

Product usage

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

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

The purchase of the pNiFty3-I-SEAP-Puro 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-Puro

IRF-inducible reporter plasmid selectable with Puromycin

Catalog code: pnf3p-sp4

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

For research use only

Version 24A17-NJ

PRODUCT INFORMATION

Contents

- 20 µg of lyophilized pNiFty3-I-SEAP-Puro (plasmid DNA)
- 1 ml of Puromycin (10 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 Puromycin 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.

Puromycin antibiotic selection cassette

- **CMV promoter & enhancer** drives the expression of the Puromycin resistance gene (*Pac*) in mammalian cells.
- **EM7** is a bacterial promoter that enables the constitutive expression of the *Pac* gene in *E. coli*.
- **Puro (resistance to the antibiotic Puromycin)** is conferred by the *Pac* gene from *Streptomyces* which encodes a N-acetyl-transferase. The *Pac* 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 *Pac*. 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-Puro 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-Puro plasmid is selectable with **Puromycin** 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.
- **Puromycin usage**

Puromycin can be used at 100-125 µg/ml in *E. coli* in liquid or solid media and at 1-10 µg/ml to select Puromycin-resistant mammalian cells.

RELATED PRODUCTS

Product	Description	Cat. Code
Puromycin	Selection antibiotic	ant-pr-1
pNiFty3-I-SEAP-Blasti	Reporter plasmid	pnf3b-sp4
pNiFty3-I-SEAP-Zeo	Reporter plasmid	pnf3-sp4
HEK-Blue™ Detection	SEAP Detection	hb-det2
QUANTI-Blue™ Solution	SEAP Detection	rep-qbs

1. Vlodjani 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

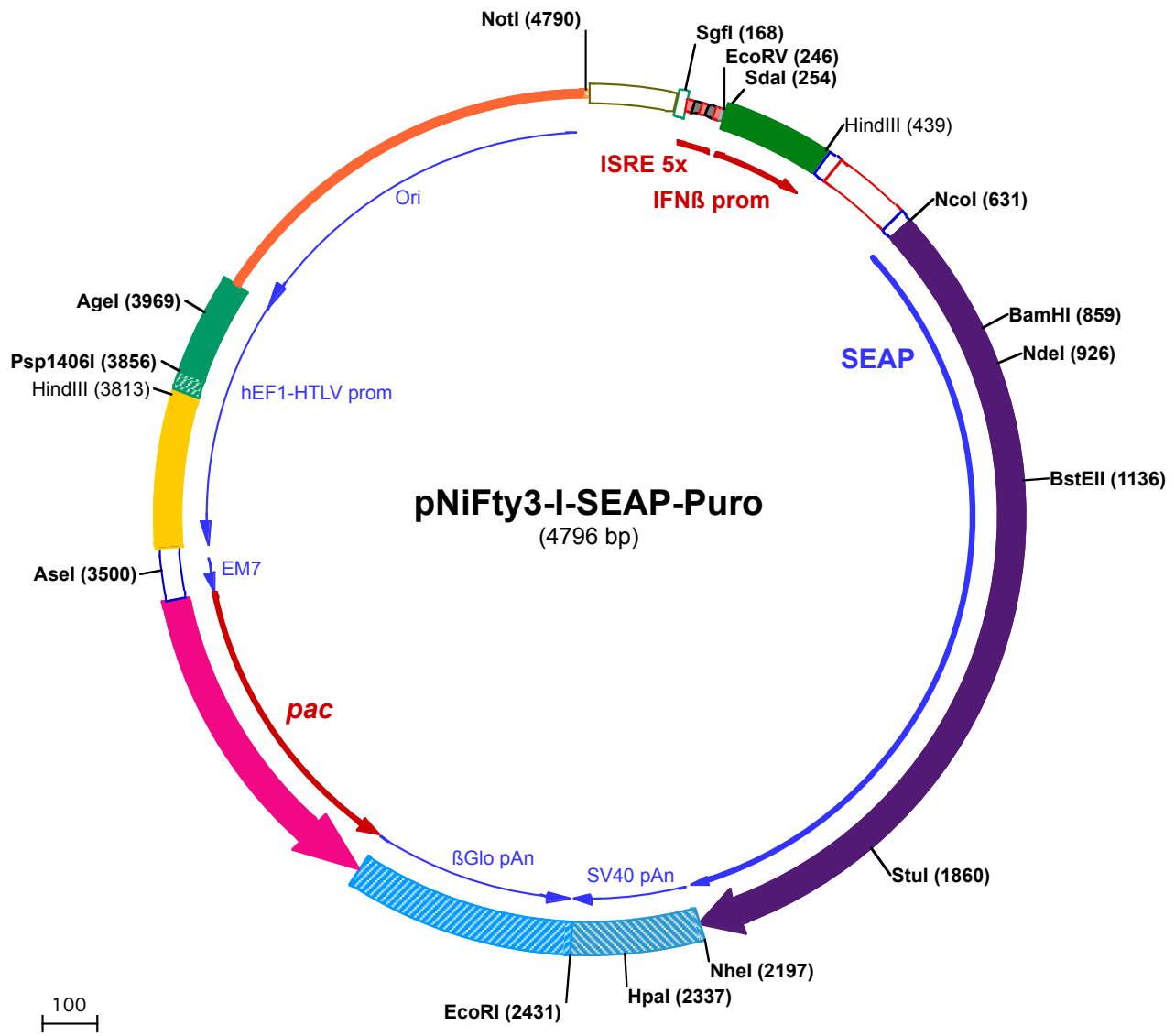
InvivoGen USA (Toll-Free): 888-457-5873

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



2825 AGCGAGCTTCTAGCTCAGGTTTAAGCTCCAGGCTTCCTTGTCATGCACCAAGTTCCTGGGCCCTTCTGGAACCTCAACATCAGCTGTCACAGTGAATCCCA
200 • A G P K R T M C W T R P G E P V E V D A T V T F G L

2925 GTCTTTTCATAAAAAGGCAGGTTTCTGGGAGCAGAAGTTTCCAGAAAAGGCAGGAAGCTCCAGCCCTTTCAGCAGCTTCAACTCCAGGCAGAACAAACAGCAGA
173 R E Y F P L N R P A S T E L F A P V G A R E A A E V G P L V V A S

3025 TCCCAGACCCTTCCCTGGTGGTCAGGGCTCACTCCAACAGTTGCCAGAAACCAAGCTGGCTCTTTTGGCCTGTGTGGTGCCAGCAGACCTCCATTTGT
140 G L G K G Q H D P S V G V T A L F W A P E K P R H P A L L G E M Q

3125 TGTTGTGCTGCCAGCCTGCTTCCAGAGAGCTCAGCCATTCTTGGTCCAATTTTCAGCAAAAACAGCACCAGCTTCAACAGACTCAGGTGTTGCCAAACTG
106 Q Q A A L R S G S L E A M R P G I E A F V A G A E V S E P T T W V A

3225 CAACAGCAGCTCCATCATCTGCAACCCAAACTTTTCCAATGTCAGTCCACTCTGGTGAGGAAGAGTTCTTGCACTTCTGCACCCTCTCAATGTGCCT
73 V A A G D D A V W V K G I D L G V R T L F L E Q L E T V R E I H R

3325 GTCAAGGTCAACTGTGTGCCTTGTGCAAGGAGTCTGCAAAAAGCAGCAGCCAGTGTCTCACAGCTTGGAAACATCATCTGTTGCCAGCCTCACT
40 D P D V T H R T A P Y D A F A A A L T R V A R P V D D R T A L R V

AseI (3500)

3425 GTGGGTTTGTACTCAGTCATGGTGGCCCTCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAACTACTGTTTGTAGG
6 T P K Y E T M

3525 CGCCGGTCACAGCTTGATCTGTAAACGGCGCAGAACAGAAAACGAAACAAAGACGTAGAGTTGAGCAAGCAGGGTCAGGCAAAGCGTGGAGAGCCGGCTG

3625 AGTCTAGGTAGGCTCCAAGGGAGCGCCGGACAAAAGGCCGGTCTCGACCTGAGCTTTAAACTTACCTAGACGGCGGACGCAGTTCAGGAGGCCACACAGG

HindIII (3813)

3725 CGGGAGGCGGCAGAACCGGACTCAACCGGCGTGGATGGCGGCCTCAGGTAGGGCGGCGGGCGCGTGAAGGAGAGATGCCAGCCCTCGAAGCTTCAGCTG

Psp1406I (3856)

3825 TGTTCTGGCGGCAAACCCGTTGCGAAAAAGAACGTTACCGGCGACTACTGCACTTATATACGGTTCTCCCCACCCCTCGGGAAAAAGGCGGAGCCAGTAC

AgeI (3969)

3925 ACGACATCACTTTCCAGTTTACCCCGCCACCTTCTTAGGCACCGTTCAATTGCCGACCCCTCCCCCAACTTCTCGGGGACTGTGGGCGATGTGC

4025 GCTCTGCCACTGACACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGA

4125 CGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCTGGAAGCTCCCTCGTGCGCTCT

4225 CCTGTTCCGACCTGCGCCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGAGGTATCTCAGTTCGG

4325 TGTAGGTCGTTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTACGCCGACCGCTGCGCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGT

4425 AAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAAC

4525 TACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCA

4625 CCGCTGGTAGCGGTGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGC

NotI (4790)

4725 TCAGTGGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGC