

STOP

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TECHNICAL SUPPORT

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pVIVO2-Lucia/SEAP

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

Catalog code: pvivo2-lucsp

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

For research use only

Version 19J02-MM

PRODUCT INFORMATION

Contents

- 20 µg of pVIVO2-Lucia/SEAP is provided as lyophilized DNA.
- 1 ml of Hygromycin B Gold at 100 mg/ml

Storage and stability

- Product is shipped at room temperature.
- Upon receipt, store lyophilized DNA at -20°C.
- Resuspended DNA should be stored at -20°C.
- Store Hygromycin B Gold at 4°C or -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

pVIVO2 is a multigenic vector with two transcription units allowing the combined expression of two genes of interest from a single vector.

- pVIVO2-Lucia/SEAP contains the reporter genes Lucia luciferase and SEAP. This plasmid can be used as a control vector.

- pVIVO2-Lucia/SEAP also can be used for cloning of open reading frames (ORFs). Both reporter genes are flanked by unique sites (*Bsp*HI/*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

- **hFerH and hFerL composite promoters:** Ferritin is a 24 subunit protein composed of two subunit types, termed H (heavy) and L (light), which perform complementary functions in the protein. Ferritin is ubiquitously expressed. Its synthesis is highly regulated by the iron status of the cell. The iron regulation is achieved at the translational level through the interaction between the iron-responsive element (IRE), located in the 5' untranslated region (5'UTR) of the ferritin mRNAs, and the iron regulatory protein¹. To eliminate the iron regulation of the ferritin promoters, the 5'UTR of FerH and FerL have been replaced by the 5'UTR of the mouse and chimpanzee elongation factor 1 (EF1) genes, respectively.
- **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 plasmids².
- **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 enhancer³.

- **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™, an assay reagent 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.*⁴

- **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 (cat. code: hb-det2) or QUANTI-Blue™ (cat. code: rep-qb1).

- **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. Dean DA. *et al.*, 1999. Sequence requirements for plasmid nuclear import. *Exp. Cell. Res.* 253:713-22. 3. 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. 4. 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.

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.

TECHNICAL SUPPORT

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

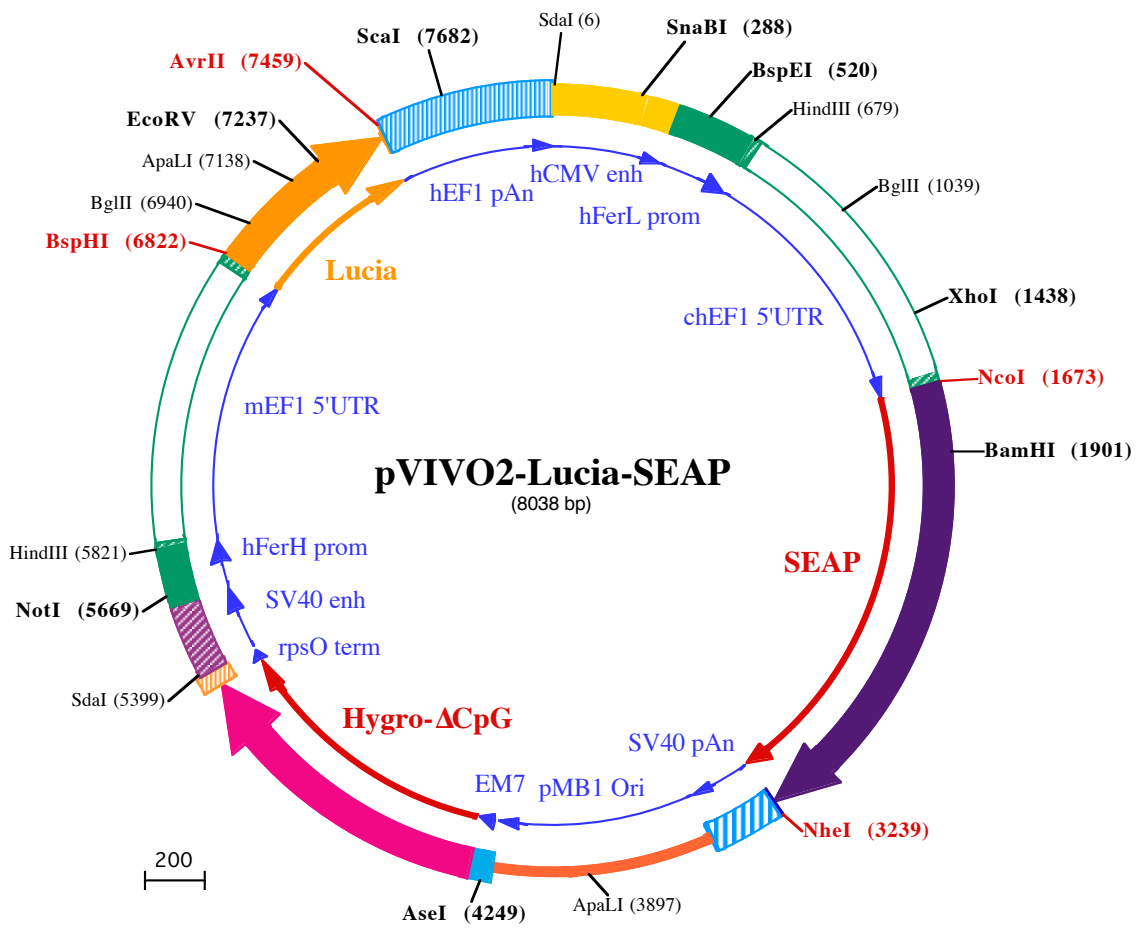
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NheI (3239)
3231 GGATTGAAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGCAAACCAACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGT
519▶ D •
3332 GATGCTATTGCTTTATTTGTAACCATATAAGCTGCAATAAAACAAGTTAAACAACAACAATTGCATTCATTTTATGTTTCAGGTTTCAGGGGGAGGTGTGGGA
3433 GGTTTTTTAAAGCAAGTAAAACTCTACAATGTGGTATGGAATGTTAATTAAGTACCATGACCAAAATCCCTAACCTGAGTTTTCTGTTCCACTGAGC
3534 GTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGTAGATCCTTTTTCTGCGCGTAATCTGCTGCTTGC AAAACAAAAAACCCCGCTACCAGCGGTGG
3635 TTTGTTTGGCGGATCAAGAGCTACCAACTCTTTTCCGAAGGTAAGTGGCTTCCAGAGCGCAGATACCAATACTGTTCTTCTAGTGTAGCCGTAGTTA
3736 GGCACCACTTCAAGAACTCTGTAGCACCGCTACATACCTCGCTCTGCTAATCTGTTACAGTGGCTGCTGCCAGTGGCGATAAGTCTGTTACCGG
ApaI (3897)
3837 GTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGCGGCTGAACGGGGGTTTCGTGCACACAGCCAGCTTGGAGCGAACGACCTACACCGAAC
3938 TGAGATACCTACAGCGTGTAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGCGGACAGGTATCCGGTAAGCGGCAGGGTGGAAACAGGAGAGCGC
4039 ACGAGGGAGCTTCCAGGGGAAACGCCTGGTATCTTTATAGTCTGTGCGGTTTCCGCACCTGACTTGAGCGTCGATTTTTGTGATGCTGTCAGGGGG
4140 GCGGAGCCTATGAAAAACGCCAGCAACCGCGCTTTTACGGTCTGCGCTTTTGTGCTGCGCTTTTGTCTACATGTTCTTAATTAATTTTTCAAAAAGTA
AseI (4249)
4241 GTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATCAGCTCACTATAGGAGGGCCACCATGAAGAAACCTGAATGCAGCAACTTCTGTTG
1▶ M K K P E L T A T S V
4342 AGAAGTTTCTCATTGAAAAATTTGATTCTGTTTCTGATCTCATGCGCTGTCTGAAGTGAAGAAAGCAGAGCCTTTTCTTTTGTGTTGGAGGAAGAGGT
12▶ E K 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
4443 TATGTTCTGAGGGTCAATTTCTGTGCTGATGGTTTTTACAAGACAGATATGTTTACAGACACTTTGCCTCTGCTGCTGCTGCAATTCAGAAAGTTCTGGA
46▶ Y V 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
4544 CATTGGAGAATTTTCTGAATCTCTCACTACTGCATCAGCAGAAGGACGACAGGAGTCACTCTCCAGGATCTCCCTGAAACTGAGCTGCCAGCTGTTCTGCG
79▶ I 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
4645 AACCTGTTGCTGAAGCAATGGATGCCATTGCGCAGCTGATCTGAGCCAACTCTGGATTGGTCTTTTGGTCCCCAAGGCATTGGTCAGTACACCACT
113▶ Q P 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
4746 TGGAGGATTTTCTTTGTCATTGCTGATCCTCATGTCTACTGGCAGACTGTGATGGATGACACAGTTTCTGTTCTGTTGCTCAGGCACTGGATGA
147▶ W R 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
4847 ACTCATGCTGTGGCAGAAGATTGCTGAAGTCAAGCAGCCTGGTCCATGCTGATTTTGAAGCAACAATGTTCTGACAGACAATGGCAGAATCACTGCAG
180▶ L 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
4948 TCATTGACTGGTCTGAAGCCATGTTTGGAGATTCTCAATATGAGGTTGCCAACATTTTTTTTTGGAGACCTGGCTGGCTTGGCAACAACAAGA
214▶ V I D W S E 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
5049 TATTTTGAAGAAGACCCAGAATCGGTTCCCGCAGACTGAGAGCCTACATGCTCAGAATTGGCCTGGACCAACTGTATCAATCTCTGGTTGATGG
248▶ Y F 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
5150 AAATTTGATGATGCTGCTTGGGCACAAGGAAGATGTGATGCCATTGTGAGGCTGGTCTGGAAGTGTGGAAGAACTCAAATGCAAGAAGGTCTGCTG
281▶ N F D D A A W A Q G R C D A I V R S G A G T V G R T Q I A R R S A
5251 CTGTTTGGACTGATGGATGTGTTGAAGTCTGGCTGACTCTGGAACAGGAGACCTCCACAAGACCCAGAGCCAAGGAATGAATTAGCTAGGAGTTTC
315▶ A V 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 (5399)
5352 AGAAAAGGGGGCTGAGTGGCCCTTTTTTCAACTTAATTAACCTGCAAGGCTGAAATAACCTCTGAAAGAGGAACCTGGTTAGGTACCTTCTGAGGCT
5452 GAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGAAAGTCCCAGGCTCCCAGCAGGAGAGTATGCAAAGCATGCATCTCAATTAGTCAGCA
5553 ACCAGGTGTGAAAGTCCCAGGCTCCCAGCAGGAGAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCACTAGTCCGCCAGAGC
NotI (5669)
5653 GCGCGAGGGCTCCAGCGGCCCTCCCCACAGCAGGGCGGGTCCCGGCCACCAGGAGCGGGCTCGGGCGGGCGGCTGATTGGCCGGG
HindIII (5821)
5754 GCGGGCTGACGCCGACGGCTATAAGAGACCACAAGCGACCCGAGGGCCAGAGCTTCTTCCGCGAAGCTTGGCGTCAGAACGCAGGTGAGGGCGGGG
5855 GTGGCTTCCGCGGCGCGAGCTGGAGGCTGCTCCGAGCGGGCGGGCCCGCTGCTGCTGCGGGGATTAGCTGCGAGCATTCCCGCTTCGAGTTGC
5956 GGGCGCGCGGGAGGAGAGTGCAGGCTAGCGCAACCCGAGCCTCCCTGCTGCTGCTGAGGCTAGCGTGTGCTGCGCGCGCGCGCGCTG
6057 CTACTCCGGCCCACTCTGGTCTTTTTTTTTTGTGTTGTTGCTGCTGCTTCCGATTGCCGTTACGAATAGGGCTAACAAAGGGAGGGTGGGGG
6158 CTTGCTGCCCGGAGCCCGGAGGTCATGTTGGGGAGGAATGGAGGACAGGAGTGGCGGCTGGGGCCCGCCGCTTGGAGCAGATGTCGAGGCCA
6259 CCTGGATGGGGCAGGCTGGGTTTTTCCGAAGCAACAGGCTGGGTTAGCGTGCCGAGCCATGTGGCCAGCACCCGACGATCTGGCTGGCG
6360 GCGCGCGTGGCTGCTCCCTAACTAGGGTGAAGCCATCCGTCGGCACCAGTTGCGTGC GTGGAAGATGGCCGCTCCCGGCGCTGTTGCAAGGAG
6461 CTCAAAATGGAGGACGCGGACGCCGCTGGAGCGGGCGGTGAGTACCCACACAAGGAAGAGGGCTGGTCCCTCACCGCTGCTGTTCTGTGACCC
6562 CGTGGTCTATCGCCGCAATAGTCACCTCGGGCTTTTGTGACACGGCTAGTGC GCGGGGGGAGGGGATGTAATGGCGTTGGAGTTTGTTCACATTTGGT
6663 GGGTGGAGACTAGTCAGGCCAGCTGGCGTGAAGTCATTTTTGAATTTGTCCCTTGAATTTTGTGAGGGAGCTAATTTCTGGGCTTCTTAGCGTTCA
BspHI (6822)
6764 AAGGTATCTTTTAAACCTTTTTTGTAGTGTGTGAAAACCCGCTAATCAAGCAATCATGATGGAATCAAGGTGCTGTTTGCCTCATCTGTATTGC
1▶ M M E I K V L F A L I C I A
BglII (6940)
6865 TGTTGCTGAGGCAAAACCACTGAAATCAATGAAGACCTCAATATAGTGTGTGGCTCCAACTTTGCCACCACAGATCTTGTGACTGACCTGTTCCACCA
14▶ 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 D L F T
6966 ACTGGGAGACCATGAATGTGATTAGCACTGACACAGAGGAGTGAACACAGATGCTGACAGGGCAAGCTGCTGGCAAAAACTCCCCAGATGCTCTG
48▶ 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 P D V L
ApaI (7138)
7067 AGGGAGCTGGAGGCAATGCCAGAAGGGCTGGTTCACAAGAGGCTGCTCATTGCTCTCCACATTAAGTGCACCCCTAAGATGAAGAAATTTATCCC
82▶ 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 K K F I P

EcoRV (7237)

7168 TGGCAGGTGCCACACTTATGAAGGTGAAAAGGAGTCTGCTCAGGGAGGGATTGGAGAGGCAATTGTTGATATCCCAGAGATTCTGGCTTCAAGGATAAGG
115▶ 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 F K D K
7269 AGCCACTGGACCAGTTTATTGCTCAAGTGGACCTCTGTGCTGATTGCACCACTGGCTGTCTGAAGGGCCTTGCCAATGTCCAGTGTCTGACCTCCTGAAG
149▶ 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 D L L K
AvrII (7459)

7370 AAGTGGCTTCCCAGAGGTGTACCACTTTTCCAGCAAGATTCAAGGGTAGGGTGGACAAAATCAAGGGTCTGGCTGGGGACAGATGATACCTAGGATTATC
183▶ 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 •
7471 CCTAATACCTGCCACCCCACTCTTAATCAGTGGTGAAGAACGGTCTCAGAACTGTTTGTTC AATTGGCCATTTAAGTTTAGTAGAAAAGACTGGTTAA
7572 TGATAACAATGCATCGTAAAACCTTCAGAAGGAAAGGAGAATGTTTTGTGGACCACTTTGGTTTTCTTTTTGCGTGTGGCAGTTTAAAGTTATTAGTTTT

ScaI (7682)

7673 TAAAATCAGTACTTTTTAATGGAACAACCTGACCAAAAATTTGTCACAGAATTTGAGACCCATTA AAAAAGTTAAATGAGAAACCTGTGTTCCTTTG
7774 GTCAACACCGAGACATTTAGGTGAAAGACATCTAATTCTGGTTTTACGAATCTGGAACTTCTTGAAAATGTAATTCTTGAGTTAACACTTCTGGGTGGAG
7875 AATAGGGTTGTTTTCCCCCACATAATTGGAAGGGGAAGGAATATCATTAAAGCTATGGGAGGTTGCTTTGATTACAACACTGGAGAGAAATGCAGCAT
7976 GTTGCTGATTGCCTGTCACTAAAACAGGCCAAAAACTGAGTCCTTGGGTTGCATAGAAAGCTG