

Product usage

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

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

The purchase of the pNiFty2-N-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.

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-Puro

NF- κ B-inducible reporter plasmid selectable with Puromycin

Catalog code: pnf2p-fluc

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

For research use only

Version 23H16-AK

PRODUCT INFORMATION

Contents

- 20 μ g of lyophilized pNiFty2-N-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

- **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) (GGGGACTTTCC)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.

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 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 pNiFty2, a collection of inducible reporter plasmids, to monitor pattern recognition receptor (PRR) activation and cytokine signaling upon ligand stimulation. The pNiFty2-N-Fluc-Puro 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-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 containing 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 .
- **Puromycin usage**

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

RELATED PRODUCTS

Product	Description	Cat. Code
Puromycin	Selection antibiotic	ant-pr-1
pNiFty2-N-Fluc-Blasti	Reporter plasmid	pnf2b-fluc
pNiFty2-N-Fluc-Zeo	Reporter plasmid	pnf2-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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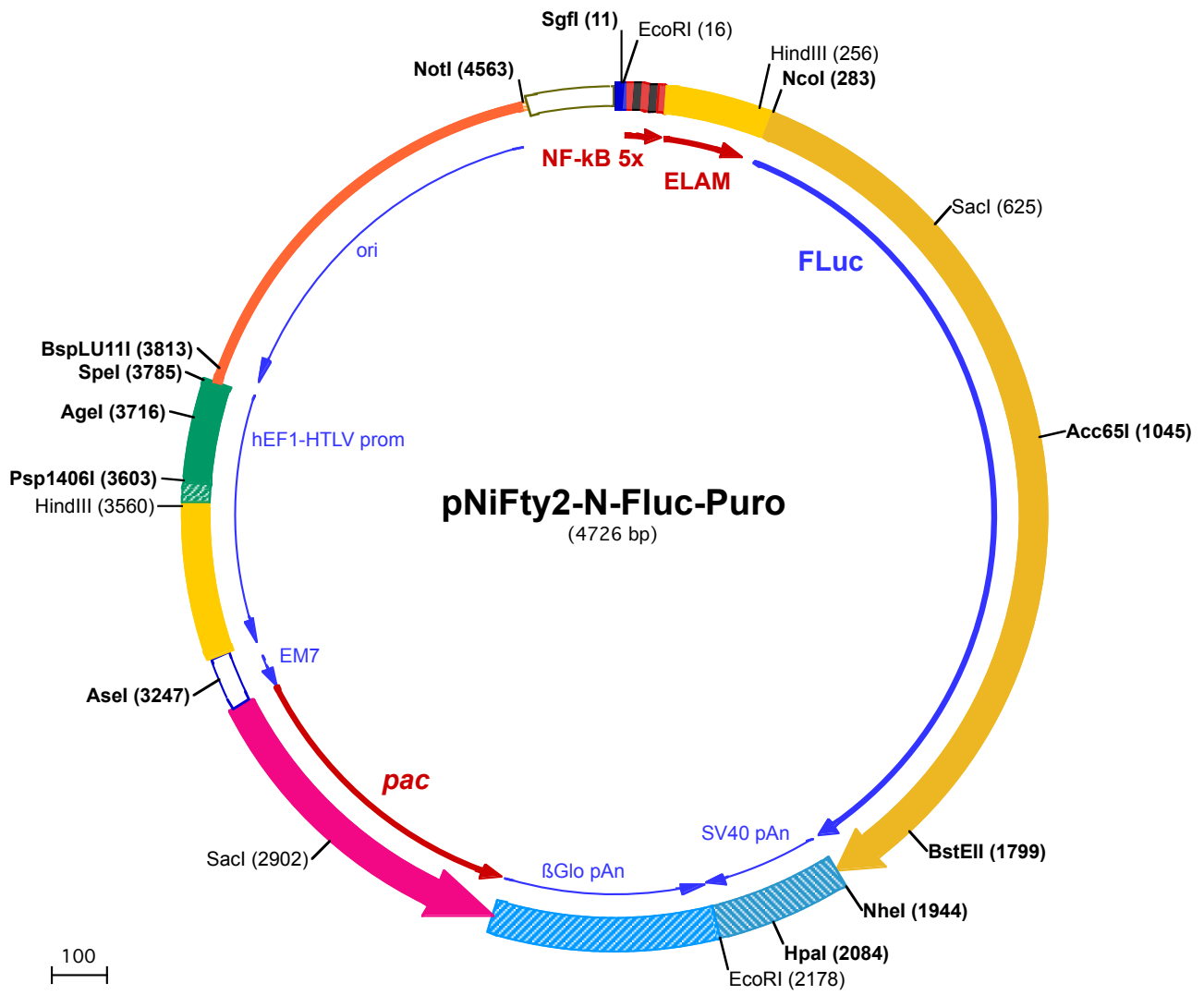
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Sgfl (11) **EcoRI (16)**
1 GGATCTGCGATCGCTGAATTC**TGGGGACTTTCCACTGGGGACTTTCCACTGGGGACTTTCCACTGGGGACTTTCCACTCCTG**CAGC
101 AGTGGATATTTCCAGAAA**ACTTTTTGGATGCAGTTGGGGATTTCCTCTTTACTGGATGTGGACAATATCCTCTATTATTCACAGGAAGCAATCCCTCCT**

HindIII (256) **NcoI (283)**
201 ATAAAAGGGCCTCAGCAGAA**GTAGTGTTCAGCTGTTCTTGGCTGACTTCACATCAAAGCTTCTATACTGACCTGAGACAGAGCCATGGAGGATGCCAAGA**
301 ATATTAAGAAAGCCCTGCC**CCATTCTACCCTCTGGAAGATGGCACTGCTGGTGAGCAACTGCACAAGGCCATGAAGAGGTATGCCCTGGTCCCTGGCAC**
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 CATTGCCTTCACTGATGCTCACATTGAGGTGGACATCACCTATGCTGAATACTTTGAGATGCTGTGAGGCTGGCAGAA**GGCCATGAAAAGATATGGACTG**
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 AACACCAACCACAGGATTGTGGTGTGCTCTGAGAACTCTCCAGTCTTCATGCCTGTGTTAGGAGCCCTGTT**CATTGGAGTGGCTGTGCCCTGCCA**
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 ATGACATCTACAATGAGAGAGACTCCTGAACAGCATGGGCATCAGCCAGCCA**ACTGTGGTCTTTGTGAGCAAGAAGGGCCTGCAAAAAGATCCTGAATGT**
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 GCAGAAGAAGCTGCCATCATCCAGAAGATCATCATCATGGACAGCAAGACTGACTACCAGGGCTTCCAGAGCATGTATAC**TTTTGTGACCAGCCACTTA**
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 CCCCTGGCTTCAATGAGTATGACTTTGTGCCTGAGAGCTTTGACAGGGACAAGACCATTGCTCTGATTATGAACAGCTCTGGCTCCACTGGACTGCCA
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 AAGGTGTGGCTCTGCC**CCACAGAAGCTTGTGTGAGATTGAGCCATGCCAGAGACCCCATCTTTGGCAACCATGATCATCCCTGACACTGCCATCCTGTC**
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 TGTGGTCCATTCCATCATGGCTTTGGCATGTTCA**CAACTGGGGTACCTGATCTGTGGCTTCCAGAGTGGTGTGATGTATAGGTTTGGAGGAGCTG**
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 TTTCTGAGGAGCCTACAAGACTACAAGATCCAGTCTGCCCTGCTGGTCCCACTCTGTT**CAGCTTCTTTGCCAAGAGCACCCCTATTGACAAGTATGACC**
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 TGAGCAACTGCATGAGATTGCCTCTGGAGGAGCACCCCTGAGCAAGGAGTGGTGGAGCTGTGGCAAGAGGTTCCATCTCCAGGAATCAGACAGGG
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 GTGGTGGACCTGGACACTGGCAAGACCCTGGGAGTGAACCAGAGGGGTGAGCTGTGTGTGAGGGTCCATGATCATGTCTGGCTATGTGAACAACCTG
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 AGGCCACCAATGCCCTGATTGACAAGGATGGCTGGCTGCACTCTGGT**GACATTGCCTACTGGGATGAGGATGAGCACTTTTTATTGTGGACAGGCTGAA**
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 GAGCCTCATCAAGTACAAGGCTACCAAGTGGCACCTGCTGAGCTAGAGAGCATCTGCTCCAGCACCCCAACATCTTTGATGCTGGTGTGGCTGGCCTG
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 CCTGATGATGATGCTGGAGAGCTGCCTGCTGCTGTTGTGGTCTG**GAGCATGGAAGACCATGACTGAGAAGGAGATTGTGGACTATGTGCCAGTCAGG**
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 TGACCACTGCCAAGAAGCTGAGGGGAGGTGGTGGTGGTGGATGAGGTGCCAAGGGTCTGACTGGCAAGCTGGATGCCAAGAA**AGATCAGAGAGATCCT**
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 GATCAAGGCCAAGAAGGGTGGCAAAATTGCTGTGTA**AACTGAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGCAAAACCACA**ACTAGA
539 I K A K K G G K I A V
HpaI (2084)
2001 ATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAA**CAAGTTAACAAACA**CAATTGCA
EcoRI (2178)
2101 TTCATTTTATGTTTCAGGTT**CAGGGGAGGTGTGGGAGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATTTCAAATACAGCATAGCA**
2201 AAACTTTAACTCCAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTT**G**
2301 CAGCCTCACCTTCTTTCATGGAGTTAAGATATAGTGTATTTCCCAAGTTTGA**ACTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCCAC**
2401 ATTCCCTTTTTAGTAAAAATTCAGAAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCC**CTTC**
2501 ATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTAATAGAAATTTGGACAGCAAGAAAGCGAGCTTCTAGCTCAGGTTTAAAGCTCC
2601 AGGCTTCTTGTGCATGCACCAAGTTCTTGGCCTTCTGGAACCTCAACATCAGCTGT**CACAGTGAATCCCAGTCTTTCATAAAAAGGCAGGTTTCTGGGA**
197 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 P
2701 GCAGAAGTTTCCAGAAAGGCAGGA**ACTCCAGCCCTTTCAGCAGCTTCAACTCCAGGCAGAACAACAGCAGATCCAGACCTTTCCCTGGTGGTCAGGGC**
163 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 P S

2801 TCACTCCAACAGTTGCCAGAAACCAAGCTGGCTCTTTTGGCCTGTGTGGTGCCAGCAGACCTTCCATTTGTTGTTGTGCTGCCAGCCTGCTTCCAGAGAG
 130 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 S L
 2901 CTCAGCCATTCTGGTCCAATTTAGCAAAAAACAGCACCAGCTTCAACAGACTCAGGTGTTGTCCAACTGCAACAGCAGCTCCATCATCTGCAACCCAA
 97 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 W
 3001 ACTTTTCCAATGTCCAGTCCCACTCTGGTGAGGAAGAGTTCTTGCAAGTCTGTCCACCTCTCAATGTGCCTGTGAGGGTCAACTGTGTGCTTGTGCGAG
 63 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 A P
 3101 GGTAGTCTGAAAAGCAGCAGCCAGTGTCTCACAGCTCTTGAACATCATCTCTGGTTGCCAGCCTCACTGTGGGTTTGTACTCAGTCATGGTGGCCCT
 30 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 (3247)

3201 CCTATAGTGAGTCGATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAACTACTGTTTGTAGGCCCGGTACAGCTTGATCTGTAACGGC
 3301 GCAGAACAGAAAACGAAACAAAGACGTAGAGTTGAGCAAGCAGGGTCAGGCAAAGCGTGGAGAGCCGGCTGAGTCTAGGTAGGCTCCAAGGGAGCGCCGG
 3401 ACAAAAGGCCCGGTCTCGACCTGAGCTTTAAACTTACCTAGACGGCGGACGCAGTTGAGGAGCACCACAGGCCGGGAGCGGCAGAACGCGACTCAACCGG

HindIII (3560)

3501 CGTGGATGGCGGCTCAGGTAGGGCGGCGGCGCTGAAGGAGAGATGCGAGCCCTCGAAGCTTCAGCTGTGTTCTGGCGCAAACCCGTTGCGAAAAA
Psp1406I (3603)
 3601 GAACGTTACGGCGACTACTGCACTTATATACGTTCTCCCCACCCTCGGGAAAAAGCGGAGCCAGTACACGACATCACTTCCAGTTTACCCCGG

AgeI (3716)

3701 CCACCTTCTCTAGGCACCGTTCAATTGCCGACCCCTCCCCCAACTTCTCGGGGACTGTGGCGATGTGCGCTCTGCCCACTGACTAGTGGGCCCTGCA

SpeI (3785)

BspLU11I (3813)

3801 GGTTAATTAAGAACATGTGAGCAAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCTTGTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGA
 3901 GCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCTGGAAGCTCCCTCGTGCCTCTCCT
 4001 GTTCCGACCTGCCGTTACCGGATACCTGTCCGCTTTCTCCCTTCCGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGT
 4101 AGGTCGTTCCGCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTCAGCCGACCGCTGCGCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGTTAAG
 4201 ACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAACTAC
 4301 GGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCG
 4401 CTGGTAGCGGTGGTTTTTTTTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTACGGGTCTGACGCTCA

NotI (4563)

4501 GTGGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAAATATCTTTATTTTCATTACATCTGT
 4601 GTGTTGGTTTTTTGTGTAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACAACTAGCAAAATAGGCTGTCCCAGTGCAAGTGC
 4701 AGGTGCCAGAACATTTCTATCGAA