

Product usage

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

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

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

IRF-inducible reporter plasmid selectable with Puromycin

Catalog code: pnf3p-fluc4

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

For research use only

Version 23H16-AK

PRODUCT INFORMATION

Contents

- 20 µg of lyophilized pNiFty3-I-Fluc-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 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 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 *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 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 promotor & 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-Fluc-Puro 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-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-zn-1
pNiFty3-I-Fluc-Blasti	Reporter plasmid	pnf3b-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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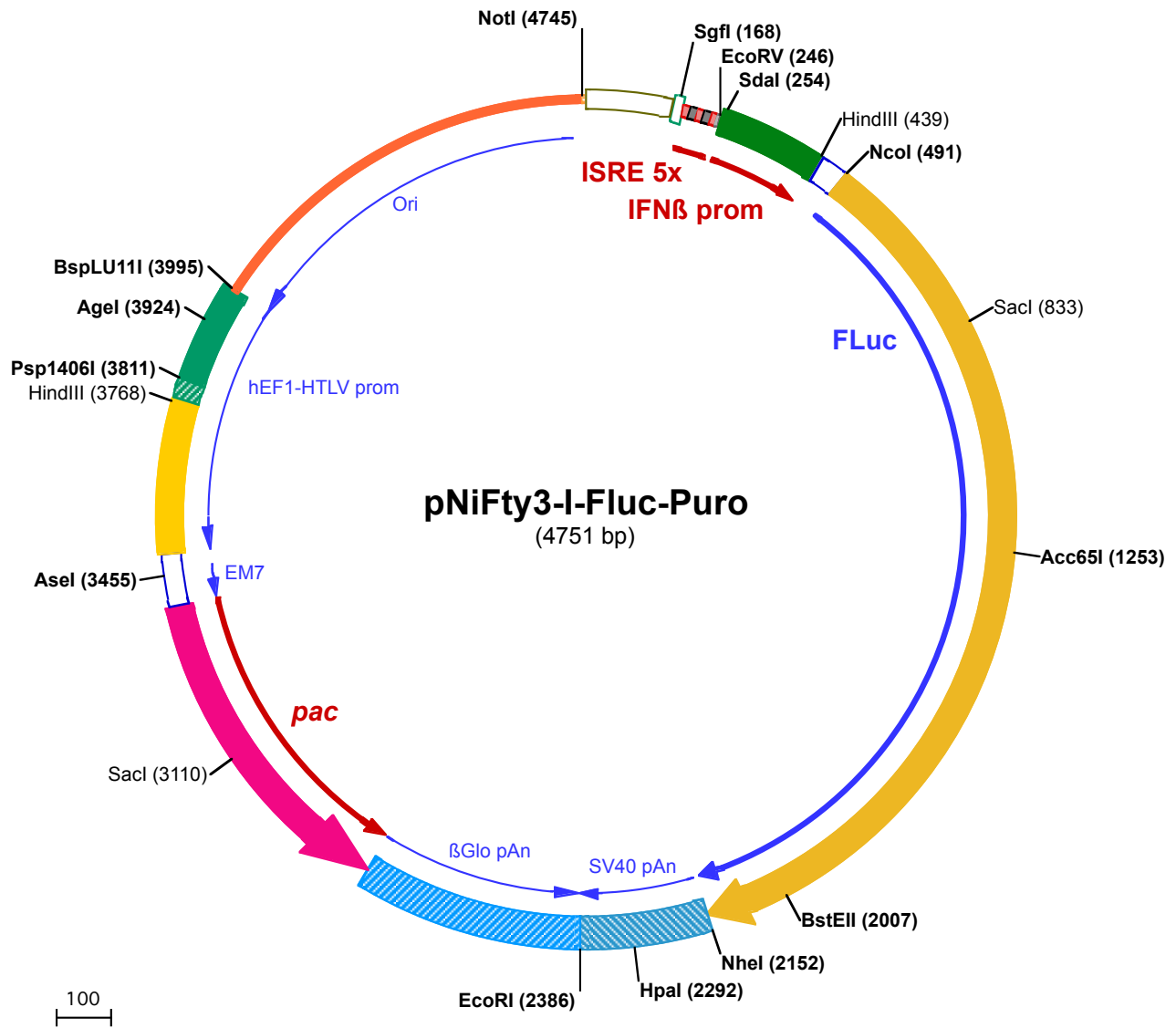
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1 AATAAAATATCTTTATTTTCATTACATCTGTGTGGTTTTTTGTGTGAATCGTAACATAACATACGCTCTCCATCAAACAAAACGAAACAAAACAAAC
101 TAGCAAATAGGCTGTCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTATCGAAGGATCGCGATCGTGAATTAGTTTCACTTTCCAGTTTCAGTT

SgfI (168)

201 TCCAGTTTCATTTCCAGTTTCATTTCCAGTTTCATTTCTGATATCCTGCAGGagcttgaataaaatgaatattagaagctgtagaataagagaaa
301 atgacagaggaAAACTGAAAGGgAGAACTGAAAGTGggaattcctctgaggcagaaggaccatccctTATAAAtagcacaggccatgaaggaagatca

SdaI (254)
EcoRV (246)

401 ttctcactgcagcctttgacagcctttgctcatcttgAAGCTTCTGCCTTCTCCCTCTGTGAGTTTGGTGGTGTACAGTAGCTTCCACCATGGAGGA
1 M E D

HindIII (439)

NcoI (491)

501 TGCCAAGAATATTAAGAAAGGCCCTGCCCATTTACCTCTGGAAGATGGCACTGCTGGTGGAGCACTGCACAAGGCCATGAAGAGGTATGCCCTGGTC
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 CCTGGCACCATTGCCTTCACTGATGCTCACATTGAGGTGGACATCACCTATGCTGAATACTTTGAGATGCTGTGAGGCTGGCAGAAGCCATGAAAAGT
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 AGGAGCTGTTTCTGAGGAGCCTACAAGACTACAAGATCCAGTCTGCCCTGCTGGTGGCCACTCTGTTCACTTCTTTGCAAGAGCACCTCATTGACAA
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 GTATGACCTGAGCAACCTGCATGAGATTGCCTCTGGAGGAGCACCCCTGAGCAAGGAGGTGGTGGAGGCTGTGGCAAGAGGTTCCATCTCCAGGAATC
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 AGACAGGCTATGGCCTGACTGAGACCCTCTGCCATCCTACCCTGAAGGAGATGACAAGCCTGGTGTGGGCAAGGTGGTTCCTTTTTTTG
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 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 AGGCTGAAGAGCCTCATCAAGTACAAAGGCTACCAAGTGGCACCTGCTGAGCTAGAGAGCATCCTGCTCCAGCACCCCAACATCTTTGATGCTGGTGTGG
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 A G V
1901 CTGGCCTGCTGATGATGCTGGAGAGCTGCCTGCTGTTGTGGTCTGGAGCATGAAAAGACCATGACTGAGAAGGAGATTGTGACTATGTGGC
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 CAATTGCATTCATTTTATGTTTCAGGTTGAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATCTAAAATACA

2401 GCATAGCAAACCTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTA

2501 GCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTAAGATATAGTGTATTTTCCAAGGTTTGAAGTACTCTTCACTTCTTTATGTTTTAAATGCACTGA

2601 CCTCCACATTCCCTTTTATGTAATAATTTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTATTAGGCAGAATCCAGATGCTCAA

2701 GGCCCTTCATAATATCCCCAGTTTAGTGTGGACTTAGGGAACAAAGAACCTTTAATAGAAATGGACAGCAAGAAGCGAGCTTCTAGCTCAGGTT

2801 TAAGTCCAGGCTTCCTTGTGCACCAAGTTCTTGGGCTTCTGGAACCTCAACATCAGCTGTACAGTGAATCCCAGTCTTTCATAAAAAGGCAGGT
199 ◀ • 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
2901 TTCTGGGAGCAGAAGTTTCCAGAAAAGGCAGGAACCTCCAGCCCTTTCAGCAGCTTCACTCCAGGCAGAAACAACAGCAGATCCCAGACCCTTTCCTGGTG
166 ◀ 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 G L G K G Q H
3001 GTCAGGGCTCACTCCAACAGTTGCCAGAAAACCAAGCTGGCTCTTTGGCCTGTGTGGTCCAGCAGACCTTCCATTTGTTGTTGTGCTGCCAGCCTGCTT
133 ◀ 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 Q Q A A L R S

SacI (3110)

3101 CCAGAGAGCTCAGCCATTCTTGGTCCAATTTCCAGAAAAACAGCACCAGCTTCAACAGACTCAGGTGTTGTCCAAACTGCAACAGCAGCTCCATCATCTG
99 ◀ 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 V A A G D D A
3201 CAACCCAAACTTTTCCAATGTCCAGTCCCACTCTGGTGAGGAAGATTCTTGCAGTCTGTACCCTCTCAATGTGCCTGTGAGGGTCAACTGTGTGCCT
66 ◀ 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 D P D V T H R
3301 TGTTCAGGGTAGTCTGCAAAAGCAGCAGCCAGTGTCTCACAGCTCTTGGAAATCATCTCTGGTTGCCAGCCTCACTGTGGGTTTGTACTCAGTCATG
33 ◀ 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 T P K Y E T M

AseI (3455)

3401 GTGGCCCTCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGCAACTACTGTTTGTAGGCGCCGGTACAGCTTGATCT
3501 GTAACGGCGCAGAACAGAAAACGAAACAAAGACGTAGAGTTGAGCAAGCAGGGTCAGGCAAAGCGTGGAGAGCCGGCTGAGTCTAGGTAGGCTCCAAGGG
3601 AGCGCCGGACAAAAGGCCCGTCTCGACCTGAGCTTTAAACTTACCTAGACGGCGACGCAATTCAGGAGGCCACAGCGGGAGCGGCAGAACCGGAC

HindIII (3768)

3701 TCAACCGCGTGGATGGCGGCCCTCAGGTAGGGCGGGCGCGCTGAAGGAGAGATGCCGAGCCCTCGAAGCTTCAGCTGTGTTCTGGCGGCAAAACCGTT

Psp1406I (3811)

3801 GCGAAAAAGAAGTTCACGGCGACTACTGCACTTATATACGGTCTCCCCACCCTCGGGAAAAAGCGGAGCCAGTACACGACATCACTTCCAGTTT

AgeI (3924)

BspLU111 (3995)

3901 ACCCCGCGCCACCTTCTCTAGGCACCGTTCAATTGCCGACCCTCCCCCAACTTCTCGGGACTGTGGCGATGTGCGCTCTGCCACTGACACATGT

4001 GAGCAAAAGGCCAGAAAAGGCCAGGAACCGTAAAAAGCCGCGTTGCTGGCGTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACG

4101 CTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGCGTTTTCCCCCTGGAAGCTCCCTCGTGCCTCTCTGTTCCGACCCTGCCGCTT

4201 ACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTGCTTCGCTCCAAGC

4301 TGGGCTGTGTGCAGAACCCCCGTTTACGCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGTAAGACACGACTTATCGCCACT

4401 GGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACA

4501 GTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGTGGTAGCGGTGTTTTT

4601 TTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGAACGAAAACCTCAGC

NotI (4745)

4701 TTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGC