

STOP

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Important Limited Use License information for pVITRO1-blasti-Lucia/SEAP

The purchase of the pVITRO1-blasti-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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pVITRO1-blasti-Lucia/SEAP

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

Catalog code: pvitro1-blucsp

<https://www.invivogen.com/pvitro1-luciaseap>

For research use only

Version 20F23-MM

PRODUCT INFORMATION

Contents:

- 20 µg of pVITRO1-blasti-Lucia/SEAP provided as lyophilized DNA
- 2 x 1 ml blasticidin at 10 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 blasticidin 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

pVITRO is a family of vectors with improved features. pVITRO plasmids allow the co-expression of two or more genes from two different transcription units. pVITRO plasmids can be stably transfected in mammalian cells and are expressed at high levels.

pVITRO1-Lucia/SEAP contains the Lucia luciferase and SEAP reporter genes. pVITRO1-Lucia/SEAP can be used as a control vector. **pVITRO1-Lucia/SEAP** also can be used for cloning of open reading frames (ORF). Both reporter genes are flanked by unique sites (*Nco* I/*Avr* II for Lucia luciferase and *Bsp*H I/*Nhe* I for SEAP) that allow for convenient cloning of ORFs.

PLASMID FEATURES

- **rEF1 and mEF1 prom:** pVITRO1-blasti-GFP/LacZ plasmid carries two elongation factor 1 alpha (EF-1α) promoters, from rat and mouse origins. Similarly to their human counterpart¹, both promoters display a strong activity that yield similar levels of expression. EF-1α promoters are expressed at high levels in all cell cycles and lower levels during G0 phase. EF-1α promoters are also non-tissue specific; they are highly expressed in all cell types.
- **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 several-fold more active than the SV40 enhancer³.
- **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.
- **FMDV IRES:** The internal ribosome entry site of the Foot and Mouth Disease Virus enables the translation of two open reading frames from one mRNA with high levels of expression⁵.
- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- **Bsr gene** confers resistance to Blasticidin both in *E. coli* and mammalian cells. In bacteria, *bsr* is expressed from the constitutive *E. coli* EM7 promoter. In mammalian cells, *bsr* is transcribed from the rat EF-1α promoter as a polycistronic mRNA and translated via the FMDV IRES.
- **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.
- **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.
- **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™.

1. Kim DW, *et al.*, 1990. Use of the human elongation factor 1α promoter as a versatile and efficient expression system. *Gene* 91(2):217-23. 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.* 9(10): 4248-58. 5. Ramesh N, *et al.*, 1996. High-iter bicistronic retroviral vectors employing foot-and-mouth disease virus internal ribosome entry site. *Nucleic Acids Res.* 24(14):2697-700.

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.

Blasticidin usage

Blasticidin should be used at 25-100 µg/ml in bacteria and 1-30 µg/ml in mammalian cells. Blasticidin is supplied at 10 mg/ml in HEPES buffer.

TECHNICAL SUPPORT

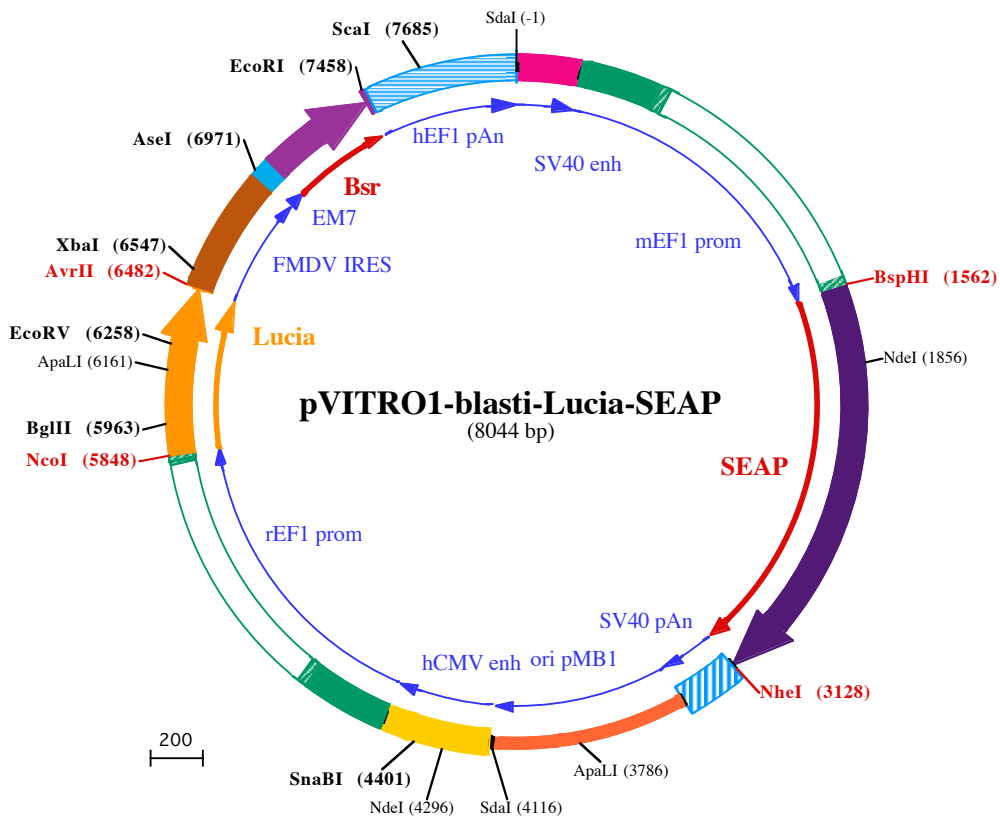
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SdaI (-1)
1 CCTGCAGGGCCCTGAAATAACCTCTGAAAGAGGAACCTTGGTTAGGTACCTTCTGAGGCGGAAAGAACCAGCTGTGAATGTGTCTAGTTAGGGTGTGGAA

101 AGTCCCCAGGCTCCCCAGCAGGCAGAAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAAG

201 TATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCACTAGTGGAGCCGAGAGTAATTCATACAAAAGGAGGGATCGCTTCGCAAGGGGAGAG

301 CCCAGGGACCGTCCCTAAATTTCTACAGACCCAAATCCCTGTAGCCGCCACGACAGCGGAGGAGCATGCGCTCAGGGCTGAGCGCGGGGAGAGCAGA

401 GCACACAAGCTCATAGACCCTGGTCGTGGGGGGAGGACCGGGGAGCTGGCGGGGCAAACCTGGGAAAGCGGTGTCGTGTCTGCTCCGCCCTTCTCC

501 CGAGGGTGGGGGAGAACGGTATATAAGTGGCGCAGTCGCTTGGACGTTCTTTTTTCGCAACGGGTTTGCCGTCAGAACCGAGGTGAGGGGCGGGTGTGGC

601 TTCCGCGGGCCCGCAGCTGGAGGTCCTGCTCCGAGCGGGCCGGCCCGCTGTCGTCGCGGGGATTAGCTGCGAGCATTCCGCTTCGAGTTGCGGGC

701 GCGCGGGGAGGAGAGTGCAGGGCTAGCGGCAACCCGTAGCCTCGCTCGTGTCCGGCTTGGAGCCTAGCGTGGTGTCCGCGCCGCCCGCGCTGCTA

801 CTCCGGCCGACTCTGCTTTTTTTTTTTTTTTTGTGTTGTTGCCCTGCTGCCTTCGATTGCCGTTACGAATAGGGGCTAACAAAGGGAGGGTGGCGGGCT

901 TGCTCGCCCGAGCCCGAGAGGTGATGTTGGGGAGGAATGGAGGGACAGAGTGGCGGCTGGGGCCCGCCCGCTTCGAGACATGTCCGAGGCCAC

1001 CTGGATGGGGCAGGCTGGGGTTTTTCCGAAGCAACCAGGCTGGGGTTAGCGTGCCGAGGCCATGTGGCCACGACCCGGCAGCATGTGGCTTGGCG

1101 GCGCCGCTTGCCTGCCTCCCTAACTAGGGTGAGGCCATCCGTCGGCCACCGTTCGCTGCGTGAAAGATGGCGCTCCCGGGCCCTGTTGCAAGGA

1201 GCTCAAAATGGAGACCGCGCAGCCGGTGGAGCGGGGGTGGAGTACCCACACAAAGGAAGAGGGCCTGGTCCCTCACGGCTGCTGCTTCTGTGAC

1301 CCCGTGCTCTATCGCCGCAATAGTCACTCGGGCTTTTGGAGCAGGCTAGTCGCGCGGGGGGAGGGATGTAATGGCGTTGGAGTTTGTTCACATTT

1401 GGTGGTGGAGACTAGTCAGGCCAGCCTGGCGCTGGAAGTATTTTTGGAATTTGTCCCTTGGATTTTGGCGGAGCTAATTTCTCGGGCTTCTTAGCGG

1501 TTCAAAGGTATCTTTTAAACCTTTTTTAGGTGTTGTGAAAACACCGCTAATTCAAAGCAATCATGATTCTGGGCGCTGCATGCTGCTGCTGCTGCTG

BspHI (1562)

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1601 CTGCTGGGCTGAGGCTACAGCTCTCCCTGGGCATCATCCAGTTGAGGAGGAGAACCAGGACTTCTGGAACCGCAGGCGAGCCGAGGCGCTGGTGCCG

13▶ L L L G 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

1701 CCAAGAAGCTGCAGCCTGCACAGACAGCCGCAAGAACCTCATCATCTTCTGGCGATGGGATGGGGGTGTCTACGGTGACAGCTGCCAGGATCCTAAA

46▶ A K K L 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

NdeI (1856)

1801 AGGGCAGAAGAAGGACAAAACCTGGGCGCTGAGATACCCCTGGCTATGGACCGCTTCCCATATGTGGCTCTGTCCAAGACATAAATGTAGACAAAATGTG

79▶ G Q K K 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

1901 CCAGACAGTGGAGCCACAGCCAGGCTACTGTGGGGGTCAAGGGCAACTTCCAGACCATTTGGCTTGGTGCAGCGCCCGCTTAAACAGTGAACA

113▶ P D S G 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

2001 CGACACGGCAACGAGGTGATCTCCGTGATGAATCGGGCCAAGAAAGCAGGAAAGTCAAGTGGGAGTGGTAACCCACACGAGTGCAGCAGCCCTCGCC

146▶ T T R G N 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

2101 AGCCGGCACCTACGCCACAGGTAACCGCAACTGTTACTCGGACCGCAGCTGCCTGCCTCGGCCCGCAGGAGGGTGCAGGACATCGCTACGCGA

179▶ A G T Y A 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

2201 CTCATCTCAACATGGACATTGATGTGATCTGGGTGGAGGCCGAAAGTACATGTTTCGATGGGAACCCAGACCCCTGAGTACCAGATGACTACAGCC

213▶ L I S N M 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

2301 AAGGTGGACAGGCTGGAGCGGAAGAACTGTTGTCAGGAATGGCTGGCGAAGCGCCAGGGTGCCTGGTATGTGTGAACCGCACGAGCTCATGACGGC

246▶ Q G G T R L 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

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2501 GAGATGACAGAGGCTGCCCTGGCGCTGCTGAGCAGGAACCCCGGGCTTCTCTCTTCTGTTGGAGGGTGGTGCATCGACCAGGTCATCACGAAAGCA

313▶ E M T E A A 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

2601 GGGCTTACCGGCACTGACTGAGACGATCATGTTGACGACGCCATTGAGAGGGCGGGCAGCTCACCAGCGAGGAGCACGCTGAGCCTCGTCACTGC

346▶ R A Y R A L T 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

2701 CGACCACTCCACGTCTTCTCTTGGAGGCTACCCCTGCGAGGGAGCTCCATCTTGGGCTGGCCCTGGCAAGGCCGGGACAGGAAGGCTACAGC

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2801 GTCCTCTATACGAAACGGTCCAGGCTATGTGCTCAAGGACGGCGCCCGGGATGTTACCGAGAGCGAGAGCGGGAGCCCGAGTATCGGACGAGT

413▶ V L L Y G N G 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

2901 CAGCAGTGGCCCTGGACGAAGAGACCCACGAGCGAGGAGCTGGCGGTGTTTCGCGCGGGCCCGCAGGCGCACCTGGTTCACGGCGTGCAGGAGCAGAC

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NheI (3128)

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513▶ R S R S K R L D •

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4101 ACATGTTCTT **SdaI (4116)** AATTAACCTGCAGGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGACG
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SnaBI (4401)
4401 CTACGTATTAGTCATCGCTATTACCATGATGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCAC
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ApaLI (6161)
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EcoRV (6258)
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1▶ M K T F N I S Q Q D L E L V E V A T E K I T M

7100 GCTCTATGAGGACAACAAGCACCATGTCGGGGCGGCCATCAGGACCAAGACTGGGGAGATCATCTCTGCTGCCACATTGAGGCCTACATTGGCAGGGTC
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EcoRI (7458)
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123▶ L V K T T I E E L I P L K Y T R N •
7500 TAATCAGTGGTGAAGAACGGTCTCAGAAGCTTTGTTTCAATTGGCCATTTAAGTTTAGTAGTAAAAGACTGGTTAATGATAACAATGCATCGTAAAC
ScaI (7685)
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