

pVIVO1-GFP/LacZ

A multigenic plasmid for high levels of expression of GFP and LacZ reporter genes in tumors

Catalog code: pvivo1-gfp-lacz

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

For research use only

Version 19J02-MM

PRODUCT INFORMATION

Content:

- 20 µg of pVIVO1-GFP/LacZ 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-GFP/LacZ contains the reporter genes GFP and LacZ and can be used as a control vector.

pVIVO1-GFP/LacZ also can be used for cloning of open reading frames (ORF). Both reporter genes are flanked by unique sites (*Bsp*HI/*Avr*II for GFP and *Nco*I/*Nhe*I for LacZ) that allow for convenient cloning of ORFs which can be selected from InvivoGen's extensive list of genes.

For more information, visit: <https://www.invivogen.com/genes>.

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.

- **LacZ-ΔCpG gene:** The *E. coli lacZ* gene codes for the enzyme β-galactosidase which catalyzes the hydrolysis of the substrate X-Gal to produce a blue color that is easily visualized under a microscope. In order to reduce the immunogenicity of this bacterial gene the 298 CpG motifs from the wildtype have been removed by chemically synthesizing the gene.

- **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** is 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.

- **LGFP** is a new allele of the green fluorescent protein. The gene has been chemically synthesized to remove the CpGs to ensure long-lasting expression.

- **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.

1. Eisenstein RS. & Munro HN. 1990. Translational regulation of ferritin synthesis by iron. *Enzyme* 44(1-4):42-58. 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/SEAP	Dual reporter plasmid	pvivo1-gfpSP
pVIVO1-Lucia/SEAP	Dual reporter plasmid	pvivo1-lucSP
pVIVO1-mcs	Multiple cloning site plasmid	pvivo1-mcs

TECHNICAL SUPPORT

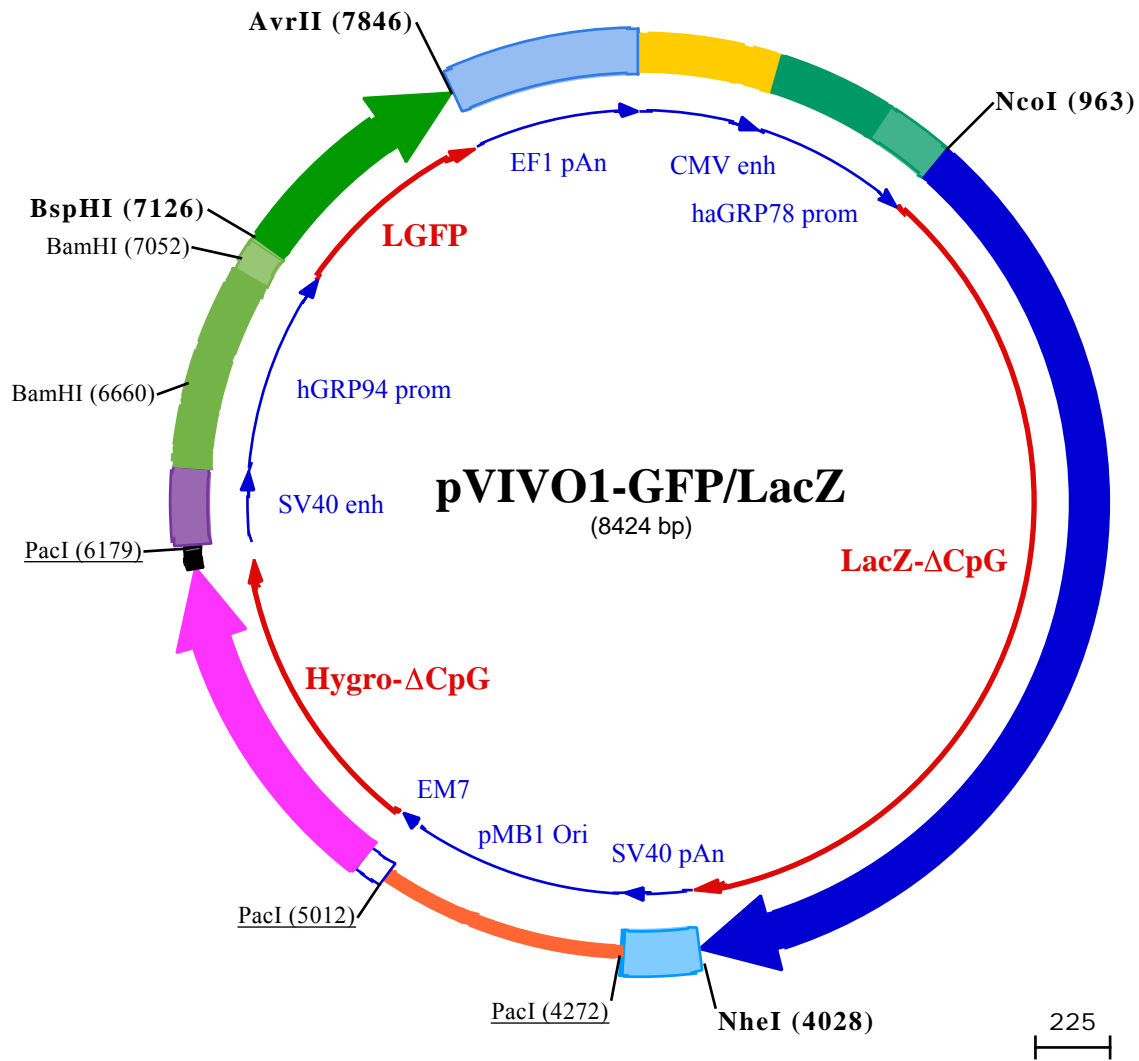
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1 CCTGCAGGCGTTACATAA...
101 CGCCAATAGGACTTTCCATTGACGTC...
201 TATTGACGTC...
301 GCTATTACCATGATGATGCGGTTT...
401 GGAGTTTGT...
501 GATGCTGCCCTCATTGGCGCGCTG...
601 ATCGGCAGCGCCAGCTTGGTGGCAT...
701 ATATAAGCCGAGTCGGCGGGCGGCT...
801 CCTTTCAGGCCAACTCGGAGCCCGT...

NcoI (963)

901 AGCCTGTTGCTGGCCCGGAGACTGCCGAAGACC...
1001 ACCCTGGAGTGACCAGTCAACAGACTGGCTGCCACCCTCCCTTGGCCTCTGGAGGA...
1101 CAGGTCTCTCAATGGAGAGTGGAGGTTGCCTGGTTCCTGCCCTGAAGCTGTGCCTGAGTCTGGCTGGAGTGTGACCTCCAGAGGCTGACACTGTT...
1201 GTGGTGCCAGCAACTGGCAGATGCATGGCTATGATGCCCATCTACACCAATGTCACCTACCCATCACTGTGAACCCCTTTGTGCCACTGAGA...
1301 ACCCCACTGGCTGCTACAGCCTGACCTTCAATGTTGATGAGAGCTGGCTGCAAGAAGCCAGACCAGGATCATCTTGTATGGAGTCAACTCTGCCTTCCA...
1401 CCTCTGGTCAATGGCAGGTGGGTTGGCTATGGCCAAGACAGCAGGCTGCCCTGAGTTTGACCTCTCTGCCTTCTCAGAGCTGGAGAGAACAGGCTG...
1501 GCTGTCATGGTGTCTCAGGTGGTCTGATGGCAGCTACCTGGAAGCAAGACATGTGGAGGATGTCTGGCATCTCAGGGATGTGAGCCTGCTGCAACAGC...
1601 CCACCACCAGATTTCTGACTTCCATGTTGCCACCAGGTTCAATGATGACTTACAGCAGAGCTGTGCTGGAGGCTGAGGTGCAGATGTGTGGAGA...
1701 AGACTACCTGAGAGTCACAGTGCAGCTCTGGCAAGGTGAGACCCAGGTGGCTCTGGCACAGCCCTTTGGAGGAGAGATCATTGATGAGAGAGGAGGC...
1801 gAspTyrLeuArgValThrValSerLeuTrpGlnGlyGluThrGlnValAlaSerGlyThrAlaProPheGlyGlyGluIleIleAspGluArgGlyGly...
1901 TATGCTGACAGAGTACCCTGAGGCTCAATGTGGAGAACC...
2001 TyrAlaAspArgValThrLeuArgLeuAsnValGluAsnProLysLeuTrpSerAlaGluIleProAsnLeuTyrArgAlaValValGluLeuHisThrA...
2101 CTGATGCCACCCTGTAAGCTGAAGCTGTGATGTTGGATTGAGAGAAGTCAGGATGAGATGGCCTGCTGCTCAATGGCAAGCCTCTGCTCAT...
2201 AlaAspGlyThrLeuIleGluAlaGluAlaCysAspValGlyPheArgGluValArgIleGluAsnGlyLeuLeuLeuLeuLeuLeuLeuIleI...
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2501 AATGCTGTCAAGTGTCTCACTACCCCAACCACCCTCTCTGGTACACCCTGTGTGACAGGATGGCCCTGTATGTTGTTGATGAGCCAACATTGAGACAC...
2601 AsnAlaValArgCysSerHisTyrProAsnHisProLeuTrpTyrTrpLeuTyrGlyLeuTyrValValGluAlaAsnIleGluThrH...
2701 ATGGCATGGTGCCATGAACAGGCTCACAGATGACCCAGGTGGCTGCCTGCCATGTCTGAGAGAGTGACCAGGATGGTGCAGAGACAGGAACCC...
2801 isGlyMetValProMetAsnArgLeuThrAspAspProArgTrpLeuProAlaMetSerGluArgValThrArgMetValGlnArgAspArgAsnHisPr...
2901 CTCTGTGATCATCTGGTCTCTGGCAATGAGTCTGGACATGGAGCAACCATGATGCTCTCTACAGGTGGATCAAGTCTGTTGACCCAGCAGACCTGTG...
3001 oSerValIleIleTrpSerLeuGlyAsnGluSerGlyHisGlyAlaAsnHisAspAlaLeuTyrArgTrpIleLysSerValAspSerArgProVal...
3101 CAGTATGAAGGCTGGAGCAGACACCACAGCACCAGACATCATGCCCATATGCCAGGGTGTATGAGGACCAGCCTTCCCTGCTGTGCCAAGT...
3201 GlnTyrGluGlyGlyGlyAlaAspThrThrAlaThrAspIleIleCysProMetTyrAlaArgValAspGluAspGlnProPheProAlaValProLysT...
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3501 GACTGGCAAGCCTTCAGACAGTACCCAGGCTGCAAGGAGGATTTGTTGGACTGGGTGGACCAATCTCTCATCAAGTATGATGAGAATGGCAACCC...
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4001 hrGluAlaLysHisGlnGlnGlnPhePheGlnPheArgLeuSerGlyGlnThrIleGluValThrSerGluTyrLeuPheArgHisSerAsnAsnGluLe...
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3901 AACATTGATGGCTTCCACATGGGCATTGGAGGAGATGACTCTTGGTCTCCTTCTGTGCTGTGAGTTCAGTTATCTGCTGGCAGGTACCACTATCAGC
980▶ AsnI leAspGlyPheHisMetGlyI leGlyGlyAspAspSerTrpSerProSerValSerAlaGluPheGlnLeuSerAlaGlyArgTyrHisTyrGlnL

NheI (4028)

4001 TGGTGTGGTGCCAGAAGTAAACCTGAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAAACCACAACCTAGAATGCAGTGAAAAAATG
1013▶ euValTrpCysGlnLys•••

4101 CTTTATTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAACAAGTTAACAACAACAAATTGCATTCAATTTATGTTTCAG

PaeI (4272)

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PaeI (5012)

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2▶ sLysProGluLeuThrAlaThrSerValGluLysPheLeuI leGluLysPheAspSerValSerAspLeuMetGlnLeuSerGluGlyGluGluSerArg
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5301 CTGCTGCTGTGCCAATTCAGAAAGTTCTGGACATTGGAGAATTTCTGAATCTCTCACCTACTGCATCAGCAGAAGAGCACAAGGAGTCACTCTCCAGGA
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5501 TTTGGTCCCAAGCATGGTCAGTACCACTGGAGGGATTTCAATTTGTGCCATTGTGATCCTATCCTGATCCTGATCCTGATCCTGATGATGATGACA
136▶ PheGlyProGlnGlyI leGlyGlnTyrThrTrpArgAspPheI leCysAlaI leAlaAspProHisValTyrHisTrpGlnThrValMetAspAspT
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6001 AACTGTTGGAAGAAGCTCAATTTGCAAGAAGTCTGCTGCTGTTTGGACTGATGGATGTTTGAAGTCTGGCTGACTCTGGAACAGGAGACCTCCACA
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PaeI (6179)

6101 AGACCAGAGCCAAGGAATGAATATTAGCTAGGAGTTTCAGAAAAGGGGCGCTGAGTGGCCCTTTTTTCAACTTAATTAACCTGCAGGGCCTGAAATAA
336▶ ArgProArgAlaLysGlu•••

6201 CCTCTGAAAGAGGAACCTGGTTAGGTACCTTCTGAGGCTGAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGAAAAGTCCCAAGGCTCCCAAGC

6301 AGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGAAAAGTCCCAAGGCTCCCAAGCAGGAGGAAGTATGCAAAGCATGCATCTC

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BamHI (6660)

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BamHI (7052)

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BspHI (7126)

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1▶ MetSerLysGlyGluGluLeuPheThrGlyValValProl leLeuValGluLeuAspGlyAspValAsnGlyHi
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59▶ ThrLeuValThrThrLeuThrTyrGlyValGlnCysPheSerArgTyrProAspHisMetLysGlnHisAspPhePheLysSerAlaMetProGluGlyT

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192 ▶ roValLeuLeuProAspAsnHisTyrLeuArgThrGlnSerAlaLeuSerLysAspProAsnGluLysArgAspHisMetValLeuLeuGluPheValTh
AvrII (7846)
7801 AGCAGCAGGAATTACTCTGGAATGGATGAGCTGTACAAGTAAACCTAGGATTATCCCTAATACCTGCCACCCCACTCTTAATCAGTGGTGAAGAACGG
225 ▶ rAlaAlaGlyI leThrLeuGlyMetAspGluLeuTyrLys•••
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▶