

pNiFty3-T-SEAP

NFAT-inducible reporter plasmid selectable with Zeocin™

Catalog code: pnf3-sp5

For research use only

Version 20L03-MM

PRODUCT INFORMATION

Content:

- 20 µg of pNiFty3-T-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

- **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.
- **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 IRF7⁴.
- **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 promoter⁵ and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat⁶. 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 transcription⁷.

1. Rao A. et al., 1997. Transcription factors of the NFAT family: regulation and function. *Annu Rev Immunol.* 15:707-47. 2. Goodridge HS. et al., 2007. Dectin-1 stimulation by *Candida albicans* yeast or zymosan triggers NFAT activation in macrophages and dendritic cells. *J Immunol.* 178(5):3107-15. 3. Yamasaki S. et al., 2009. C-type lectin Mincle is an activating receptor for pathogenic fungus, *Malassezia*. *PNAS.* 106(6):1897-902. 4. Vojdani G. et al., 1988. Structure and characterization of a murine chromosomal fragment containing the interferon beta 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.

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.

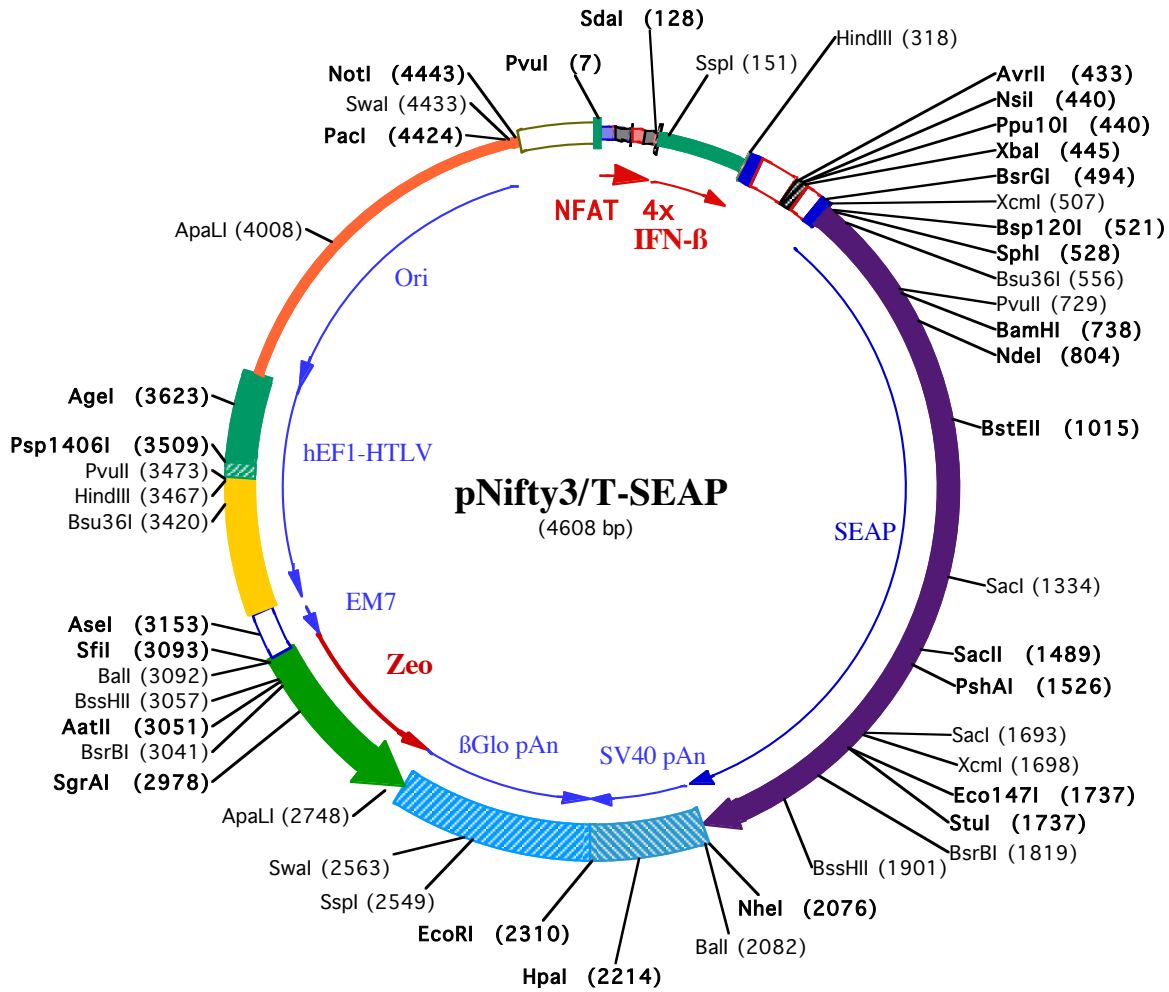
Zeocin™ usage

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
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





125

PvuI (7)
1 GGATCTGCGATCGCGGAGGAAAACTGTTTCATACAGAAGGGTGGAGGAAAACTGTTTCATACAGAAGGGGTGTACCCAAGAGGAAAAATTTGTTTC

SdaI (128) SspI (151)
101 ATACAGAAAGGAGGAAAACTGTTTCATACCTGCAAGGagcttgaataaaatgaatattagaagctgttagaataagagaaatgacagaggaAACTGAAA

GgAGAACTGAAAGTggaattctctgaggcagaagaccatccctTATAAAtagcacaggccatgaaggaagatcattctcactgcagcctttg

HindIII (318)
299 acagcctttgcctcatcttgAGGCTTCTGCTTCTCCCTCTGTGAGTTTgtaagtcactgactgtctatgcctgggaaggggtgggcaggagatgggg

Ppu10I (440)
NsiI (440)
AvrII (433) XbaI (445) BsrGI (494)
399 cagtgcaggaagtgccactatgaaccTGCAGCCCTAGGAATGCATCTAGAcattgactaaccttcttctcttctctctctgacagGTTGGTGTA

XcmI (507) Bsp120I (521) Bsu36I (556)
499 CAGTAGCTTCCACCATGATTCTGGGGCCCTGCATGCTGCTGCTGCTGCTGCTGGGCTGAGGCTACAGCTCCTCCCTGGGCATCATCCCAGTTGAGGA
1 M I L G P C M L L L L L L L G L R L Q L S L G I I P V E E
599 GGAAACCCGGACTTCTGGAACCCGAGGAGGCGAGCCCTGGTCCGCAAGAAGCTGCAGCCTGCACAGACAGCCGCAAGAACCTCATCATCTTC
29 E N P D F W N R E A A E A L A K K L Q P A Q T A A K N L I I F

PvuII (729) BamHI (738)
699 CTGGGCGATGGGATGGGGGTGTCTACGGTACAGCTGCCAGGATCCTAAAAGGGCAGAAGAAGGACAAACTGGGCGTGGAGATACCCCTGGCTATGGACC
63 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 A M D

NdeI (804)
799 GCTTCCCATATGTGGCTCTGTCCAAGACATACATGTAGACAAACATGTGCCAGACAGTGGAGCCACAGCCAGCCCTACCTGTGCGGGGTCAAGGGCAA
96 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 K G N
899 CTTCCAGACCATTTGGCTTGTAGTGCAGCCGCGCTTAAACCAGTGAACACAGACAGCAGCCGCAACGAGGTCATCTCCGTGATGAATCGGGCCAAAGAAAGCA
129 F Q T I G L S A A A R F N Q C N T T R G N E V I S V M N R A K K A

BstEII (1015)
999 GGGAAAGTCAAGTGGGAGTGGTAAACACACACAGTGCAGCAGCCTGCCAGCCGACCTACGCCACACGGTGAACCGCAACTGGTACTCGGACGCGG
163 G K S V G V V T T R V Q H A S P A G T Y A H T V N R N W Y S D A
1099 ACGTGCTGCTCGGCCCGCAGGAGGGGTGCCAGGACATCGCTACGAGCTCATCTCAACATGGACATTTGATGTGATCCTGGTGGAGCCGAAAGTA
196 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 R K Y
1199 CATGTTTCGCATGGGAACCCAGACCCCTGAGTACCCAGTACTACAGCAAGGTGGGACAGGCTGGACGGGAAGAATCTGGTGCAGGAATGGCTGGCG
229 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 W L A

SacI (1334)
1299 AAGCGCCAGGGTCCCGGTATGTGTGAACCGCACTGAGTCTATGCAGGGTTCCTGGACCGTCTGTGACCCATCTCATGGTCTCTTTGAGCCTGGAG
263 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 E P G

SacII (1489)
1399 ACATGAAATACGAGATCCACCGAGACTCCACACTGGACCCCTCCTGATGGAGATGACAGAGGCTGCCCTGCGCTGTGAGCAGGAACCCCGCGCTT
296 D M K Y E I H R D S T L D P S L M E M T E A A L R L L S R N P R G F

PshAI (1526)
1499 CTTCTCTTCTGGAGGGTGGTGCATGACACAGGTCATACGAAAGCAGGGCTTACCGGGCACTGACTGAGACGATCATGTTGACGACGCCATTGAG
329 F L F V E G G R I D H G H C H E S R A Y R A L T E T I M F D D A I E

SacI (1693)
1599 AGGGCGGCCAGCTCACCAGCGAGGAGGACAGCTGAGCCTCGTCACTGCCCAGCAGCTCCACGCTTCTCTCTCGGAGGCTACCCCTGCGAGGGAGCT
363 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 R G S

XcmI (1698) Eco147I (1737) Stul (1737)
1699 CCATCTTCGGGCTGGCCCTGGCAAGGCCCGGACAGGAAGGCTACAGGCTCCTCTATACGAAACGGTCCAGGCTATGTGCTCAAGGACGGCGCCG
396 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 G A R

BsrBI (1819)
1799 GCCGGATGTTACCGAGAGGAGCGGGAGCCCGATATCGGCAGCAGTCAAGCAGTCCCTGGACGAAGAGACCCACGAGCGAGGACGTGGCGGTG
429 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 V A V

BssHII (1901)
1899 TTCGCGCGCGCCCGCAGGCGCACCTGGTTCACGGCGTGCAGGAGCAGACCTTCATAGCGCAGTCAATGGCTTCGCGCGCTGCTGGAGCCCTACACCG
463 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 P Y T

NheI (2076) Ball (2082)
1999 CCTGCGACCTGGCGCCCGCCCGCCAGCAGCGCGCACCCGGGGCGTCCCGGTCCAAGCGTCTGGATTGAAGCTAGCTGGCCAGACATGATAA
496 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 GATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAATAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTA

HpaI (2214)
2199 AAGCTGCAATAAACAAGTTAAACAACAACAATTGCATTCATTTTATGTTTCAGGTTTCAGGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTAC

EcoRI (2310)
2299 AAATGTGGTATGGAATCTAAAATACAGCATAGCAAACTTAACTCCAATCAAGCCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGC
2399 ATCAGGGGCTGTTGCCAATGTGCAATTAGCTGTTTGCAGCCTCACCTCTTTTCATGGAGTTAAGATATAGTGATTTTTCCAAGGTTTGAAGTACTCTT

SspI (2549) SmaI (2563)
2499 CATTCTTTATGTTTAAATGCACTGACCTCCACATTCCTTTTTAGTAAAATATTCAGAAAATAATTAATACATCATGCAATGAAAATAAATGTTT
2599 TTTATTAGGCAGAAATCCAGATGCTCAAGGCCCTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATGGACAG

ApaLI (2748)
2699 CAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCTGCTCCTCTGCCACAAAGTGACGAGTGTGGCGCCGGGTGCGCGAGGGCGAACTCCCGCCCCACG
1274 • G • D Q E E A V F H V C N G A P D R L A F E R G W P
2799 GCTGCTCGCGATCTCGGTTCATGGCCGCGGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGCGGTACAGCTCGTCCAGCGCGCGCAC
1004 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 G R V

SgrAI (2978)
2899 CCACACCCAGGCCAGGTTGTTGTCGGCACCACCTGGTCTGGACCGCGTGATGAACAGGGTACGTCGTCGCCGACACACCGGCAAGTCTGCTCC
674 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 D D E

BssHII (3057) SfiI (3093)
2999 ACGAAGTCCCGGAGAACCCGAGCCGGTCCGAGCAAGTCCGCGGCGAGTCCGCGCGGTGAGCACCAGGCAAGGCACTGGTCAACTTGGCCA
334 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 K A M

AseI (3153)

3099 TGATGGCCCTCCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAACTACTGTTTGTAGGCGCCGGTCACAGCTTGAT
04 ←
3199 CTGTAACGGCGCAGAACAGAAAACGAAACAAAGACGTAGAGTTGAGCAAGCAGGGTCAGGCCAAAGCGTGGAGAGCCGGCTGAGTCTAGGTAGGCTCCAAG
3299 GGAGCGCCGGACAAAGGCCCGGCTCGACCTGAGCTTTAAACTTACCTAGACGGCGGACGCGAGTTCAGGAGGCACCACAGCGGGAGGCGGCAGAACCGG

Bsu36I (3420)

PvuII (3473)

HindIII (3467)

3399 ACTCAACCGGCGTGGATGGCGGCCCTCAGGTAGGGCGGGCGCGCTGAAGGAGAGATGCGAGCCCTCGAAAGCTTCAGCTGTGTTCTGGCGCAAACCGG

Psp1406I (3509)

3499 TTGCGAAAAGAAGCGTTACGGCGACTACTGCACCTTATATACGGTTCTCCCCACCCCTCGGGAAAAGGCGGAGCCAGTACACGACATCACTTTCCAGT

AgeI (3623)

3599 TTACCCCGCGCCACCTTCTCTAGGCACCGGTTCAATTGCCGACCCCTCCCCCAACTTCTCGGGACTGTGGCGATGTGCGCTCTGCCACTGACACAT

3699 GTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAGGCCGCGTGTGCGGTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGA

3799 CGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTCCCCCTGGAAGCTCCCTCGTGCCTCTCTGTTCCGACCCCTGCCG

3899 TTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTGTTGCTCCAA

ApaI (4008)

3999 GCTGGGCTGTGTGCACGAACCCCGGTTACGCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCA

4099 CTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAA

4199 CAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTTTGATCCGGCAAACAACACCGCTGGTAGCGGTGGTTT

4299 TTTTGTTCGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACCTCA

Swal (4433)

PacI (4424)

NotI (4443)

4399 CGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGCCGCAATAAAATATCTTTATTTTTCATTACATCTGTGTGGTTTTTTGTG

4499 TGAATCGTAACTAACATACGCTCTCCATCAAACAAAACGAAACAAAACAACTAGCAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACATTT
4599 CTCTATCGAA