

Product usage

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

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

The purchase of the pNiFty2-N-Rluc-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.

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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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pNiFty2-N-Rluc-Puro

NF- κ B-inducible reporter plasmid selectable with Puromycin

Catalog code: pnf2p-rluc

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

For research use only

Version 23H16-AK

PRODUCT INFORMATION

Contents

- 20 μ g of lyophilized pNiFty2-N-Rluc-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) (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.
- **Rluc**: The *Renilla luciferase (Rluc)* gene encodes for an intracellular luciferase from the sea pansy *renilla reniformis*. This enzyme catalyzes coelenterazine oxidation leading to bioluminescence and the production of light emission peaking at 480 nm³. After cell lysis, the activity can be evaluated using QUANTI-Luc™ 4 Renilla (cat. code: rep-qlc4r1), an assay reagent containing all the components required to quantitatively measure the activity of Renilla 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 mRNA⁴.
- **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 promoter⁵ and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat⁶. 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 signal⁷.

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-Rluc-Puro plasmid features an NF- κ B-inducible *Renilla luciferase (Rluc)* 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. Of note, the Renilla luciferase remains intracellular, and requires cell lysis in order to measure bioluminescence. The subsequent expression of Rluc upon receptor activation is readily measurable after cell lysis when using QUANTI-Luc™ 4 Renilla, a Renilla luciferase detection kit, that also includes a lysis buffer. The pNiFty2-N-Rluc-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 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-Rluc-Blasti	Reporter plasmid	pnf2b-rluc
pNiFty2-N-Rluc-Zeo	Reporter plasmid	pnf2-rluc
QUANTI-Luc™ 4 Renilla	Luciferase Detection	rep-qlc4r1

1. Schindler U., Baichwal VR., 1994. Mol Cell Biol. 14(9):5820-31. 2. Jensen LE. & Whitehead AS., 2003. Biotechniques 35:54-58. 3. Bhaumik S, Gambhir SS. Proc Natl Acad Sci U S A. 2002 Jan 8;99(1):377-82 4. Carswell S. & Alwine J., 1989. Mol Cell Biol. 9(10):4248-58. 5. Kim D. et al., 1990. Gene 91 (2): 217-223. 6. Takebe Y. et al., 1988. Mol. Cell Biol. 1: 466-472. 7. Yu J. & Russell J., 2001. Mol Cell Biol, 21(17):5879-88.

TECHNICAL SUPPORT

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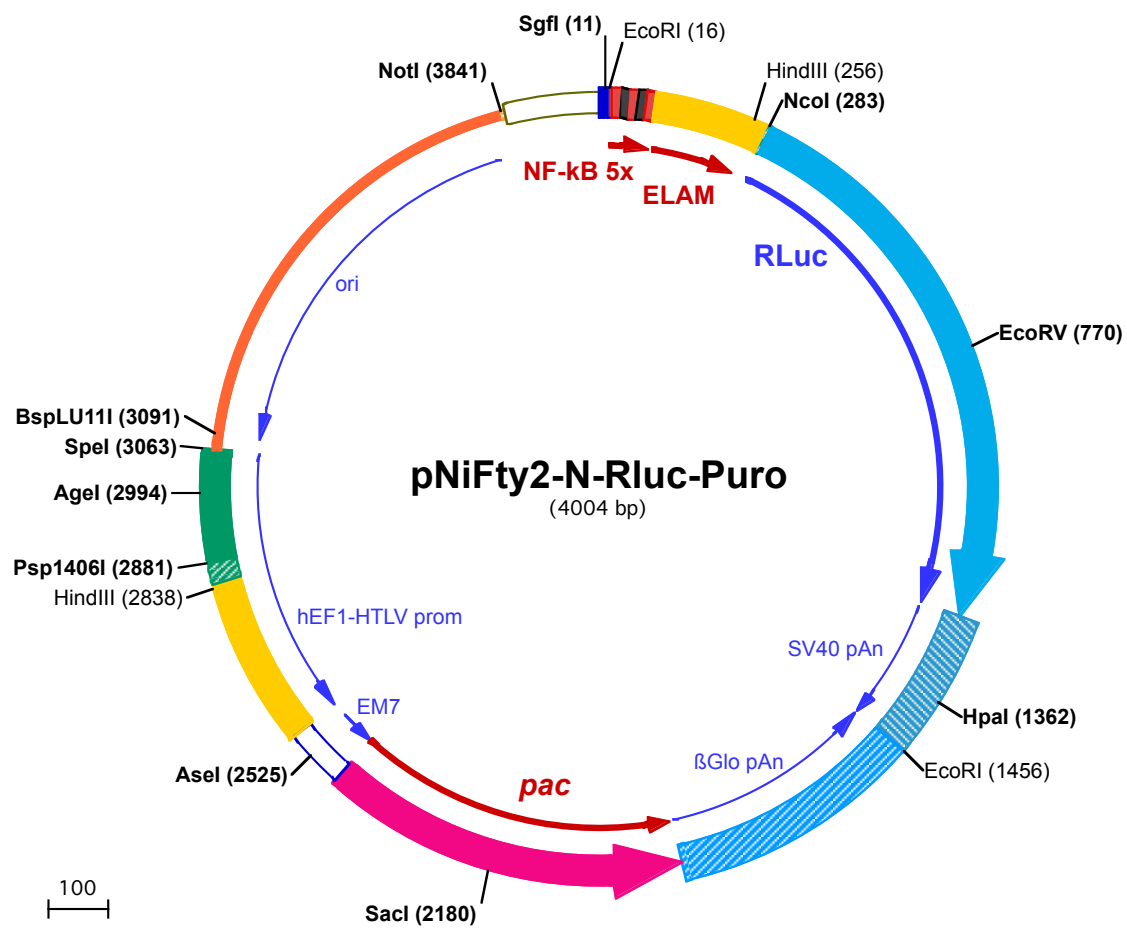
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Sgfl (11) EcoRI (16)
1 GGATCTGCGATCGCTGAATTC**TGGGGACTTTCCACTGGGGACTTTCCACTGGGGACTTTCCACTGGGGACTTTCCACTCCTGCAGC**
101 AGTGGATATTTCCAGAAAACTTTTTGGATGCAGTTGGGGATTCTCTTTACTGGATGTGGACAATATCTCTATTATTACAGGAAGCAATCCCTCCT

HindIII (256) NcoI (283)
201 AAAAAAGGGCCTCAGCAGAAAGTAGTGTTCAGCTGTTCTTGGCTGACTT**CACATCAAAGCTTCTATACTGACCTGAGACAGAGC**CATGGCTTCCAAGGTGT
301 ACGACCCCAGCAACGCAAACGCATGATCACTGGGCCTCAGTGGTGGGCTCGCTGCAAGCAAATGAACGTGCTGGACTCCTTCATCAACTACTATGATTC
6▶ Y D P E Q R K R M I T G P Q W W A R C K Q M N V L D S F I N Y Y D S
401 CGAGAAGCACGCCGAGAACGCCGTGATTTTTCTGCATGGTAACGCTGCCTCCAGCTACCTGTGGAGGCACGCTGCTGCCTCACATCGAGCCCGTGGCTAGA
39▶ E K H A E N A V I F L H G N A A S S Y L W R H V V P H I E P V A R
501 TGCATCATCCCTGATCTGATCGGAATGGTAAGTCCGGCAAGAGCGGAATGGCTCATATCGCTCCTGGATCACTACAAGTACCTCACCGCTTGGTTG
73▶ C I I P D L I G M G K S G K S G N G S Y R L L D H Y K Y L T A W F
601 AGCTGCTGAACCTTCAAAGAAAATCATCTTTGTGGCCACGACTGGGGGCTTGTCTGGCCTTCTACTACTCTACGAGCACCAAGACAAGTCAAGGC
106▶ E L L N L P K K I I F V G H D W G A C L A F H Y S Y E H Q D K I K A

EcoRV (770)
701 CATCGTCCATGCTGAGAGTGTCTGGACGTGATCGAGTCTGGGACGAGTGGCCTGACATCGAGGAGGATATCGCCCTGATCAAGAGCGAAGAGGGCGAG
139▶ I V H A E S V V D V I E S W D E W P D I E E D I A L I K S E E G E
801 AAAATGGTCTTGAGAATAACTTCTTCGTCGAGACCATGCTCCAAGCAAGATCATGCGGAACTGGAGCCTGAGGAGTTCGCTGCCTACCTGGAGCCAT
173▶ K M V L E N N F F V E T M L P S K I M R K L E P E E F A A Y L E P
901 TCAAGGAGAAGGGCGAGGTTAGACGGCTACCTCTCTGGCTCGCGAGATCCCTCTCGTTAAGGGAGGCAAGCCCGACGCTCGTCCAGATTGTCCGCAA
206▶ F K E K G E V R R P T L S W P R E I P L V K G G K P D V V Q I V R N
1001 CTACAACGCTACCTTCGGGCCAGCGACGATCTGCCTAAGATGTTTCATCGAGTCCGACCTGGGTTCTTTTCCAACGCTATTGTGAGGGAGCTAAGAAG
239▶ Y N A Y L R A S D D L P K M F I E S D P G F F S N A I V E G A K K
1101 TTCCTAACACCGAGTTCGTGAAGGTGAAGGGCTCCACTTCAGCCAGGAGGACGCTCCAGATGAAATGGTAAGTACATCAAGAGCTTCGTGGAGCGCG
273▶ F P N T E F V K V K G L H F S Q E D A P D E M G K Y I K S F V E R
1201 TGCTGAAGAACGAGCAGTAATCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTA
306▶ V L K N E Q •

HpaI (1362)
1301 TTTGTAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAAACAAGTTAAACAACAACAAATTGCATTATTTTATGTTTCAGGTTCA

EcoRI (1456)
1401 GGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATTC**AAAAATACAGCATAGCAAACCTTAACTCCAATCAAG**
1501 CCTCTACTTGAATCCTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGAGCCTCACCTTCTTTCATGGA
1601 GTTTAAGATATAGTGATTTTTCCAAGGTTTGAAGTCTTCTATTCTTTATGTTTTAAATGCACTGACCTCCACATTCCCTTTTATGAAAAATATT
1701 CAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGTAG
1801 TTGGACTTAGGGAACAAAGAACCTTTAATAGAAATTTGGACAGCAAGAAAGCGAGCTTCTAGCTCAGGTTAAGCTCCAGGCTTCTTGTGCATGCACCAA
200▶ A G P K R T M C W
1901 GTTCTTGGCCTTCTGGAACCTCAACATCAGCTGTACAGTGAATCCAGTCTTTTATAAAAAGGCAGGTTTCTGGGAGCAGAAGTTCCAGAAAGGCAG
189▶ 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 A S T E L F A P
2001 GAACTCCAGCCCTTTCAGCAGCTTCACTCCAGGCAGAAACAGCAGATCCAGACCCTTCCCTGGTGGTCAGGGCTCACTCCAACAGTTGCCAGAAA
156▶ 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 V G V T A L F

SacI (2180)
2101 CCAAGCTGGCTCTTTTGGCTGTGTGGTGCAGCAGACCTCCATTTGTTGTTGTGCTGCCAGCCTGCTCCAGAGAGCTCAGCCATTCTTGGTCCAATT
123▶ 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 E A M R P G I
2201 TCAGCAAAAACAGCACCAGCTTCAACAGACTCAGGTGTGTCCAAACTGCAACAGCAGCTCCATCATCTGCAACCAAACTTTTCCAATGTCCAGTCCCA
89▶ 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 V K G I D L G V
2301 CTCTGGTGAAGAGATTCTGAGTCTGTACCCTCTCAATGTGCCTGTGAGGTCAGGTCAGTGTGCCTTGTGAGGGTAGTCTGAAAAGCAGCAGC
56▶ 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 Y D A F A A A
2401 CAGTGTCTCACAGCTCTTGAACATCATCTCTGGTTGCCAGCCTCACTGTGGGTTTGTACTCAGTCA**GGTGGCCCTCTATAGTGAAGTCTGATTATA**
23▶ L T R V A R P V D D R T A L R V T P K Y E T M

Asel (2525)
2501 TATGCCGATATACTATGCCGATGATTAATTGTCAACTACTGTTTGTAGGCGCCGGTCAAGCTTGGATCTGTAACGGCGCAGAACAGAAAACGAAACAAA
2601 GACGTAGAGTTGAGCAAGCAGGGTCAGGCAAAGCGTGGAGAGCCGGCTGAGTCTAGGTAGGCTCAAGGGAGCGCCGACAAAGGCCCGGTCTCGACCTG
2701 AGCTTTAAACTTACCTAGACGGCGGACGCAGTTCAGGAGGCCACAGCGGGAGGCGGACAGAACCGGACTCAACCGGCGTGGATGGCGGCCTCAGGTAG

HindIII (2838) Psp1406I (2881)
2801 GCGGGCGGGCGCGTGAAGGAGAGATGCGAGCCCTCGAAGCTT**CAGCTGTGTTCTGGCGGCAACCCGTTGCGAAAAAGAACGTTACGGCGACTACTGC**

Agel (2994)

2901 ACTTATATACGGTTCTCCCCACCCTCGGGAAAAAGGCGGAGCCAGTACACGACATCACTTTCCAGTTTACCCCGGCCACCTTCTCTAGGCACCGGTT

SpeI (3063)

BspLU11I (3091)

3001 CAATTGCCGACCCTCCCCCAACTTCTCGGGGACTGTGGGCGATGTGCGCTCTGCCACTGACTAGTGGGCCCTGCAGGTTAATTAAGAACATGTGAGC

3101 AAAAGCCAGCAAAGGCCAGGAACCGTAAAAAGGCCGCTTGTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCA

3201 AGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCTGTTCCGACCCTGCCGTTACCG

3301 GATACCTGTCCGCCTTCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCAAGCTGGG

3401 CTGTGTGCACGAACCCCGTTAGCCCGACCGCTGCGCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCA

3501 GCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTAT

3601 TTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAACCACCGCTGGTAGCGGTGGTTTTTTTGT

3701 TTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTGGAACGAAAACACTCACGTTAA

NotI (3841)

3801 GGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATC

3901 GTAACATAACGCTCTCCATCAAACAAAACGAAACAAAACAACTAGCAAATAGGCTGTCCCCAGTGAAGTGCAGGTGCCAGAACATTTCTCTAT

4001 CGAA