

# Product usage

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

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

The purchase of the pNiFty2-N-SEAP-Blasti 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.

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### TECHNICAL SUPPORT

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# pNiFty2-N-SEAP-Blasti

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

Catalog code: pnf2b-sp1

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

For research use only

Version 24A17-NJ

## PRODUCT INFORMATION

### Contents

- 20  $\mu$ g of lyophilized pNiFty2-N-SEAP-Blasti (plasmid DNA)
- 2 x 1 ml of Blasticidin (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 Blasticidin 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.

### Blasticidin antibiotic selection cassette

- **CMV promoter & enhancer** drives the expression of the Blasticidin resistance gene (*Bsr*) in mammalian cells.
- **EM7** is a bacterial promoter that enables the constitutive expression of the *Bsr* gene in *E. coli*.
- **Blasti (resistance to the antibiotic Blasticidin)** is conferred by the *Bsr* gene from *Bacillus cereus*. It 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 *Bsr*. 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-Blasti 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-Blasti plasmid is selectable with **Blasticidin** 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 $\alpha$ .
- **Blasticidin usage**

Blasticidin can be used at 25-100  $\mu$ g/ml in *E. coli* in liquid or solid media and at 1-30  $\mu$ g/ml to select Blasticidin-resistant mammalian cells.

## RELATED PRODUCTS

Product	Description	Cat. Code
Blasticidin	Selection antibiotic	ant-bl-1
pNiFty2-N-SEAP-Puro	Reporter plasmid	pnf2p-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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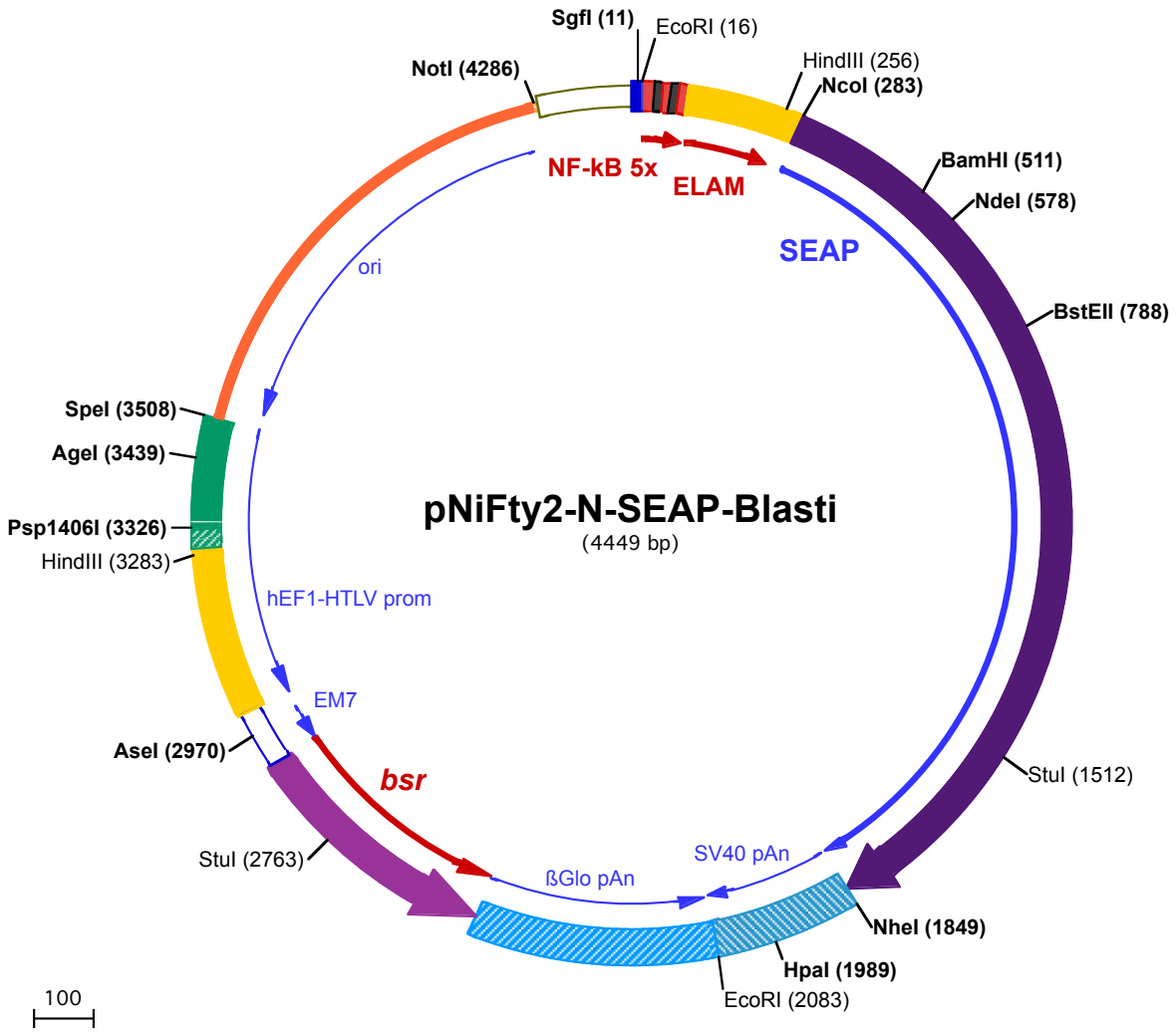
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**Sgfl (11) EcoRI (16)**  
1 GGATCTGCGATCGCTGAATTCTGGGGACTTTCCACTGGGGACTTTCCACTGGGGACTTTCCACTGGGGACTTTCCACTCCTGCAGC

101 AGTGGATATTTCCAGAAAACTTTTTGGATGCAGTTGGGGATTTCCTCTTTACTGGATGTGGACAATATCCTCTATTATTACAGGAAGCAATCCTCTCT

**HindIII (256) NcoI (283)**  
201 AAAAAAGGCGCTCAGCAGAAGTAGTGTTTCAGCTGTTCTTGCTGACTTACATCAAAGCTTCTATACTGACCTGAGACAGAGC CATGGTTCTGGGGCCCT  
1 M V L G P

301 GCATGCTGCTGCTGCTGCTGCTGCTGCTGGGCGTGGGCTACAGCTCTCCCTGGGCGATCATCCCAGTTGAGGAGGAGAACCCGACTTCTGGAACCGGAGGC  
6 C M L L 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 AGCCGAGGCCCTGGTGCCGCAAGAAGCTGCAGCCTGCACAGACAGCCGCAAGAAGCTCATCATCTTCTGGCGATGGGATGGGGGTGTCTACGGTG  
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 ACAGTGCCAGGATCCTAAAAGGCGAGAAGAAGGACAAACTGGGCGTGGATACCCCTGGCTATGGACCGCTTCCATATGTGGCTCTGTCCAAGACAT  
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 ACAATGTAGACAAACATGTGCCAGACAGTGGAGCCACAGCCACGGCTACCTGTGCGGGTCAAGGGCACTTCCAGACCATTTGGCTGAGTGACGCCG  
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 CCGCTTTAACAGTGCAACACGACAGCGGGCAACGAGGTCATCTCCGTGATGAATCGGGCCAAGAAAGCAGGGAAGTCACTGGGAGTGGAACCAACACA  
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 CGAGTGCAGCAGCGCTCGCCAGCCGCACTACGCCACCGTGAACCCGCACTGGTACTCGGACGCGCAGCTGCCTGCCTCGGCCCGCCAGGAGGGGT  
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 GCCAGGACATCGCTACGCACTCATCTCAACATGGACATTGATGATCCTGGTGGAGGCCGAAAGTACATGTTTCGCATGGGAACCCCACTCTGA  
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 GTACCCAGATGACTACAGCCAAGGTGGAGCCAGGCTGGACGGGAAGATCTGGTGCAGGAATGGTGGCAAGCGCCAGGGTGCCTGGTGTGTGGAAC  
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  
1101 CGCACTGAGCTCATGCAGGCTTCCCTGGACCGTGTGTGACCCATCTCATGGGTCTCTTTGAGCCTGGAGACATGAAATACGAGATCCACCGAGACTCA  
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 CACTGGACCCCTCCCTGATGGAGATGACAGAGGCTGCCCTGCGCTGCTGAGCAGGAACCCCGCGGCTTCTCTCTTCTGTGGAGGTTGGTGCATCGA  
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 CCACGGTCATCACGAAAGCAGGGCTTACCGGGCACTGACTGAGACGATCATGTTCCAGCAGCATTGAGAGGGCGGGCCAGCTCACAGAGGAGGAC  
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  
1401 ACCTGAGCCTCGTCACTGCCGACCACTCCACGTCTTCTCTCGGAGGCTACCCCTCGGAGGGAGCTCCATCTCGGGCTGGCCCTGGCAAGGCC  
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 GGGACAGGAAGGCTACACGGTCTCCTATAACGAAACGGTCCAGGCTATGTGCTCAAGGACGGCCGCGCCGATGTTACCAGAGCGAGAGCGGGAG  
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 CCCGAGTATCGGCAGCAGTGCAGTCCCCCTGGACGAAGAGACCCACGAGGCGAGGACGTGGCGGTTCGCGCGCGCCCGAGGCACCTGGTT  
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 CACGGCGTGCAGGAGCAGACCTCATAGCGCACGTATGGCTTCGCGCCTGCTGGAGCCCTACACCGCTGCGACCTGGCGCCCCCGCCGCCACA  
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 CCGACCCGCGCACCCGGGGCGTCCCGTCCAAGCGTCTGGATTGAAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGACAAACCA  
506 T D A A H P G R S R S K R L D •

**HpaI (1989)**  
1901 CTAGAATGCAGTGAATAAATGCTTTATTTGTAATTTGTATGCTATTGCTTTATTTGTAACCATTAAGCTGCAATAAAACAAGTAAACAACA  
•

**EcoRI (2083)**  
2001 TTGCATTCATTTATGTTTCAGGTTAGGGGGAGTGTGGAGGTTTTTAAAGCAAGTAAAACCTCTACAAATGGTATGGAATTCTAAAATACAGCA  
•

2101 TAGCAAACTTTAACCCTCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCT  
•

2201 GTTTCAGCCTCAGTCTCTTTCATGAGTTTTAAGATATAGTGTATTTCCCAAGTGGTAACTAGCTTTCATTTCTTTATGTTTTAAATGACTGACCT  
•

2301 CCCAATTCCTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGCAGAATCCAGATGCTCAAGGC  
•

2401 CTTTCATAATATCCCCAGTTTGTAGTTGACTTGGGGAACAAAGAACCTTAAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTTAGTTCCT  
•

2501 GGTGACTTGAGGGGATGAGTTCCTCAATGGTGGTTTTGACCAGTTGCCATTCATCTCAATGAGCACAAGCAGTCAGGAGCATAGTCAGAGATGAGC  
138 T Y K L P I L E E I T T K V L K G N M E I L V F C D P A Y D S I L  
2601 TCTCTGCACATGCCACAGGGGCTGACCACCCTGATGGATCTGTCCACCTCATCAGAGTAGGGGTGCTGACAGCCACAATGGTGTCAAAGTCCTTCTGCC  
104 E R C M G C P S V V R I S R D V E D S Y P H R V A V I T D F D K Q G

**StuI (2763)**  
2701 CGTTGCTCAGCAGACCCAATGGCAATGGCTCAGCAGACAGTACCCTGCAATGTAGGCTCAATGTGGACAGCAGAGATGATCTCCCAGTCTT  
71 N S V A S G I A I A E A C V T V R G I Y A E I H V A S I I E G T K

2801 GGCCTGATGGCCGCCCGACATGGTGCTTGTGTCTCATAGAGCATGGTGATCTTCTCAGTGGCGACCTCCACCAGCTCCAGATCCTGCTGAGAGATG  
38 T R I A A G V H H K N D E Y L M T I K E T A V E V L E L D Q Q S I  
AseI (2970)  
2901 TTGAAGTCTTCATGGTGGCCCTCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAACTACTGTTGTAGGCGCCGG  
4 N F T K M  
3001 TCACAGCTTGATCTGTAACGGCGCAGAACAGAAAACGAAACAAAGACGTAGAGTTGAGCAAGCAGGGTCAGGCAAAGCGTGGAGAGCCGGCTGAGTCTA  
3101 GGTAGGCTCAAGGGAGCGCCGACAAAGGCCGGTCTCGACCTGAGCTTTAAACTTACCTAGACGGCGGACGCAGTTCAGGAGGCACCACAGGCGGGAG  
HindIII (3283)  
3201 GCGGCAGAACCGGACTCAACCGCGTGGATGGCGGCCTCAGGTAGGGCGGGCGCGTGAAGGAGAGATGCGAGCCCTCGAAGCTTCAGCTGTGTCT  
Psp1406I (3326)  
3301 GCGGCAAACCCGTTGCGAAAAAGAACGTTACGGCGACTACTGCACTTATATACGGTCTCCCCACCCTCGGAAAAAGCGGAGCCAGTACACGACA  
AgeI (3439)  
3401 TCACTTTCCAGTTTACCCCGCCACCTTCTTAGGCACCGTTCAATTGCCGACCCTCCCCCAACTTCTCGGGACTGTGGCGATGTGCGCTCTG  
SpeI (3508)  
3501 CCCACTGACTAGTGGCCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAGGCCAGGAACCGTAAAAAGGCCGCTTCTGGCGTTTTTC  
3601 CATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTCCCCCTG  
3701 GAAGTCCCTCGTGCCTCTCCTGTTCCGACCCTGCCGTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCAG  
3801 CTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTCAGCCCGACCCTGCGCTTATCCGGTAACTAT  
3901 CGTCTTGAGTCCAACCCGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGT  
4001 TCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTC  
4101 TTGATCCGGCAAACAACCACCGCTGGTAGCGGTGTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATC  
NotI (4286)  
4201 TTTTCTACGGGTCTGACGCTCAGTGAACGAAAACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGAATAAAAT  
4301 ATCTTTATTTTATTACATCTGTGTGTTGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAACAAAACGAAACAAAACAACTAGCAAAA  
4401 TAGGCTGTCCCGAGTGAAGTGCAGGTGCCAGAACATTTCTCTATCGAA