

Product usage

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

Important Limited Use information for pTiGer3-SEAP

The purchase of the pTiGer3-SEAP 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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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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pTiGer3-SEAP

A multigenic plasmid for inducible SEAP expression, selectable with Hygromycin

Catalog code: ptg3-sp

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

For research use only

Version 24A16-NJ

PRODUCT INFORMATION

Contents:

- 20 µg of pTiGer3-SEAP 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-SEAP plasmid encodes the secreted embryonic alkaline phosphatase (SEAP) 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 SEAP expression is only possible upon transfection of cells featuring the tetracycline repressor (TetR) protein¹, 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 SEAP-encoding gene is transcribed.

pTiGer-Lucia and pTiGer-eGFP 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 α (EF-1 α)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.
- **SEAP:** The secreted embryonic alkaline phosphatase catalyzes the hydrolysis of pNitrophenyl phosphate (pNpp), producing a yellow end product. SEAP levels can be evaluated qualitatively with the naked eye, and quantitatively using a spectrophotometer in combination with SEAP detection media, such as HEK-Blue™ Detection, or QUANTI-Blue™ Solution, a SEAP detection reagent.

- **SV40 pAn:** The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA³.

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*.
- **mEF15'UTR:** The 5'UTR (untranslated region) of the murine EF-1 α enhances *hph*-encoding mRNA stability and protein translation.
- **h β Glo 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 1 α 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 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 DH5 α .

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
QUANTI-Blue™	SEAP detection reagent	rep-qbs

TECHNICAL SUPPORT

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

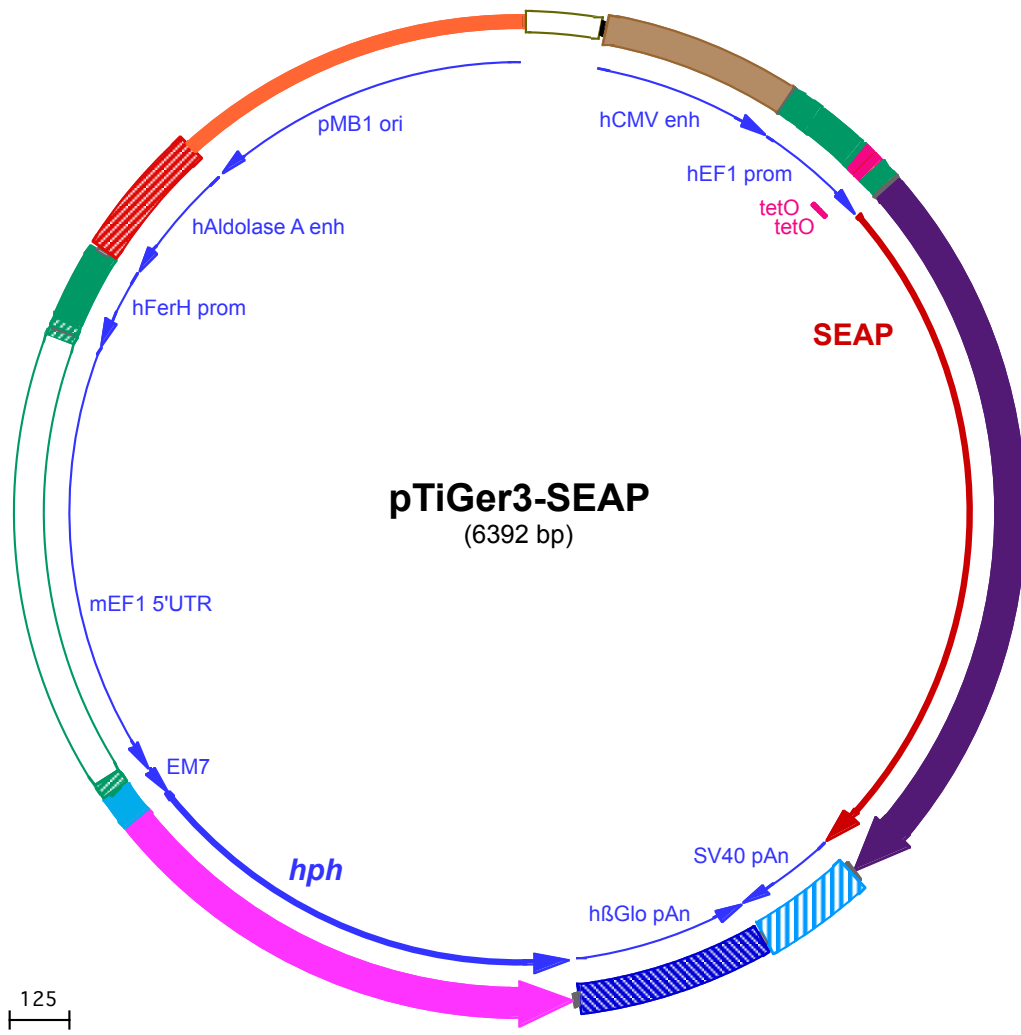
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1 CTCGAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACG
101 AAACAAAACAACTAGCAAAATAGGCTGTCCCAAGTCAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGACCTGCAGGCGTTACATAACTTACGGTA
201 AATGGCCCCGCTGGTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTC
301 AATGGGTGGAGTATTTACGGTAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCC
401 CGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGATGATGCGGTTTTGG
501 CAGTACATCAATGGGCGTGGATAGCGGTTTACTCACGGGATTTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTTGTGTTTACTAGTCAGTGGCC
601 AGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGGAGGGTTCGGCAATTGATCCGGTGCCTAGAGAAGTGGCGCGGGTAACTGGGAAAGTGAT
701 GTCGTGTAAGTGGCTCCGCTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTTGCCGTGAACGTTTCCCTATCAGTGATAGAGATCTCCC
801 TATCAGTGATAGAGATCTTTCGCAACGGGTTTCCGCCAGAACACAGCTGAAGCTTACCAGGTCACCATGGTTCTGGGGCCCTGCATGCTGCTGCTGCTG
12 M V L G P C M L L L L
901 CTGCTGCTGGCCTGAGGCTACAGCTCTCCCTGGGCATCATCCAGTTGAGGAGGAGAACCAGGACTTCTGGAACCGCGAGGCAGCCGAGGCCCTGGGTG
12 L L L G L R L Q L S L G I I P V E E E N P D F W N R E A A E A L G
1001 CCGCAAGAAGCTGCAGCCTGCACAGACAGCCGCAAGAACCTCATCATCTTCTGGGCGATGGGATGGGGGTGTCTACGGTACAGCTGCCAGGATCCT
45 A A K K L Q P A Q T A A K N L I I F L G D G M G V S T V T A A R I L
1101 AAAAGGGCAGAAGAAGGACAACTGGGGCCTGAGATACCCCTGGCTATGGACCGTTCATATGTGGCTCTGTCCAAGACATACAATGTAGACAAACAT
78 K G Q K K D K L G P E I P L A M D R F P Y V A L S K T Y N V D K H
1201 GTGCCAGACAGTGGAGCCACAGCCACGGCTACCTGTGCGGGTCAAGGGCAACTCCAGACCATTGGCTTGAAGTGCAGCCGCGCCGTTTAAACAGTGCA
112 V P D S G A T A T A Y L C G V K G N F Q T I G L S A A A R F N Q C
1301 ACACGACACGCGCAACGAGGTATCTCCGTGATGAATCGGGCCAAGAAAGCAGGGAAGTCAAGTGGGAGTGGTAACCACCACACGAGTGCAGCACGCCCTC
145 N T T R G N E V I S V M N R A K K A G K S V G V V T T T R V Q H A S
1401 GCCAGCCGGCACCTACGCCACAGGTGAACCGCAACTGGTACTCGGACGCCGACGTGCTGCCTCGGCCCGCAGGAGGGTGCAGGACATCGCTACG
178 P A G T Y A H T V N R N W Y S D A D V P A S A R Q E G C Q D I A T
1501 CAGCTCATCTCCAACATGGACATTGATGTGATCCTGGTGGAGGCCGAAAGTACATGTTTCGCATGGGAACCCAGACCCTGAGTACCAGATGACTACA
212 Q L I S N M D I D V I L G G G R K Y M F R M G T P D P E Y P D D Y
1601 GCCAAGTGGGACCAGGTGGACGGGAAGAATCTGGTGCAGGAATGGTGGCGAAGCGCCAGGGTGCCTGGTATGTGTGAACCGCACTGAGCTCATGCA
245 S Q G G T R L D G K N L V Q E W L A K R Q G A R Y V W N R T E L M Q
1701 GGCTTCCCTGGACCCGTCTGTGACCCATCTCATGGTCTCTTTGAGCCTGGAGACATGAAATACGAGATCCACCGAGACTCCACACTGGACCCCTCCCTG
278 A S L D P S V T H L M G L F E P G D M K Y E I H R D S T L D P S L
1801 ATGGAGATGACAGAGGCTGCCCTGCGCCTGCTGAGCAGGAACCCCGGGCTTCTTCTCTCTGAGGGTGGTGCATCGACCACGGTATCACGAAA
312 M E M T E A A L R L L S R N P R G F F L F V E G G R I D H G H H E
1901 GCAGGGCTTACCGGCACTGACTGAGACGATCATGTTGACGACGCCATTGAGAGGGCGGGCCAGCTCACCAGCGAGGAGGACACGCTGAGCCTCGTAC
345 S R A Y R A L T E T I M F D D A I E R A G Q L T S E E D T L S L V T
2001 TGCCGACCCTCCACGCTTCTTCTCGGAGGCTACCCCTGCGAGGAGCTCCATCTCGGGCTGGCCCTGGCAAGGCGGGACAGGAGGCGCTAC
378 A D H S H V F S F G G Y P L R G S S I F G L A P G K A R D R K A Y
2101 ACGGCTCTCTATACGAAACGGTCCAGGCTATGTGCTCAAGGACGGCGCCCGGGCGGATGTTACCGAGAGCGAGAGCGGGAGCCCGAGTATCGGCAGC
412 T V L L Y G N G P G Y V L K D G A R P D V T E S E S G S P E Y R Q
2201 AGTCAGCAGTGCCTGGACGAAGAGACCCACGAGGCGAGGAGTGGCGGTGTTGCGCGCGGCCCGAGGCGACCTGGTTCACGGCGTGCAGGAGCA
445 Q S A V P L D E E T H A G E D V A V F A R G P Q A H L V H G V Q E Q
2301 GACCTTATAGCGCAGTGCATGGCCTTCCGCGCTGCTGGAGCCCTACACCGCTGCGACCTGGCGCCCCCGCGGACACCACGCGCCGCGACCCG
478 T F I A H V M A F A A C L E P Y T A C D L A P P A G T T D A A H P
2401 GGGCGTCCCGTCCAAGCGTCTGGATTGAAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTGGACAAACCACAACACTAGAATGCAGTAAAA
512 G R S R S K R L D •
2501 AAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTATTTTATGT
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2701 CCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGACGCTCACCTT
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3201 CAGTCCCGGCTCCGGATCGGACGATTGCGTGCATCGACCCTGCGCCCAAGCTGCATCATCGAAATGCGCTCAACCAAGCTCTGATAGAGTTGGTCAAG
303 T G A G S R V I A D C R G Q A W A A D D F N G D V L S Q Y L Q D L

3301 ACCAATGCGGAGCATATACGCCCGAGCCGCGGCGATCCTGCAAGTCCGGATGCCTCCGCTCGAAGTAGCGGTCTGCTGCTCCATACAAGCCAACCAC
270 G I R L M Y A R L R P S G A L E P H R R E F Y R T Q Q E M C A L W
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236 P R W F F I N A V E Y Q S D G F M A E S W D I V A T I R G N D T L V
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203 N N S G F D A H V L H R V E P C D E A W L M L E D L A Q A V S A S
3601 GACGGTGTCTCCATCACAGTTTCCAGTGATACATGGGATCAGCAATCGCGCATATGAAATCACGCCATGTAGTGTATTGACCATTCTTGCGGT
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136 G F P G F G S T Q S L D A A A I A D M L E A V P Q L V A P L E T E P
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70 A S A F H R Y V Y R D K Y F G D A C S N V R L V Y G R G G V D F S
4001 AAAGCACGAGATTCTCGCCCTCGAGAGTGCATCAGGTGCGAGACGCTGTGCAACTTTTCGATCAGAACTTCGCGACAGACGTCGCGGTGAGTTCAG
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3 K K M
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