

Product usage

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

Important Limited Use information for pNiFty2-N-Fluc-Blasti

The purchase of the pNiFty2-N-Fluc-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.

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

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pNiFty2-N-Fluc-Blasti

NF- κ B-inducible reporter plasmid selectable with Blasticidin

Catalog code: pnf2b-fluc

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

For research use only

Version 23H16-AK

PRODUCT INFORMATION

Contents

- 20 μ g of lyophilized pNiFty2-N-Fluc-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- κ B-5x ELAM** is an engineered ELAM (endothelial cell-leukocyte adhesion molecule) promoter combined with five NF- κ B repeated transcription factor binding sites (TFBS) (GGGGACTTCC)¹. This minimal promoter is truly NF- κ B-specific, as it lacks an AP-1/CREB site found in the full-length promoter^{1,2}. The addition of the five TFBS enhances the NF- κ B-mediated transcription of the SEAP reporter gene.
- **Fluc:** The *firefly luciferase (Fluc)* gene encodes for an intracellular luciferase of fireflies and click beetles. This enzyme interacts with D-luciferin as a chemiluminescent substrate to produce light emission peaking at 560 nm. After cell lysis, the reaction can be measured and detected simply, rapidly and with good sensitivity by means of a luminescence-measuring instrument.
- **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-1 α /HTLV hybrid promoter** is a composite promoter comprising the Elongation Factor-1 α (EF-1 α) 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-1 α 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 α 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*.
- **Bsr (Blasticidin resistance gene)** is conferred by the *Bsr* gene from *Bacillus cereus*. It is driven by the CMV promoter/enhancer 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 *Bsr*. The use of β -globin pAn minimizes interference and possible recombination events with the SV40 pAn signal⁶.

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-Fluc-Blasti plasmid features an NF- κ B-inducible *Firefly Luciferase (Fluc)* reporter gene under the control of an engineered ELAM promoter. This promoter comprises five NF- κ B repeated TFBS to enhance the NF- κ B-mediated transcription. The subsequent expression of Fluc can be measured and detected simply, rapidly and with good sensitivity by means of a luminescence-measuring instrument. Of note, the Firefly luciferase remains intracellular, and requires cell lysis in order to measure bioluminescence. The pNiFty2-N-Fluc-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 μ g/ μ l, resuspend the DNA in 20 μ 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 α .
- **Blasticidin usage**

Blasticidin should be used at 25-100 μ g/ml in bacteria and 1-30 μ g/ml in mammalian cells. Blasticidin is supplied at 10 mg/ml in HEPES buffer.

RELATED PRODUCTS

Product	Description	Cat. Code
Blasticidin	Selection antibiotic	ant-bl-1
pNiFty2-N-Fluc-Zeo	Reporter plasmid	pnf2-fluc
pNiFty2-N-Fluc-Puro	Reporter plasmid	pnf2p-fluc

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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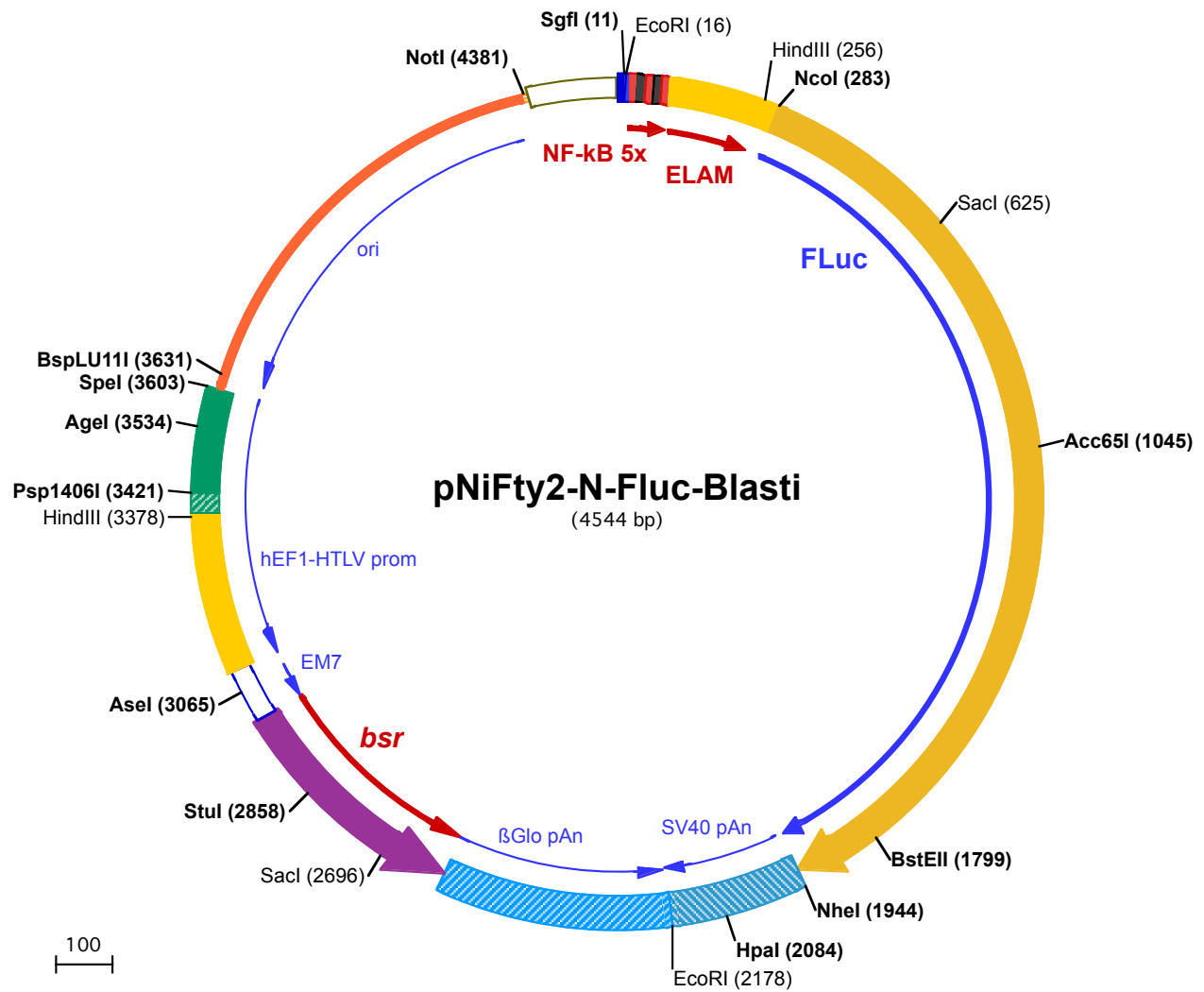
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Sgfl (11) **EcoRI (16)**
 1 GGATCTGCATCGCTGAATTC**TGGGACTTTCCACTGGGACTTTCCACTGGGACTTTCCACTGGGACTTTCCACTCCTGCAGC**
 101 AGTGGATATTTCCAGAAAAC**TTTTGGATGCAGTTGGGATTTCCTCTTTACTGGATGTGGACAATATCCTCTATTATTCACAGGAAGCAATCCCTCCT**

HindIII (256) **NcoI (283)**
 201 ATAAAAGGGCCTCAGCAGAAGTAGTGTTCAGCTGTTCTTGGCTGACTTCACATCAAAGCTTCTATACTGACCTGAGACAGAGCCATGGAGGATGCCAAGA
 301 ATATTAAGAAAGGCCCTGCCCATTTCTACCCTCTGGAAGATGGCACTGCTGGTGAGCAACTGCACAAGGCCATGAAGAGGTATGCCCTGGTCCCTGGCAC
 6N I K K G P A P F Y P L E D G T A G E Q L H K A M K R Y A L V P G T
 401 CATTGCCCTTCACTGATGCTCACATTGAGGTGGACATCACCTATGCTGAATACTTTGAGATGCTGTGAGGCTGGCAGAAGCCATGAAAAGATATGGACTG
 39 I A F T D A H I E V D I T Y A E Y F E M S V R L A E A M K R Y G L
 501 AACACCAACCACAGGATTGTGGTGTGCTCTGAGAACTCTCCAGTCTTCATGCCTGTGTTAGGAGCCCTGTTCACTGGAGTGGCTGTGCCCTGCCA
 73 N T N H R I V V C S E N S L Q F F M P V L G A L F I G V A V A P A
SacI (625)
 601 ATGACATCTACAATGAGAGAGCTCCTGAACAGCATGGGCATCAGCCAGCCAACCTGTGGTCTTTGTGAGCAAGAAGGCCCTGCAAAAAGATCCTGAATGT
 106 N D I Y N E R E L L N S M G I S Q P T V V F V S K K G L Q K I L N V
 701 GCAGAAGAAGCTGCCCATCATCCAGAAGATCATCATCATGGACAGCAAGACTGACTACCAGGGCTTCCAGAGCATGTATACCTTTGTGACCAGCCACTTA
 139 Q K K L P I I Q K I I I M D S K T D Y Q G F Q S M Y T F V T S H L
 801 CCCCTGGCTTCAATGAGTATGACTTTGTGCCCTGAGAGCTTTGACAGGGACAAGACCATTGCTCTGATTATGAACAGCTCTGGCTCCACTGGACTGCCA
 173 P P G F N E Y D F V P E S F D R D K T I A L I M N S S G S T G L P
 901 AAGGTGTGGCTCTGCCCCACAGAAGCTTGTGTGAGATTGAGCCATGCCAGAGACCCCATCTTTGGCAACCATGATCATCCCTGACTGCCATCCTGTG
 206 K G V A L P H R T A C V R F S H A R D P I F G N Q I I P D T A I L S
Acc65I (1045)
 1001 TGTGGTCCATTCCATCATGGCTTTGGCATGTTCAACAACCTGGGGTACCTGATCTGTGGCTTCCAGAGTGGTGTGATGTATAGGTTTGGAGGAGGCTG
 239 V V P F H H G F G M F T T L G Y L I C G F R V V L M Y R F E E E L
 1101 TTTCTGAGGAGCCTACAAGACTACAAGATCCAGTCTGCCCTGCTGGTCCCACTCTGTTCCAGTCTTTTGGCAAGAGCACCCCTATTGACAAGTATGACC
 273 F L R S L Q D Y K I Q S A L L V P T L F S F F A K S T L I D K Y D
 1201 TGAGCAACTGCATGAGATTGCTCTGGAGGAGCACCCCTGAGCAAGGAGTGGTGGAGGCTGTGGCAAGAGGTTCCATCTCCAGGAATCAGACAGGG
 306 L S N L H E I A S G G A P L S K E V G E A V A K R F H L P G I R Q G
 1301 CTATGGCCTGACTGAGACCACCTCTGCCATCCTCATACCCCTGAAGGAGATGACAAGCCTGGTGTGTTGGGCAAGTGGTCCCTTTTTTGGAGCCAAG
 339 Y G L T E T T S A I L I T P E G D D K P G A V G K V V P F F E A K
 1401 GTGGTGGACCTGGACACTGGCAAGACCCTGGGAGTGAACCAGAGGGGTGAGCTGTGTGTGAGGGTCCCATGATCATGTCTGGCTATGTGAACAACCTG
 373 V V D L D T G K T L G V N Q R G E L C V R G P M I M S G Y V N N P
 1501 AGGCCACCAATGCCCTGATTGACAAGGATGGCTGGCTGCACTCTGGTACATTGCCTACTGGGATGAGGATGAGCACTTTTTTATTGTGGACAGGCTGAA
 406 E A T N A L I D K D G W L H S G D I A Y W D E D E H F F I V D R L K
 1601 GAGCCTCATCAAGTACAAAGGCTACCAAGTGGCACCTGCTGAGCTAGAGAGCATCTGCTCCAGCACCCCAACATCTTTGATGCTGGTGTGGCTGGCCTG
 439 S L I K Y K G Y Q V A P A E L E S I L L Q H P N I F D A G V A G L
BstEII (1799)
 1701 CCTGATGATGATGCTGGAGAGCTGCCTGCTGCTGTTGTGGTCTGGAGCATGGAAGACCATGACTGAGAAGGAGATTGTGGACTATGTGGCCAGTCAGG
 473 P D D D A G E L P A A V V V L E H G K T M T E K E I V D Y V A S Q
 1801 TGACCACTGCCAAGAAGCTGAGGGGAGGTGGTGGTGTGGATGAGGTGCCAAGGGTCTGACTGGCAAGCTGGATGCCAAGAAGATCAGAGAGATCCT
 506 V T T A K K L R G G V V F V D E V P K G L T G K L D A R K I R E I L
NheI (1944)
 1901 GATCAAGGCCAAGAAGGGTGGCAAAATTGCTGTGTAACCTGAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGCAAAACCACAATA
 539 I K A K K G G K I A V •
HpaI (2084)
 2001 ATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCA
EcoRI (2178)
 2101 TTCATTTTATGTTTCAGGTTCCAGGGGAGGTGTGGGAGTTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAATTTCAAATACAGCATAGCA
 2201 AAACCTTAACCTCCAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTG
 2301 CAGCCTCACCTTCTTTCATGGAGTTAAGATATAGTGTATTTCCCAAGTTTGAACCTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCCAC
 2401 ATTCCCTTTTTAGTAAAAATTCAGAAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTC
 2501 ATAATATCCCCAGTTTAGTAGTTGGACTTAGGAACAAGGAACCTTAATAGAAAATGGACAGCAAGAAAGCGAGCTTCTAGCTTTAGTTCCTGGTGT
 141 ◀ • N R T Y
SacI (2696)
 2601 ACTTGAGGGGATGAGTTCCTCAATGGTGGTTTTGACCAGCTTGCATTCTCAATGAGCACAAGCAGTCAGGAGCATAGTCAGAGATGAGCTCTCT
 136 ◀ 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 E R
 2701 GCACATGCCACAGGGGCTGACCACCTGATGGATCTGTCCACCTCATCAGAGTAGGGTGCCTGACAGCCACAATGGTGTCAAAGTCTTCTGCCCTGTG
 103 ◀ 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 N

StuI (2858)

2801 CTACAGCAGACCCAATGGCAATGGCTTCAGCACAGACAGTGACCCTGCCAATGTAGGCCTCAATGTGGACAGCAGAGATGATCTCCCCAGTCTTGGTCC
69 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 T R
2901 TGATGGCCGCCCCGACATGGTGTCTGTCTCATAGAGCATGGTGTCTCTCAGTGGCGACCTCCACCAGCTCCAGATCTGTGAGAGATGTTGAA
36 I A A G V 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 N F

AseI (3065)

3001 GGTCTTCATGGTGGCCCTCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAACTACTGTTGTAGGCGCCGTCACA
3 T K M
3101 GCTTGATCTGTAACGGCGCAGAACAGAAAACGAAACAAAGACGTAGAGTTGAGCAAGCAGGGTCAGGCAAAGCGTGGAGAGCCGGCTGAGTCTAGGTAG
3201 GCTCCAAGGGAGCGCCGACAAAGGCCGGTCTCGACCTGAGCTTTAAACTTACCTAGACGGCGGACGCAGTTCAGGAGGCACCACAGCGGGAGCGCGC

HindIII (3378)

3301 AGAACCGGACTCAACCGCGTGGATGGCGGCCTCAGGTAGGGCGGGCGCGCTGAAGGAGAGATGCGAGCCCTCGAAGCTTCAGCTGTGTCTGGCGG

Psp1406I (3421)

3401 CAAACCCGTTGCGAAAAAGAACGTTACGGCGACTACTGCACTTATATACGGTTCTCCCCACCCTCGGAAAAAGCGGAGCCAGTACACGACATCACT

AgeI (3534)

3501 TTCCCAGTTTACCCCGCCACCTTCTCTAGGCACCGTTCAATTGCCGACCCTCCCCCAACTTCTCGGGGACTGTGGCGATGTGCGCTCTGCCAC

SpeI (3603)

BspLU11I (3631)

3601 TGACTAGTGGGCCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCTTGTGGCGTTTTTCCATAG

3701 GCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCTTTCCCCCTGGAAGC

3801 TCCCTCGTGCCTCTCCTGTTCCGACCCTGCCGTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTA

3901 GGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCAAGCTGGGCTGTGTGCACGAACCCCGTTCAGCCCGACCCTGCGCTTATCCGGTAACTATCGTCT

4001 TGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGTACAGAGTTCTTG

4101 AAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGAT

4201 CCGGCAAAACAAACCACCGCTGGTAGCGGTGGTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTC

NotI (4381)

4301 TACGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTATGGCTAGTTAATTAACATTTAAATCAGCGGCCGAATAAAATATCTT

4401 TATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACAACTAGCAAATAGGC

4501 TGTCGCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA