

# pVITRO1-blasti-GFP/SEAP

An expression plasmid coding for the GFP and SEAP reporter genes

Catalog code: pvitro1-bgfpsp

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

For research use only

Version 20F23-MM

## PRODUCT INFORMATION

### Contents:

- 20 µg of pVITRO1-blasti-GFP/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 that 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-GFP/SEAP contains the reporter genes GFP and SEAP and can be used as a control vector. pVITRO1-GFP/SEAP also can be used for cloning of open reading frames (ORF). Both reporter genes are flanked by unique sites (NcoI/AvrII for GFP and BspHI/NheI for SEAP) that allow for convenient cloning of ORFs.

## PLASMID FEATURES

- **rEF1 and mEF1 prom:** pVITRO1-blasti-GFP/SEAP plasmid carries two elongation factor 1 alpha (EF-1α) promoters, from rat and mouse origins. Similarly to their human counterpart<sup>1</sup>, 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 plasmids<sup>2</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 several-fold more active than the SV40 enhancer<sup>3</sup>.
- **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>4</sup>

- **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 expression<sup>5</sup>.
- **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.
- **GFP gene:** This red-shifted variant of the jellyfish GFP gene encodes a green fluorescent protein that absorbs blue light (major peak at 480 nm) and emits green light (major peak at 505 nm).
- **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-riter 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

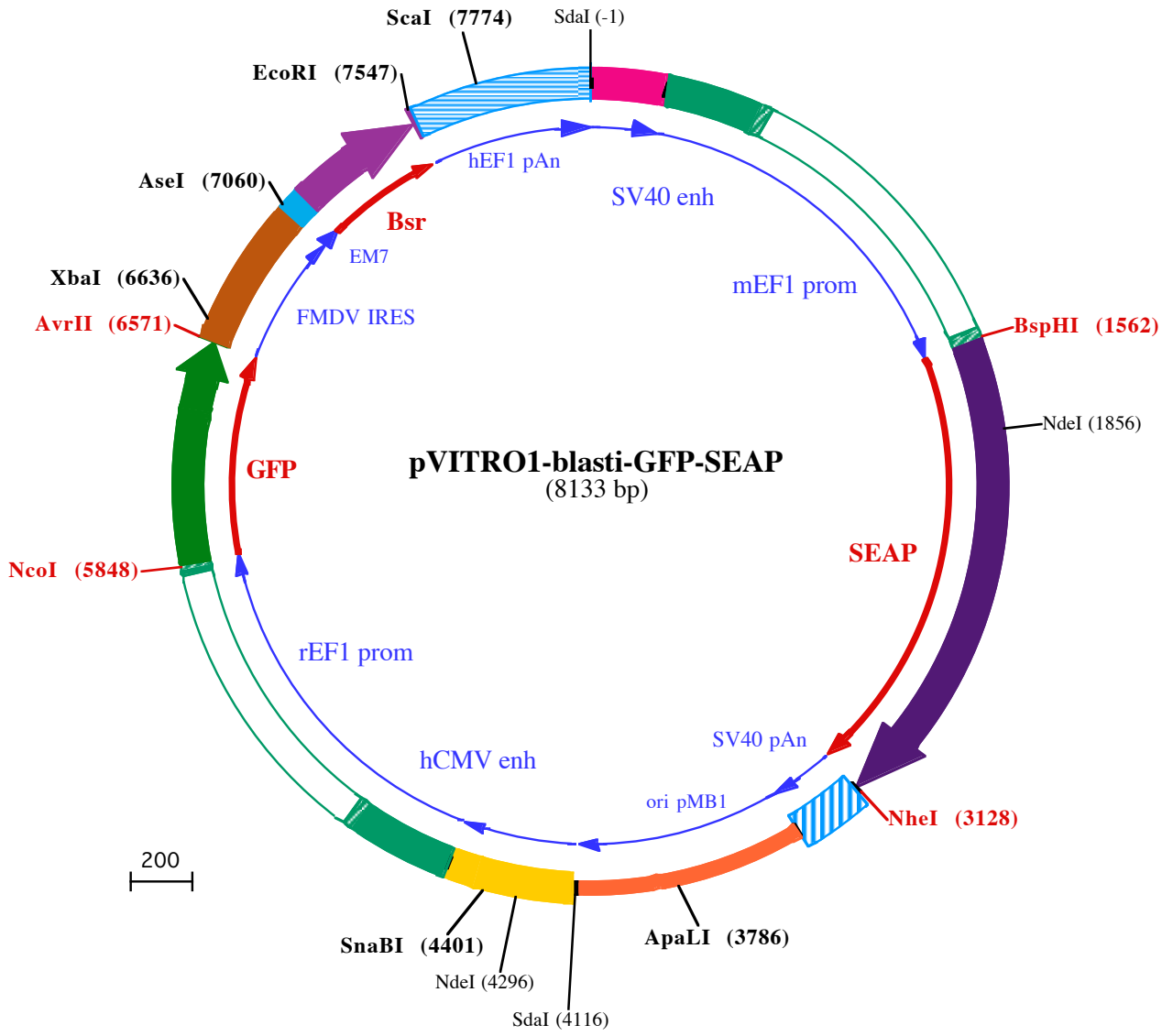
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SdaI (-1)  
1 CCTGCAGGGCCCTGAAATAACCTCTGAAAGAGGAACCTTGGTTAGGTACCTTCTGAGGCGGAAAGAACCAGCTGTGAATGTGTTCAGTTAGGGTGTGGAA

101 AGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAG

201 TATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCACTAGTGGAGCCGAGAGTAATTCATACAAAAGGAGGGATCGCTTCGCAAGGGGAGAG

301 CCCAGGGACCGTCCCTAAATTTCTACAGACCCAAATCCCTGTAGCCGCCACGACAGCGGAGGAGCATGCGCTCAGGGCTGAGCGCGGGGAGAGCAGA

401 GCACACAAGCTCATAGACCCTGGTCTGGGGGGAGGACCGGGGAGCTGGCGGGGCAAACCTGGGAAAGCGGTCTGCTGTCTGCTCCGCCCTTCTCC

501 CGAGGGTGGGGGAGAACGGTATATAAGTGGCGCAGTCGCTTGGACGTTCTTTTTTCGCAACGGGTTTGCCGTCAGAACCGAGGTGAGGGGCGGGTGTGGC

601 TTCCGCGGGCCCGCAGCTGGAGGTCCTGCTCCGAGCGGGCCGGCCCGCTGCTGCTCGCGGGGATTAGCTGCGAGCATTCCGCTTCGAGTTGCGGGC

701 GCGCGGGAGGCAGAGTGCAGGCGCTAGCGGCAACCCGTAGCCTCGCTCGTGTCCGGCTTGGAGCCTAGCGTGGTGTCCGCGCCGCCCGCGCTGCTA

801 CTCCGGCCGACTCTGCTTTTTTTTTTTTTTTTGTGTTGTTGCCCTGCTGCCTTCGATTGCCGTTACGAATAGGGGCTAACAAAGGGAGGGTGGCGGGCT

901 TGCTCGCCCGAGCCCGAGAGGTGATGTTGGGGAGGAATGGAGGGACAGAGTGGCGGCTGGGGCCCGCCCGCTTCGAGACATGTCCGAGGCCAC

1001 CTGGATGGGGCAGGCTGGGGTTTTTCCGAAGCAACCAGGCTGGGGTTAGCGTGCCGAGGCCATGTGGCCACGACCCGGCAGCATGTGGCTTGGCG

1101 GCGCCGCTTGCCTGCCTCCCTAACTAGGGTGGAGCCATCCGTCGGCACCAGTTGCGTGCCTGAAAGATGGCGCTCCCGGGCCCTGTTGCAAGGA

1201 GCTCAAATGGAGACCGCGCAGCCGGTGGAGCGGGCGGTGAGTACCCACACAAAGGAAGAGGGCCTGGTCCCTACCGGCTGCTGCTTCTGTGAC

1301 CCGTGTCTATCGCCGCAATAGTCACTCGGGCTTTTGGAGCAGGCTAGTCCGCGGGGGGAGGGATGTAATGGCGTTGGAGTTTGTTCACATTT

1401 GGTGGTGGAGACTAGTCAGGCCAGCTGGCGCTGGAAGTATTTTTGGAATTTGCCCTTGGTTTTGAGCGGAGCTAATTTCTCGGGCTTCTTAGCGG

1501 TTCAAAGTATCTTTTAAACCTTTTTTAGGTGTTGTGAAAACACCGCTAATTCAAAGCAATCATGATTCTGGGCGCTGCATGCTGCTGCTGCTGCTG

**BspHI (1562)**

1▶ M I L G P C M L L L L L L

1601 CTGCTGGGCGTGGGCTACAGCTCTCCCTGGGCATCATCCAGTTGAGGAGGAGAACCAGGACTTCTGGAACCGCAGGCGAGCCGAGGCGCTGGGTGCCG

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 CCAAGAAGCTGCAGCTGCACAGACAGCCGCAAGAACCTCATCATCTTCTGGCGATGGGATGGGGGTGTCTACGGTGACAGCTGCCAGGATCCTAAA

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 AGGGCAGAAGAAGGACAAAAGTGGGCGCTGAGATACCCCTGGCTATGGACCGCTTCCCATATGTGGCTCTGTCCAAGACATAAATGTAGACAAAATGTG

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 CCAGACAGTGGAGCCACAGCCAGGCGCTACCTGTGGGGGTCAAGGGCAACTTCCAGACCATTTGGCTTGGTGCAGCGCCCGCTTAAACAGTGAACA

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 CGACACGGCAACGAGGTGATCTCCGTCATGAATCGGGCAAGAAAGCAGGAAAGTCAAGTGGGAGTGGTAACCCACACGAGTGCAGCAGCGCTCGCC

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 CTCATCTCAACATGGACATTTGATGTGATCTGGGTGGAGGCCGAAAGTACATGTTTCGATGGGAACCCAGACCCCTGAGTACCAGATGACTACGCG

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 AAGGTGGACAGGCTGGAGCGGAAGAACTGTTGTCAGGAATGGCTGGCGAAGCGCCAGGGTGCCTGGTATGTGGAACCGCACGAGCTCATGACGGC

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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279▶ S L D P S V 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

2501 GAGATGACAGAGGCTGCCCTGGCGCTGAGCAGGAACCCCGGGCTTCTCTCTTCTGTTGGAGGGTGGTGCATCGACCAGGTCATCACGAAAGCA

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2601 GGGCTTACCGGCACTGACTGAGACGATGTTGTCGACGACCCATTGAGAGGGCGGGCCAGCTCACCAGCGAGGAGCACGCTGAGCCTCGTCACTGC

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

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379▶ D H S H V F S 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

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 CAGCAGTGGCCCTGGACGAAGAGACCCACGAGCGAGGAGCTGGCGGTGTTTCGCGCGGGCCCGCAGGCGCACCTGGTTCACGGCGTGCAGGAGCAGC

446▶ S A V P L D E E 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

3001 CTTATAGCGCAGTGCATGGCTTCGCGCTGCTGGAGCCCTACACCGCTGCGACCTGGCGCCCGCCGGCACCACCGACCGCGCCGACCCGGG

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513▶ R S R S K R L D •

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3301 AGGTTTCAGGGGAGGTTGGGGAGGTTTTTAAAGCAAGTAAACCTCTACAATATGGTATGGAATGTTAATTAACACTAGCCATGACCAAAATCCCTTAA

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4201 TATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATA **NdeI (4296)**  
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**SnaBI (4401)**  
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6001 17 E L D G D V N G H K F S V S G E G E G D A T Y G K L T L K F I C T  
6101 51 T G K L P V P W P T L V T T L T Y G V Q C F S R Y P D H M K Q H D  
6201 84 F F K S A M P E G Y V Q E R T I F F K D D G N Y K T R A E V K F E G  
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6401 151 V Y I M A D K Q K N G I K V N F K I R H N I E D G S V Q L A D H Y  
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6701 217 H M V L L E F V T A A G I T L G M D E L Y K •  
**XbaI (6636)**  
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7001 TGTGTGCAACCCAGCAGCGGACTTTACTGCGAAACCACTTTAAAGTGACATTGAAACTGGTACCCACACACTGGTACAGGCTAAGGATGCCCTTCA **AseI (7060)**  
7100 GGTACCCCGAGGTAACACCGGCACTCGGGATCTGAGAAGGGGACTGGGGCTTCTATAAAAGCGCTCGGTTTAAAAAGCTTCTATGCCTGAATAGGTGAC  
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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 L Y E

7200 ACAACAAGCACCATGTCGGGGCGGCCATCAGGACCAAGACTGGGGAGATCATCTCTGCTGTCCACATTGAGGCCTACATTGGCAGGGTCACTGTCTGTGC  
27▶ D N K H H V G A A I R T K T G E I I S A V H I E A Y I G R V T V C A  
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7400 ATCAGGGTGGTCAGCCCCTGTGGCATGTGCAGAGAGCTCATCTGACTATGCTCCTGACTGCTTTGTGCTCATTGAGATGAATGGCAAGCTGGTCAAAA  
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EcoRI (7547)  
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127▶ T T I E E L I P L K Y T R N •  
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ScaI (7774)  
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8100 CAAAACTGAGTCCTTGGGTTGCATAGAAAGCTG ▶