

pNiFty3-SEAP

Secreted alkaline phosphatase reporter plasmid selectable with Zeocin™

Catalog code: pnf3-sp1

For research use only

Version 20L03-MM

PRODUCT INFORMATION

Content:

- 20 µg of pNiFty3-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.

PLASMID FEATURES

- **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 IRF7¹.
- **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 levels can be evaluated qualitatively with the naked eye and quantitatively using SEAP detection media, such as HEK-Blue™ Detection (catalog code: hb-det1), or the SEAP Reporter Assay Kit (catalog code: rep-sap).
- **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 promoter² and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat³. 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 transcription⁴.

1. **Vodjani G. et al., 1988.** Structure and characterization of a murine chromosomal fragment containing the interferon beta gene. *J Mol Biol.* 204(2):221-31. 2. **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. 3. **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. 4. **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.

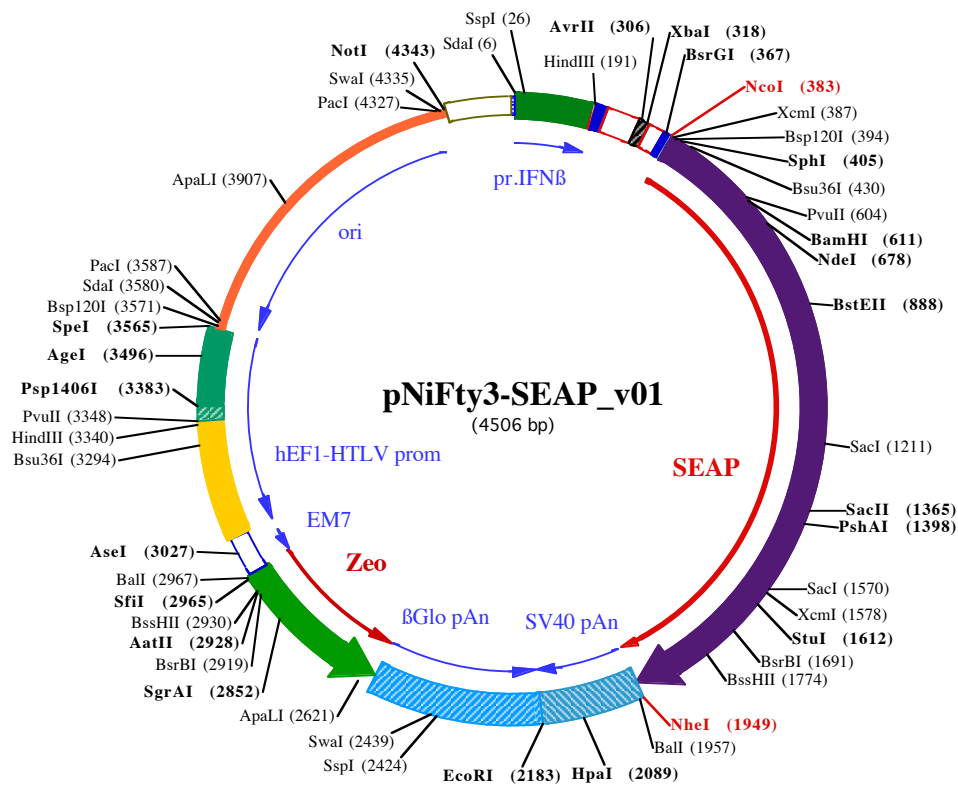
Zeocin™ usage

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
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InvivoGen Hong Kong : +852 3-622-34-80
E-mail: info@invivogen.com





SdaI (6) SspI (26)
1 CTGCTGAGGagcttgaataaaaatgaatattagaagctgttagaataagagaaaatgacagaggaAACTGAAAGGgAGAACTGAAAGTGggaattcctct
HindIII (191)
101 gaggcagaaaggaccatccctTATAAAtagcacaggccatgaaggaagatattctcactgcagcctttgacagcctttgctcatcttgAAGCTTCT
199 GCCTTCTCCCTCTGTGAGTTTgtaagtcaactgactgtctatgcttgggaagggtgggcaggagatggggcagtcaggaagaagtgccactatgaacc
AvrII (306) XbaI (318) BsrGI (367) XcmI (387) NcoI (383) Bsp120I (394)
299 CTGCAGCCCTAGGAATGCATCTAGAcattgtactaaccttcttctcttctcctgacagGTTGGTGTACAGTAGCTTCCACCATGGTCTGGGGCC
1 M V L G P
SphI (405) Bsu36I (430)
399 CTGCATGCTGCTGCTGCTGCTGCTGGGCTGAGGCTACAGCTCTCCCTGGGCATCATCCCAGTTGAGGAGGAGAACCAGGACTTCTGGAACCCGAG
5 C M L L L L L L L 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
499 GCAGCCGAGGCCCTGGGTGCCCAAGAAGCTGCAGCTGCACAGACAGCCGCCAAGAACCTCATCTTCTGGCGATGGGATGGGGGTGTCTACGG
39 A A E A L G A A 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
PvuII (604) BamHI (611) NdeI (678)
599 TGACAGTGCAGGATCCTAAAAGGGCAGAAGAAGGACAACTGGGGCTGAGATACCCTGGCTATGGACCCGCTTCCCATATGTGGCTCTGTCCAAGAC
72 V T A A R I L K G Q K K D K L G P E I P L A M D R F P Y A L S K T
699 ATACAATGTAGACAAACATGTGCCAGACAGTGGAGCCACAGCCAGCCCTACCTGTGCGGGTCAAGGGCAACTTCCAGACCATTGGCTTGTGAGTCAGCC
105 Y N V D K H V 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
BstEII (888)
799 GCCCCTTAAACAGTGAACACGACACGCGGCAACGAGGTCTCCGTGATGAATCGGGCAAGAAAGCAGGGAAGTCAGTGGGAGTGGTAACCA
139 A R F N Q C N T 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
899 CACGAGTGCAGCAGCCCTGCCAGCCGACCTACGCCACAGGTAACCGCAACTGGTACTCGGACGCCGACGTGCTGCTCGCCCGCCAGGAGG
172 T R V Q H A S P 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
999 GTGCCAGGACATCGTACGACGCTCATCTCCAACATGGACATTGATGTGATCCTGGTGGAGCCGAAAGTACATGTTTCGATGGAAACCCAGACCT
205 C Q D I A T Q Q 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
1099 GAGTACCCAGATGACTACAGCCAAGGTGGGACAGGCTGGACGGGAAGAATCTGGTGCAGGAATGGCTGGCGAAGCCAGGTTGCCGGTATGTGTGA
239 E Y P D D Y S Q 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
SaeI (1211)
1199 ACCGCACTGAGCTCATGCAGGCTTCCCTGGACCCGTCTGTGACCCATCTCATGGTCTCTTTGAGCCTGGAGACATGAAATACGAGATCCACCGAGACT
272 N R T E L M Q A 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
SaeII (1365)
1299 CACACTGGACCCCTCCCTGATGGAGATGACAGAGGCTGCCCTGCGCTGCTGAGCAGGAACCCCGCGCTTCTTCTTCTGAGGAGGTGGTGCATC
305 T L D P S L M E M T E A A L R L L S R N P R G F F L F V E G G R I
PshAI (1398)
1399 GACCAGGTGCATCAGAAAGCAGGCTTACCGGGCACTGACTGAGACGATCATGTTTCAGCAGCCATTGAGAGGGGGCCAGCTCACCAGCGAGGAG
339 D H G H H E S R 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
XcmI (1578)
SaeI (1570)
1499 ACACGCTGAGCCTCGTCACTGCCACCACTCCCACGTCTTCTCTCGGAGGCTACCCCTGCGAGGGAGCTCCATCTTGGGCTGGCCCTGGCAAGGC
372 D T L S L V T A 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
StuI (1612)
1599 CCGGGACAGGAAGGCTACACGGTCTCCTATACGAAACGGTCCAGGCTATGTGCTCAAGGACGGCGCCCGCCGGATGTTACCAGAGCGGAGCGGG
405 R D R K A Y T 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
BsrBI (1691)
1699 AGCCCCGAGTATCGGCAGCAGTCAAGCAGTGCCTGGACGAAGAGACCCACGAGGCGAGGACGTGGCGGTGTTCCGCGCGCCGAGCGCACCTGG
439 S P E Y R Q Q S 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
1799 TTCACGGCGTGCAGGAGCAGACCTTATAGCGCACGTATGGCTTCCGCGCTGAGCCCTACACCCGCTGCGACCTGGCGCCCGCCCGCCGCGCAC
472 V H G V Q E Q T F I A H V M A F A A C C L E P Y T A C D L A P P A G T
Ball (1957)
NheI (1949)
1899 CACCGACCCGCGCACCCGGGGCGTCCCGTCCAAGCGTCTGGATTGAAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACAC
505 T D A A H P G R S R S K R L D •
HpaI (2089)
1999 AACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACATTATAAGCTGCAATAAAACAGTTAAACAACA
EcoRI (2183)
2099 AATTGCATTCAATTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAATTTCAAAATACAG
2199 CATAGCAAACTTTAACTCCAAATCAAGCCTCTACTTGAATCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAG
2299 CTGTTTGCAGCCTCACCTTCTTCATGGAGTTTAAGATATAGTGATTTTTCCCAAGTTTGAACCTCTTCTTCTTTATGTTTTAAATGCAGTAC
SspI (2424) SnaI (2439)
2399 CTCCACATTCCTTTTTTAGTAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAATAAATGTTTTTTATTAGGCAGAAATCCAGATGCTCAAG
2499 GCCCTTCATAATATCCCCAGTTTAGTGTGACTTAGGGAACAAAGAACCTTTAATAGAAATTTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTC
1274 • G •
ApaLI (2621)
2599 AGTCTGCTCCTCTGCCACAAAGTGACGCAAGTTGCCGCGGGTGCAGGCGGCAACTCCCGCCACCGGCTGCTCGCGATCTCGGTATGGCCGG
1244 D Q E E A V F 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
2699 CCGGAGGCGTCCGGAAGTTCTGGACACGACCTCCGACACTCGCGTACAGCTCGTCCAGGCGCGCACCCACCCAGGCCAGGTTGTGTCGGC
914 G S A D R F N 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
SgrAI (2852)
2799 ACCACTGGTCTGGACCGCTGATGAACAGGTCACGCTGCTCCGACACACCGGCAAGTCTCTCCACGAAGTCCGGGAGAACCCGAGCCGGT
574 V V Q D Q V A 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
BssHII (2930) SfiI (2965)
2899 CGGTCCAGAAGTCCAGCCGCTCCGCGCAGCTCGCGCGGTTGAGCACCAGGACCGGCAACTGGTCAACTTGGCCATGATGGCCCTCTATAGTGAAGTCTATT
244 T W F E V A G A V D R A T L V P V A S T L K A M
BsrBI (2919) AatII (2928) Ball (2967)
2999 ATACTATGCCGATATACTATGCCGATGATTAATTGCAACTACTGTTTGTAGGCGCGGTCACAGCTTGGATCTGTAAACGGCGCAGAACAGAAAACGAAA
AseI (3027)
3099 CAAAGACGTAGAGTTGAGCAAGCAGGGTTCAGGCAAGCGTGGAGAGCCGGCTGAGTCTAGGTAGGCTCAAGGGAGCGCCGGCAAGGCGCGTCTCGA

3199 CCTGAGCTTTAAACTTACCTAGACGGCGGACGCAGTTTCAGGAGGCCACAGGCCGGGAGGCCGAGAACCGGACTCAACCGGCGTGGATGGCGGCTCAG Bsu36I (3294)

3299 GTAGGGCGGGCGCGTGAAGGAGAGATGCGAGCCCCTCGAAGCTTCAGCTGTGTTCTGGCGGCAAACCGTTGCGAAAAAGAACGTTACCGGCGACTA PvuII (3348)
HindIII (3340) Psp1406I (3383)

3399 CTGCACTTATATACGTTTCTCCCCACCCTCGGAAAAAGCGGAGCCAGTACACGACATCACTTTCCAGTTTACCCGCGCCACCTTCTCTAGGCACC AgeI (3496)

3499 GGTTC AATTGCCGACCCCTCCCCCAACTTCTCGGGGACTGTGGGCGATGTGCGCTCTGCCACTGACTAGTGGGCCCTGCAGGTTAATTAAGAACATGT Bsp120I (3571)
SpeI (3565) SdaI (3580) PacI (3587)

3599 GAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACG

3699 CTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAGATACCAGGCGTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCTGCCGCTT

3799 ACCGGATACCTGTCCGCTTTCTCCCTCGGGAAGCGTGCCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTGCTTCCGCTCAAGC

3899 TGGGCTGTGTGCACGAACCCCGTTAGCCCGACCGCTGCGCCTTATCCGGTAACATATCGTCTTGAGTCCAACCCGTAAGACACGACTTATCGCCACT ApaLI (3907)

3999 GGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACA

4099 GTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAACACCCTGGTAGCGGTGGTTTTT

4199 TTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGTCTGACGCTCAGTGAACGAAAACTCAGC

4299 TTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTGTGTG PacI (4327) SwaI (4335) NotI (4343)

4399 AATCGTAACATAACGCTCTCCATCAAACAAAACGAAACAAAACAACTAGCAAATAGGCTGTCCCCAGTCAAGTGCAAGTGCCAGAACATTTCT

4499 CTATCGAA