

pNiFty3-N-SEAP

A NF- κ B-inducible reporter plasmid selectable with Zeocin™

Catalog code: pnf3-sp2

For research use only

Version 20L03-MM

PRODUCT INFORMATION

Content:

- 20 μ g of pNiFty3-N-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

• **NF- κ B binding site:** Nuclear factor (NF)- κ B is a “rapid-acting” primary transcription factor activated by a wide variety of PRRs. NF- κ B is a protein complex that belongs to the Rel-homology domain-containing protein family. The prototypical NF- κ B is composed of the p65(RelA) and p50 subunits¹. NF- κ B 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- κ B, IRF3 and IRF7².

• **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-1 α (EF-1 α) 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-1 α promoter exhibits a strong activity and yields long lasting expression of a transgene *in vivo*. The R-U5' has been coupled to the EF-1 α 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*.

• **β Glo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription⁵.

1. **Kawai T. & Akira S., 2007.** Signaling to NF-kappaB by Toll-like receptors. *Trends Mol Med.* 13(11):460-9. 2. **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. 3. **Kim D. et al., 1990.** Use of the human elongation factor 1 α promoter as a versatile and efficient expression system. *Gene* 91(2): 217-23. 4. **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. 5. **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

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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

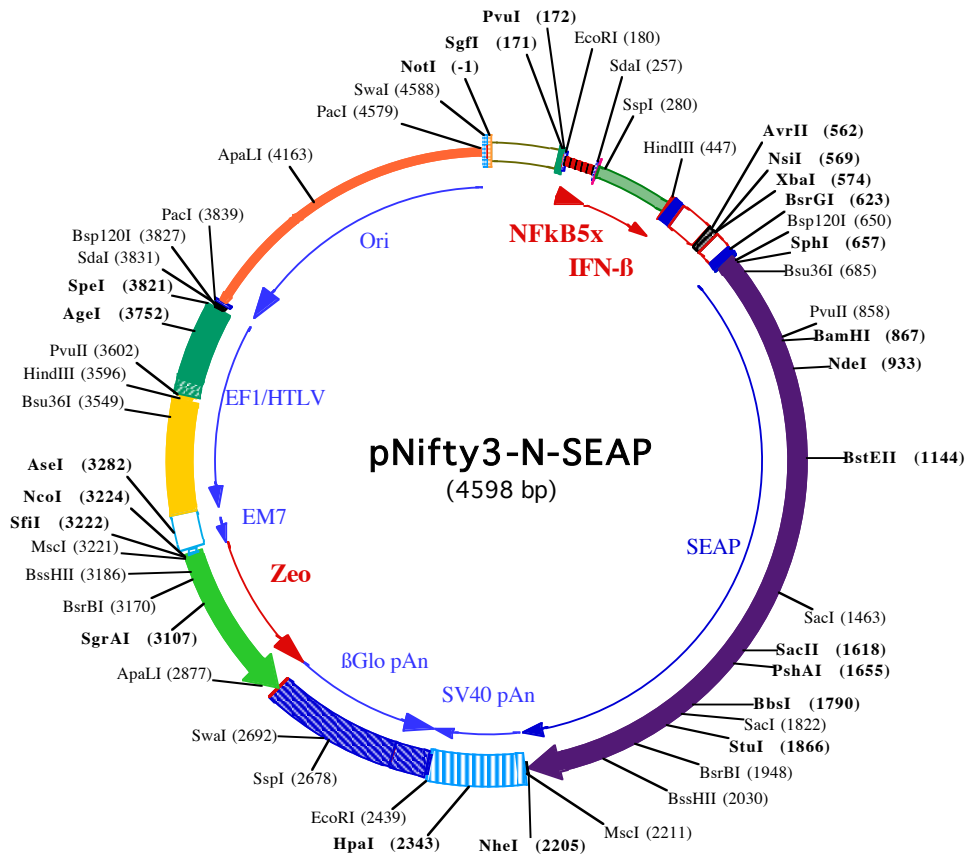
This antibiotic can be used for *E. coli* at 25 µg/ml in liquid or solid media and at 50-200 µg/ml to select Zeocin™-resistant mammalian cells.

RELATED PRODUCTS

Product	Catalog Code
ChemiComp GT116	gt116-11
Zeocin™	ant-zn-1

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NotI (-1)
1 CCGGCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGGTTTTTTTGTGTGAATCGTAACTAACATACGCTCCTCCATCAAACAACAAACGAAACA

                PvuI (172)
101 AAACAACTAGCAAATAGGCTGTCCCGAGTGCAAGTGCAAGTGCCAGAACATTTCTCTATCGAAGGATCTGCGATCGTGAATTCGGGGACTTCCAC

                SgfI (171) EcoRI (180)
201 TGGGGACTTTCCACTGGGGACTTTCCACTGGGGACTTTCCACTGGGGACTTTCCACTCTGCGAGgagcttgaataaaatgaatattagaagctgttagaa

                SdaI (257)                    SspI (280)
301 taagagaaaatgacagaggaAACTGAAAGGgAGAAGCTGAAAGTGGaaattcctctgaggcagaaggaccatccctTATAAAtagacagggcctg

                HindIII (447)
399 aaggaagatcatttctcactgcagcctttgacagcctttgcctcactcttAAAGCTTCTGCCTTCTCCCTCCTGTGAGTTTGtaagtcactgactgtctat

                NsiI (569)
                AvrII (562) XbaI (574)
499 gcctgggaaaggggtggcaggagatgggagctgcaggaaggtggcactatgaaccTGCAGCCCTAGGAATGCATCTAGAc-aattgtactaaccttct

                SphI (657)
599 tctcttttcttctctgacagGTTGGTGTACAGTAGCTTCCACCATGATTCTGGGGCCCTGCATGCTGTGTGCTGCTGCTGCTGGGCCCTGAGGCTACAG

                BsrGI (623)                    Bsp120I (650)                    Bsu36I (685)
699 CTCTCCCTGGGCATCATCCAGTTGAGGAGGAGAACCCGGACTTCTGAAAGCGCGAGGAGCCGAGGCCTGGGTGCCCAAGAAGCTGACGCTGCAC
                10M I L G P C M L L L L L L L G L R L Q
200 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 A K K L Q P A

                PvuII (858) BamHI (867)
799 AGACAGCCGCCAAGAACCTCATCATCTTCTGGCGATGGGATGGGGTGCTACGGTGACAGCTGCCAGGATCCTAAAAGGCCAGAAGAAGGACAAACT
530 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 K G Q K K D K L

                NdeI (933)
899 GGGCCCTGAGATACCCCTGGCTATGGACCCTTCCCATATGTTGGCTCTGTCCAAGACATAAATGTAGACAAACATGTGCCAGACAGTGGAGCCACAGCC
860 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 V P D S G A T A
999 ACGGCCTACCTGTGCGGGGTCAGGGCAACTTCCAGACCATTGGCTTGAGTGCAGCCGCCCTTAAACAGTGCAACACGACACGCGGCAACGAGGTC
1200 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 T T R G N E V

                BstEII (1144)
1099 TCTCCGTGATGAATCGGGCCAAGAAGCAGGGAAGTCACTGGGAGTGGTAAACCCACACGAGTGCAGCACCGCTCGCCAGCCGGCACCTACGCCACAC
1530 I S V M N R A K K A G K S V G V V T T R V Q H A S P A G T Y A H T
1199 GGTGAACCGCAACTGGTACTCGGACGCGGAGTGCCTGCCTCGGCCCCAGGAGGGTGCAGGACATCGCTACGCAGCTCATCTCCAACATGGACATT
1860 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 Q L I S N M D I
1299 GATGTGATCCTGGGTGGGCGGGAAGTACATGTTCGATGGGAACCCAGACCTTGAGTACCCAGATGACTACAGCAAGTGGGACCAGGCTGGACG
2200 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 Q G G R T L D

                SacI (1463)
1399 GGAAGAATCTGGTCAGGAATGGCTGGCAAGCCAGGCGCTTGTGTGAACCGCACTGAGCTCATGCAGGCTTCCCTGGACCCGCTGTGTGAC
2530 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 A S L D P S V T
1499 CCATCTCATGGGTCTCTTGGACCTGGAGACATGAAATACGAGATCCACCGAGACTCCACTGGACCCCTCCCTGATGGAGATGACAGAGGCTGCCCTG
2860 H L M G L F 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 L

                SacII (1618)                    PshAI (1655)
1599 CGCTGCTGAGCAGGAACCCCGGGCTTCTCTCTCTCGTGGAGGGTGGTCGATCGACCAGGTATCACGAAAGCAGGGTTACCGGGCACTGACTG
3200 R L L S R N P 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

                BbsI (1790)
1699 AGACGATCATGTTTCAGCAGCCATTGAGAGGGGGGGCAGCTACCAGCGAGGAGGACACGCTGAGCTCGTCACTGCCACACTCCACGTCTTCTC
3530 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 A D H S H V F S

                SacI (1822)                    StuI (1866)
1799 CTTCGGAGGCTACCCCTCGGAGGAGCTCCATCTTCGGGCTGGCCCTGGCAAGCCCGGGACAGGAAGCCCTACAGGTCCTCTATACGGAAACGGT
3860 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 T V L L Y G N G

                BsrBI (1948)
1899 CCAGGCTATGCTCAAGGACGGCGCCCGGGTATTACCGAGAGCGAGAGCGGGAGCCCGAGTATCGGCAGCAGTACGAGTCCCTTGACGAAG
4200 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 S A V P L D E

                BssHII (2030)
1999 AGACCCACGACGGCAGGAGCTGGCGGTGTTGCGCGCGCGCCGAGCGCACCTGGTTACGGCGTGCAGGAGCAGACCTTCATAGCCACGTATGGC
4530 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 T F I A H V M A
2099 CTTCCCGGCTGCTGGAGCCTACACGCTGCAGCTGGCGCCCCCGCGGCACCACGAGCCGCGCACCCGGGGGGTCCCGGTCGAAGCGCTG
4860 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 G R S R S K R L

                MscI (2211)
2199 GATTGAAGCTAGCTGGCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAAGTGAAGGAAATGCTTTATTTGTGAAATTTGT
5200 D .

                HpaI (2343)
2299 GATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAACAAGTTAAACAACAATTGCATTCAATTTATGTTTCAGGTTACAGGGGGAGGTGGG

                EcoRI (2439)
2399 AGGTTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAATTTCTAAAATACAGCATAGCAAAACCTTAAACCTCCAAATCAAGCCCTCACTTGAATC

                SspI (2678)                    SwaI (2692)
2599 GTATTTTCCAAGGTTTGAAGTAGCTTCTTCTTATGTTTTAAATGCACTGACCTCCACATTCCTTTTATGATAAATATTCAGAAAATAATTTAA
2699 ATACATCATTGCAATGAAAATAAATGTTTTTATTAGGAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGACTTAGGAA

                ApaI (2877)
2799 CAAAGGAACCTTAAATAGAATTTGACAGCAAGAAGCGAGCTTCTAGCTTATCCTCAGTCTGCTCCTGTGCCAAAGTGACAGCTTGCCGGCGGG
                1274 . G . D Q E E A V F H V C N G A P
2899 GTCGCGCAGGGCAACTCCCGCCCCACGGCTGCTCGCGATCTCGGTCATGCGCGCGGAGGCGTCCCGGAAAGTTCTGGACACGACTCCGACACC

                SgrAI (3107)                    BsrBI (3170)                    BssHII (3186)
3099 CCGGACACACCGCGAAGTCTCTCCACGAAGTCCCGGGAACCCGAGCGGTCGTTCCAGAAGCTCAGCCGCTCCGGCGAGCTCGCGCGGGTGG
764 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 S I F L T V D D
3099 TCGGCGTACAGCTCGTCCAGCGCGCACCCACACCCAGGCGAGGTGTTCCCGCACCCCTGGTCTGGACCGCGCTGATGAACAGGGTCAGTCTGT
764 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 S I F L T V D D
3099 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 G A V D R A T L

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NeoI (3224)
 SfiI (3222)
 MscI (3221)

AseI (3282)

3199 CACCGAACGGCACTGGTCAACTTGGCCATGGTGGCCCTCCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGCAACTAC

104 V P V A S T L K A M

3299 TGTTTGTAGGCGCCGGTCACAGCTTGATCTGTAACGGCGCAGAACGAAAAACAAAGACGTAGAGTTGAGCAAGCAGGGTCAGGCAAAAGCGTGGAA

3399 GAGCCGGCTGAGTCTAGGTAGGCTCAAGGGAGCGCCGACAAAGGCCGGTCTCGACCTGAGCTTTAAACTTACCTAGACGGCGGACGAGTTCAGGAG

Bsu36I (3549) HindIII (3596)

3499 GCACCACAGGCGGGAGGCGGAGAACGCGACTCAACCGCGTGGATGGCGGCTCAGGTAGGGCGGGCGCGTGAAGGAGAGATGCCAGCCCTCGAA

PvuII (3602)

3599 GCTTCAGCTGTGTTCTGGCGGCAACCCGTTGCGAAAAAGAACGTTACGGCGACTACTGCACTTATATACGGTTCTCCCCACCCTCGGGAAAAAGGCG

AgeI (3752)

3699 GAGCCAGTACAGACATCACTTTCCAGTTTACCCCGCCACCTTCTCTAGGCACCGTTCAATTGCCGACCCCTCCCCCAACTTCTCGGGACTGTG

Bsp120I (3827)

SpeI (3821) SdaI (3831) PacI (3839)

3799 GCGATGTGCGTCTGCCACTGACTAGTGGCCCTGCAGGTTAATTAGAACAATGTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAAAGGCCGC

3899 GTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATAC

3999 CAGGCGTTTCCCCTGGAAGCTCCCTCGTGGCTCTCTGTCCGACCTGCCGTTACCGGATACCTGTCCGCTTTCTCCCTTGGGAAGCGTGGCGC

ApaLI (4163)

4099 TTTCTCATAGCTACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTGCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTAGCCCGACCGCTGGCG

4199 CTTATCCGGTAACTATCGTCTTGTGCTCAACCCGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGGCGAGGTATGTA

4299 GGCGGTGCTACAGAGTCTTGAAGTGGTGGCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAA

4399 AAAGAGTTGGTAGCTCTTGATCCGGCAACAACACCGCTGGTAGCGGTGGTTTTTTTTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCA

PacI (4579) SmaI (4588)

4499 AGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTGAACGAAAACTCAGTTAAGGGATTTTGGTCATGGCTAGTTAATTAATTAATCA