

# Product usage

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

## Important Limited Use information for pTiGer4-eGFP

The purchase of the pTiGer4-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.

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### TECHNICAL SUPPORT

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# pTiGer4-eGFP

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

Catalog code: ptg4-gfp

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

For research use only

Version 24A16-NJ

## PRODUCT INFORMATION

### Contents:

- 20 µg of pTiGer4-eGFP provided as lyophilized 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 should be stored at -20 °C and is stable for up to 1 year.
- Store Puromycin 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 pTiGer4-eGFP plasmid encodes the enhanced green fluorescent protein (eGFP) and the Puromycin 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<sup>1</sup>, 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-1 $\alpha$  (EF-1 $\alpha$ )<sup>2</sup>.
- **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)<sup>1</sup>.
- **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<sup>3</sup>.

### Puromycin 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 *pac* gene in *E. coli*.
- **pac:** The resistance to Puromycin is conferred by the *pac* gene from *Streptomyces* which encodes a N-acetyl-transferase. The *pac* 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-1 $\alpha$  enhances *pac*-encoding mRNA stability and protein translation.
- **hβGlo pAn:** The human  $\beta$ -Globin pAn is a strong polyadenylation signal placed downstream of *pac*<sup>4</sup>.

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 1 $\alpha$  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 high stability of human  $\beta$ -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 DH5 $\alpha$ .

### Puromycin usage

Puromycin can be used at 100-125 µg/ml in *E. coli* in liquid or solid media and at 1-10 µg/ml to select Puromycin-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
Puromycin	Selection antibiotic	ant-pr1
HEK-RepTor™ cells	TetR-expressing cells	hk-rtor

## TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873

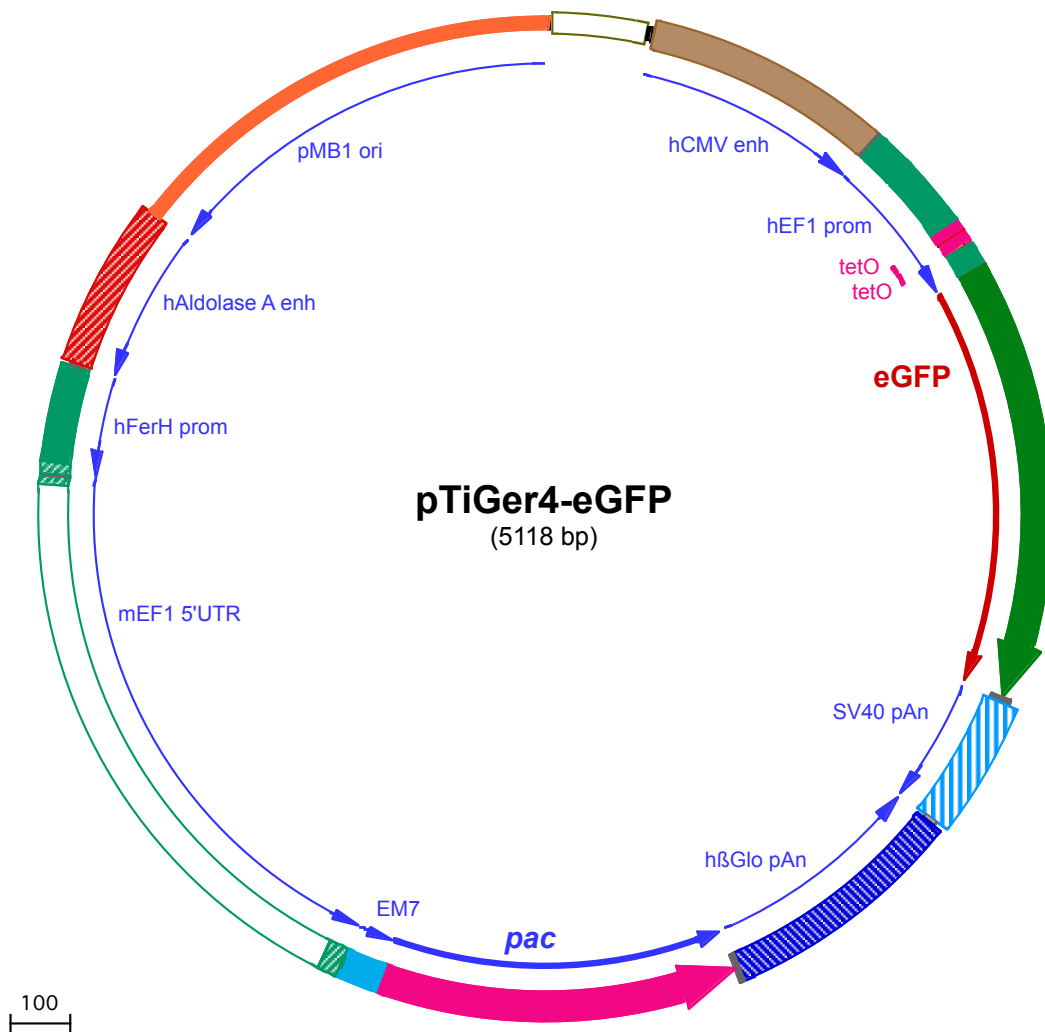
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1 CTCGAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACG  
101 AAACAAAACAACTAGCAAAATAGGCTGTCCCAAGTCAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGACCTGCAGGCGTTACATAACTTACGGTA  
201 AATGGCCCGCTGGTGACCCGCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTC  
301 AATGGGTGGAGTATTTACGGTAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCC  
401 CGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGATGATGCGGTTTTGG  
501 CAGTACATCAATGGGCGTGGATAGCGGTTTACTCACGGGATTTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTTGTGTTTACTAGTCAGTGGCC  
601 AGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGGTCCGCAATTGATCCGGTGCCTAGAGAAGTGGCGCGGGTAACTGGGAAAGTGAT  
701 GTCGTGTAAGTCCGCTTTTCCGAGGTTGGGGGAGAACCCTATATAAGTGCAGTAGTTGCCGTGAACGTTTCCCTATCAGTGATAGAGATCTCCC  
801 TATCAGTGATAGAGATCTTTCGAACGGGTTTCCGCCAGAACACAGCTGAAGCTTACCAGTCCACCATGGTGAAGCAAGGGCGAGGAGCTGTTACCAGGG  
120 M V S K G E E L F T G  
901 GTGGTGCCATCTGGTTCGAGCTGGACGGCGACGTAACGGCCACAAGTTCAGCGTGTCCGGCAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCC  
120 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 TGAAGTTCATCTGCCACCACGGCAAGTGCCTGTCCCTGGCCACCCTCGTACCACCTGACCTACGGCGTGCAGTGTTCAGCCGCTACCCCGACCA  
145 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 CATGAAGCAGCAGACTTCTTCAAGTCCGCCATGCCCGAAGGTACGTCCAGGAGCGACCATCTTCTTCAAGGACGACGGCAACTACAAGCCCGCGCC  
178 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 GAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGCGACAAGCTGGAGTACA  
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 ACTACAACAGCCACAACGTCTATATCATGCGCCACAAGCAGAAGAACGGCATCAAGTGAACCTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCA  
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 GCTCGCCGACCACTACCAGCAGAACACCCCATCGGCGACGGCCCGTGTGTGCGCCACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGAC  
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 CCCAACGAGAAGCGGATCACATGGTCTGCTGGAGTTCGTGACCGCCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGCTAGCTGGCCA  
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 GACATGATAAGATACATTGATGAGTTTGACAAACCACAAC TAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTG  
1701 TAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGTTTTTTAAAGCAAGTA  
1801 AAACCTCTACAAATGTGGTATGGAATCTAAAATACAGCATAGCAAACTTTAACCTCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATA  
1901 AGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTCTTTTATGAGTAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAA  
2001 ACTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCACATTCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAA  
2101 ATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAGGAACCTTTAATAGA  
2201 AATTGGACAGCAAGAAAGCGAGCTTCTAGCTCAGGTTAAGCTCCAGGCTTCTTGTGCATGCACCAAGTTCCTGGGCCTTCTGGAACCTCAACATCAGCT  
200 A G P K R T M C W T R P G E P V E V D A  
2301 GTCACAGTGAATCCAGTCTTTTATAAAAAGCAGGTTTCTGGGAGCAGAAGTTTCCAGAAAGGCAGGAATCCAGCCCTTTCAGCAGCTTCAACTCCAG  
178 T V T F G L R E Y F P L N R P A S T E L F A P V G A R E A A E V G P  
2401 GCAGAACAAAGCAGATCCAGACCCTTTCCCTGGTGGTCAGGGCTCACTCCAACAGTTGCCAGAAACCAAGCTGGCTTTTGGCCTGTGGTGCCAG  
145 L V V A S G L G K G Q H D P S V G V T A L F W A P E K P R H P A L  
2501 CAGACCTTCCATTTGTTGTTGTGCTGCCAGCCTGCTTCCAGAGAGCTCAGCCATTCTGGTCCAATTTCCAGCAAAAACAGCACCAGCTTCAACAGACTCA  
112 L G E M Q Q Q A A L R S G S L E A M R P G I E A F V A G A E V S E  
2601 GGTGTTGTCAAACCTGCAACAGCAGCTCCATCATCTGCAACCCAACTTTTCCAATGTCCAGTCCCCTCTGGTGAGGAAGAGTTCTTGCAGTCTGTCA  
78 P T T W V A V A A G D D A V W V K G I D L G V R T L F L E Q L E T V  
2701 CCCTCTCAATGTGCTGTGAGGGTCACTGTGTGCTTGTGTCAGGGTGTGCTGCAAAAAGCAGCAGCCAGTGTCTCACAGCTCTTGGAAATCATCTCT  
45 R E I H R D P D V T H R T A P Y D A F A A A L T R V A R P V D D R  
2801 GGTGCCAGCCTCAGTGGGTTTGTACTAGTCTGTTGGCCCTCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTC  
12 T A L R V T P K Y E T M  
2901 AATCCGGTTGCTTTGAATTAGCGGTGGTTTTCAACAACCTAAAAAGGGTTAAAAGATACCTTTGAACCGTAAGAAGCCCGAGAATTAGCTCCGCTC  
3001 AAAACTCAAGGGGACAAATTCAAAAATGACTTCCAGCGCCAGGCTGGCCTGACTAGTCTCCACCCACCAATGTGAACAACTCCAACGCCATTACATC  
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3301 TTCCACGCACGCAACTGGTGCCGGACGGGATGGCCTCACCTAGTTAGGGAGGCAGGGCAACGCGGCCGCCAAGCCAGATCGTGCCGGGTGCTGGGGC  
3401 CACATGGCCTCGGCACGCTAACCCAGCCTGGTTGCTTCGGGAAAAACCCAGGCCTCGCCCATCCAGGTGGCGTCGGACATGTGCTCCGAAGCGGGC  
3501 GGGCCCCAGCCGCACTCCTGTCCCTCATTCTCCCAACCATGACTCTCCGGGCTCCGGGCGAGCAAGCCCCGCACCCTCCCTTTGTTAGCCCCTAT  
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3901 GCAAGCTTCGGCGAAGAAGCTCTGGCCCTGCGGGTCGCTTGTGGTCTCTTATAGCCGCTCGGCGTCAGGCCCGCCCGCCAATCAGCGCCGCCGCC  
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4101 CTGGGTACGGGCCGCCCGAGAGGCGAGCCAGAGGCCGCTAGGAAGGGGCGGGCGCCGAGAACACGATCCCTCCCCACCCCTCGGACGTGAC  
4201 TCGGACCACATCCCGGGTTCGCTAGGGCCCTCCCTTCTGCTCTTTCCCCAGCCTGGCGCTCTGGGGCGCCGTGACTCAGCCAGAATGTTGGCAAT  
4301 GGGGGAGGGCGGAACGGGGAAGTGGAGGACCGGATGGAAAAGTCGAAACGAAGGAAGCTGAGTTTCGCCTGCAGGTTAATTAAGAACATGTGAGCAAA  
4401 AGGCCAGCAAAGGCCAGGAACCGTAAAAAGCCGCTTGGTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGT  
4501 CAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTTCCCTGGAAGCTCCCTCGTGCCTCTCCTGTTCCGACCCTGCCGTTACCGAT  
4601 ACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTGCTCCAAGCTGGGCTG  
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4801 GCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTCTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTG  
4901 GTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTTTGATCCGGCAAACAACACCGCTGGTAGCGGTGTTTTTTGTTTG  
5001 CAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTGAACGAAAACTCACGTTAAGGG  
5101 ATTTTGGTCATGGCTAGT