Product usage

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

Important Limited Use information for pTiGer3-eGFP

The purchase of the pTiGer3-eGFP 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.

pTiGer3-eGFP

A multigenic plasmid for inducible enhanced green fluorescent protein expression, selectable with Hygromycin

Catalog code: ptg3-gfp

https://www.invivogen.com/tet-on-ptiger-reporter

For research use only

Version 24A16-NJ

PRODUCT INFORMATION

Contents:

- **20 µg of pTiGer3-eGFP** provided as lyophilized DNA
- **1 ml of Hygromycin** (100 mg/ml)

Storage and Stability:

- Product is shipped at room temperature.
- Lyophilized DNA should be stored at -20 °C.
- Resuspended DNA should be stored at -20 °C and is stable for up to 1 year.
- Store Hygromycin at 4 °C or at -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.

PRODUCT DESCRIPTION

InvivoGen provides a family of plasmids featuring a tetracycline-inducible reporter gene. The **pTiGer3-eGFP** plasmid encodes the enhanced green fluorescent protein (eGFP) and the Hygromycin resistance marker for selection in both mammalian cells and bacteria. This plasmid can be used as a transfection control for plasmids of the pTiGer-mcs family.

The eGFP expression is only possible upon transfection of cells featuring the tetracycline repressor (TetR) protein[1], such as InvivoGen's HEK-RepTor™ or A549-RepTor™ cells. These cells express TetR constitutively in the nucleus, where it binds to tetracycline operator (tetO) sequences and represses gene transcription. Upon incubation with doxycycline (a synthetic tetracycline derivative), TetR is released from the tetO sequences and the eGFP-encoding gene is transcribed.

pTiGer-SEAP and pTiGer-Lucia plasmids are also available.

PLASMID FEATURES

SEAP expression cassette

- **hCMV enh/ hEF1 prom:** This composite promoter combines the human cytomegalovirus (HCMV) enhancer and the core promoter of the human elongation factor-1α (EF-1α)[2].
- **tetOtetO:** This sequence is also known as TRE (Tetracycline Response Element). It is a repeat of the 19-nucleotide sequence of the tetracycline operator (tetO)[1].
- **eGFP (enhanced green fluorescent protein)** is a synthetic variant of the wild-type GFP. It features mutations that allow for better folding efficiency at 37°C and higher-intensity emission, compared to GFP. Moreover its codon sequence is optimized for expression in mammalian cells. The eGFP excitation peak is at ~ 490 nm, and its emission peak at ~ 509 nm.
- **SV40 pAn:** The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA[3].

Hygromycin antibiotic selection cassette

- **pMB1 Ori:** This minimal *E. coli* origin of replication with the same activity as the longer Ori.
- **hAldA enh/ hFerH prom:** This composite promoter combines the human aldehyde dehydrogenase (aldA) enhancer and the core promoter of the human ferritin heavy chain gene (FerH).
- **EM7**: This bacterial promoter enables the constitutive expression of the *hph* gene in *E. coli*.
- ***hph*:** The resistance to Hygromycin is conferred by the *hph* gene. The *hph* gene is driven by the human AldA/FerH promoter in tandem with the bacterial EM7 promoter allowing selection in both mammalian cells and *E. coli*.
- **mEF1 5'UTR:** The 5'UTR (untranslated region) of the murine EF-1α enhances *hph*-encoding mRNA stability and protein translation.
- **hβGlo pAn:** The human β-Globin pAn is a strong polyadenylation signal placed downstream of *hph*[4].

1. Hillen, W., Wissmann, A. (1989). Tet repressor-tet operator interaction. Protein-Nucleic Acid Interaction. DOI: 10.1007/978-1-349-09871-2_7. **2. Kim DW. *et al.*, 1990.** Use of the human elongation factor 1α promoter as a versatile and efficient expression system. Gene 91(2):217-23. **3. Carswell S. & Alwine JC., 1989.** Efficiency of utilization of the simian virus 40 late polyadenylation site:effects of upstream sequences. Mol Cell Biol. 9(10):4248-58. **4. Yu J. & Russell JE., 2001.** Structural and functional analysis of an mRNP complex that mediates the highstability of human β-globin mRNA. Mol Cell Biol. 21(17):5879-88.

METHODS

- **Plasmid resuspension**
  - Quickly spin the tube 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.

- **Hygromycin usage**

Hygromycin can be used at 50-100 µg/ml in *E. coli* in liquid or solid media and at 50-500 µg/ml to select Hygromycin-resistant mammalian cells.

- **Generation of Tet-inducible expression cells**

For a general procedure using InvivoGen's RepTor™ cell lines, please visit: https://www.invivogen.com/reptor-cells.

RELATED PRODUCTS

Product	Description	Cat. Code
Hygromycin	Selection antibiotic	ant-hg-1
HEK-RepTor™ cells	TetR-expressing cells	hk-rtor

TECHNICAL SUPPORT
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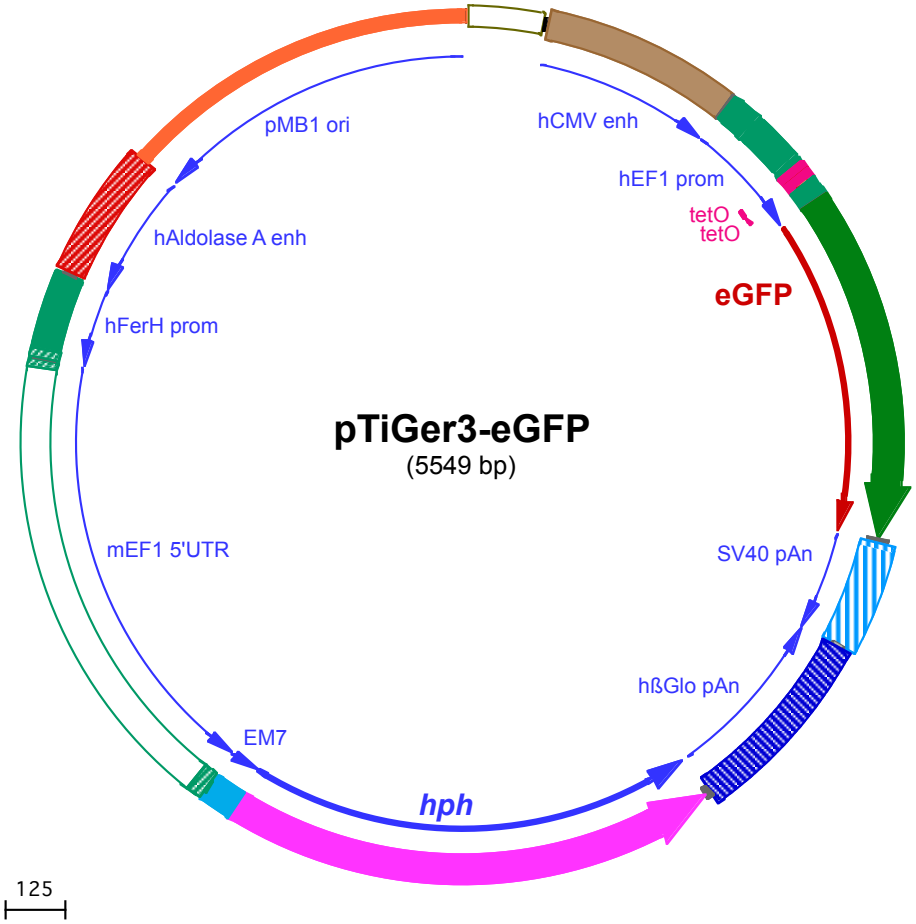
pMB1 ori
hCMV enh
hEF1 prom
tetO
tetO
eGFP
hAldolase A enh
hFerH prom
pTiGer3-eGFP
(5549 bp)
mEF1 5'UTR
SV40 pAn
hßGlo pAn
EM7
hph
125


1 CTCGAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACG
101 AAACAAAACAAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGACCTGCAGGCGTTACATAACTTACGGTA
201 AATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTC
301 AATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCC
401 CGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGATGATGCGGTTTTGG
501 CAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGACTAGTCAGTGGGC
601 AGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGATCCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGAT
701 GTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTTGCCGTGAACGTTTCCCTATCAGTGATAGAGATCTCCC
801 TATCAGTGATAGAGATCTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCACCGGTCACCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGG
1 M V S K G E E L F T G
901 GTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCC
12 V V P I L V E L D G D V N G H K F S V S G E G E G D A T Y G K L T
1001 TGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCA
45 L K F I C T T G K L P V P W P T L V T T L T Y G V Q C F S R Y P D H
1101 CATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCC
78 M K Q H D F F K S A M P E G Y V Q E R T I F F K D D G N Y K T R A
1201 GAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACA
112 E V K F E G D T L V N R I E L K G I D F K E D G N I L G H K L E Y
1301 ACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCA
145 N Y N S H N V Y I M A D K Q K N G I K V N F K I R H N I E D G S V Q
1401 GCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGAC
178 L A D H Y Q Q N T P I G D G P V L L P D N H Y L S T Q S A L S K D
1501 CCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGCTAGCTGGCCA
212 P N E K R D H M V L L E F V T A A G I T L G M D E L Y K •
1601 GACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTG
1701 TAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTA
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2201 AATTGGACAGCAAGAAAGCGAGCTTCTAGCGAATTCTCGACTCATTCCTTTGCCCTCGGACGAGTGCTGGGGCGTCGGTTTCCACTATCGGCGAGTACTT
342 • E K A R P R T S P R R N G S D A L V E
2301 CTACACAGCCATCGGTCCAGACGGCCGCGCTTCTGCGGGCGATTTGTGTACGCCCGACAGTCCCGGCTCCGGATCGGACGATTGCGTCGCATCGACCCTG
322 V C G D T W V A A S R R A I Q T R G V T G A G S R V I A D C R G Q
2401 CGCCCAAGCTGCATCATCGAAATTGCCGTCAACCAAGCTCTGATAGAGTTGGTCAAGACCAATGCGGAGCATATACGCCCGGAGCCGCGGCGATCCTGCA
289 A W A A D D F N G D V L S Q Y L Q D L G I R L M Y A R L R P S G A
2501 AGCTCCGGATGCCTCCGCTCGAAGTAGCGCGTCTGCTGCTCCATACAAGCCAACCACGGCCTCCAGAAGAAGATGTTGGCGACCTCGTATTGGGAATCCC
255 L E P H R R E F Y R T Q Q E M C A L W P R W F F I N A V E Y Q S D G
2601 CGAACATCGCCTCGCTCCAGTCAATGACCGCTGTTATGCGGCCATTGTCCGTCAGGACATTGTTGGAGCCGAAATCCGCGTGCACGAGGTGCCGGACTTC
222 F M A E S W D I V A T I R G N D T L V N N S G F D A H V L H R V E
2701 GGGGCAGTCCTCGGCCCAAAGCATCAGCTCATCGAGAGCCTGCGCGACGGACGCACTGACGGTGTCGTCCATCACAGTTTGCCAGTGATACACATGGGGA
189 P C D E A W L M L E D L A Q A V S A S V T D D M V T Q W H Y V H P
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122 I A D M L E A V P Q L V A P L E T E P L D Q L T V G Q A R R S I C
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89 Y T L S E S F E G I D L V E P I P L A A S A F H R Y V Y R D K Y F
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55 G D A C S N V R L V Y G R G G V D F S F A R S E E G E S L Q M L D S
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22 V S D F K E I L F K A V S T A T L E P K K M


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