

Product usage

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

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

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

IRF-inducible reporter plasmid selectable with Zeocin®

Catalog code: pnf3-fluc4

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

For research use only

Version 23H08-NJ

PRODUCT INFORMATION

Contents

- 20 µg of lyophilized pNiFty3-I-Fluc-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 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.

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 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-Zeo 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-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>	gt115-11

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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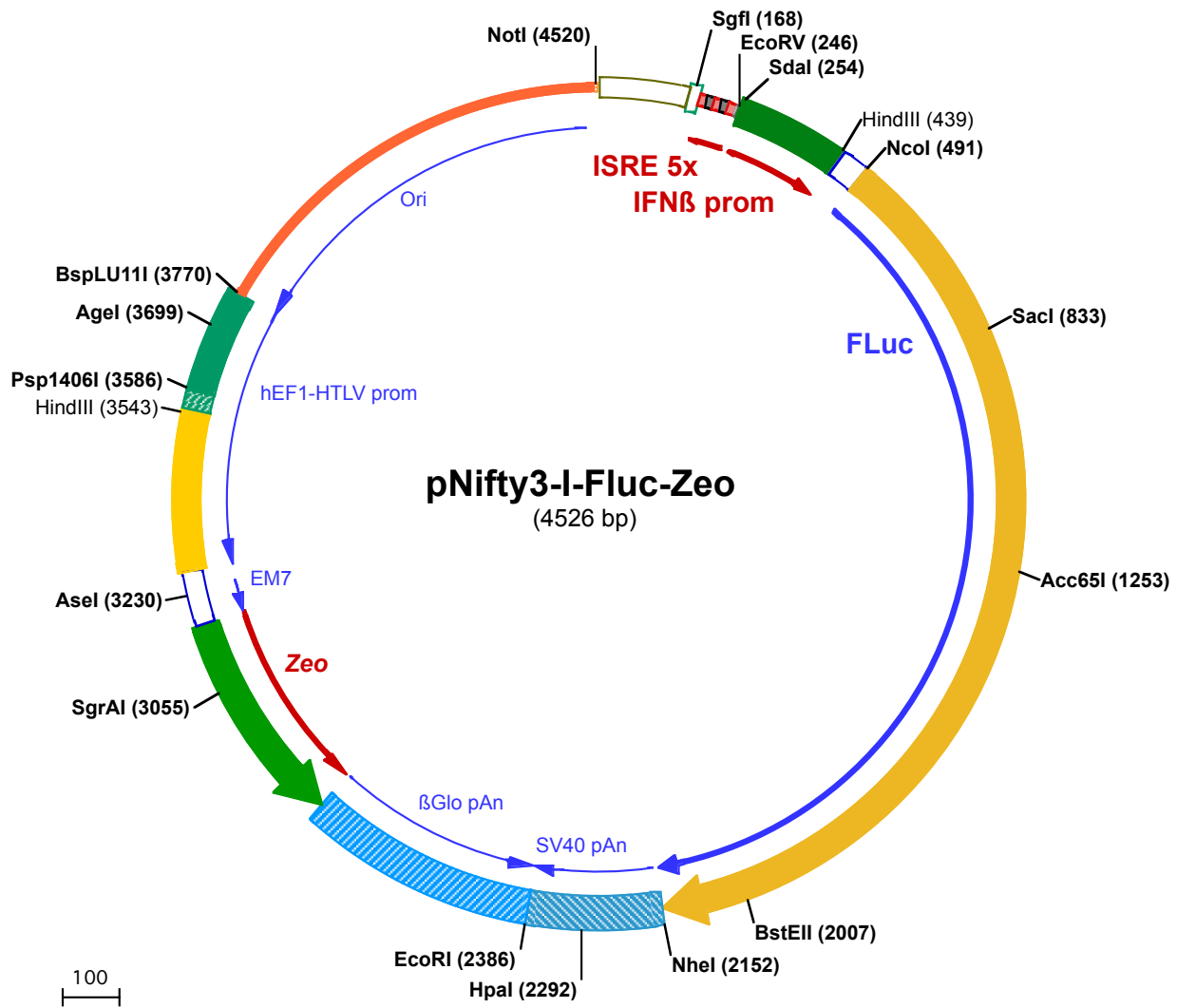
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1 AATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTGTGTGAATCGTAACATAACGCTCTCCATCAAAACAAAACGAAACAAAACAAAC
101 TAGCAAAATAGGCTGTCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTATCGAAGGATCGCGATCGTGAATTAGTTTCACTTTCCAGTTTCAGTT

SgfI (168)

201 TCCAGTTTCATTTCCAGTTTCATTTCCAGTTTCATTTCTGATATCCTGCAGGagcttgaataaaatgaatattagaagctgtagaataagagaaa
301 atgacagaggaAAACTGAAAGGgAGAACTGAAAGTGggaattcctctgaggcagaaggaccatccctTATAAatagcacaggccatgaaggaagatca

SdaI (254)
EcoRV (246)

401 ttctcactgcagcctttgacagcctttgctcatcttgAAGCTTCTGCCTTCTCCCTCTGTGAGTTTGGTTGGTGTACAGTAGCTTCCACCATGGAGGA
1 M E D

HindIII (439)

NcoI (491)

501 TGCCAAGAATATTAAGAAAGGCCCTGCCCATTTACCTCTGGAAGATGGCACTGCTGGTGGAGCAACTGCACAAGGCCATGAAGAGGTATGCCCTGGTC
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 CCTGGCACCATTGCCTTCACTGATGCTCACATTGAGGTGGACATCACCTATGCTGAATACTTTGAGATGCTGTGAGGCTGGCAGAAGCCATGAAAAGAT
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 CCCTGCCAATGACATCTACAATGAGAGAGAGCTCCTGAACAGCATGGGCATCAGCCAGCCAAGTGGTCTTTGTGAGCAAGAAGGGCCTGCAAAAGATC
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 I M D S K T D Y Q G F Q S M Y T F V T
1001 GCCACTTACCCCTGGCTTCAATGAGTATGACTTTGTGCTGAGAGCTTTGACAGGGACAAGACCATTGCTCTGATTATGAACAGCTCTGGCTCCACTGG
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 ACTGCCAAAAGGTGTGGCTCTGCCCCACAGAAGTCTGTGTGAGATTGAGCCATGCCAGAGACCCCATCTTTGGCAACCAGATCATCCCTGACACTGCC
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 ATCCTGTCTGTGGTTCCATTCCATCATGGCTTTGGCATGTTCAACAACACTGGGGTACCTGATCTGTGGCTTCCAGAGTGGTGTGATGTATAGGTTTGGAG
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 GTATGACCTGAGCAACCTGCATGAGATTGCCTCTGGAGGAGCACCCCTGAGCAAGGAGGTGGGTGAGGCTGTGGCAAGAGGTTCCATCTCCAGGAATC
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 CTGGCCTGCTGATGATGCTGGAGAGCTGCCTGCTGTTGTGGTCTGGAGCATGAAAAGACCATGACTGAGAAGGAGATTGTGGACTATGTGGC
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 GAGATCCTGATCAAGGCCAAGAAGGTTGGCAAAATTGCTGTGTAACCTGAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCA
537 E I L I K A K K G G K I A V

HpaI (2292)

2201 CAACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAAGTTAAACAACA

EcoRI (2386)

2301 CAATTGCATTCATTTTATGTTTCAGGTTTCAAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATCTAAAATACA

2401 GCATAGCAAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTA

2501 GCTGTTTGCAGCCTCACCTTCTTTTCATGGAGTTAAGATATAGTGTATTTTCCAAGGTTTGAAGTACTCTTCATTTCTTTATGTTTAAATGCACTGA

2601 CCTCCACATTCCCTTTTATGTAATAATTTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTATTAGGCAGAATCCAGATGCTCAA

2701 GGCCCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGAACCTTTAATAGAAATTTGGACAGCAAGAAGCGAGCTTCTAGCTTATCCT

2801 CAGTCCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGTTCGCGCAGGGCGAACTCCCGCCCCACGGTGCTCGCCGATCTCGGTCATGGCCG
124◀• D Q E E A V F 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
2901 GCCCGGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACACCCAGGCCAGGGTGTGTCCGG
91◀ G S A D R F N 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
SgrAI (3055)
3001 CACCACCTGGTCTGACC GCGCTGATGAACAGGGTACGTCGTCGCCGACCAACCCGGCGAAGTCGTCCTCCACGAAGTCCCGGGAGAACCAGCCGAGCCGG
58◀ V V Q D Q V A 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
3101 TCGGTCCAGAACTCGACCGTCCGGCGACGTCGCGCGGTTGAGCACC GGAACGGCACTGGTCAACTTGGCCATGATGGCCCTCTATAGTGAGTCGTAT
24◀ D T W F E V A G A V D R A T L V P V A S T L K A M ←
AseI (3230)
3201 TATACTATGCCGATATACTATGCCGATGATTAATTGTCAACTACTGTTTGTAGGCGCCGGTACAGCTTGATCTGTAACGGCGCAGAACAGAAAACGAA
3301 ACAAAGACGTAGAGTTGAGCAAGCAGGGTCAGGCAAAGCGTGAGAGCCGGCTGAGTCTAGGTAGGCTCAAGGGAGCGCCGGACAAAGGCCCGGTCTCG
3401 ACCTGAGCTTTAAACTTACCTAGACGGCGGACGCAGTT CAGGAGGCCACACAGCGGGAGGCGCAGAACCGCACTCAACCGCGTGGATGGCGGCCCTCA
HindIII (3543) Psp1406I (3586)
3501 GGTAGGGCGGGCGCGCTGAAGGAGAGATGCGAGCCCTCGAAGCTTCAGCTGTGTCTGCGCGCAAACCCGTTGCGAAAAAGAAGCTTACCGGCGACT
Agel (3699)
3601 ACTGCACTTATATACGTTCTCCCCACCCTCGGAAAAAGCGGAGCCAGTACACGACATCACTTTCCAGTTTACCCCGGCCACCTTCTCTAGGCAC
BspLU11I (3770)
3701 CGGTTCAATTGCCGACCCTCCCCCAACTTCTCGGGACTGTGGCGATGTGCGCTCTGCCACTGACACATGTGAGCAAAGGCCAGCAAAGGCCAG
3801 GAACCGTAAAAAGGCCGCTTGTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCAGAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCG
3901 ACAGGACTATAAAGATACCAGCGTTTTCCCCCTGGAAGCTCCCTCGTGCCTCTCCTGTTCCGACCCTGCCGTTACCGGATACCTGTCCGCTTTCTCC
4001 CTTCCGGAAGCGTGGCGTTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCCGGTGTAGGTCGTTCCGCTCCAAGCTGGGCTGTGTGCAGAACCCCGT
4101 TCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGTAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAAACAGGATT
4201 AGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGA
4301 AGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTGTTGCAAGCAGCAGATTACGG
4401 CAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGTCTGACGCTCAGTGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGGCTAGT
NotI (4520)
4501 TAATTAACATTTAAATCAGCGGCCGC