

Product usage

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

Important Limited Use information for pNiFty3-I-RLuc-Zeo

The purchase of the pNiFty3-I-RLuc-Zeo 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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pNiFty3-I-Rluc-Zeo

IRF-inducible reporter plasmid selectable with Zeocin®

Catalog code: pnf3-rluc4

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

For research use only

Version 23H08-NJ

PRODUCT INFORMATION

Contents

- 20 µg of lyophilized pNiFty3-I-Rluc-Zeo (plasmid DNA)
- 1 ml of Zeocin® (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 Zeocin® 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 mIFN-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 *Rluc* reporter gene.
- **Rluc**: The *Renilla luciferase (Rluc)* gene encodes for an intracellular (non secreted) 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-1α/HTLV hybrid promoter** is a composite promoter comprising the Elongation Factor-1α (EF-1α) 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-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.

Zeocin® antibiotic selection cassette

- **EM7** is a bacterial promoter that enables the constitutive expression of the Zeocin® resistance gene (*Sh ble*) in *E. coli*.
- **Zeo (resistance to the antibiotic Zeocin®)** is conferred by the *Sh ble* gene from *Streptoalloteichus hindustanus*. The *Sh ble* 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 *bsr*. The use of β-globin pAn minimizes interference and possible recombination events with the SV40 pAn signal⁷.

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-Rluc-Zeo plasmid features an IRF-inducible *Renilla luciferase (Rluc)* reporter gene under the control of an engineered mIFN-β promoter. This promoter comprises several ISRE repeated TFBS to enhance the IRF-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 pNiFty3-I-Rluc-Zeo plasmid is selectable with Zeocin® in both *E. coli* and mammalian cells, and can be used to generate stable clones.

METHODS

- **Plasmid resuspension**
 - Quickly spin the tube containing the lyophilized plasmid 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.
- **Zeocin® usage**

Zeocin® can be used at 25 µg/ml in *E. coli* in liquid or solid media and at 50-200 µg/ml to select Zeocin®-resistant mammalian cells.

RELATED PRODUCTS

Product	Description	Cat. Code
Zeocin®	Selection antibiotic	ant-zn-1
ChemiComp GT115	Competent <i>E. coli</i>	115-11
QUANTI-Luc™ 4 Renilla	Luciferase Detection	rep-qlc4r1

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. 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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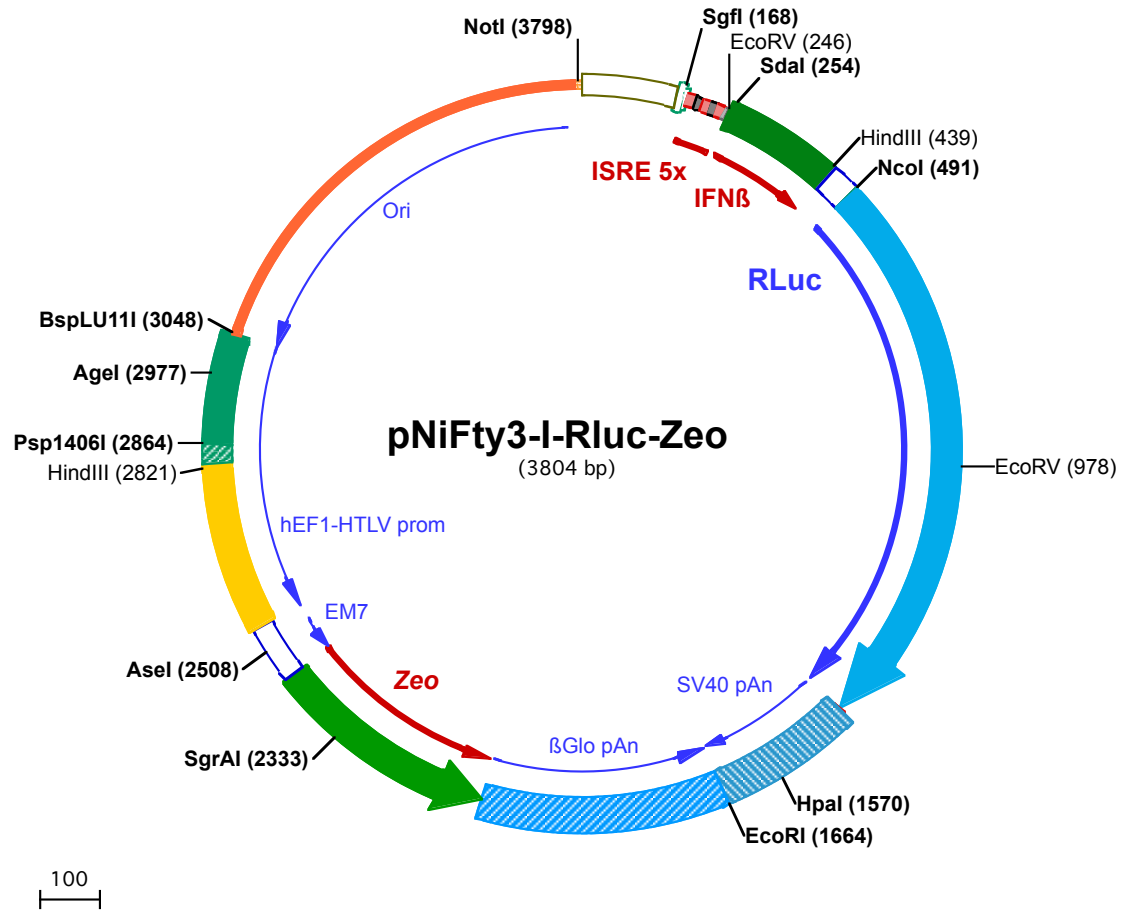
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1 AATAAAATATCTTTATTTTCATTACATCTGTGTGTTGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAACAAAACGAAACAAAACAAAC
SgfI (168)
101 TAGCAAATAGGCTGTCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTATCGAAGGATCGCGATCGTGAATTAGTTTCACTTTCCAGTTTCAGTT

SdaI (254)
EcoRV (246)
201 TCCAGTTTCATTTTCCAGTTTCATTTTCCAGTTTCATTTTCTGATATCCTGCAGGagcttgaataaatgaatattagaagctgtagaataagagaaa

301 atgacagaggaAAACTGAAAGGgAGAACTGAAAGTGggaattcctctgaggcagaaggaccatccctTATAAAtagcacaggccatgaaggaagatca

HindIII (439) NcoI (491)
401 ttctcactgcagcctttgacagcctttgctcatcttgAAGCTTCTGCCTTCTCCCTCCTGTGAGTTTGGTTGGTGTACAGTAGTCCACCATGGCTTC
1 M A S
501 CAAGGTGTACGACCCCGAGCAACGCAACGCATGATCACTGGGCTCAGTGGTGGGCTCGCTGCAAGCAAATGAACGTGTGGACTCCTTCATCAACTAC
3 K V 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
601 TATGATTCGAGAAGCACGCCGAGAAGCGCTGATTTTCTGCATGGTAACGCTGCCTCCAGCTACCTGTGGAGGCACGTCTGCCTCACATCGAGCCCG
37 Y D S 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
701 TGGCTAGATGCATCATCCTGATCTGATCGGAATGGTAAAGTCGGCAAGAGCGGGAATGGCTCATATCGCCTCCTGGATCACTACAAGTACCTCACCGC
70 V A R 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
801 TTGGTTCGAGCTGCTGAACCTTCAAAGAAAATCATCTTTGTGGGCCACGACTGGGGGGCTTGTCTGGCTTTCCTACTCTACGAGCACCAGACAAG
103 W F 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
EcoRV (978)
901 ATCAAGGCCATCGTCCATGCTGAGAGTGTCTGGAGCTGATCGAGTCTGGGACGAGTGGCCTGACATCGAGGAGGATATCGCCTGATCAAGAGCGAAG
137 I K A 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
1001 AGGGCGAGAAAATGGTGTGAGAATAACTTCTCGTCGAGACCATGCTCCAAGCAAGATCATCGGAAACTGGAGCCTGAGGAGTTCGCTGCCTACCT
170 E G E 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
1101 GGAGCCATTCAAGGAGAAGGGCGAGGTTAGACGGCCTACCCTCTCCTGGCCTCGCGAGATCCCTCTCGTTAAGGGAGGCAAGCCCGACGCTGTCCAGATT
203 E P 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
1201 GTCCGCAACTACAACGCCTACCTCGGGCCAGCGACGATCGCTAAGATGTTTCATCGAGTCCGACCTGGGTTCTTTCCAACGCATTGTCGAGGGAG
237 V R N 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
1301 CTAAGAAGTTCCTAACACCGAGTTCGTGAAGGTGAAGGGCTCCACTTCAGCCAGGAGGACGCTCCAGATGAAATGGGTAAGTACATCAAGAGCTTCGT
270 A K K 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
1401 GGAGCGCTGTGAAGAACGAGCAGTAACTCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACTAGAATGCAGTGAAAAA
303 E R V L K N E Q •

HpaI (1570)
1501 ATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTATGTTT

EcoRI (1664)
1601 CAGGTTCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATTCATAAATACAGCATAGCAAACTTTAACCTCC
1701 AAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCT
1801 TTCATGGAGTTTAAGATATAGTGATTTTCCCAAGGTTTGAAGTACTGCTCTTCAATTTCTTATGTTTTAAATGCACTGACCTCCACATTCCCTTTTATG
1901 AAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAG
2001 TTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCTGCTCTGCCACAA
125 D Q E E A V F
2101 AGTGCACGAGTTGCCGGCCGGTGCAGGCGAAGTCCCGCCCCACGGTCTGCTCGCGATCTCGGTCTGCGGATGCGCGGATGCGCGGAGGCGTCCCGAAGTT
117 H V C N G A P D R L A F E R G W P Q E G I E T M A P G S A D R F N
2201 CGTGGACACGACTCCGACCACTCGGCGTACAGCTCGTCCAGGCGCGCACCCACACCCAGGCCAGGGTGTGTCGCGCACCACTGGTCTGGACCGCG
84 T S V V E S W E A Y L E D L G R V W V W A L T N D P V V Q D Q V A
SgrAI (2333)
2301 CTGATGAACAGGGTCACGTCTGCCGACCAACCGGCGAAGTCTGCTCCACGAAGTCCCGGAGAACCAGGCGGTTCGAGTCCAGAACTCGACCGCTC
50 S I F L T V D D R V V G A F D D E V F D R S F G L R D T W F E V A G
2401 CGGCGAGTCTCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTTGGCCATGATGCCCTCTATAGTGAGTCGATTATACTATGCCGATATACTATG
17 A V D R A T L V P V A S T L K A M
AseI (2508)
2501 CCGATGATTAATTGTCAACTACTGTTTGTAGGCCCGGTACACAGTTGGATCTGTAACGGCGCAGAACAGAAAACGAAACAAAGACGTAGAGTTGAGCAA
2601 GCAGGGTCAGGCAAAGCGTGGAGAGCCGGTCTGAGTCTAGGTAGGCTCCAAGGGAGCGCCGACAAAGGCCCGGTCTCGACCTGAGCTTTAAACTTACCTA
2701 GACGGCGGACGAGTTTCAAGAGGCACCAAGCGGGAGCGGCGAGAACCGACTCAACCGGCTGGATGGCGGCTCAGGTAGGGCGGCGGGCGGTGAA

2801 GGAGAGATGCGAGCCCCTCGAAGCTTCAGCTGTGTTCTGGCGCAAACCCGTTGCGAAAAAGAACGTTACGCGGACTACTGCACTTATATACGGTCTC
HindIII (2821) Psp1406I (2864)

2901 CCCCACCCTCGGGAAAAAGGCGGAGCCAGTACACGACATCACTTCCAGTTTACCCCGCGCCACCTTCTCTAGGCACCGTTCAATTGCCGACCCCTCC
Agel (2977)

3001 CCCCAACTTCTCGGGACTGTGGGCGATGTGCGCTCTGCCACTGACACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCTTG
BspLU11I (3048)

3101 CTGGCGTTTTTTCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGG

3201 CGTTTCCCCTGGAAAGCTCCCTCGTGCCTCTCCTGTTCCGACCCTGCCGTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGTTTC

3301 TCATAGCTCAGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTCAGCCCGACCGCTGCGCCTTA

3401 TCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCG

3501 GTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAG

3601 AGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAA
NotI (3798)

3701 GATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGG

3801 CCGC