

# Product usage

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

## Important Limited Use information for pNiFty2-N-SEAP-Puro

The purchase of the pNiFty2-N-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.

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### TECHNICAL SUPPORT

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# pNiFty2-N-SEAP-Puro

NF- $\kappa$ B-inducible reporter plasmid selectable with Puromycin

Catalog code: pnf2p-sp1

<https://www.invivogen.com/pnifty2-family-puro>

For research use only

Version 24A17-NJ

## PRODUCT INFORMATION

### Contents

- 20  $\mu$ g of lyophilized pNiFty2-N-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

- **NF- $\kappa$ B-5x ELAM** is an engineered ELAM (endothelial cell-leukocyte adhesion molecule) promoter combined with five NF- $\kappa$ B repeated transcription factor binding sites (TFBS) (GGGGACTTTCC)<sup>1</sup>. This minimal promoter is truly NF- $\kappa$ B-specific, as it lacks an AP-1/CREB site found in the full-length promoter<sup>1,2</sup>. The addition of the five TFBS enhances the NF- $\kappa$ B-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 catalyzes 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 mRNA<sup>3</sup>.
- **Ori** is a minimal *E. coli* origin of replication with the same activity as the longer Ori.
- **EF-1 $\alpha$ /HTLV hybrid promoter** is a composite promoter comprising the Elongation Factor-1 $\alpha$  (EF-1 $\alpha$ ) core promoter<sup>4</sup> and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat<sup>5</sup>. The EF-1 $\alpha$  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 $\alpha$  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  $\beta$ -Globin pAn** is a strong polyadenylation (pAn) signal placed downstream of *Pac*. The use of  $\beta$ -globin pAn minimizes interference and possible recombination events with the SV40 pAn signal<sup>6</sup>.

## PRODUCT INFORMATION

InvivoGen has designed pNiFty2, a collection of inducible reporter plasmids, to monitor pattern recognition receptor (PRR) activation and cytokine signaling upon ligand stimulation. The pNiFty2-N-SEAP-Puro plasmid features an NF- $\kappa$ B-inducible SEAP reporter gene under the control of an engineered ELAM promoter. This promoter comprises five NF- $\kappa$ B repeated TFBS to enhance the NF- $\kappa$ B-mediated 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 pNiFty2-N-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  $\mu$ g/ $\mu$ l, resuspend the DNA in 20  $\mu$ l 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 **DH5 $\alpha$** .
- **Puromycin usage**

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

## RELATED PRODUCTS

Product	Description	Cat. Code
Puromycin	Selection antibiotic	ant-pr-1
pNiFty2-N-SEAP-Blasti	Reporter plasmid	pnf2b-sp1
pNiFty2-N-SEAP-Zeo	Reporter plasmid	pnf2-sp1
QUANTI-Blue™ Solution	SEAP Detection	rep-qbs

1. Schindler U., Baichwal VR., 1994. Mol Cell Biol. 14(9):5820-31. 2. Jensen LE. & Whitehead AS., 2003. Biotechniques 35:54-58. 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

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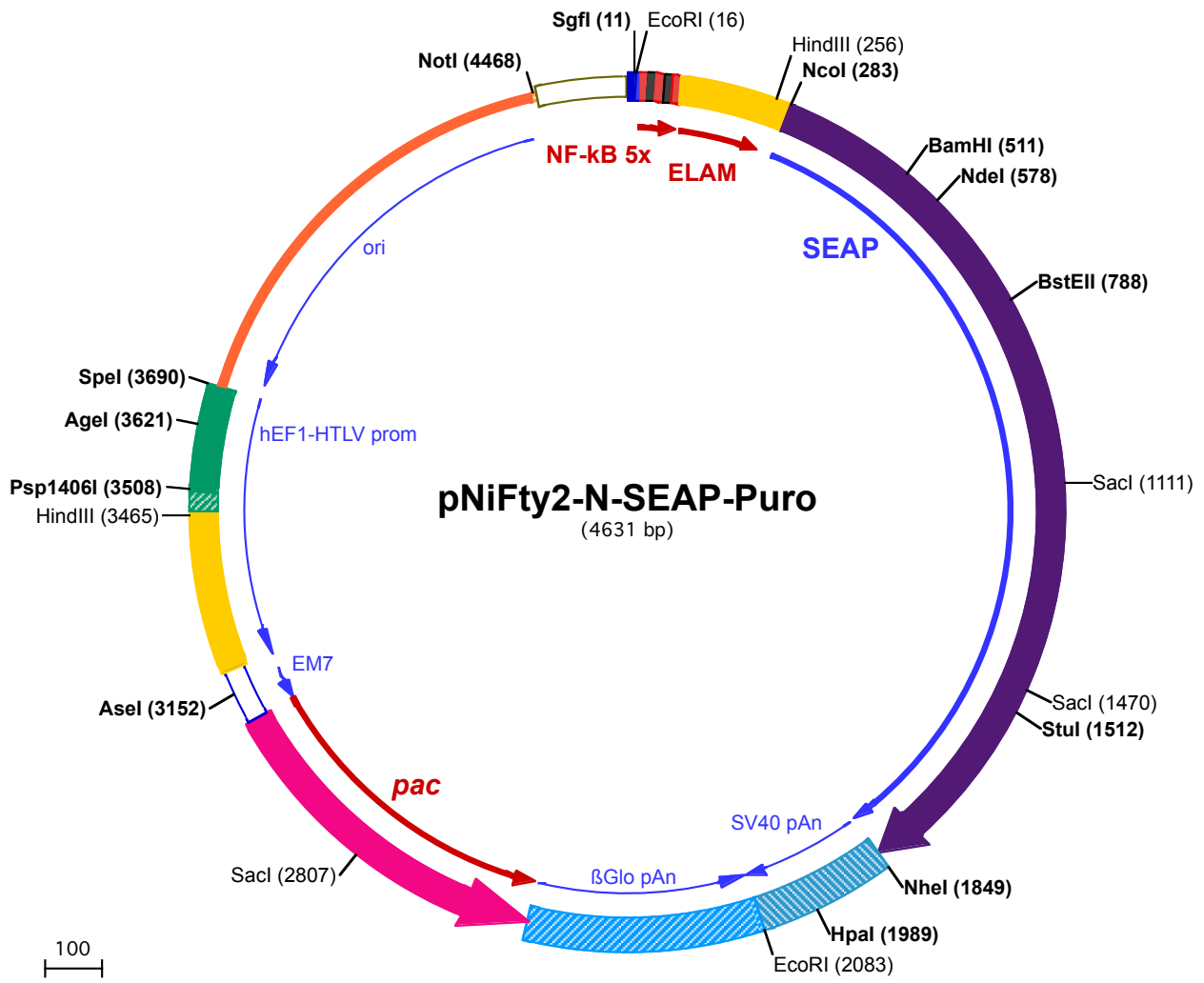
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**Sgfl (11)** **EcoRI (16)**  
1 GGATCTGCATCGCTGAATTC**TGGGGACTTTCCACTGGGGACTTTCCACTGGGGACTTTCCACTGGGGACTTTCCACTCCTGCAGC**  
101 **AGTGGATATTTCCAGAAAAC**TTTTTGGATGCAGTTGGGGATTCTCTTTACTGGATGTGGACAATATCTCTCTATTATTCACAGGAAGCAATCCCTCCT

**HindIII (256)** **NcoI (283)**  
201 **ATAAAAAGGGCCTCAGCAGAAGTAGTGTTCAGCTGTTCTTGGCTGACTTCA**CATCAAAGCTTCTATACTGACCTGAGACAGAGC**CATGGTTCTGGGGCCCT**  
301 GCATGCTGCTGCTGCTGCTGCTGCTGGCCTGAGGCTACAGCTCTCCCTGGGCATCATCCAGTTGAGGAGGAGAACCCGGACTTCTGGAACCGGAGGC  
6▶ **C M L 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 A**  
401 AGCCGAGGCCCTGGTGCCGCAAGAAGCTGCAGCCTGCACAGACAGCCGCAAGAACCTCATCATCTTCTGGGCGATGGGATGGGGGTGTCTACGGTG  
39▶ **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 V**

**BamHI (511)** **NdeI (578)**  
501 ACAGCTGCCAGGATCCTAAAAGGGCAGAAGAAGGACAAACTGGGGCCTGAGATACCCCTGGCTATGGACCGCTTCCATATGTGGCTCTGTCCAAGACAT  
73▶ **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 V A L S K T**  
601 ACAATGTAGACAAACATGTGCCAGACAGTGGAGCCACAGCCACGGCCTACCTGTGCGGGTCAAGGGCAACTTCCAGACCATTTGGCTTGAAGTCCAGCCG  
106▶ **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 A**

**BstEII (788)**  
701 CCGCTTTAACAGTGCAACACGACACGCGGCAACGAGGTCATCTCCGTGATGAATCGGGCCAAGAAAGCAGGGAAGTCAAGTGGGAGTGGTAACCAACACA  
139▶ **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 T**  
801 CGAGTGCAGCAGCCCTCGCCAGCCGGCACCTACGCCACCGTGAACCGCAACTGGTACTCGGACGCCGACGTGCCCTCGGCCCGCCAGGAGGGGT  
173▶ **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**  
901 GCCAGGACATCGCTACGCACTCATCTCCAACATGGACATTGATGTATCCTGGTGGAGGCCGAAAGTACATGTTTCGCATGGGAACCCAGCCCTGA  
206▶ **C Q D I A T 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 E**  
1001 GTACCCAGATGACTACAGCAAGGTGGGACCAGGCTGGACGGGAAGATCTGGTGCAGGAATGGTGGCGAAGCGCCAGGTTGCCCGTATGTGTGGAAC  
239▶ **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 N**

**SacI (1111)**  
1101 CGCATGAGCTCATGCAGGCTTCCCTGGACCCGTCTGTGACCCATCTCATGGTCTCTTTGAGCCTGGAGACATGAAATACGAGATCCACCGAGACTCCA  
273▶ **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**  
1201 CACTGGACCCCTCCCTGATGGAGATGACAGAGGCTGCCTGCGCTGCTGAGCAGAAACCCCGCGGCTTCTTCTCTTCTGAGGGGTGGTGCATCGA  
306▶ **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 D**  
1301 CCACGGTCATCACGAAAGCAGGGCTTACCGGGCACTGACTGAGACGATCATGTTGACGACGCCATTGAGAGGGCGGGCCAGCTCACCAGCGAGGAGGAC  
339▶ **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 D**

**SacI (1470)**  
1401 ACCTGAGCCTCGTCACTGCCGACCACTCCACGTCTTCTCCTCGGAGGCTACCCCTGCGAGGGAGCTCCATCTTGGGCTGGCCCTGGCAAGGCC  
373▶ **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 (1512)**  
1501 GGGACAGGAAGGCTACACGGTCTCCTATACGGAAACGGTCCAGGCTATGTGCTCAAGGACGGCGCCCGCCGGATGTTACCGAGAGCGAGAGCGGGAG  
406▶ **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 S**  
1601 CCCCGAGTATCGGAGCAGTCAAGTCCCTGGACGAAGAGACCCACGCGAGGCGAGGAGTGGCGGTGTTGCGCGCGGCCCGCAGGCGCACCTGGTT  
439▶ **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 V**  
1701 CACGGCGTGCAGGAGCAGACCTTATAGCGCACGTATGGCCTTCCGCGCTGCTGGAGCCCTACACCGCTGCGACCTGGCGCCCGCCCGCCGACCA  
473▶ **H G V Q E Q T F I A H V M A F A A C L E P Y T A C D L A P P A G T**

**NheI (1849)**  
1801 CCGACCCGCGCACCCGGGGCGGTCCCGTCAAGCGTCTGGATTGAAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAA  
506▶ **T D A A H P G R S R S K R L D •**

**HpaI (1989)**  
1901 **CTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAAACAAGTTAACAAACAACA**

**EcoRI (2083)**  
2001 **TTCATTCAATTTATGTTTCAGTTTCAGGGGAGGTGTGGGAGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATTC**TAATAACAGCA  
2101 **TAGCAAACTTTAACCTCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCT**

2201 **GTTTGCAGCCTCACCTTCTTTATGAGTAAAGATATAGTGTATTTTCCCAAGTGTGAACTAGCTTTCATTTCTTTATGTTTTAAATGACTGACCT**

2301 **CCCACATTCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGC**

2401 **CCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTTGGACAGCAAGAAAGCGAGCTTCTAGCTCAGGTTTAA**  
200◀ •

2501 **GCTCCAGGCTTCTTGTGCATGCACCAAGTTCTTGGCCTTCTGGAACCTCAACATCAGCTGTACAGTGAATCCAGTCTTTTATAAAAAGGACAGGTTT**  
198◀ **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 R E Y F P L N R**  
2601 **TGGGAGCAGAAGTTTCCAGAAAGGCAGGAATCCAGCCCTTTCAGCAGCTTCAACTCCAGGCAGAACACAGCAGATCCAGACCTTTTCCCTGGTGGTC**  
165◀ **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 G L G K G Q H D**  
2701 **AGGGCTCACTCCAACAGTTGCCAGAAACCAAGTGGCTCTTTTGGCCTGTGTGGTGCCAGCAGACCTCCATTTGTTGTTGTGCTGCCAGCCTGCTTCCA**  
132◀ **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 Q Q A A L R S G**

SacI (2807)

2801 GAGAGCTCAGCCATTCTTGGTCCAATTTGAGCAAAAACAGCACCAGCTTCAACAGACTCAGGTGTTGTCCAAACTGCAACAGCAGCTCCATCATCTGCAA  
98 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 V A A G D D A V  
2901 CCCAAACTTTTCCAATGTCAGTCCCCTCTGGTGAGGAAGAGTTCTTGAGTCTGTCCACCTCTCAATGTGCCTGTCAGGGTCAACTGTGTGCCTTGT  
65 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 D P D V T H R T  
3001 TGCAGGGTAGTCTGCAAAAGCAGCAGCCAGTGTCTCACAGCTCTTGGAAACATCATCTCTGGTTGCCAGCCTCACTGTGGGTTTGTACTCAGTCATGGTG  
32 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 T P K Y E T M

Asel (3152)

3101 GCCCTCTATAGTGAGTCGATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAACTACTGTTTGTAGGCGCCGGTCACAGCTTGATCTGTGA  
3201 ACGGCGCAGAACAGAAAACGAAACAAAGACGTAGAGTTGAGCAAGCAGGGTCAGGCAAAGCGTGGAGAGCCGGCTGAGTCTAGGTAGGCTCCAAGGGAGC  
3301 GCCGGACAAAAGGCCCGTCTCGACCTGAGCTTTAAACTTACCTAGACGGCGGACGCAGTTCAGGAGGCCACCACAGGCGGGAGGCGGCAGAACGCGACTCA

HindIII (3465)

3401 ACCGGCGTGGATGGCGGCCTCAGGTAGGGCGGCGGCGCGTGAAGGAGAGATGCGAGCCCCCTGAAGCTTCAGCTGTGTTCTGGCGGCAAACCCGTTGCG

Psp1406I (3508)

3501 AAAAAGAAGCTTACGGCGACTACTGCCTTATATACGGTTCTCCCCACCCTCGGGAAAAGGCGGAGCCAGTACACGACATCACTTTCCAGTTTACC

AgeI (3621)

SpeI (3690)

3601 CCGCGCCACCTTCTTAGGCACCGGTTCAATTGCCGCCCTCCCCCAACTTCTCGGGACTGTGGCGATGTGCGCTCTGCCACTGACTAGTGGGCC  
3701 CTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGCCGCTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCT  
3801 GACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTTCCCCTGGAAGCTCCCTCGTGCCT  
3901 CTCCTGTTCCGACCCTGCCGTTACCGGATACCTGTCCGCTTCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTATCTCAGTTC  
4001 GGTGTAGGTCGTTCCGCTCAAGCTGGCTGTGTGCACGAACCCCGTTCAGCCCGACCGCTGCGCTTATCCGGTAACTATCGTCTTGAGTCCAACCCG  
4101 GTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTCTACAGAGTCTTGAAGTGGTGGCCTA  
4201 ACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAC  
4301 CACCGCTGGTAGCGGTGGTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGAC

NotI (4468)

4401 GCTCAGTGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAATATCTTTATTTTCATTACA  
4501 TCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAAACAAAACGAAACAAAACAACTAGCAAAATAGGCTGTCCCAGTGCA  
4601 AGTGCAGGTGCCAGAACATTTCTATCGAA