

# STOP

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

## **Important Limited Use License information for pVIVO1-Lucia/SEAP**

The purchase of the pVIVO1-Lucia/SEAP 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.

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### TECHNICAL SUPPORT

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# pVIVO1-Lucia/SEAP

A multigenic plasmid for high levels of expression of Lucia luciferase and SEAP reporter genes in tumors

Catalog code: pvivo1-lucsp

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

For research use only

Version 19J02-MM

## PRODUCT INFORMATION

### Contents

- 20 µg of pVIVO1-Lucia/SEAP 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-Lucia/SEAP contains two reporter genes (Lucia luciferase and SEAP) and can be used as a control vector.

pVIVO1-Lucia/SEAP also can be used for cloning of open reading frames (ORF). Both reporter genes are flanked by unique sites (*Bsp*H I/*Avr* II for Lucia luciferase and *Nco* I/*Nhe* I for SEAP) 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<sup>1</sup>. Within the tumor micro-environment, the GRP promoters yield persistent expression whereas the activity of viral promoters declines rapidly<sup>2</sup>.
- **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<sup>3</sup>.
- **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<sup>4</sup>.

- **Lucia luciferase** is a synthetic CpG-free gene that codes for a secreted coelenterazine-utilizing luciferase.

ORF size (from the ATG to the stop codon): 634 bp.

Lucia luciferase activity can be evaluated using QUANTI-Luc™ or QUANTI-Luc™, assay reagents containing all the components required to quantitatively measure the activity of Lucia luciferase and other coelenterazine-utilizing luciferases.

- **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.*<sup>5</sup>

- **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.

- **SEAP** is a secreted form of human embryonic alkaline phosphatase. Unlike endogenous alkaline phosphatases, SEAP is extremely heat stable and resistant to the inhibitor L-homoarginine. It catalyses the hydrolysis of pNitrophenyl phosphate (pNpp) producing a yellow end product. SEAP expression can be readily quantified by collecting samples of culture medium and measuring the hydrolysis of pNpp with a spectrophotometer at 405 nm. SEAP activity that can be readily assessed qualitatively and quantitatively using HEK-Blue™ Detection or QUANTI-Blue™ Solution.

- **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.

## REFERENCES

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
HEK-Blue™ Detection	SEAP detection reagent	hb-det2
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-mcs	Multiple cloning site plasmid	pvivo1-mcs
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs
QUANTI-Luc™	Lucia detection reagent	rep-qlc1
QUANTI-Luc™ Gold	Lucia detection reagent	rep-qlcg1

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### TECHNICAL SUPPORT

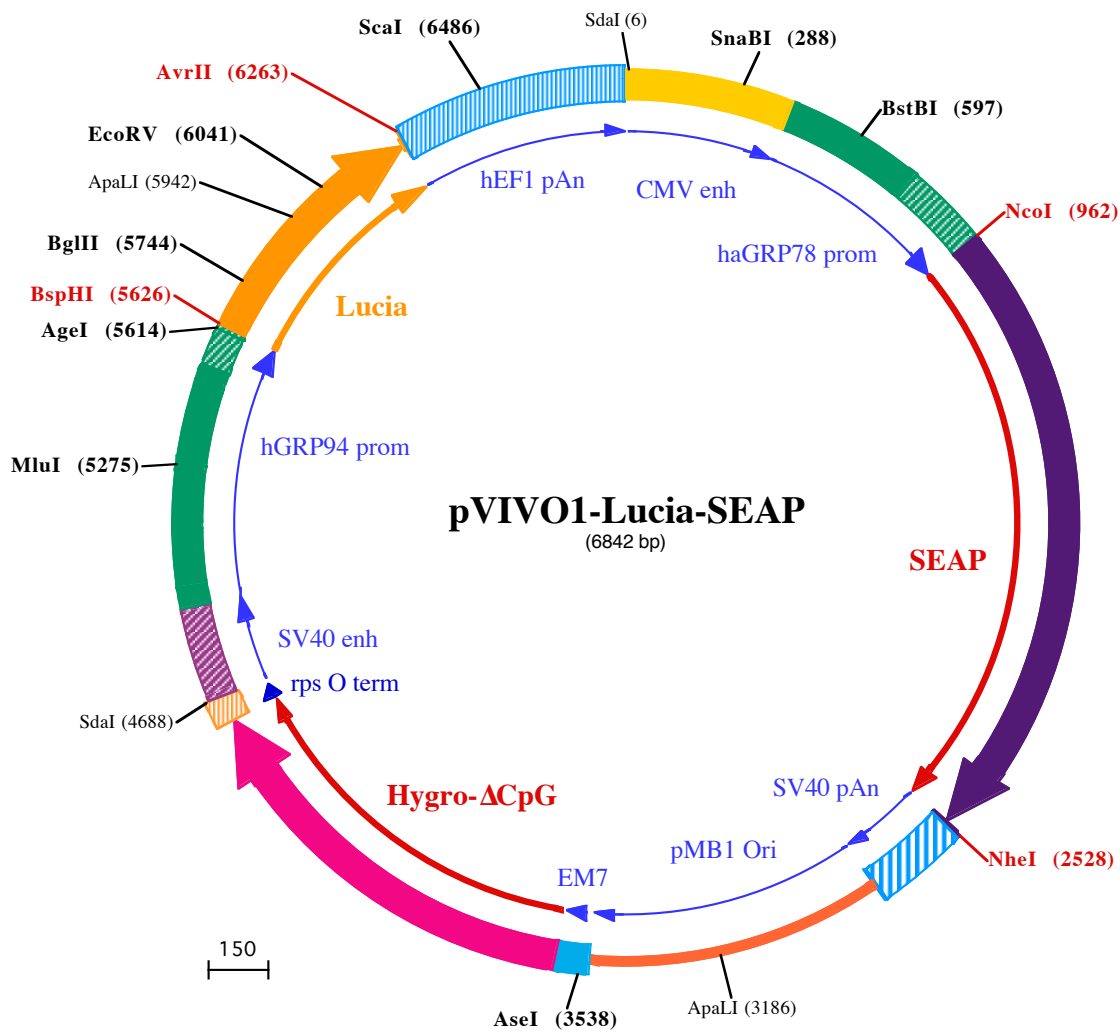
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SdaI (6)  
1 CCTGCAGGCGTTACATAA...  
101 CGCCAATAGGGACTTTC...  
SnaBI (288)  
202 ATTGACGTCAATGACGGT...  
303 TATTACCATGATGATGCG...  
404 GTTTGTTTTGACTAGTT...  
BstBI (597)  
505 CTGCCCTCATTGGCGGCG...  
606 CAGCGCCAGCTTGGTGGC...  
707 GCCGAGTCGGCGGCGGCT...  
808 AGGCCAACTCGGAGCCGT...  
NeoI (962)  
909 GCTGGCCCGGAGACTGCC...  
1010 TGAGGCTACAGCTCTCC...  
161 L R L Q L S L G I I P V E E E N P D F W N R E A A E A L G A A K K L  
1111 CAGCCTGCACAGACAGCC...  
501 Q P A Q T A A K N L I I F L G D G M G V S T V T A A R I L K G Q K K  
1212 GGACAAACTGGGCGTGA...  
831 D K L G P E I P L A M D R F P Y V A L S K T Y N V D K H V P D S G  
1313 CCACAGCCACGGCTACT...  
1171 A T A T A Y L C G V K G N F Q T I G L S A A A R F N Q C N T T R G N  
1414 GAGTCATCTCCGTGATG...  
1511 E V I S V M N R A K K A G K S V G V V T T T R V Q H A S P A G T Y A  
1515 CCACAGGTGAACCGCACT...  
1841 H T V N R N W Y S D A D V P A S A R Q E G C Q D I A T Q L I S N M  
1616 ACATTGATGTATCCTGG...  
2181 D I D V I L G G G R K Y M F R M G T P D P E Y P D D Y S Q G G T R L  
1717 GACGGGAAGAAATCTGT...  
2521 D G K N L V Q E W L A K R Q G A R Y V W N R T E L M Q A S L D P S V  
1818 GACCATCTCATGGGCTC...  
2851 T H L M G L F E P G D M K Y E I H R D S T L D P S L M E M T E A A  
1919 TGCCTCTGTGAGCAGGA...  
3191 L R L L S R N P R G F F L F V E G G R I D H G H H E S R A Y R A L T  
2020 GAGAGCATGTTCGACGAC...  
3531 E T I M F D D A I E R A G Q L T S E E D T L S L V T A D H S H V F S  
2121 CTTGGAGGTACCCCTGC...  
3861 F G G Y P L R G S S I F G L A P G K A R D R K A Y T V L L Y G N G  
2222 CAGGCTATGTCTAAGGAC...  
4201 P G Y V L K D G A R P D V T E S E S G S P E Y R Q Q S A V P L D E E  
2323 ACCACGCAGCGAGGACGT...  
4541 T H A G E D V A V F A R G P Q A H L V H G V Q E Q T F I A H V M A F  
2424 CGCCGCTGCCTGGAGCCT...  
4871 A A C L E P Y T A C D L A P P A G T T D A A H P G R S R S K R L D  
NheI (2528)  
2525 GAAGCTAGTGGCAGACAT...  
5211 •  
2626 TATTGCTTTATTGTAACC...  
2727 TTTAAAGCAAGTAAACCT...  
2828 ACCCCGTAGAAAAGATCA...  
2929 TTGCCGATCAAGAGCTAC...  
3030 CCACTTCAAGAACTCTGT...  
ApaLI (3186)  
3131 ACTCAAGACGATAGTTACC...  
3232 TACCTACAGCGTGAAGTA...

3333 GGAGCTTCCAGGGGAAACGCTGGTATCTTTATAGTCTGCTGGGTTCCGCCACCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGCCGA  
3434 GCCTATGAAAAACGCCAGCAACGCGGCTTTTTACGGTTCCTGGCCTTTTGTGCTCACATGTTCTTAATTAATTTTCAAAGTAGTTGA  
AseI (3538)  
3535 CAATTAATCATCGCATAGTATATCGGCATAGTATAATACGACTCACTATAGGAGGCCACCATGAAGAAACCTGAACTGACAGCAACTTCTGTTGAGAAG  
1 M K K P E L T A T S V E K  
3636 TTTCTCATTGAAAAATTTGATTCTGTTTCTGATCTCATGCAGTGTCTGAAGTGAAGAAAGCAGAGCCTTTTCTTTGATGTTGGAGGAAGAGGTTATGT  
14 F L I E K F D S V S D L M Q L S E G E E S R A F S F D V G G R G Y V  
3737 TCTGAGGTCAATTCTGTGCTGATGGTTTTACAAAGACAGATATGTTACAGACACTTTGCCTCTGCTGCTGCAATCCAGAAGTCTGGACATTG  
47 L R V N S C A D G F Y K D R Y V Y R H F A S A A L P I P E V L D I  
3838 GAGAATTTCTGAATCTCTCACCTACTGCATCAGCAGAAGAGCACAAGGAGTCACTCTCCAGGATCTCCCTGAACTGAGCTGCCAGCTGTTCTGCAACT  
81 G E F S E S L T Y C I S R R A Q G V T L Q D L P E T E L P A V L Q P  
3939 GTTGCTGAAGCAATGGATGCCATTGCAGCAGCTGATCTGAGCCAAACCTCTGGATTTGGTCTTTTGGTCCCAAGGCATTGGTCAGTACACCATTGGAG  
115 V A E A M D A I A A A D L S Q T S G F G P F G P Q G I G Q Y T T W R  
4040 GGATTTTCATTTGTCCATTGTGATCCTCATGCTATCACTGGCAGACTGTGATGGATGACACAGTTTCTGCTTCTGTTGCTCAGGCCTGGATGAACTCA  
148 D F I C A I A D P H V Y H W Q T V M D D T V S A S V A Q A L D E L  
4141 TGCTGTGGGCAGAAGATTGCTGAAGTCAAGACCTGGTCCATGCTGATTTTGAAGCAACAATGTTCTGACAGACAATGGCAGAATCACTGCAGTCAAT  
182 M L W A E D C P E V R H L V H A D F G S N N V L T D N G R I T A V I  
4242 GACTGGTCTGAAGCCATGTTTGGAGATTCTCAATATGAGGTTGCCAACATTTTTTTTTGGAGACCTGGCTGGCTTGCATGGAACAACAACAAGATATT  
216 D W S E A A M F G D S Q Y E V A N I F F W R P W L A C M E Q Q T R Y F  
4343 TGAAGAAGACACCAAGTGGTGGTCCCCAGACTGAGAGCTACATGCTCAGAATTGGCTGGCAACTGTATCAATCTCTGGTTGATGAAACT  
249 E R R H P E L A G S P R L R A Y M L R I G L D Q L Y Q S L V D G N  
4444 TTGATGATGCTGTTGGGCACAAGGAAGATGTGATGCCATTGTGAGGTCTGGTCTGGAACCTGTTGGAAGAAGTCAAATGCAAGAAGGCTGCTGCTGTT  
283 F D D A A W A Q G R C D A I V R S G C A G T V G R T Q I A R R S A A V  
4545 TGGACTGATGGATGTTGAAATTTCTGGCTGACTCTGGAACAGGAGACCTCCACAAGACCAGGCAAGGAATGAATATTAGCTAGGAGTTTCAGAAA  
317 W T D G C V E V L A D S G N R R P S T R P R A K E •  
SdaI (4688)  
4646 AGGGGGCCTGAGTGGCCCTTTTTTCAACTTAATTAACCTGCAGGGCCTGAAATAACCTCTGAAAGAGGAACCTGGTTAGGTACCTTCTGAGGCTGAAAGA  
4747 ACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAGCATGCATCTCAATTAGTCAGCAACCAGG  
4848 TGTGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCACTAGTTTCATCACCACCCACC  
4949 CCCCCGCCCCCGCCATCTGAAAGGTTCTAGGGGATTTGCAACCTCTCTCGTGTGTTTCTTCTTTCCGAGAAGCGCCGCCACACGAGAAAGCTGGCCG  
5050 GAAAGTCTGCTGGAATCACTTCCAACGAAACCCAGGCATAGATGGAAAGGTTGAAGAACACGTTGTATGGCTACCGTTTCCCGGTCACGGAATAAA  
5151 CGCTCTCTAGGATCCGGAAGTAGTTCGCCCGACCTCTCTAAAAGGATGGATGTTTCTCTGTTACATTCATTGGACGTTTTCCCTAGAGGCAAGGC  
MluI (5275)  
5252 CGCCAGGCAAAGGGCGGTCCCACGCGTGAGGGGCCCGGAGCCATTTGATTGGAGAAAAGCTGCAAACCTGACCAATCGGAAGGAGCCACGCTTCGG  
5353 GCATCGGTCAACCGACCTGGACAGCTCCGATTGGTGGACTTCCGCCCCCCCTCACGAATCCTCATTGGGTGCCGTGGGTGCGTGGTGGCGCGGATTGGTG  
5454 GGTTCATGTTTCCCGTCCCCCGCCGCAAGAGTGGGGGTGAAAGCGGCCGACCTGCTTGGGTGTAGTGGCGGACCGCGGGCTGGAGGTGTGAGGA  
AgeI (5614) BspHI (5626)  
5555 TCCGAACCCAGGGGTGGGGGTGGAGGCGCTCTGCGATCGAAGGGGACTTGAGACTCACCGGTCGCACGTCATGATGGAATCAAGGTGCTGTTTGCC  
1 M M E I K V L F A  
BglIII (5744)  
5656 TCATCTGTATTGCTGTTGCTGAGGCAAACCCACTGAAATCAATGAAGACCTCAATATAGCTGCTGTGGCCTCCAACCTTGGCACCACAGATCTTGAGACT  
10 L I C I A V A E A K P T E I N E D L N I A A V A S N F A T T D L E T  
5757 GACCTGTTCAACCACTGGGAGACCATGAATGTGATTAGCACTGACACAGAGCAGGTGAACACAGATGCTGACAGGGGCAAGCTGCTGGCAAAAACCTCC  
44 D L F T N W E T M N V I S T D T E Q V N T D A D R G K L P G K K L P  
ApaLI (5942)  
5858 CCCAGATGCTCTGAGGGAGCTGGAGGCCAATGCCAGAAGGCTGGTGCACAAGAGGCTGCTCATTGCTCTCCACATTAAGTGCACCCCTAAGATGA  
77 P D V L R E L E A N A R R A G C T R G C L I C L S H I K C T P K M  
EcoRV (6041)  
5959 AGAAATTTATCCCTGGCAGGTGCCACACTTATGAAGGTGAAAGGAGTCTGCTCAGGGAGGATTGGAGAGGCAATTGTTGATATCCAGAGATTCTGGC  
111 K K F I P G R C H T Y E G E K E S A Q G G I G E A I V D I P E I P G  
6060 TTCAAGGATAAGGAGCCATGGACAGTATTATTGCTCAAGTGGACCTCTGTGCTGATTGCACCACTGGCTGTGGAAGGGCCTTCCCAATGTCCAGTGTCT  
145 F K D K E P L D Q F I A Q V D L C A D C T T G C L K G L A N V Q C S  
6161 TGACCTCTGAAGAAGTGGTTCAGAGGTTACCACCTTTTCCAGCAAGATTGAGGTTAGGTTGACAAAATCAAGGGTCTGGCTGGGACAGATGAT  
178 D L L K K W L P Q R C T T F A S K I Q G R V D K I K G L A G D R •  
AvrII (6263)  
6262 ACCTAGGATTATCCCTAATACCTGCCACCCACTCTTAATCAGTGGTGAAGAAGGCTCTCAGAAGTCTGTTTCAATTGGCCATTAAAGTTAGTAGTA  
6363 AAAGACTGGTTAATGATAAATGCATCGTAAACCTCAGAAGGAAAGGAGAATGTTTTGTGGACCCTTTGGTTTTCTTTTTGCGTGTGGCAGTTTTA  
ScaI (6486)  
6464 AGTTATTAGTTTTTAAATCAGTACTTTTTAATGAAACAACCTGACCAAAAATTTGTACAGAATTTTGTGAGACCCATTAAAAAGTTAAATGAGAAACCT  
6565 GTGTGTTCTTTGGTCAACACCGAGACATTTAGTGAAGACATCTAATCTGGTTTTACGAATCTGGAACTTCTGAAAATGTAATCTTGAGTTAACA  
6666 CTTCTGGGTGGGAATAGGTTGTTTTCCCCACATAATTGGAAGGGAAGGAATATCATTAAAGCTATGGGAGGTTGCTTTGATTACACACTGGAG  
6767 AGAAATGCAGCATGTTGCTGATTGCTGCTACTAAAACAGGCCAAAACTGAGTCTTGGTTCATAGAAAGCTG