

Product usage

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

Important Limited Use information for pTiGer4-mcs

The purchase of the pTiGer4-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



pTiGer4-mcs

A multigenic cloning plasmid for inducible expression, selectable with Puromycin

Catalog code: ptg4-mcs

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

For research use only

Version 24A15-NJ

PRODUCT INFORMATION

Contents:

- 20 µg of pTiGer4-mcs 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 multiple cloning site for the tetracycline-inducible expression of a gene of interest (GOI). The pTiGer4-mcs plasmid contains the Puromycin 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.

pTiGer2-mcs and pTiGer3-mcs plasmids, selectable using Zeocin® and Hygromycin, 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.

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 α enhances *pac*-encoding mRNA stability and protein translation.
- **hβGlo pAn:** The human β -Globin pAn is a strong polyadenylation signal placed downstream of *pac*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 .

• **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/tet-on-cell-lines>.

RELATED PRODUCTS

Product	Description	Cat. Code
Puromycin	Selection antibiotic	ant-pr1
ChemiComp GT115	Competent <i>E. coli</i>	gt115-1
HEK-RepTor™ cells	TetR-expressing cells	hk-rtor

TECHNICAL SUPPORT

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

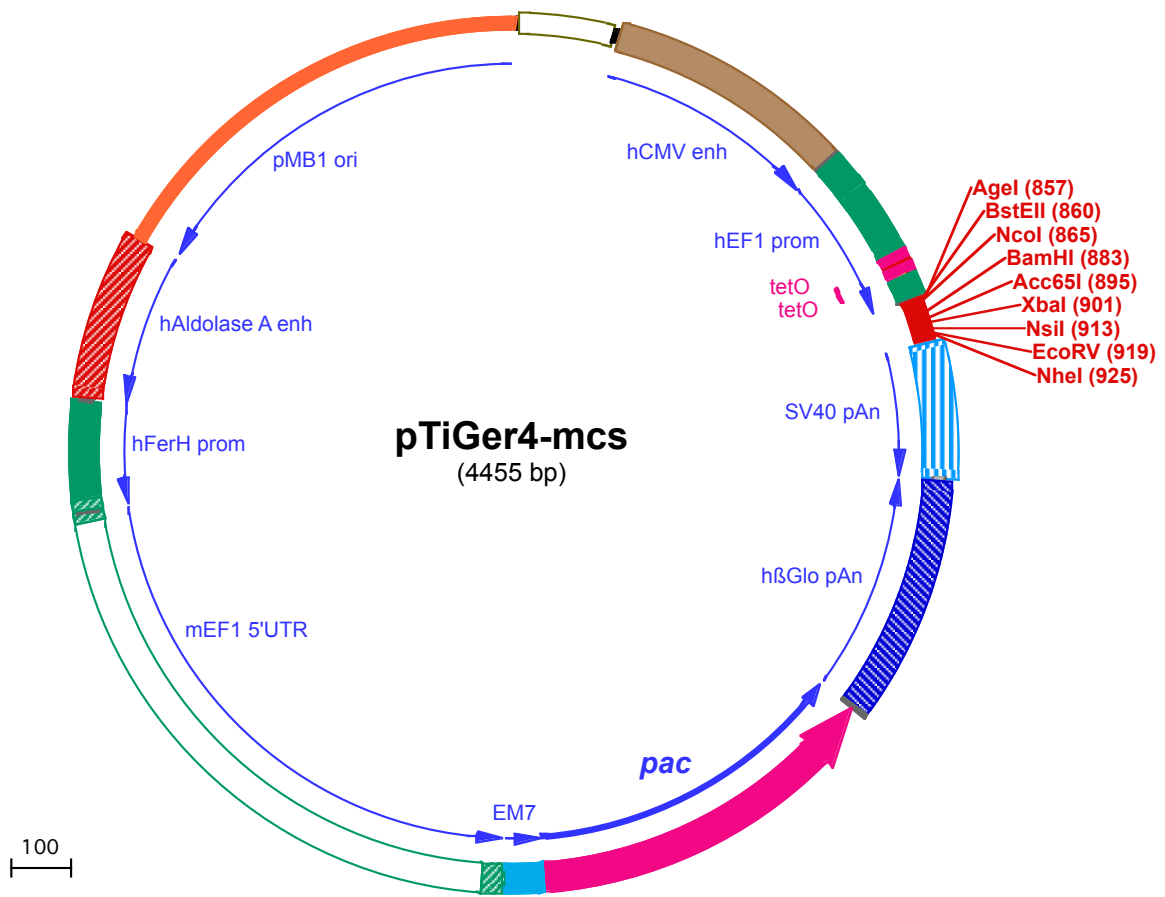
InvivoGen USA (International): +1 (858) 457-5873

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

InvivoGen Asia: +852 3622-3480

E-mail: info@invivogen.com

 **InvivoGen**
www.invivogen.com



1 CTCGAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACG
101 AAACAAAACAAACTAGCAAAATAGGCTGTCCCAAGTCAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGACCTGCAGGCGTTACATAACTTACGGTA
201 AATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTC
301 AATGGGTGGAGTATTTACGGTAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCC
401 CGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGATGATGCGGTTTTGG
501 CAGTACATCAATGGGCGTGGATAGCGGTTTACTCACGGGATTTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTTGTGTTTACTAGTCAGTGGCC
601 AGAGCGCACATCGCCACAGTCCCGAGAAAGTTGGGGGAGGGGTCCGCAATTGATCCGGTGCCTAGAGAAGTGGCGCGGGGTAACTGGGAAAGTGAT
701 GTCGTGTACTGGCTCCGCTTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTTGCCGTGAACGTTTCCCTATCAGTGATAGAGATCTCCC
801 TATCAGTGATAGAGATCTTTCGCAACGGGTTTCCGCCAGAACACAGCTGAAGCTTACCCGGTACCATGGGAATTC AAGCTTGGATCCAGATCTGGTAC
901 CTCTAGACTCGAGATGCATGATATCGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAAAAAATGC
1001 TTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAAACAAGTTAAACAACAACAAATTCATTCTTTTATGTTTCAGG
1101 TTCAGGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAATCTAAAATACAGCATAGCAAACTTAACTCCAAT
1201 CAAGCCTCTACTTGAATCCTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTGCCAATGTGCATTAGCTGTTGACGCTCACCTCTTTCA
1301 TGGAGTTTAAGATATAGTGATTTTTCCAAGGTTTGAAGTACTGCTTTCATTTCTTTATGTTTTAAATGCAGTACCTCCACATTCCCTTTTTAGTAAAA
1401 TATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTA
1501 GTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATGGACAGCAAGAAAGCGAGCTTCTAGCTCAGGTTAAGCTCCAGGCTTCTTGTGATGCA
1601 CCAAGTCTTGGGCTTCTGGAACCTCAACATCAGCTGTACAGTGAATCCAGTCTTTTCATAAAAAAGGCGAGTTTCTGGGAGCAGAAGTTCCAGAAGG
191 W T R P G E P V E V D A T V T F G L R E Y F P L N R P A S T E L F
1701 GCAGAACTCCAGCCCTTTCAGCAGTTC AACTCCAGGCAGAACACAGCAGATCCCAGACCTTTCCCTGGTGGT CAGGGCTCACTCCAACAGTTGCCA
157 A P V G A R E A A E V G P L V V A S G L G K G Q H D P S V G V T A L
1801 GAAACCAAGCTGGCTCTTTGGCCTGTGTGGTCCAGCAGACCTCCATTTGTTGTTGTGCTCCAGAGCTGCTCCAGAGAGCTCAGCCATTCTTGGTCC
124 F W A P E K P R H P A L L G E M Q Q Q A A L R S G S L E A M R P G
1901 AATTTAGCAAAAACAGCACCAGCTTCAACAGACTCAGGTGTTGTCCAAGTCAACAGCAGCTCCATCATCTGCAACCCAACTTTTCCAATGTCCAGT
91 I E A F V A G A E V S E P T T W V A V A G D D A V W V K G I D L
2001 CCCACTGGTGAGGAAGAGTCTTGCAGTTCCTCACCTCTCAATGTGCCTGT CAGGTCAGACTGTGCTTGTG CAGGGTAGCTGCAAAAGCAG
57 G V R T L F L E Q L E T V R E I H R D P D V T H R T A P Y D A F A A
2101 CAGCCAGTGTCTCACAGCTCTTGAACATCATCTCTGGTTCAGCCTCACTGTGGGTTTGTACTCAGTCATGGTGGCCCTCTATAGTGAGTCGTATT
24 A L T R V A R P V D D R T A L R V T P K Y E T M
2201 ATACTATGCCGATATACTATGCCGATGATTAATGTCAATCCGTTGCTTTGAATTAGCGGTGGTTTTCAACACCTAAAAAGGGTTAAAAGATACC
2301 TTTGAACCGTAAGAAGCCGAGAATTAGTCCGCTCAAACTCAAGGGGACAAATCCAAAATGACTTCCAGCGCCAGGCTGGCCTGACTAGTCTCCA
2401 CCCACCAATGTGAACAACTCCAACGCCATTACATCCCCTCCCCCGCGGACTAGCCGTGCTCAAAAGCCCGAGGTGACTATTGCGGCCGATAGGAC
2501 CACGGGGTACAGGAAGCAGCAGCCGGT GAGGGACCAGGCCCTCTTCTTTGTGTTGGTGACTCACCCGCCGCTCCACCGGGCTGCCGCTCTCCATT
2601 TTGAGTCTTTGCAACAGGGCCCGGAGCGCCATCTTTCCACGCACGCAACTGGTGGCGGACGGGATGGCCTCACCTAGTTAGGGAGGCAGGGCAACG
2701 CGGCGCCCAAGCCAGATCGTGCCGGTGTGGGGCCACATGGCCTCGGCACGCTAACCCAGCCTGGTTGCTTCGGGAAAAACCCAGGCTCGCCCC
2801 ATCCAGTGGCGTCGACATGTGCTCCGAAGCGGGGGGCCAGCCGCACTCCTGTCCCTCCATTCTCCCAACCATGACCTCTCCGGCTCCGGG
2901 CGAGCAAGCCCCGACCCTCCCTTTGTTAGCCCTATTGCTGAACGGCAATCGAAGGCAGCAGGGCAACAACAACAAAAAAGACCAGAGTGCCG
3001 CCGGAGTAGCACGCGGCGGCGGACACCACGCTAGGCCTCAAGCCGACACGAGGCGAGGCTACGGGTTGCCGCTAGGCTCGCACTCTGCCTCC

3101 CGCGCCGCCGCAACTCGAAGCGGGAATGCTCGCAGCTAATCCCCGCCGACGACAGCGGGGCCGCGCTCGGAGCAGGACCTCCAGCTCGGGGCC
3201 GCGGAAGCCACACCCGCCCTCACCTGCGTTCTGACGGCAAGCTTCGGCGAAGAACGTCTGGCCCTGCGGGTCGTTGTGGTCTTTATAGCCGCTCGG
3301 CGTCAGGCCGCCCGGCAATCAGCGCCGCCCGAGCCCGCTCTCCGGTGGGCGCGGGACCCGCCCTGCTGTGGGGAGGGGCGCGCTG
3401 GAGGCCCTCGCGCTCTGGCGGAAGTACTAGTACGACGGACTGGGCTACGGGCCGCCCGAGAGGCGCAGCCCAGAGGCCGCGCTAGGAAGGGCGGGCGCC
3501 GAGAACACGATCCCTCCCACCCCTCGGACGTGACTCGGACCACATCCCGGGTCGCTAGGGCCCTCCCTTCTGCTCCTTCCCCAGCCTGGCGG
3601 CTCTGGGGCGCCGTGACTCAGCCAGAATGTTGGCAATGGGGAGGGCGGAACGGGGAAGTGGAGGACGCGGATGGAAAAGTCGAAACGAAGGAAGCTGA
3701 GTTTCGCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGAAAAGGCCAGGAACCGTAAAAAGGCCGCTTGTGGCGTTTTTCCATAGGCTCCG
3801 CCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTTCCCTGGAAGCTCCCTC
3901 GTGCGCTCTCCTGTTCCGACCCTGCCGTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGTTTTCTCATAGCTCACGCTGTAGGTATC
4001 TCAGTTCGGTGTAGGTCGTTGCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTC
4101 CAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGG
4201 TGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCA
4301 AACAAACCACCGCTGGTAGCGGTGGTTTTTTTGGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGG
4401 GTCTGACGCTCAGTGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGGCTAGT