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 hypoxia[1]. Within the tumor micro-environment, the GRP promoters yield persistent expression whereas the activity of viral promoters declines rapidly[2].

• **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 plasmids[3].

• **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 enhancer[4].

• **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 *E. coli*	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

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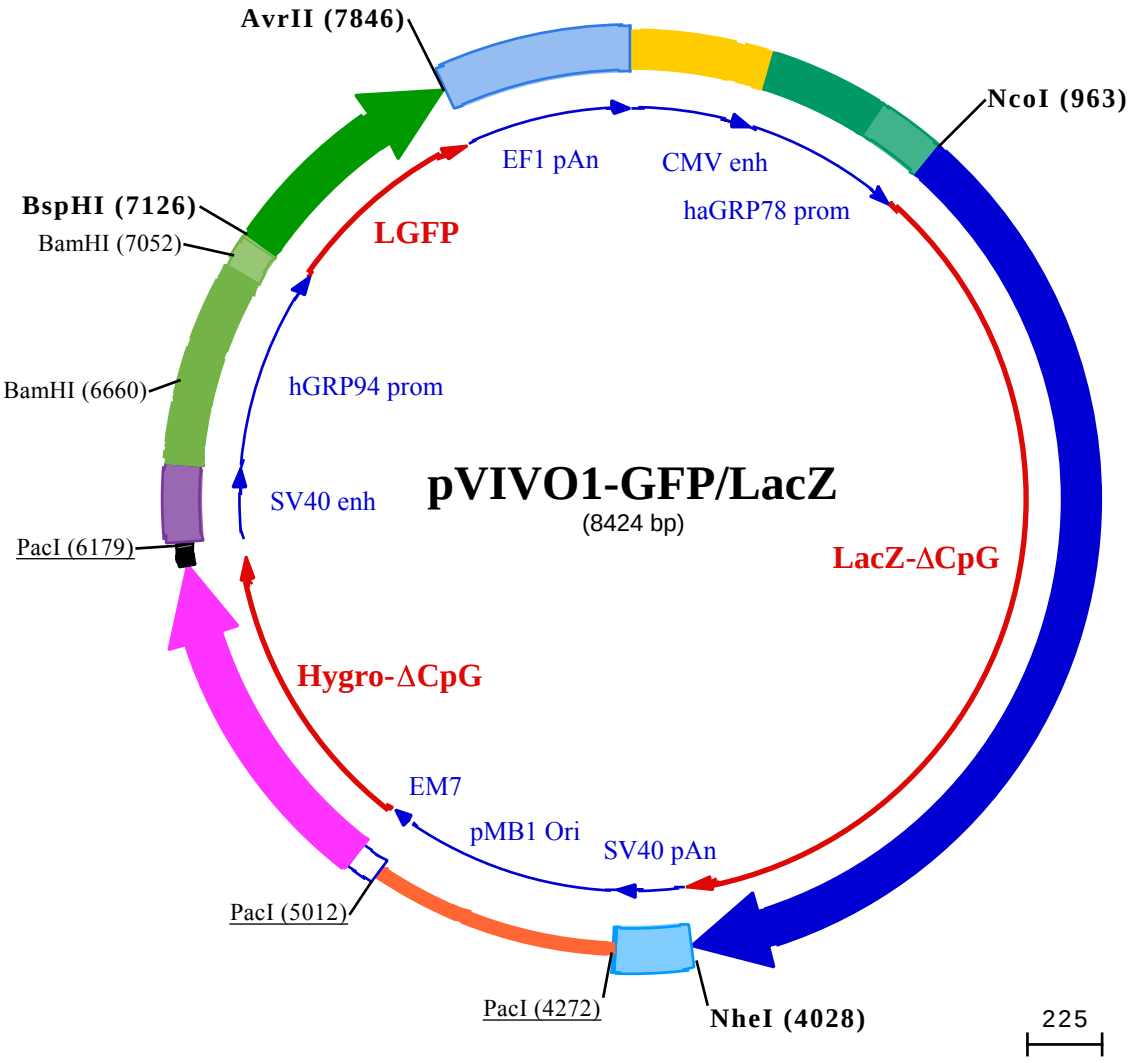
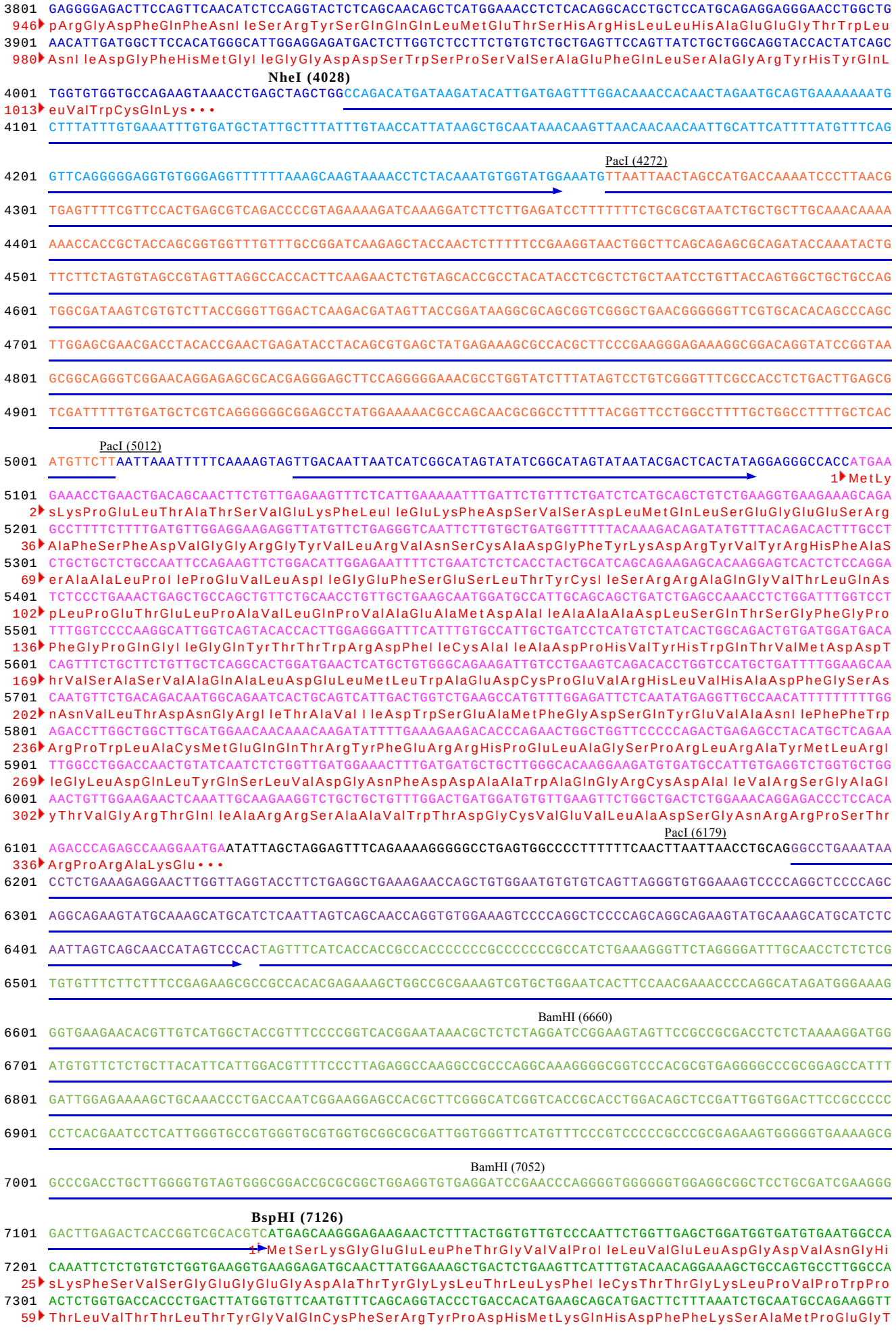

AvrII (7846)
NcoI (963)
BspHI (7126)
BamHI (7052)
BamHI (6660)
PacI (6179)
PacI (5012)
PacI (4272)
NheI (4028)
EF1 pAn
CMV enh
haGRP78 prom
LGFP
hGRP94 prom
SV40 enh
pVIVO1-GFP/LacZ
(8424 bp)
LacZ-ΔCpG
Hygro-ΔCpG
EM7
pMB1 Ori
SV40 pAn
225

InvivoGen


NcoI (963)


NheI (4028)
PacI (4272)
PacI (5012)
PacI (6179)
BamHI (6660)
BamHI (7052)
BspHI (7126)


7401 ATGTTCAGGAGAGGACAATCTTCTTTAAGGATGATGGAAATTATAAGACAAGGGCAGAAGTGAAGTTTGAAGGTGATACACTGGTTAACAGAATTGAGCT
92 yrValGlnGluArgThrI lePhePheLysAspAspGlyAsnTyrLysThrArgAlaGluValLysPheGluGlyAspThrLeuValAsnArgI leGluLe
7501 GAAAGGCATTGATTTTAAGGAAGATGGAAACATTCTGGGTCACAAGCTGGAGTACAACTATAATTCTCACAATGTTTACATTATGGCAGATAAGCAGAAG
125 uLysGlyI leAspPheLysGluAspGlyAsnI leLeuGlyHisLysLeuGluTyrAsnTyrAsnSerHisAsnValTyrI leMetAlaAspLysGlnLys
7601 AATGGAATTAAGGTTAATTTCAAGATTAGACACAACATTGAGGATGGATCTGTCCAACTGGCAGACCATTACCAGCAGAACACCCCTATTGGTGATGGCC
159 AsnGlyI leLysValAsnPheLysI leArgHisAsnI leGluAspGlySerValGlnLeuAlaAspHisTyrGlnGlnAsnThrProI leGlyAspGlyP
7701 CAGTTCTCCTCCCAGATAATCACTATCTCCGCACTCAATCTGCTCTGTCCAAAGACCCTAATGAGAAAAGAGACCACATGGTCCTCCTGGAGTTTGTGAC
192 roValLeuLeuProAspAsnHisTyrLeuArgThrGlnSerAlaLeuSerLysAspProAsnGluLysArgAspHisMetValLeuLeuGluPheValTh
AvrII (7846)
7801 AGCAGCAGGAATTACTCTGGGAATGGATGAGCTGTACAAGTAAACCTAGGATTATCCCTAATACCTGCCACCCCACTCTTAATCAGTGGTGGAAGAACGG
225 rAlaAlaGlyI leThrLeuGlyMetAspGluLeuTyrLys•••
7901 TCTCAGAACTGTTTGTTTCAATTGGCCATTTAAGTTTAGTAGTAAAAGACTGGTTAATGATAACAATGCATCGTAAAACCTTCAGAAGGAAAGGAGAATG
8001 TTTTGTGGACCACTTTGGTTTTCTTTTTTGCGTGTGGCAGTTTTAAGTTATTAGTTTTTAAAATCAGTACTTTTTAATGGAAACAACTTGACCAAAAATT
8101 TGTCACAGAATTTTGAGACCCATTAAAAAAGTTAAATGAGAAACCTGTGTGTTCCTTTGGTCAACACCGAGACATTTAGGTGAAAGACATCTAATTCTGG
8201 TTTTACGAATCTGGAAACTTCTTGAAAATGTAATTCTTGAGTTAACACTTCTGGGTGGAGAATAGGGTTGTTTTCCCCCCACATAATTGGAAGGGGAAGG
8301 AATATCATTTAAAGCTATGGGAGGGTTTCTTTGATTACAACACTGGAGAGAAATGCAGCATGTTGCTGATTGCCTGTCACTAAAACAGGCCAAAAACTGA
8401 GTCCTTGGGTTGCATAGAAAGCTG