

pVIVO1-mcs

A multigenic cloning plasmid for strong and sustained expression in tumors

Catalog code: pvivo1-mcs

<https://www.invivogen.com/pvivo-mcs>

For research use only

Version 19J02-MM

PRODUCT INFORMATION

Contents

- 20 µg of pVIVO1-mcs provided as lyophilized DNA
- 1 ml of Hygromycin B Gold (ultrapure Hygromycin B; 100 mg/ml)

Storage and Stability

- Product is shipped at room temperature.
- Store lyophilized DNA at -20 °C.
- Resuspended DNA is stable for 12 months when stored at -20 °C. Avoid repeated freeze-thaw cycles.
- Store Hygromycin B Gold at 4 °C or at -20 °C. The expiry date is specified on the product label.

Quality control

- Plasmid construct has been confirmed by restriction analysis and sequencing.
- Plasmid DNA was purified by ion exchange chromatography and lyophilized.

GENERAL PRODUCT USE

pVIVO1 is a multigenic vector with two transcription units allowing the combined expression of two genes of interest from a single vector. pVIVO1-mcs may be used for:

- **Cloning two genes of interest.** pVIVO1-mcs contains two multiple cloning sites (MCS) for convenient insertion of two genes.
- **Co-expression of two genes *in vivo*.** Both multiple cloning sites are located downstream of the glucose regulated protein (GRP) 78 and 94 promoters, allowing high levels of expression of the cloned transgenes in tumors. Expression may be achieved *in vivo* and *in vitro* in transient transfection experiments.

PLASMID FEATURES

- **haGRP78 and hGRP94 prom:** The hamster GRP78 and human GRP94 promoters drive weak levels of expression in normal conditions and are induced in stress conditions prevailing inside tumors, such as glucose deprivation and hypoxia¹. Within the tumor micro-environment, the GRP promoters yield persistent expression whereas the activity of viral promoters declines rapidly².
- **SV40 enhancer** which is comprised of a 72-base-pair repeat allows the enhancement of gene expression in a large host range. The enhancement varies from 2-fold in non-permissive cells to 20-fold in permissive cells. Furthermore, the SV40 enhancer is able to direct nuclear localization of plasmids³.
- **CMV enhancer:** The major immediate early enhancer of the human cytomegalovirus (HCMV), is composed of unique and repeated sequence motifs. The HCMV enhancer can substitute for the 72-bp repeats of SV40 and is severalfold more active than the SV40 enhancer⁴.

- **SV40 pAn:** the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA. The efficiency of this signal was first described by Carswell *et al.*⁵
- **pMB1 ori:** a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.
- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- **Hygro-ΔCpG** is a new allele of the *hph* gene conferring resistance to hygromycin B. In order to reduce the immunogenicity of this bacterial gene all CpG motifs have been removed by chemically synthesizing the gene. The *Hygro-ΔCpG* gene allows the selection of *E. coli* clones transformed with a pVIVO plasmid.
Note: Stable transfection of mammalian clones cannot be performed due to the absence of a eukaryotic promoter upstream of the Hygro-ΔCpG gene.
- **Term:** The *E. coli rps O* terminator allows efficient transcription termination of the *Hygro-ΔCpG* gene.
- **EF1 pAn** is a strong polyadenylation signal. InvivoGen uses a sequence starting after the stop codon of the EF1 cDNA and finishing after a bent structure rich in GT.
- **MCS:** Each multiple cloning site contains several restriction sites that are compatible with many other enzymes, thus facilitating cloning.

MCS1 contains the following restriction sites:

Age I, *Bsp* HI, *Xba* I, *Hind* III, *Bst* 1107I, and *Avr* II
Age I is compatible with *Ava*I, *Xma*I, *Bsa*WI, *Bsp*EI, *Bsr*FI, *Sgr*AI and *Ngo*MIV
Bsp HI is compatible with *Nco* I and *Bsp* LU11I
Xba I is compatible with *Nhe* I, *Spe* I and *Avr* II
Bst 1107I is compatible with any other blunt-end restriction enzymes
Avr II is compatible with *Xba* I, *Spe* I and *Nhe* I

MCS2 contains the following restriction sites:

*Ngo*M I, *Nco* I, *Bgl* II, *Eco*R I, and *Nhe* I
Nco I is compatible with *Bsp*HI I and *Bsp*LU11 I
Bgl II is compatible with *Bam*HI I, *Bst*Y I and *Bcl* I
*Eco*R I is compatible with *Apo* I, *Mfe* I and *Tsp*509 I
Nhe I is compatible with *Xba* I, *Spe* I and *Avr* II

Important: *Bsp* HI and *Nco* I restriction sites, located at the 5' end of MCS1 and MCS2 respectively, contain an ATG in their sequence.

1. Little E. *et al.*, 1994. The glucose-regulated proteins (GRP78 and GRP94): functions, gene regulation, and applications. *Crit. Rev. Eukaryot. Gene Expr.* 4:1-18. 2. Gazit G. *et al.* 1999. Use of the glucose starvation-inducible glucose-regulated protein 78 promoter in suicide gene therapy of murine fibrosarcoma. *Cancer Res* 59: 3100-6. 3. Dean DA. *et al.*, 1999. Sequence requirements for plasmid nuclear import. *Exp. Cell. Res.* 253:713-22. 4. Boshart M. *et al.*, 1985. A very strong enhancer is located upstream of an immediate early gene of human cytomegalovirus. *Cell* 41(2):521-30. 5. Carswell S. & Alwine JC. 1989. Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. *Mol. Cell Biol.* 10: 4248-4258.

TECHNICAL SUPPORT

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METHODS

Plasmid resuspension:

Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1 µg/µl, resuspend the DNA in 20 µl of sterile water. Store resuspended plasmid at -20°C.

Plasmid amplification and cloning

Plasmid amplification and cloning can be performed in *E. coli* GT116 or other commonly used laboratory *E. coli* strains, such as DH5α.

Hygromycin B usage

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

RELATED PRODUCTS

Product	Description	Cat. Code
ChemiComp GT116	Competent <i>E. coli</i>	gt116-11
Hygromycin B Gold	Selection antibiotic	ant-hg-1
pVIVO1-GFP/LacZ	Dual reporter plasmid	pvivo1-gfplacz
pVIVO1-GFP/SEAP	Dual reporter plasmid	pvivo1-gfppsp
pVIVO1-Lucia/SEAP	Dual reporter plasmid	pvivo1-lucsp

TECHNICAL SUPPORT

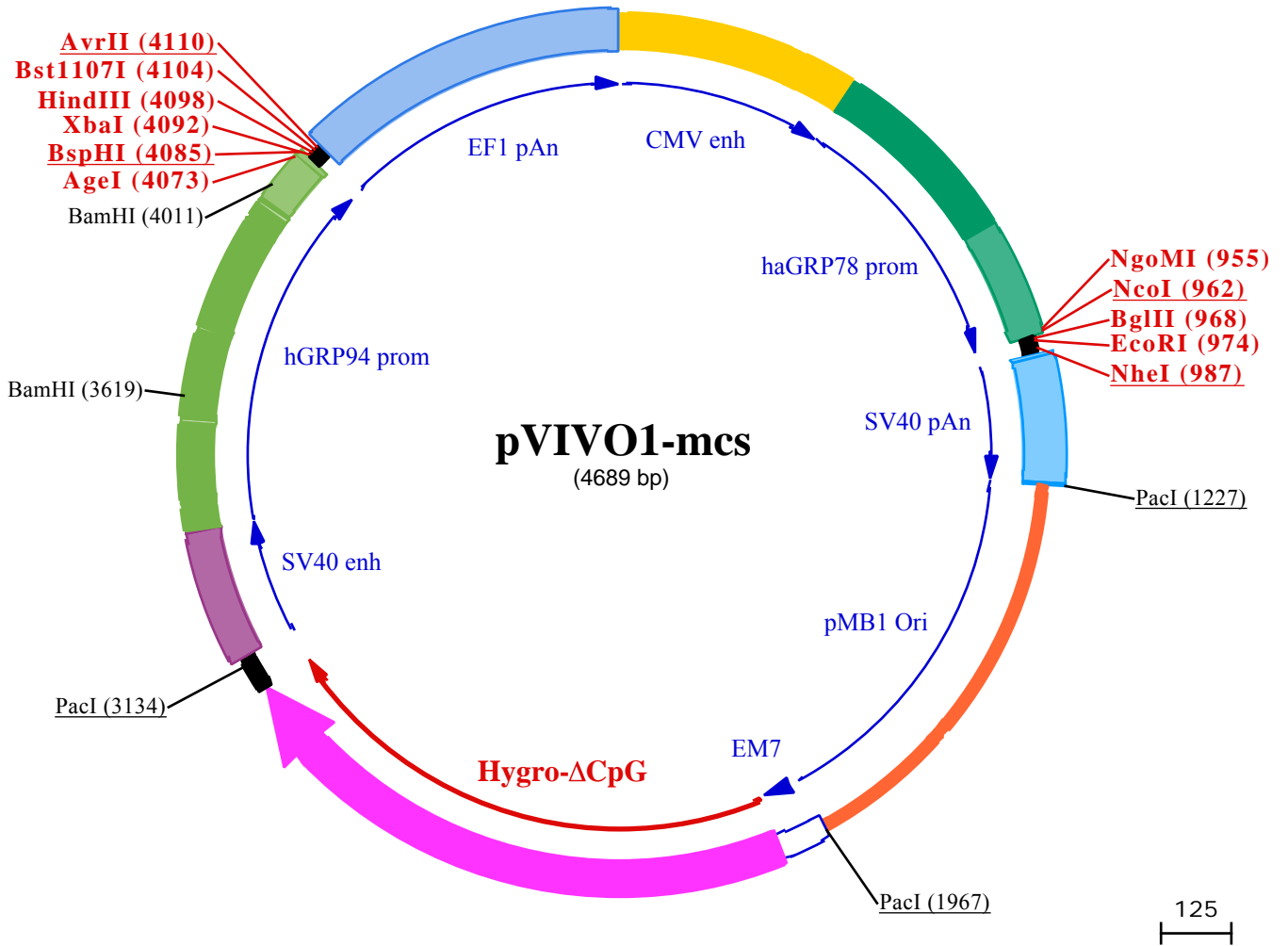
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1 CCTGCAGGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA
101 CGCCAATAGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCC
201 TATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATC
301 GCTATTACCATGATGATGCGGTTTTGGCAGTACATCAATGGCGCTGGATAGCGGTTTGACTCACGGGGATTTCGAAGTCTCCACCCCACTTACGCTCAATG
401 GGAGTTTGTTTGACTAGTTACCGCGGAAACGGTCTCGGGTTGAGAGGTACCCGAGGGACAGGCAGCTGTGAACCAATAGGACCGCGCACAGGGCG
501 GATGCTGCCCTCATTGGCGCGCTTGAGAGTGACCAAGAGCCAATGAGTCAGCCCGGGGGCGTAGCAGTACGTAAGTTGCGGAGGAGCCGCTTCGA
601 ATCGGCAGCGCCAGCTTGGTGGCATGGACCAATCAGCGTCTCCAACGAGGAGCGCTTCGCCAATCGGAGGCCTCCACGACGGGGCTGGGGGAGGGT
701 ATATAAGCCGAGTGGCGGGCGCGCTCCACACGGGCCGAGACCACAGCGACGGGAGCGTCTGCCTCTGCGGGCCGAGAGGTAAGCGCCGGCGCTGC
801 CCTTCCAGGCCAACTCGGAGCCGCTCTCGTGGCTCCGCTGATCGGGGCTCCTGTCCCTCAGATCGGTGGAAACGCCGTCGGCTCCGGGACTACA
901 AGCCTGTTGCTGGGCCGGAGACTGCCGAAGGACCCTGAGCACTGTCTCAGCGCCGGCAccATGGAGATCTGAATTCTAGGCCTGCTAGCTGGCCAGA
1001 CATGATAAGATACATTGATGAGTTTGGACAAACCACAACAGTAAGTGCAGTGAAAAAATGCTTTATTGTGAAATTTGTGATGCTATTGCTTTATTTGTA
1101 ACCATTATAAGCTGCAATAAACAAAGTAAACAACAACAATTGCATTCAATTTATGTTTCAGGTTACAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAA
1201 ACCTCTACAAATGTGATGAAATGTTAATTAAGTCCATGACCAAAATCCCTTAACGTGAGTTTTCTGTTCCACTGAGCGTCAGACCCGTAGAAAAG
1301 ATCAAAGGATCTTCTTGGATCCTTTTTTCTGCGGTAATCTGCTGCTGCAAAACAAAAAACCACCGCTACCAGCGGTGGTTTTGTTGCCGGATCAAG
1401 AGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTACGAGAGCGCAGATACAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTCAAGAA
1501 CTCTGTAGCACCGCTACATACCTCGCTCTGCTAATCTGTTACCAGTGGCTGTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGA
1601 TAGTTACCGGATAAAGCGCAGCGGTGGGCTGAACGGGGGTTCTGTGCACACAGCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGC
1701 GTGAGCTATGAGAAAGCGCCACGCTTCCGAAGGGAGAAAGCGGCAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACAGGGAGCTTCC
1801 AGGGGAAACGCCGTGATCTTTATAGTCTGTGCGGTTTTGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTACGGGGGGCGGAGCCTATGG
1901 AAAAAAGCCAGCAACGCGGCTTTTTACGGTTCCTGGCCTTTTGTGCGCTTTTGTCTCACATGTTCTTAAATTAATTTTTCAAAGTAGTTGACAATTA
2001 TCATCGGCATAGTATATCGGCATAGTATAATACGACTCACTATAGGAGGGCCACCATGAAGAAACCTGAACTGACAGCAACTTCTGTTGAGAAGTTTCTC
2101 ATTGAAAAATTTGATTCTGTTTCTGATCTCATGCAGCTGTCTGAAGGTGAAGAAAGCAGAGCCTTTTCTTTTGTGATGTTGGAGGAAGAGTTATGTTCTGA
16▶ I leGluLysPheAspSerValSerAspLeuMetGlnLeuSerGluGlyGluGluSerArgAlaPheSerPheAspValGlyGlyArgGlyTyrValLeuA
2201 GGTCAATTCTTGTGCTGATGGTTTTTACAAGACAGATATGTTTACAGACACTTGGCTCTGCTGCTCGCAATTCAGAAGTTCTGGACATTGGAGA
49▶ rgValAsnSerCysAlaAspGlyPheTyrLysAspArgTyrValTyrArgHisPheAlaSerAlaAlaLeuProl leProGluValLeuAspI leGlyGI
2301 ATTTCTGATCTCTCACCTACTGCATCAGCAGAAGAGCACAAGGAGTCACTCTCCAGGATCTCCCTGAAACTGAGCTGCCAGCTTCTGCAACCTGTT
82▶ uPheSerGluSerLeuThrTyrCysl leSerArgArgAlaGlnGlyValThrLeuGlnAspLeuProGluThrGluLeuProAlaValLeuGlnProVal
2401 GCTGAAGCAATGGATGCCATTGCAGCAGCTGATCTGAGCCAACTCTGGATTTGGTCCCTTTGGTCCCAAGGCTATGGTCAGTACACCACTGGAGGG
116▶ AlaGluAlaMetAspAlaI leAlaAlaAlaAspLeuSerGlnThrSerGlyPheGlyProPheGlyProGlnGlyI leGlyGlnTyrThrTrpArgA
2501 ATTTCAATTTGTCATTTGCTGATCCTCATGCTATCACTGGCAGACTGTGATGGATGACACAGTTTCTGCTTCTGTTGCTCAGGCACTGGATGAATCAT
149▶ spPheI leCysAlaI leAlaAspProHisValTyrHisTrpGlnThrValMetAspAspThrValSerAlaSerValAlaGlnAlaLeuAspGluLeuMe
2601 GCTGTGGGCAGAAGATTGCTCGAAGTCAGACACCTGGTCCATGCTGATTTTGAAGCAACAATGTTCTGACAGACAATGGCAGAATCACTGCAGTCATT
182▶ tLeuTrpAlaGluAspCysProGluValArgHisLeuValHisAlaAspPheGlySerAsnAsnValLeuThrAspAsnGlyArgI leThrAlaVal I le
2701 GACTGGTCTGAAGCCATGTTGGAGATTCTCAATATGAGGTTGCCAACATTTTTTTTTGGAGACCTTGGCTGGCTTGCATGGAACAACAAACAAGATATT
216▶ AspTrpSerGluAlaMetPheGlyAspSerGlnTyrGluValAlaAsnI lePhePheTrpArgProTrpLeuAlaCysMetGluGlnGlnThrArgTyrP
2801 TTGAAAGAAGACACCCAGAAGTGGCTGGTCCCCAGACTGAGAGCCTACATGCTCAGAATTGGCCTGGACCAACTGTATCAATCTCTGGTTGATGGAAA
249▶ heGluArgArgHisProGluLeuAlaGlySerProArgLeuArgAlaTyrMetLeuArgI leGlyLeuAspGlnLeuTyrGlnSerLeuValAspGlyAs
2901 CTTTGTATGCTGCTTGGCCACAAGGAGTGTGATGCCATTTGGAGTCTGGTCTGGTGGAACTGTTGGAAGAAGTCAAATTTGGAAGAAGGCTGCTGCT
282▶ nPheAspAspAlaAlaTrpAlaGlnGlyArgCysAspAlaI leValArgSerGlyAlaGlyThrValGlyArgThrGlnI leAlaArgArgSerAlaAla
3001 GTTTGACTGATGGATGTTGAAGTTCTGGCTGACTCTGGAACAGGAGACCTCCACAAGACCCAGGCAAGGAATGAATATTAGCTAGGAGTTTCA
316▶ ValTrpThrAspGlyCysValGluValLeuAlaAspSerGlyAsnArgArgProSerThrArgProArgAlaLysGlu•••
3101 GAAAAGGGGGCTGAGTGGCCCTTTTTTCAACTTAATTAACCTGCAGGGCCTGAAATAACCTCTGAAAGAGGAAGTGGTTAGGTACCTTCTGAGGCTG
3201 AAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCA
3301 ACCAGGTGTGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCACTAGTTTATCACCAC
3401 CGCCACCCCGCCCGCCCGCCATCTGAAAGGTTCTAGGGGATTTGCAACCTCTCTCGTGTGTTTCTTTTCCGAGAAGCGCCGCCACAGAAAAG
3501 CTGGCCGGCAAAGTCGTGCTGGAATCACTTCCAACGAAACCCAGGCATAGATGGAAAGGGTGAAGAACACGTTGTCATGGCTACCGTTTCCCGGTCA

BamHI (3619)

3601 CGGAATAAACGCTCTCTAGGATCCGGAAGTAGTTCGCCCGCGACCTCTCTAAAAGGATGGATGTGTCTCTGCTTACATTCATTGGACGTTTTCCCTTAG
3701 AGGCCAAGGCCGCCAGGCAAAGGGGCGGTCCACGCGTGAGGGGCCGCGGAGCCATTTGATTGGAGAAAAGCTGCAAACCCTGACCAATCGGAAGGAG
3801 CCACGCTTCGGGCATCGGTACCCGCACCTGGACAGCTCCGATTGGTGGACTTCGCCCCCTCACGAATCCTCATTGGGTGCCGTGGTGGCTGGTGGC
3901 CGCGATTGGTGGTTTCATGTTTCCCGTCCCCGCCCGCGAGAAGTGGGGGTGAAAAGCGGCCGACCTGCTTGGGGTGTAGTGGGCGGACCGCGGGCT

BamHI (4011)

XbaI (4092)
AgeI (4073) **BspHI (4085)** **HindIII (4098)**

4001 GGAGGTGTGAGGATCCGAACCCAGGGGTGGGGGTGGAGGCGGCTCCTGCGATCGAAGGGGACTTGAGACTCACCGGTGCGACGTCATGAATCTAGAAAG

AvrII (4110)

Bst1107I (4104)

4101 CTTGTATACCCTAGGATTATCCCTAATACCTGCCACCCCACTCTTAATCAGTGGTGAAGAACGGTCTCAGAACTGTTTGTTC AATTGGCCATTTAAGT
4201 TTAGTAGTAAAAGACTGGTTAATGATAACAATGCATCGTAAAACCTTCAGAAGGAAAGGAGAATGTTTTGTGGACCACTTGTTTCTTTTTGCCTGT
4301 GGCAGTTTTAAGTTATTAGTTTTTAAAATCAGTACTTTTTAATGAAACAACCTTGACCAAAAATTTGTCACAGAATTTTGAGACCCATTAAAAAGTTAA
4401 ATGAGAAACCTGTGTGTTCTTTGGTCAACACCGAGACATTTAGGTGAAAGACATCTAATTCTGGTTTTACGAATCTGGAAACTTCTTGAAAATGTAATT
4501 CTTGAGTTAACACTTCTGGGTGGAGAATAGGGTTGTTTTCCCCCACATAATTGGAAGGGGAAGGAATATCATTAAAGCTATGGGAGGGTTTCTTTGAT
4601 TACAACACTGGAGAGAAATGCAGCATGTTGCTGATTGCCTGTCACTAAAACAGGCCAAAAACTGAGTCCTTGGGTTGCATAGAAAAGCTG