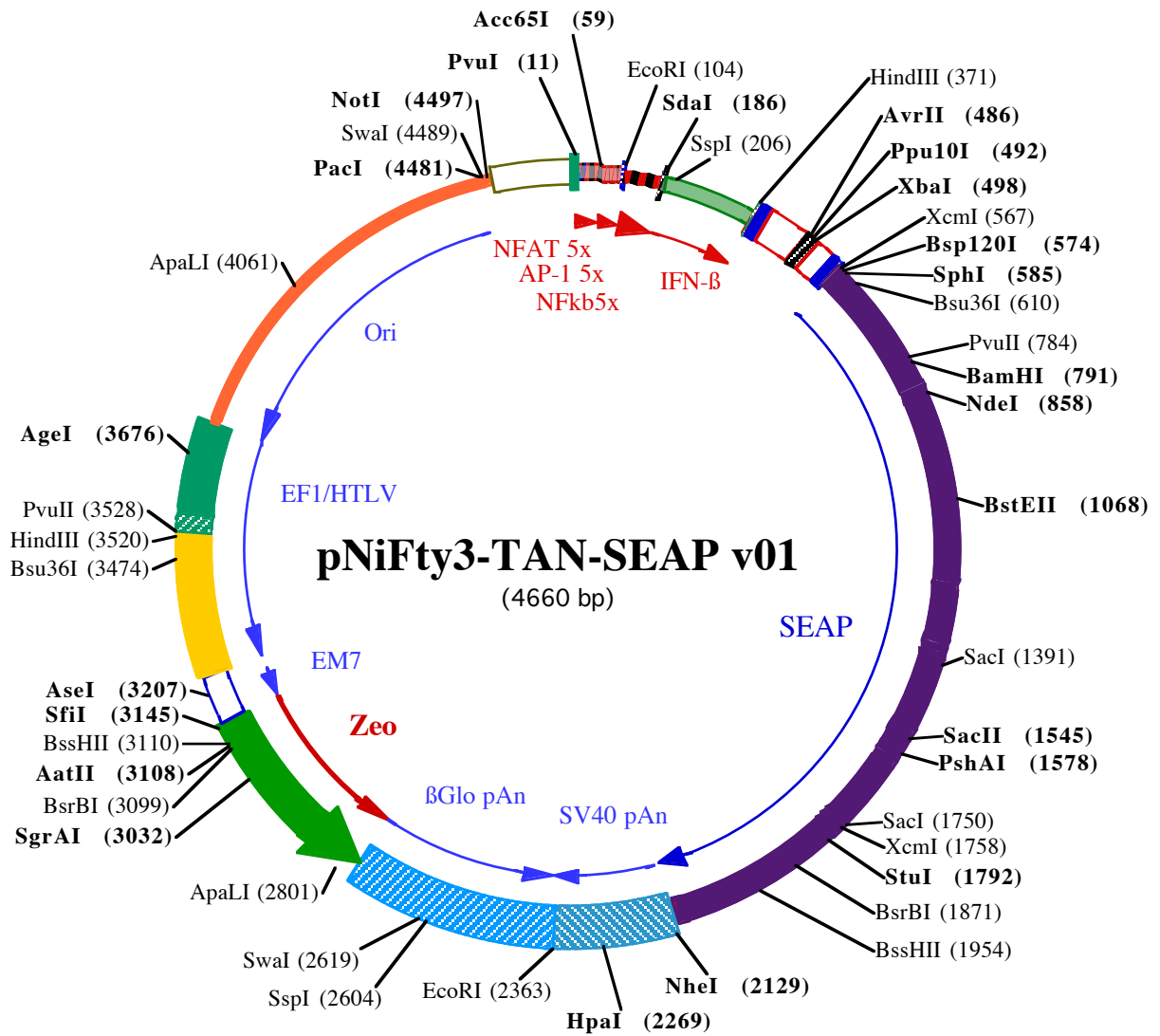
This antibiotic can be used for *E. coli* at 25 µg/ml in liquid or solid media and at 50-200 µg/ml to select Zeocin™-resistant mammalian cells.

References

1. Rao A. et al., 1997. Transcription factors of the NFAT family: regulation and function. *Annu Rev Immunol.* 15:707-47.
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3. Yamasaki S. et al., 2009. C-type lectin Mincle is an activating receptor for pathogenic fungus, *Malassezia* PNAS. 106(6):1897-902.
4. Hess J, et al., 2004. AP-1 subunits: quarrel and harmony among siblings. *J Cell Sci.* 117(Pt 25):5965-73.
5. Kawai T. & Akira S., 2007. Signaling to NF-κB by Toll-like receptors. *Trends Mol Med.* 13(11):460-9.
6. Vojdani G. et al., 1988. Structure and characterization of a murine chromosomal fragment containing the interferon β gene. *J Mol Biol.* 204(2):221-31.
7. Kim et al., 1990. Use of the human elongation factor 1α promoter as a versatile and efficient expression system. *Gene* 91(2): 217-223.
8. Takebe 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.
9. 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.

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1 GGATCTGCGATCGCTGGAAGATTGGAAGACTGGAAGATAGGAAACACTGGAAGAGGTACCTGAGTCACTGACTCAGTGAGTCACTGACTCAGTGAG
PvuI (11) Acc65I (59)
101 TAAGAATTCCTGGGACTTTCCACTGGGACTTTCCACTGGGACTTTCCACTGGGACTTTCCACTCCTGCAGGagcttgaataaa
EcoRI (104) SdaI (186)
201 atgaatattagaagctgttagaataagagaaaatgacagaggaAAACTGAAAGGgAGAAGTAAAGTggaattcctctgaggcagaaggaccatccc
SspI (206)
301 tTATAAtagcacaggccatgaaggaagatcattctcactgcagcctttgacagcctttgacctcttgAGCTTCTGCCCTTCCCTCCTGTGAGT
HindIII (371)
399 TTGgtaagtcaactgactgtctatgctctggaaaggtgggcaggagatggggcagtgccaggaagtggcactatgaacctGCAGCCCTAGGAATGCAT
Ppu10I (492) AvrII (486) XbaI (498)
499 CTAGAcattgtactaaccttcttctcttctctctctctgacagGTTGGTGTACAGTAGCTTCCACCATGATTCTGGGCCCTGCATGCTGCTGCTGCTC
SphI (585) XcmI (567) Bsp120I (574)
599 TGCTGCTGGCCTGAGGCTACAGCTCTCCCTGGGCATCATCCAGTTGAGGAGGAGAACCAGGACTTCTGGAACCGCAGGCGAGCCAGGCCCTGGGTG
12L L L G L R L Q L S L G I I P V E E E N P D F W N R E A A E A L G A
Bsu36I (610)
699 CGCCAAGAAGCTGCAGCCTGCACAGACAGCCGCAAGAACCTCATCATCTTCTGGCGATGGGATGGGGTGTCTACGGTGACAGCTGCCAGGATCCTA
45A K K L Q P A Q T A A K N L I I F L G D G M G V S T V T A A R I L
PvuII (784) BamHI (791)
799 AAAGGGCAGAAGAAGGACAACTGGGCTGAGATACCCTGGCTATGGACCGCTTCCATATGTGGCTCTGTCCAAGACATCAATGTAGACAAACATG
79K G Q K K D K L G P E I P L A M D R F P Y V A L S K T Y N V D K H
899 TGCCAGACAGTGGAGCCACAGCCACGGCCTACCTGTGCGGGTCAAGGGCAACTCCAGACCACTTGGCTTGAAGTGCAGCCGCGCTTAAACCAAGTGC
112V P D S G A T A T A Y L C G V K G N F Q T I G L S A A A R F N Q C N
NdeI (858)
999 CACGACACGGGCAACAGGTCATCTCCGTGATGAATCGGCCAAGAAGCAGGGAAGTCAAGTGGGAGTGGTAACCAACACAGTGCAGCAGCCCTCG
145T T R G N E V I S V M N R A K K A G K S V G V V T T T R V Q H A S
1099 CCAGCCGGCCTACGCCACAGCGTGAACCGCAACTGGTACTCGGACCGCAGCTGCTCCCTCGGCCCGCAGGAGGGGTGCCAGGACATCGCTACGC
179P A G T Y A H T V N R N W Y S D A D V P A S A R Q E G C Q D I A T
1199 AGCTCATCCTCAACATGGACATTGATGTGATCCTGGGTGGAGGCCGAAAGTACATGTTTCGCATGGGAACCCAGACCTGAGTACCCAGATGACTACAG
212Q L I S N M D I D V I L G G G R K Y M F R M G T P D P E Y P D D Y S
BstEII (1068)
1299 CCAAGTGGGACAGGCTGGACGGGAAGAATCTGGTGCAGGAATGGCTGGCAGGCGCAGGGTGCCTGGTATGTGTGAACCGCACTGAGCTCATGCAG
245Q G G T R L D G K N L V Q E W L A K R Q G A R Y V W N R T E L M Q
1399 GCTTCCCTGGACCGTCTGTGACCCATCTCATGGTCTCTTGGACCTGGAGACATGAAATACGAGATCCACCGAGACTCCACACTGGACCCCTCCCTGA
279A S L D P S V T H L M G L F E P G D M K Y E I H R D S T L H R D S L
SacII (1545) PshAI (1578)
1499 TGGAGTGCAGAGGCTGCCCTGCGCCTGTGAGCAGGAAACCCCGGGCTTCTTCTTCTCGTGGAGGGTGGTGCATCGACACCGGTTCATCACGAAAG
312M E M T E A A L R L L S R N P R G F F L F V E G R I D H G H E S
1599 CAGGGCTTACCGGCACTGACTGAGACGATCATGTTGACGACGCCATTGAGAGGGGGGGCAGCTCACCAGCGAGGAGGACACGCTGAGCCTGCTCACT
345R A Y R A L T E T I M F D D A I E R A G Q L T S E E D T L S L V T
XcmI (1758)
1699 GCCGACCACTCCACGCTTCTCCTTCCGAGGCTACCCCTGCGAGGGAGTCCATCTTCCGGCTGGCCCTGGCAAGGCCCGGACAGGAAGGCTTACA
379A D H S H V F S F G G Y P L R G S S I F G L A P G K A R D R K A Y
SacI (1750) StuI (1792)
1799 CGGTCTCTATACGAAACGGTCCAGGCTATGTGCTCAAGGACGGCGCCCGGGCGGATGTTACCGAGAGCGAGAGCGGGAGCCCGAGTATCGGCAGCA
412T V L L Y G N G P G Y V L K D G A R P D V T E S E S G S P E Y R Q Q
BsrBI (1871)
1899 GTCAGCAGTCCCTGGACGAAGAGACCCACGAGGCGAGGACGTGGCGGTGTTCCGCGCGGGCCCGAGGCGCACCTGGTTCACGGCTGCAGGAGCAG
445S A V P L D E E T H A G E D V A V F A R G P Q A H L V H G V Q E Q
1999 ACCTTCATAGCGCACGTGATGGCTTCGCGCCTGCTGGAGCCCTACACCGCTGCGACCTGGCGCCCGCCGCGGACACCGACCGCCGACCCGCG
479T F I A H V M A F A A C L E P Y T A C D L A P P A G T T D A A H P
NheI (2129)
2099 GGCGGTCCCGTCAAGCGTCTGATTGAAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTGGACAAACCACAAGTGAATGCAGTGAAAAA
512G R S R S K R L D •
HpaI (2269)
2199 AATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAAACAACAATTGCATTCATTTTATGTT
EcoRI (2363)
2299 TCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAATTTAAATAACAGCATAGCAAACTTTAACCTC
3399 CAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTC
2499 TTTCATGGAGTTTAAAGATATAGTGTATTTTCCCAAGTTTGAACCTAGCTTTCATTTCTTTATGTTTTAAATGCACTGACCTCCCACTTCCCTTTTATG
SspI (2604) SmaI (2619)
2599 TAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAAATAATGTTTTTATTAGGCAGAAATCCAGATGCTCAAGGCCCTTATAATATCCCCCA
2699 GTTTAGTAGTTGGACTTAGGGAACAAAGAACCTTTAATAGAAATTTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCTGCTCCTCTGCCACA
1274 • G • D Q E E A V
ApaLI (2801)
2799 AAGTGCACGAGTTGCCGCCGGGTGCGCGAGGGGCAACTCCCGCCCGGCTGCTCGCGATCTCGGTTCATGGCCGGCCCGGAGGCGTCCCGGAAGT
1174F H V C N G A P D R L A F E R G W P Q E G I E T M A P G S A D R F N
2899 TCGTGGACACGACCTCCGACCACTCGCGTACAGCTCGTCCAGCCGCGCACCCACAGCCAGGGTGTGTCGGCACCACTGGTCTGGACCGC
844 T S V V E S W E A Y L E D L G R V W V W A L T N D P V V Q D Q V A
SgrAI (3032) SbrBI (3099)
2999 GCTGATGAACAGGTCACGTCGTCGCGGACCAACCGCGAAGTCTGCTCCACGAAGTCCCGGAGAACCCGAGCCGGTCCGAGCACTCGACCGCT
514 S I F L T V D D R V V G A F D D E V F D R S F G L R D T W F E V A

BssHII (3110)
 AatII (3108) SfiI (3145)
 3099 CCGGCACGTCGCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTTGGCCATGATGGCCCTCTATAGTGGTCTATTATACTATGCCGATATACTAT
 174 G A V D R A T L V P V A S T L K A M ←

AseI (3207)
 3199 GCCGATGATTAATTGTCAACTACTGTTTGTAGGCGCCGGTACAGCTTGATCTGTAACGGCGCAGAACAGAAAACGAAACAAAGACGTAGAGTTGAGCA

3299 AGCAGGGTCAGGCAAAGCGTGGAGAGCCGGCTGAGTCTAGGTAGGCTCCAAGGGAGCGCCGGACAAAGGCCCGTCTCGACCTGAGCTTTAAACTTACCT

Bsu36I (3474)
 3399 AGACGGCGGACGCAGTTCAGGAGGCCACACAGGCGGGAGGCGGAGAACCGGCACTCAACCGCGTGGATGGCGGCTCAGGTAGGGCGGCGGCGGTGA

PvuII (3528)
 HindIII (3520)
 3499 AGGAGAGATGCGAGCCCTCGAAGCTTCAGCTGTGTTCTGGCGGCAACCCGTTGCGAAAAAGAACGTTACGCGGACTACTGCACCTTATATACGGTTCT

AgeI (3676)
 3599 CCCCCACCTCGGGAAAAAGGCGGAGCAGTACACGACATCACTTCCAGTTTACCCGCGCCACCTTCTCTAGGCACCGGTTCAATTGCCGACCCCTC
 3699 CCCCCAATTCTCGGGGACTGTGGCGATGTGCGCTCGCCACTGACACATGTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAGGCCGCGTT
 3799 GCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCAAAAATCGACGCTCAAGTCAGAGGTGCCGAAACCCGACAGGACTATAAAGATACCAG
 3899 GCGTTTCCCTGGAAGCTCCCTCGTGCCTCTCTGTTCCGACCTGCCGTTACCGGATACCTGTCCGCTTCTCCCTTCGGGAAGCGTGGCGTTT

ApaLI (4061)
 3999 CTCATAGCTCAGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTGCTCCAAGCTGGGCTGTGTGCAGAACCCCGTTAGCCCGACCGCTGCCCTT
 4099 ATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGC
 4199 GGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAA
 4299 GAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGA

SwaI (4489)
 PacI (4481) NotI (4497)
 4399 AGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTGAACGAAACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCG

4499 GCCGCAATAAAATATCTTTATTTTATTACATCTGTGTGTTGTTTTTTGTGTAATCGTAACTAACATACGCTCTCCATCAAACAAAACGAAACAAAA
 4599 CAAACTAGCAAAATAGGCTGTCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA