

# Product usage

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

## Important Limited Use information for pNiFty3-I-Fluc-Blasti

The purchase of the pNiFty3-I-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.

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### TECHNICAL SUPPORT

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# pNiFty3-I-Fluc-Blasti

IRF-inducible reporter plasmid selectable with Blastcidin

Catalog code: pnf3b-fluc4

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

For research use only

Version 23H16-AK

## PRODUCT INFORMATION

### Contents

- 20 µg of lyophilized pNiFty3-I-Fluc-Blasti (plasmid DNA)
- 1 ml of Blastcidin (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 Blastcidin 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 IFN-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 IRF7<sup>1</sup>. 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)<sup>2</sup>. This feature also enhances the IRF-mediated transcription of the *Fluc* reporter gene.
- **Fluc:** The *firefly luciferase (Fluc)* gene encodes for an intracellular (non secreted) 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 mRNA<sup>3</sup>.
- **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 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-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.

### Blasticidin antibiotic selection cassette

- **CMV promotor & 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 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 signal<sup>6</sup>.

## 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-Fluc-Blasti plasmid features an IRF-inducible *Firefly luciferase (Fluc)* 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 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 pNiFty3-I-Fluc-Blasti plasmid is selectable with Blastcidin 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.
- **Blasticidin usage**

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

## RELATED PRODUCTS

Product	Description	Cat. Code
Blasticidin	Selection antibiotic	ant-zn-1
pNiFty3-I-Fluc-Puro	Reporter plasmid	pnf3p-fluc4
pNiFty3-I-Fluc-Zeo	Reporter plasmid	pnf3-fluc4

1. Vodjdani 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

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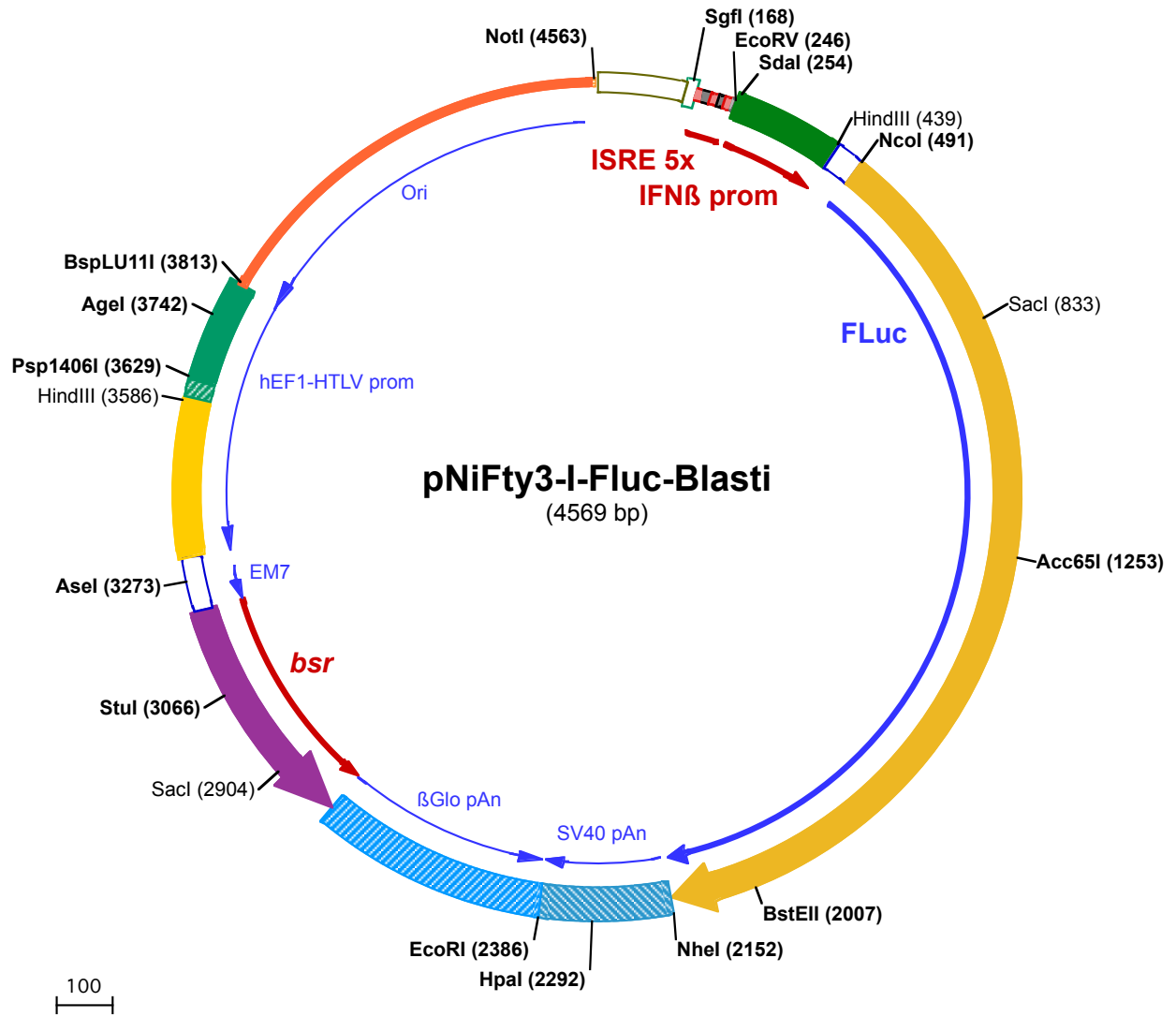
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1 AATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTGTGTGAATCGTAACATAACATACGCTCTCCATCAAACAAAACGAAACAAAACAAAC  
101 TAGCAAATAGGCTGTCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTATCGAAGGATCGCGATCGTGAATTAGTTTCACTTTCCAGTTTCAGTT

SgfI (168)

201 TCCAGTTTCATTTCCAGTTTCATTTCCAGTTTCATTTCTGATATCCTGCAGGagcttgaataaaatgaatattagaagctgtagaataagagaaa  
301 atgacagaggaAAACTGAAAGGgAGAACTGAAAGTGggaattcctctgaggcagaaggaccatccctTATAAatagcacaggccatgaaggaagatca

SdaI (254)  
EcoRV (246)

401 ttctcactgcagcctttgacagcctttgctcatcttgAAGCTTCTGCCTTCTCCCTCTGTGAGTTTGGTTGGTGTACAGTAGTCCACCATGGAGGA  
1 M E D

HindIII (439)

NcoI (491)

501 TGCCAAGAATATTAAGAAAGGCCCTGCCCATTTACCTCTGGAAGATGGCACTGCTGGTGGAGCAACTGCACAAGGCCATGAAGAGGTATGCCCTGGTC  
3 A K N 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  
601 CCTGGCACCATTGCCTTCACTGATGCTCACATTGAGGTGGACATCACCTATGCTGAATACTTTGAGATGCTGTGAGGCTGGCAGAAGCCATGAAAAGAT  
37 P G T 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  
701 ATGGACTGAACACCAACCACAGGATTGGTGTGCTCTGAGAAGTCTCCAGTTCTTCATGCTGTGTAGGAGCCCTGTTCACTGGAGTGGCTGTGGC  
70 Y G L 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

SacI (833)

801 CCCTGCCAATGACATCTACAATGAGAGAGAGCTCTGAACAGCATGGGCATCAGCCAGCCAAGTGGTCTTTGTGAGCAAGAAGGCCCTGCAAAAGATC  
103 P A 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  
901 CTGAATGTGAGAAGAAGCTGCCCATCATCCAGAAGATCATCATGGACAGCAAGACTGACTACCAGGGCTTCCAGAGCATGTATACCTTTGTGACCA  
137 L N V Q K K L P I I Q K I I M D S K T D Y Q G F Q S M Y T F V T  
1001 GCCACTTACCCCTGGCTTCAATGAGTATGACTTTGCTGCTGAGAGCTTTGACAGGGACAAGACCATTGCTCTGATTATGAACAGCTCTGGCTCCACTGG  
170 S H L 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  
1101 ACTGCCAAAAGGTGTGGCTCTGCCCCACAGAAGTGTGTGAGATTGAGCCATGCCAGAGACCCCATCTTTGGCAACCAGATCATCCCTGACACTGCC  
203 L P 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

Acc65I (1253)

1201 ATCCTGTCTGTGGTTCCATTCCATCATGGCTTTGGCATGTTCAACAACACTGGGGTACCTGATCTGTGGCTTCCAGAGTGGTGTGATGTATAGGTTTGAGG  
237 I L S 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  
1301 AGGAGCTGTTTCTGAGGAGCCTACAAGACTACAAGATCCAGTCTGCCCTGCTGGTGGCCACTCTGTTGAGTCTTTGCAAGAGCACCTCATTGACAA  
270 E E L 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  
1401 GTATGACCTGAGCAACCTGCATGAGATTGCCTCTGGAGGAGCACCCCTGAGCAAGGAGGTGGGTGAGGCTGTGGCAAGAGGTTCCATCTCCAGGAATC  
303 Y D 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  
1501 AGACAGGCTATGGCCTGACTGAGACCCTCTGCCATCCTCACCCCTGAAGGAGATGACAAGCCTGGTGTGGGCAAGGTGGTTCCTTTTTTTG  
337 R Q G Y G L T E T T S A I L I T P E G D D K P G A V A V G K V V P F F  
1601 AGGCCAAGGTGGTGGACCTGGACACTGGCAAGACCCTGGGAGTGAACCAGAGGGGTGAGCTGTGTGTGAGGGTCCCATGATCATGTCTGGCTATGTGAA  
370 E A K 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  
1701 CAACCCTGAGGCCACCAATGCCCTGATTGACAAGGATGGCTGGCTGCACTCTGGTACATTGCCTACTGGGATGAGGATGAGCACTTTTTCATTGTGGAC  
403 N P 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  
1801 AGGCTGAAGAGCCTCATCAAGTACAAGGCTACCAAGTGGCACCTGCTGAGCTAGAGAGCATCCTGCTCCAGCACCCCAACATCTTTGATGCTGGTGTGG  
437 R L K 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 G A G V  
1901 CTGGCCTGCTGATGATGCTGGAGAGCTGCCTGCTGTTGTGGTCTGGAGCATGAAAAGACCATGACTGAGAAGGAGATTGGACTATGTGGC  
470 A G L 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

BstEII (2007)

2001 CAGTCAGGTGACCACTGCAAGAAGCTGAGGGGAGGTGTGGTGTGTTGTGGATGAGGTGCCAAAGGCTGACTGGCAAGCTGGATGCCAGAAAGATCAGA  
503 S Q 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

NheI (2152)

2101 GAGATCCTGATCAAGGCCAAGAAGGTTGGCAAATTGCTGTGTAACCTGAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCA  
537 E I L I K A K K G G K I A V

HpaI (2292)

2201 CAACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAAGTTAAACAACA

EcoRI (2386)

2301 CAATTGCATTCATTTTATGTTTCAGGTTTCAAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATCTAAAATACA

2401 GCATAGCAAACCTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTA

2501 GCTGTTTGCAGCCTCACCTTCTTTTCATGGAGTTAAGATATAGTGTATTTTCCAAGGTTTGAAGTACTCTTCATTTCTTTATGTTTAAATGCACTGA

2601 CCTCCACATTCCCTTTTATGTAATAATTTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTATTAGGCAGAATCCAGATGCTCAA

2701 GGCCCTTCATAATATCCCCAGTTTAGTGTGGACTTAGGGAACAAAGAACCTTTAATAGAAATGGACAGCAAGAAGCGAGCTTCTAGCTTTAGTT

2801 CCTGGTGTACTTGAGGGGGATGAGTTCCTCAATGGTGGTTTTGACCAGCTTGCCATTCATCTCAATGAGCACAAAGCAGTCAGGAGCATAGTCAGAGATG  
 139 R 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  
 2901 AGCTCTCTGCACATGCCACAGGGGTGACCACCCTGATGGATCTGCCACCTCATCAGAGTAGGGTGCCTGACAGCCACAATGGTGTCAAAGTCCTTCT  
 105 L 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

**StuI (3066)**

3001 GCCCGTTGCTCACAGCAGACCAATGGCAATGGCTTCAGCACAGACAGTGACCCTGCCAATGTAGGCCTCAATGTGGACAGCAGAGATGATCTCCCCAGT  
 72 G 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  
 3101 CTTGGTCTGATGGCCGCCCCGACATGGTGCTTGTCTCATAGAGCATGGTGATCTTCTCAGTGGCGACCTCCACCAGCTCCAGATCCTGCTGAGAG  
 39 K 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

**AseI (3273)**

3201 ATGTTGAAGGTCTTCATGGTGGCCCTCTATAGTGAGTCGATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAACTACTGTTTGTAGGGCC  
 5 I N F T K M  
 3301 CGGTCACAGCTTGATCTGTAACGGCGCAGAACAGAAAACGAAACAAAGACGTAGAGTTGAGCAAGCAGGGTCAGGCAAAGCGTGAGAGCCGGCTGAGT  
 3401 CTAGGTAGGCTCCAAGGGAGCGCCGGACAAAGGCCCGGTCTCGACCTGAGCTTTAAACTTACCTAGACGGCGGACGCGAGTTTCAGGAGGCCACACAGCCG

**HindIII (3586)**

3501 GAGGGCGCAGAACCGGACTCAACCGCGTGGATGGCGGCCTCAGGTAGGGCGGGCGCGTGAAGGAGAGATGCGAGCCCCCTCGAAGCTTCAGCTGTGT

**Psp1406I (3629)**

3601 TCTGGCGGCAAACCCGTTGCGAAAAAGAACGTTACGGCGACTACTGCACTTATATACGGTCTCCCCACCCTCGGAAAAAGCGGAGCCAGTACAG

**AgeI (3742)**

3701 ACATCACTTTCCAGTTTACCCCGGCCACCTTCTTAGGCACCGTTCAATTGCCGACCCTCCCCCAACTTCTCGGGACTGTGGCGATGTGCGCT

**BspLU11I (3813)**

3801 CTGCCACTGACACATGTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAAAGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGA

3901 GCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCTGGAAGCTCCCTCGTGCCTCTCCT

4001 GTTCCGACCTGCCGTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGT

4101 AGGTCGTTGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTTACGCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAG

4201 ACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAACTAC

4301 GGCTACACTAGAAGAACAGTATTTGGTATCTGCCTGCTGTAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCG

4401 CTGGTAGCGGTGTTTTTTTGTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCA

**NotI (4563)**

4501 GTGGAACGAAACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGC