

Product usage

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

Important Limited Use information for pTiGer2-mcs

The purchase of the pTiGer2-mcs 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

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

InvivoGen USA (International): +1 (858) 457-5873

InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Asia: +852 3622-3480

E-mail: info@invivogen.com



pTiGer2-mcs

A multigenic cloning plasmid for inducible expression, selectable with Zeocin®

Catalog code: ptg2-mcs

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

For research use only

Version 24A15-NJ

PRODUCT INFORMATION

Contents:

- 20 µg of pTiGer2-mcs provided as lyophilized 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 should be stored at -20 °C and is stable for up to 1 year.
- Store Zeocin® 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 multiple cloning site for the tetracycline-inducible expression of a gene of interest (GOI). The pTiGer2-mcs plasmid contains the Zeocin® resistance marker for selection in both mammalian cells and bacteria.

The expression of the GOI 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 gene of interest is transcribed.

pTiGer3-mcs and pTiGer4-mcs plasmids, selectable using Hygromycin and Puromycin, respectively, are also available.

PLASMID FEATURES

Mutli-cloning site 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.
- **MCS:** The multiple cloning site comprises the following restriction sites: AgeI, BstEII, NcoI, BamHI, Acc65I, XbaI, NsiI, EcoRV, and NheI.
- **SV40 pAn:** The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA³.

Zeocin® 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 *Sh ble* gene in *E. coli*.
- **Sh ble:** The resistance to Zeocin® is conferred by the *Sh ble* gene from *Streptoalloteichus hindustanus*. The *Sh ble* 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 α enhances *Sh ble*-encoding mRNA stability and protein translation.
- **hβGlo pAn:** The human β -Globin pAn is a strong polyadenylation signal placed downstream of *Sh ble*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 .

• **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.

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

For a general procedure using InvivoGen's RepTor™ cell lines, please visit: <https://www.invivogen.com/tet-on-cell-lines>.

RELATED PRODUCTS

Product	Description	Cat. Code
Zeocin®	Selection antibiotic	ant-zn-1
ChemiComp GT115	Competent <i>E. coli</i>	gt115-1
HEK-RepTor™ cells	TetR-expressing cells	hk-rtor
pTiGer2-SEAP	Reporter plasmid	ptg2-sp

TECHNICAL SUPPORT

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

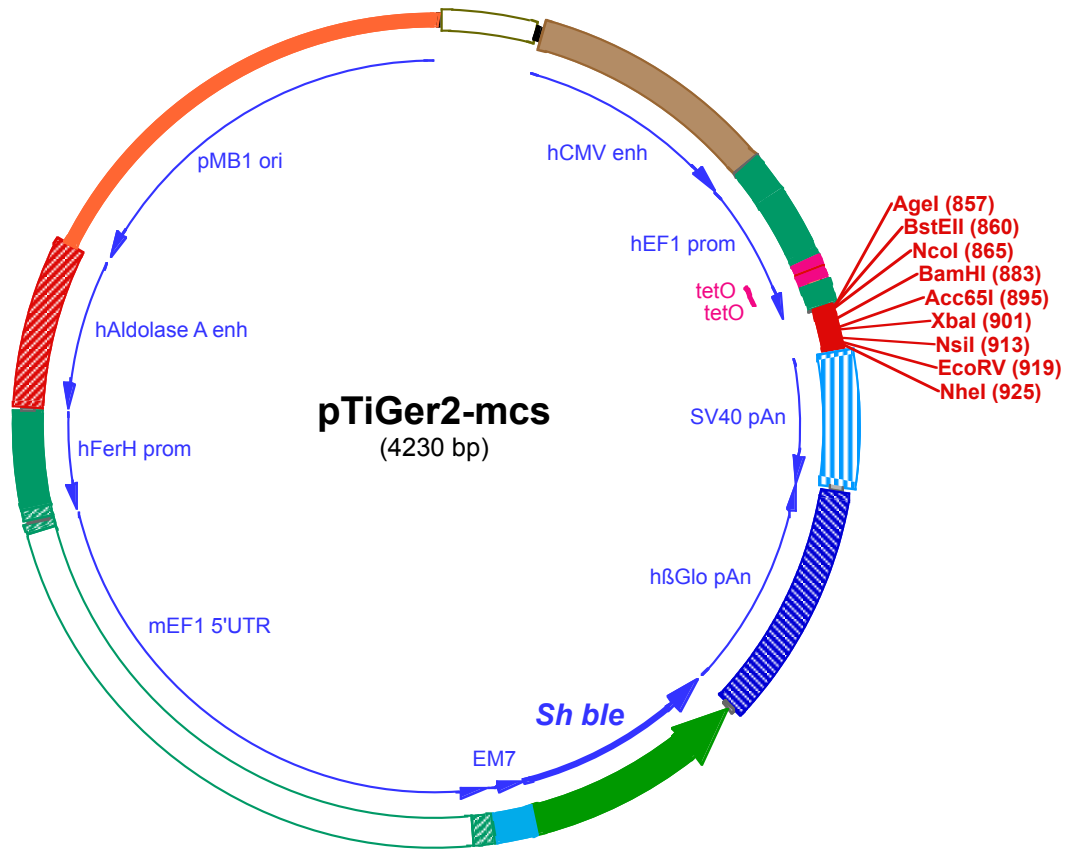
InvivoGen USA (International): +1 (858) 457-5873

InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Asia: +852 3622-3480

E-mail: info@invivogen.com

 **InvivoGen**
www.invivogen.com



100

1 **CTCGAGC**GGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACG
101 AAACAAAACAAACTAGCAAAATAGGCTGTCCCAAGTCAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGACCTGCAGGCGTTACATAACTTACGGTA
201 AATGGCCCGCTGGTGACCCGCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTC
301 AATGGGTGGAGTATTTACGGTAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCTATTGACGTCAATGACGGTAAATGGCC
401 CGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGTATTACCATGATGATGCGGTTTTGG
501 CAGTACATCAATGGGCGTGGATAGCGGTTTACTCACGGGATTTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTTGTGTTTACTAGTCAGTGGCC
601 AGAGCGCACATCGCCACAGTCCCGAGAAAGTTGGGGGAGGGGTGCGCAATTGATCCGGTGCCTAGAGAAGTGGCGCGGGTAAACTGGGAAAGTGAT
701 GTCGTGTAAGTGGCTCCGCTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTTGCCGTGAACGTT**CCCTATCAGTGATAGAGATCTCC**
801 **TATCAGTGATAGAGATCTTTCGCAACGGGTTT**GCCGCCAGAACACAGCTGAAGCTT**CCCGGTACCCATGGGAATTC**AAGCTTGGATCCAGATCTGGTAC
901 **CTCTAGACTCGAGATGCATGATATCGCTAGCTGG**CCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAAAAAATGC
1001 TTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAAACAAGTTAAACAACAACAAATGCATTCATTTTATGTTTCAGG
1101 TTCAGGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAATTC**AAAATACAGCATAGCAAAACTTAACTCCAAT**
1201 CAAGCCTCTACTTGAATCCTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTGCCAATGTGCATTAGCTGTTGCAGCCTCACCTTCTTTCA
1301 TGGAGTTTAAGATATAGTGATTTTTCCAAGGTTTGAAGTACTGCTTTCATTTCTTTATGTTTTAAATGCAGTACCTCCACATTCCCTTTTTAGTAAAA
1401 TATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTA
1501 GTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCTGCTCCTCTGCCACAAAGTG
1601 CACGCAGTTGCCGGCCGGTGCAGCAGGGCGAACTCCCGCCCGCAGGCTGCTCGCGATCTCGGTATGGCCGGCCGGAGGCGTCCCGAAAGTTCGTG
116 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 T
1701 GACACGACCTCCGACCACTCGCGTACAGTCTGTCAGGCGCGCACCCACAGCCAGGGTGTGTCGGCACCACCTGGTCTGGACCGCGCTGA
82 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 S I
1801 TGAACAGGGTACGTCGTCCCGACACACGGCGAAGTCTGCTCCACGAAGTCCCGGGAGAACCAGCCGGTCCGATCAGAACTGACCGCTCCGGC
49 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 A
1901 GACGTCGCGCGGGTGAACCGGAAACGGCACTGGTCAACTTGGCCATGATGGCCCTCCTATAGTGAGTCTGATTATACTATGCCATATACTATGCCGA
16 V D R A T L V P V A S T L K A M
2001 TGATTAATTGTCAATCCGGTTGCTTTGAATTAGCGGTGTTTTACAAACACCTAAAAAGGGTTTAAAGATACCTTTGAACCCTAAGAAGCCCGAGAA
2101 TTAGTCCGCTCAAACTCAAGGGGACAAATCCAAAATGACTTCCAGCGCCAGGCTGGCCTGACTAGTCTCACCCACCAATGTGAACAAACTCCAA
2201 CGCCATTACATCCCTCCCCCGCCGACTAGCCGTCTCAAAGCCGAGGTGACTATTGCGGCCGATAGGACCACGGGTACAGGAAGCAGCAGCC
2301 GGTGAGGGACCAGGCCCTCTTCTTTGTGGGTGACTCACCCGCCGCTCCACCGGGCTGCCGCTCCTCATTGAGCTCCTTGAACAGGGCCCGG
2401 GAGCGCCATCTTCCACGCACGCAACTGGTGCCGACGGGATGGCCTCACCTAGTTAGGGAGGCAGGGCAACGCGGCCGCCAAGCCAGATCGTGCC
2501 GGGTGTGGGGCCACATGGCTCGGCACGCTAACCCAGCCTGGTTGCTTCGGGAAAAACCCAGGCCTGCCCATCCAGGTGGCGTGGACATGTGCT
2601 CCGAAGCGGGCGGGCCAGCCGCACTCCTGTCCCTCATTCTCCCAACCATGACTCTCCGGCTCCGGGCGAGCAAGCCCGCACCTCCCTTT
2701 GTTAGCCCTATTGCTGAACGGCAATCGAAGGAGCAGGGCAACAACAACAAAAAAGACCAGAGTGGCGCGGAGTAGCACGGCGGGCGGCGC
2801 GGACACCAGCTAGGCTCAAGCCGACACGAGGCGAGGCTACGGGTTGCCGCTAGGCTCGCACTCTGCTCCCGCGCCGCCCAACTCGAAGCGGG
2901 AATGCTCGAGCTAATCCCCGCGACGACAGCGGGCCGGCCGCTCGGAGCAGGACTCCAGCTCGGGCGCCGGGAAGCCACACCCGCCCTCAC
3001 TGCGTTCTGACGGCAAGCTTCGGCGAAGAAGCTCTGGCCCTGCGGGTCTGTTGGTCTTTATAGCCGCTCGGGTCTAGGCCCGCCCGCCCAATCAG

3101 CGCCGCCCGCCCCGAGCCGCTCCTCCGGTGGGCGCGGGACCCGCCCTGCTGTGGGGAGGGGCGGCGCTGGAGGCCCTCGCGCGCTCTGGCGGAA
3201 CTAGTCGACGGACTGGGCTACGGGCGCCCCGAGAGGCGCAGCCCAGAGGCCGCGCTAGGAAGGGGCGGCGCCGAGAACACGATCCCTCCCACCCCC
3301 CTCGGACGTGACTCGGACCACATCCCGGGGTCGCTAGGGCCCTCCCTTCTGCTCCTTCCCCAGCCTGGCGCGCTCTGGGGCGCCGTGACTCAGCCAG
3401 AATGTTGGCAATGGGGAGGGCGGAACGGGAAGTGGAGGACGCGGATGGAAAAGTCGAAACGAAGGAAGCTGAGTTTCGCCTGCAGGTTAATTAAGAA
3501 CATGTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAT
3601 CGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTTCCCCCTGGAAGCTCCCTCGTGCCTCTCCTGTTCCGACCCTGC
3701 CGCTTACCGGATACCTGTCCGCCTTCTCCCTTCGGAAGCGTGGCGCTTTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTTCGCTC
3801 CAAGCTGGGCTGTGTGCACGAACCCCCGTTCCAGCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGTAAGACACGACTTATCG
3901 CCACTGGCAGCAGCCACTGGTAAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAA
4001 GAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGG
4101 TTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTGAACGAAAC
4201 TCACGTAAAGGGATTTTGGTCATGGCTAGT