

Product usage

Before using this product, please read the Limited Use statement below

Important Limited Use information for pNiFty3-I-SEAP-Zeo

The purchase of the pNiFty3-I-SEAP-Zeo vector conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes.

Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic, or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research.

If the purchaser is unwilling to accept the limitations of this limited use statement, InvivoGen is willing to accept return of the product with a full refund. The product must be returned in resaleable condition. For information on purchasing a license to this product for purposes other than research, contact us at outlicensing@invivogen.com.

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 Asia: +852 3622-3480

E-mail: info@invivogen.com



pNiFty3-I-SEAP-Zeo

IRF-inducible reporter plasmid selectable with Zeocin®

Catalog code: pnf3-sp4

<https://www.invivogen.com/pnifty3-family>

For research use only

Version 24A17-NJ

PRODUCT INFORMATION

Contents

- 20 µg of lyophilized pNiFty3-I-SEAP-Zeo (plasmid 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 is stable for 1 year at -20°C.
- Store Zeocin® at 4°C or -20°C. The expiry date is specified on the product label.

Quality control

- Plasmid construct is confirmed by restriction analysis and full-length open reading frame (ORF) sequencing.
- After purification by ion exchange chromatography, predominant supercoiled conformation is verified by electrophoresis.

PLASMID FEATURES

- **ISRE-5x mIFN-β** is an engineered murine interferon beta (mIFN-β) promoter comprising different positive regulatory domains that bind transcription factors such as NF-κB, IRF3 and IRF71. This minimal promoter is truly IRF-specific due to the addition of several interferon-stimulated response elements (ISRE) repeated transcription factor binding sites (TFBS) (AGTTTCNNTTCC)². This feature also enhances the IRF-mediated transcription of the SEAP reporter gene.
- **SEAP** is a secreted form of human embryonic alkaline phosphatase. It 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 a spectrophotometer in combination with SEAP detection media, such as **HEK-Blue™ Detection**, or **QUANTI-Blue™ Solution**, a SEAP detection reagent.
- **SV40 pAn** is the Simian Virus 40 late polyadenylation (pAn) signal and it enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA³.
- **Ori** is a minimal *E. coli* origin of replication with the same activity as the longer Ori.
- **EF-1α/HTLV hybrid promoter** 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 DNA and RNA. This modification not only increases steady state transcription, but also significantly increases translation efficiency.

Zeocin® antibiotic selection cassette

- **CMV promoter & enhancer** drives the expression of the Zeocin® resistance gene (*Sh ble*) in mammalian cells.
- **EM7** is a bacterial promoter that enables the constitutive expression of the *Sh ble* 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*.
- **Human β-Globin pAn** is a strong polyadenylation (pAn) signal placed downstream of *Sh ble*. The use of β-globin pAn minimizes interference and possible recombination events with the SV40 pAn signal⁶.

PRODUCT INFORMATION

InvivoGen has designed pNiFty3, a collection of inducible reporter plasmids, to monitor pattern recognition receptor (PRR) activation and cytokine signaling upon ligand stimulation. The pNiFty3-I-SEAP-Zeo plasmid features an IRF-inducible SEAP reporter gene under the control of an engineered mIFN-β promoter. This promoter comprises several ISRE repeated TFBS to enhance the IRF-specific transcription. The subsequent expression of SEAP upon receptor activation is readily measurable in the cell culture supernatant when using **QUANTI-Blue™ Solution**, a SEAP detection reagent. The pNiFty3-I-SEAP-Zeo plasmid is selectable with Zeocin® in both *E. coli* and mammalian cells, and can be used to generate stable clones.

METHODS

- **Plasmid resuspension**
 - Quickly spin the tube to pellet the DNA.
 - To obtain a plasmid solution at 1 µg/µl, resuspend the DNA in 20 µl of sterile water. Store the resuspended plasmid at -20°C.
- **Plasmid amplification and cloning**

Plasmid amplification and cloning can be performed in *E. coli* GT115 or other commonly used laboratory *E. coli* strains, such as DH5α.
- **Zeocin® usage**

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

RELATED PRODUCTS

Product	Description	Cat. Code
Zeocin®	Selection antibiotic	ant-zn-1
pNiFty3-I-SEAP-Blasti	Reporter plasmid	pnf3b-sp4
pNiFty3-I-SEAP-Puro	Reporter plasmid	pnf3p-sp4
HEK-Blue™ Detection	SEAP Detection	hb-det2
QUANTI-Blue™ Solution	SEAP Detection	rep-qbs

1. Vojdani G. *et al.*, 1988. J Mol Biol. 204(2):221-31. 2. Wesoly J. *et al.*, 2007. Acta Biochim Pol. 54(1):27-38 3. Carswell S. & Alwine J., 1989. Mol Cell Biol. 9(10):4248-58. 4. Kim D. *et al.*, 1990. Gene 91 (2): 217-223. 5. Takebe Y. *et al.*, 1988. Mol. Cell Biol. 1: 466-472. 6. Yu J. & Russell J., 2001. Mol Cell Biol, 21(17):5879-88.

TECHNICAL SUPPORT

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

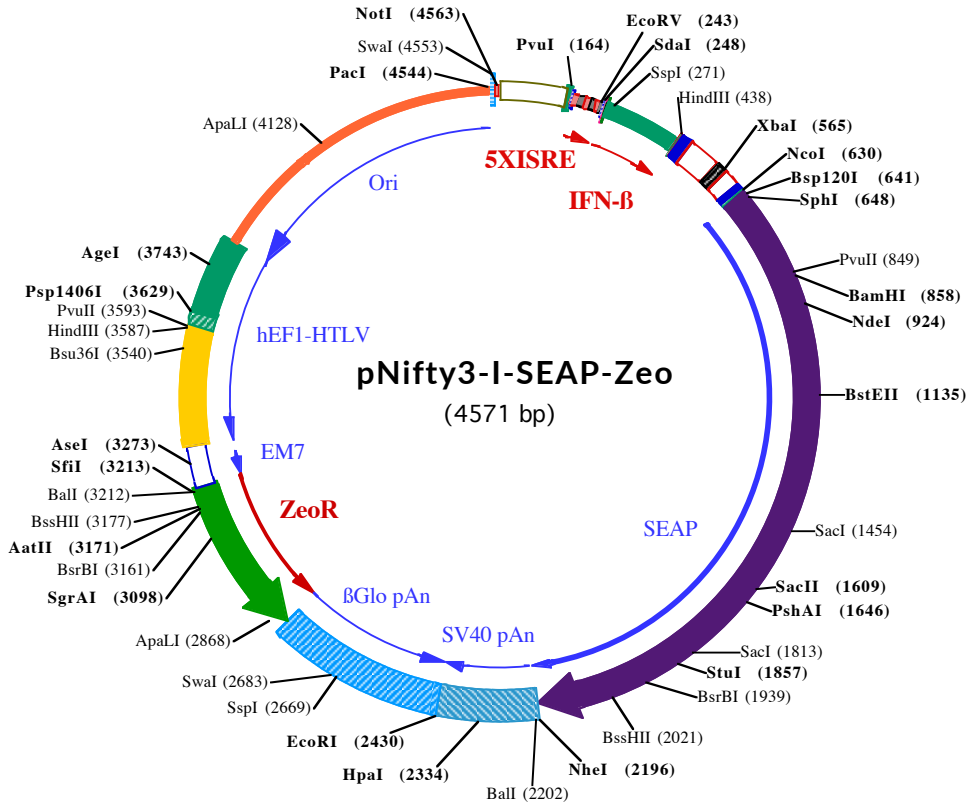
InvivoGen USA (International): +1 (858) 457-5873

InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Asia: +852 3622-3480

E-mail: info@invivogen.com

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www.invivogen.com



1 AATAAAATATCTTTATTTTCATTACATCTGTGTGGTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACAAAC
101 TAGCAAATAGGCTGTCCCCAGTGAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGATCTGCGATCGCTGAATTAGTTTCACTTCCAGTTTCAGTT
PvuI (164)
201 TCCAGTTTCACTTTCAGTTTCATTTTCCAGTTTCACTTTCCTGATATCTCTGACGAGcagcttgaataaaatgaatattagaagctgttagaataagagaaa
SdaI (248) EcoRV (243) SspI (271)
301 atgacagaggaAAACTGAAAGGgAGAAGTGAAGTGGgaaattcctctgaggcagaaaggaccatccctTATAAAtagcacaggccatgaaggaagat
HindIII (438)
399 catttctactgcagcctttgacagcctttgctcatcttAAGCTTCTGCTTCTCCCTCTGTGAGTTTgtaagtcactgactgtctatgctgggaa
XbaI (565)
499 aggggtgggcaggagatggggcagtcaggaaaagtggcactatgaaccTGCAGCCCTAGGAATGCATCTAGAcattgtactaaccttcttctctttcc
SphI (648)
599 tctcctgacagGTTGGTGTACAGTAGCTCCACCATGGTTCTGGGCCCTGCATGTGCTGTGCTGTGCTGGGCTGAGGCTACAGCTCTCCCTG
1 M V L G P C M L L L L L L L L L L G L R L Q L S L
699 GGCATCATCCAGTTGAGGAGGAGAACCCGGACTTCTGGAACCCGAGGCGAGCCGAGGCCCTGGGTGCCGCAAGAAGCTGCAGCTGCACAGACAGCCG
23 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 Q T A
PvuII (849) BamHI (858)
799 CCAAGAACCTCATCATCTTCTGGGCGATGGGATGGGGGTGTCTACGGTGACAGCTGCCAGGATCTAAAAGGGCAGAAGAAGGACAACTGGGGCTGA
56 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 G P E
NdeI (924)
899 GATACCCCTGGCTATGGACCGCTTCCCATATGTGGCTCTGTCCAAGACATACAATGTAGACAAACATGTGCCAGACAGTGGAGCCACAGCCACGGCTAC
89 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 T A Y
999 CTGTGGGGGTCAAGGGCAACTTCCAGACCATTTGGCTTGTGAGTGCAGCCGCTTTAACCAGTGCAACACGACACGGCGCAACGAGGTCATCTCCGTGA
123 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 I S V
BstEII (1135)
1099 TGAATCGGGCCAAGAAAGCAGGGAAGTCAAGTGGAGTGGTAACACACAGTGCAGCAGCCCTCGCCAGCCGGCACCTACGCCACACGGTGAACCG
156 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 V N R
1199 CAACTGGTACTCGGACGCCAGCTGCCCTCGGCCCGCAGGAGGGTGCAGGACATCGCTACGAGCTCATCTCAACATGGACATTGATGTGATC
189 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 D V I
1299 CTGGGTGGAGGCCAAAATACATGTTTCGCATGGGAACCCAGACCCCTGAGTACCCAGATGACTACAGCCAAGGTGGGACAGGCTGGACGGGAAGATC
223 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 T R L D G K N
SacI (1454)
1399 TGGTGCAGGAATGGCTGGCGAAGCCAGGGTGGCCGGTATGTGTGAAACCGCACTGAGCTCATGCAGGCTTCCCTGGACCCGTGTGACCCATCTCAT
256 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 H L M
1499 GGGTCTCTTTGAGCCTGGAGACATGAAATACGAGATCCACCGAGACTCCACTGGACCCCTCCCTGATGGAGATGACAGAGGCTGCCCTGCGCTGTG
289 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 R L L
SacII (1609) PshAI (1646)
1599 AGCAGGAACCCCGCGGCTTCTTCTCTTCTGTTGGGGTGGTGCATCGACCCGGTGCATCACGAAAGCAGGGCTTACCGGGCACTGACTGAGACGATCA
323 S R N P R G F F L V E G F R I D H G H H E S R A Y R A L T E T I
1699 TGTTCCAGCAGCCATTGAGAGGGCGGCCAGCTCACCAGCAGGAGGACAGCTGAGCCTCGTCACTGCCACCACTCCACGCTTCTTCTTCTCGGAGG
356 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 F G G
SacI (1813) StuI (1857)
1799 CTACCCCTGCGAGGGAGCTCCATCTTCCGGCTGGCCCTGGCAAGCCCGGGACAGGAAGCCCTACACGGTCTCTATACGAAACGGTCCAGGCTAT
389 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 P G Y
BsrBI (1939)
1899 GTGCTCAAGGACGGCGCCGGCCGGATGTTACCAGAGCGAGAGCGGGAGCCCGGAGTATCGGCAGCAGTACGAGTCCCTGGACGAAGAGACCCACG
423 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 E T H
BssHII (2021)
1999 CAGGCGAGGACGTGGCGGTGTTCCGCGCGGGCCCGCAGGCGCACCTGTTTACGGCGTGCAGGAGCAGACCTTATAGCGCACGTCATGGCCTTCCGGCG
456 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 F A A
NheI (2196)
2099 CTGCCTGGAGCCCTACACCGCTGCGACCTGGCGCCCGCGGCACCCAGCCGCGCACCCGGGGCGGTCCCGGTCCAAGCGTCTGGATTGAAGC
489 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 D •
Ball (2202)
2199 TAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAATAAATGCTTTATTTGTGAAATTTGTGATGCTATT
HpaI (2334)
2299 GCTTTATTTGTAACCATTATAAGCTGCAATAAACAAAGTTAAACAACAACAAATGCAATTCATTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTT
EcoRI (2430)
2399 AAAGCAAGTAAACCTCTACAATGTGGTATGGAATCTAAATACAGCATAGCAAAAATTTAACTCCAATCAAGCCTCTACTTGAATCCTTTTCTGA
2499 GGGATGAATAAGGCATAGGCATCAGGGGCTGTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTCATGGAGTTAAGATATAGTGATTTTTCC
SspI (2669) SmaI (2683)
2599 CAAGGTTTGAAGTACTTCTTCTTATGTTTAAATGCATGACCTCCACATTCCTTTTTAGTAAATATTCAGAAATAATTTAAATACATCAT
2699 TGCAATGAAATAAATGTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTCATAATATCCCCAGTTTAGTAGTTGACTTAGGGAACAAAGGAAC
ApaLI (2868)
2799 CTTTAATAGAAATTTGACAGCAAGAAAGCGAGCTTCTAGCTTATCTCTCAGTCTGCTCTGCCACAAAGTGACGAGTTGCCGGCCGGTCCGCGAG
1274 • G • D Q E E A V F H V C N G A P D R L
2899 GCGAAGTCCCGCCCGCCAGGCTGCTCGCGATCTCGGTATGCGCCGCGGAGGCGTCCCGAAGTTCTGTGGACACGACCTCCGACCACTCGCGTAC
1074 A F E R 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
2999 AGCTCGTCCAGGCGCGCACCCACCCAGGCGAGGTTGTCCGGCACCCTGGTCTGGACCGCTGATGAACAGGTCACGTCGTCGGGACCA
734 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 R V V
BssHII (3177)
SgrAI (3098) BsrBI (3161) AatII (3171)
3099 CACCGCGAAGTCTCTCCACGAAGTCCCGGGAACCCGAGCCGGTCCGTTCCAGAAGTCCGCGCTCCGCGCAGCTCGCGCGCGGTGAGCACCGGAAC
404 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 V P V
SfiI (3213)
Ball (3212) AseI (3273)
3199 GGCAGTGGTCAACTTGGCCATGATGGCCCTCTATAGTGTGCTGTTATTACTATGCCGATATACTATGCCGATGATTAATGTCAACTACTGTTGTAG
74 A S T L K A M
3299 GCGCGGTCACAGCTTGACTGTAAAGCGGCGAGAACGAAACAAAGACGTAGAGTTGAGCAAGCAGGGTCAGGCAAGCGTGGAGAGCCGGCT

3399 GAGTCTAGGTAGGCTCCAAGGGAGCGCCGGACAAAGGCCGGTCTCGACCTGAGCTTTAAACTTACCTAGACGGCGGACGCAGTTCAGGAGGCACCACAG
PvuII (3593)
3499 GCGGGAGGCGGCAGAACCGGACTCAACCGGCGTGGATGGCGGCCCTCAGGTAGGGCGGGCGCGTGAAGGAGAGATGCGAGCCCTCGAAGCTTCAGCT
Bsu36I (3540) HindIII (3587)
3599 GTGTTCTGGCGCAAACCCGTTGCGAAAAAGAACGTTACGGCGACTACTGCACCTTATATACGGTTCTCCCCACCCCTCGGGAAAAAGCGGAGCCAGTA
Psp1406I (3629)
3699 CACGACATCACTTTCCAGTTTACCCCGCGCCACCTTCTCTAGGCACCGTTCAATTGCCGACCCCTCCCCCAACTTCTCGGGGACTGTGGGCGATGTG
AgeI (3743)
3799 CGCTCTGCCACTGACACATGTGAGCAAAAGCCAGCAAAAGCCAGGAACCGTAAAAAGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTG
3899 ACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGCGTTTTCCCTGGAAGCTCCCTCGTGCCTC
3999 TCCGTTCGACCCCTGCCGCTTACCGGATACCTGTCCGCCTTCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCAGCTGTAGGTATCTCAGTTCC
ApaLI (4128)
4099 GTGTAGGTCGTTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTACGCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGG
4199 TAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAA
4299 CTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAACAACCC
4399 ACCGCTGGTAGCGGTGGTTTTTTTGGTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGTCTGACG
SwaI (4553)
4499 CTCAGTGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGC
PaeI (4544) NotI (4563)