

# Product usage

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

## Important Limited Use information for pNiFty2-N-Lucia-Puro

The purchase of the pNiFty2-N-Lucia-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](mailto:outlicensing@invivogen.com).

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### TECHNICAL SUPPORT

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# pNiFty2-N-Lucia-Puro

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

Catalog code: pnf2p-lc

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

For research use only

Version 23H16-AK

## PRODUCT INFORMATION

### Contents

- 20  $\mu$ g of lyophilized pNiFty2-N-Lucia-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- $\kappa$ 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) (GGGGACTTCC)<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 *Lucia* reporter gene.
- **Lucia** is a secreted coelenterazine luciferase encoded by a synthetic gene developed by InvivoGen. It generates 1000-fold higher bioluminescent signal compared to the commonly used Firefly and Renilla luciferases. Lucia luciferase activity can be evaluated using QUANTI-Luc™ 4 Lucia/Gaussia (cat. code: rep-qlc4lg1), an assay reagent containing all the components required to quantitatively measure the activity of Lucia luciferase and other coelenterazine-utilizing luciferases.
- **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.

### 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  $\beta$ -Globin pAn** is a strong polyadenylation (pAn) signal placed downstream of *Pac*. 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-Lucia-Puro plasmid features an NF- $\kappa$ B-inducible *luciferae* 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 Lucia upon receptor activation is readily measurable in the cell culture supernatant when using QUANTI-Luc™ 4 Lucia/ Gaussia, a Lucia luciferase detection reagent. The pNiFty2-N-Lucia-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  $\mu$ 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 $\alpha$ .
- **Puromycin usage**

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

## RELATED PRODUCTS

Product	Description	Cat. Code
Puromycin	Selection antibiotic	ant-pr-1
pNiFty2-N-Lucia-Blasti	Reporter plasmid	pnf2b-lc
pNiFty2-N-Lucia-Zeo	Reporter plasmid	pnf2-lc
QUANTI-Luc™ 4 Lucia/Gaussia	Luciferase Detection	rep-qlc4lg1

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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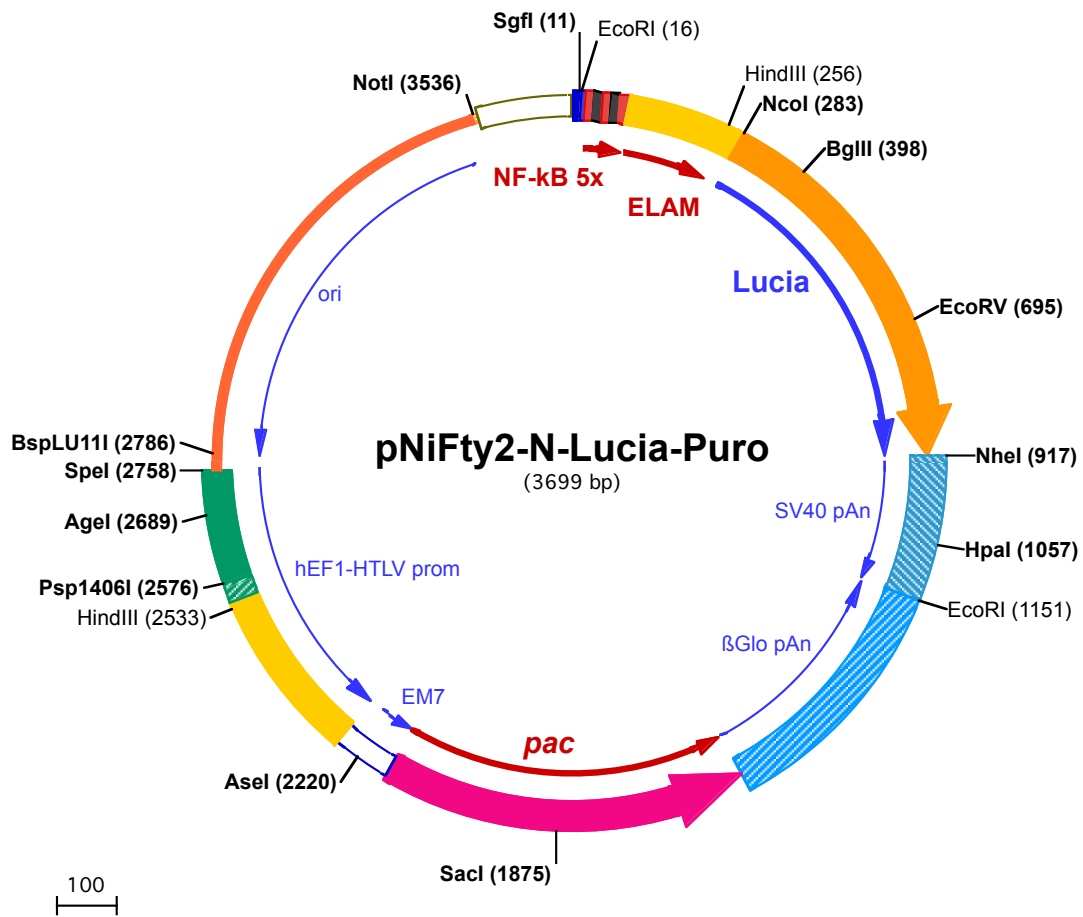
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**Sgfl (11)** **EcoRI (16)**  
1 GGATCTGCGATCGCTGAATTC**TGGGGACTTTCCACTGGGGACTTTCCACTGGGGACTTTCCACTGGGGACTTTCCACTCCTGCAGC**

101 AGTGGATATTTCCAGAAA**ACTTTTTGGATGCAGTTGGGGATTCTCTTTACTGGATGTGGACAATATCTCTATTATTACAGGAAGCAATCCCTCCT**

**HindIII (256)** **NcoI (283)**  
201 A**TA**AAAAGGGCCTCAGCAGAA**GTAGTGTTCAGCTGTTCTTGGCTGACTTCACATCAAAGCTTCTATACTGACCTGAGACAGAGCCATGGAAATCAAGGTGC**  
1 M E I K V **BglIII (398)**

301 TGT**TTGCCTCATCTGTATTGCTGTTGCTGAGGCAAACCCACTGAAATCAATGAAGACCTCAATATAGCTGTGTGGCTCCA**ACTTTGCCACCACAG**A**  
6 L F A L I C I A V A E A K P T E I N E D L N I A A V A S N F A T T D  
401 TCTT**GAGACTGACCTGTTCA**CCA**ACTGGGAGACCATGAATGTGATTAGCACTGACACAGAGCAGGTGAACACAGATGCTGACAGGGGCAAGCTGCCTGGC**  
39 L E T D L F T N W E T M N V I S T D T E Q V N T D A D R G K L P G  
501 A**AAAAACTCCCCCAGATGTCCTGAGGGAGCTGGAGGCCAATGCCAGAAGGGCTGTTGCACAAGAGGCTGCCTCATT**GGCTCTCC**ACATTAAGTGA**  
73 K K L P P D V L R E L E A N A R R A G C T R G C L I C L S H I K C **EcoRV (695)**

601 CC**CTAAGATGAAGAAATTTATCC**TGGCAGGTGCC**CACTTATGAAGGTGAAAAGGAGTCTGCTCAGGGAGGGATTGGAGAGGCAATTGTTGATATCCC**  
106 T P K M K K F I P G R C H T Y E G E K E S A Q G G I G E A I V D I P  
701 A**GAGATTCCTGGCTTCAAGGATAAAGGAGCCACTGGACCA**GT**TTATTGCTCAAGTGGACCTCTGTGCTGATTGACCACTGGCTGTCTGAAGGGCCTT**GCC  
139 E I P G F K D K E P L D Q F I A Q V D L C A D C T T G C L K G L A  
801 A**ATGTCCAGTGTCTGACCTCTGAAGAAGTGGCTTCCCAGAGGTGTACC**ACTTTT**GCCAGCAAGATTCAAGGGTGGGCAAAATCAAGGGTCTGG**  
173 N V Q C S D L L K K W L P Q R C T T F A S K I Q G R V D K I K G L

**NheI (917)**  
901 CTGGGGACAGATGATAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAA**CCACA**ACTAGAA**TGCAGTGA**AAAAATGCTTTATTTGT  
206 A G D R •

**HpaI (1057)**  
1001 GAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAA**CAAGTTAA**CAACA**CAATTCATT**TTTTATGTTTCAGGTT**CAGGGG**

**EcoRI (1151)**  
1101 AGGTGTGGAGGTTTTTAAAGCAAGTAA**ACCTCTACAAATGTGGTATGGAATCTAAAATACAGCATAGCAA**AACTTTAACCTCCA**ATCAAGCCTCT**  
1201 ACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTG**CAGCCTCAC**TTCTTT**CATGGAGTTA**  
1301 AGATATAGTGTATTTTCCAAGT**TTGAACTAGCTTTCATTTCTTTATGTTTTAAATGCACTGACCTCCCACATTCC**TTTTTAGTAA**ATATT**CAGAA  
1401 ATAATTTAAATACATCATTGCAATGAAATA**AAATGTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCC**CCAGTTTAGT**AGTTGGA**  
1501 CTTAGGGA**CAAAAGGAAC**CTTTAATAGAA**ATTGACAGCAAGAAAGCGAGCTTCTAGCTCAGGTTTAAAGCTCCAGGCTTCCTTGT**CATGCACCAAGTTCT  
200 A G P K R T M C W T R

1601 TGGCCTTCTGAACTCAACATCAGCTGT**CACAGTGAATCCAGTCTTTCATAAAAAGGCAGGTTTCTGGGAGCAGAAGTTTCCAGAAAGGCAGGAACT**  
188 P G E P V E V D A T V T F G L R E Y F P L N R P A S T E L F A P V  
1701 CCAGCC**TTTTCAGCAGCTTCAACTCCAGGCAGAACAACAGCAGATCCAGACC**TTTCCCTGGTGGT**CAGGGCTCACTCAACAGTTGCCAGAAACCAAG**  
154 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 V G V T A L F W A **SacI (1875)**

1801 CTGGCTCTTTGGCTGTGTGGT**GCCAGCAGACCTCCATTTGTTGTTGTGCTGCCAGCCTGTTCCAGAGAGCTCAGCCATTCTTGGTCCA**ATTT**CAGC**  
121 P E K P R H P A L L G E M Q Q Q A A L R S G S L E A M R P G I E A  
1901 A**AAAA**CAGCAGCTTCAACAGACTCAGGTGTTGT**CCAACTGCAACAGCAGCTCCATCATCTGCAACCCAACTTTTCCAATGTCCAGTCCC**ACTCTG  
88 F V A G A E V S E P T T W V A V A A G D D A V W V K G I D L G V R  
2001 GTGAGGAAGAGTTCTTGCAGTTCTGT**CACCCTCAATGTGCCTGTCAAGGTCAACTGTGCTTGTGAGGGTAGTCTGCAAAAGCAGCAGCCAGTG**  
54 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 Y D A F A A A L T  
2101 TTCTCAGCTCTT**GGAACATCATCTCTGGTTGCCAGCCTCACTGTGGTTTGTACTCAGTCATGGTGGCCCTCTATAGT**GAGTCGTATTACTATGC  
21 R V A R P V D D R T A L R V T P K Y E T M

**Asel (2220)**  
2201 CGATATACTATGCCGATGATTAATTGTCAACTACTGTTTGTAGGCGCCGGT**CACAGCTTGATCTGTAACGGCGAGAACAGAAAACGAAACAAAGACGT**

2301 AGAGTTGAGCAAGCAGGGT**CAGGCAAAGCGTGGAGAGCCGGCTGAGTCTAGGTAGGCTCAAGGGAGCGCCGGACAAAGGCCCGGTCTCGACCTGAGCTT**

2401 TAAACTTACTAGACGGCGACGAGTT**CAGGAGGCCACAGCGGGAGCGGCAGAACCGGACTCAACCGCGTGGATGGCGGCCTCAGGTAGGGCGG**

**HindIII (2533)** **Psp1406I (2576)**  
2501 CGGGCGGTGAAGGAGAGATGCGAGCCCTCGAAGCTT**CAGCTGTGTTCTGGCGCAAACCCGTTGCGAAAAAGAAGTTACGGCGACTACTGCACTTA**

2601 TATACGGTTCTCCCCACCTCGGGAAAAGCGGAGCCAGTACACGACATCACTTTCCAGTTTACCCCGCCACCTTCTTAGGCACCGGTT**CAATT** **Agel (2689)**

Spel (2758)

BspLU111 (2786)

2701 GCGACCCTCCCCCACTTCTCGGGACTGTGGCGATGTGCGCTGCCCACTGACTAGTGGCCCTGCAGGTTAATTAAGAACATGTGAGCAAAG  
2801 GCCAGCAAAGGCCAGGAACCGTAAAAAGCCGCTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCA  
2901 GAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCTGTTCCGACCCTGCCGTTACCGGATAC  
3001 CTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGTTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCAAGCTGGGCTGTG  
3101 TGCACGAACCCCCGTTACGCCGACCCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGC  
3201 CACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGT  
3301 ATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAACCACCGCTGGTAGCGGTGGTTTTTTGTTTGCA  
3401 AGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGTCTGACGCTCAGTGGAACGAAAACACTCACGTTAAGGGAT

NotI (3536)

3501 TTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAAC  
3601 TAACATACGCTCTCCATCAAACAAAACGAAACAAAACAACTAGCAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA