

# pVITRO2-blasti-GFP/SEAP

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

Catalog code: pvitro2-bgfpsp

<https://www.invivogen.com/pvitro2-gfpseap>

For research use only

Version 20H18-MM

## PRODUCT INFORMATION

### Contents

- 20 µg of pVITRO2-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 plasmids developed mainly for *in vitro* studies. They allow the ubiquitous and constitutive co-expression of two genes of interest. pVITRO plasmids can be stably transfected in mammalian cells and the genes of interest are expressed at high levels. Each pVITRO plasmid is available with either two multiple cloning sites or two reporter genes.

**pVITRO2-blasti-GFP/SEAP** contains the GFP and SEAP reporter genes and can be used as a control vector.

**pVITRO2-blasti-GFP/SEAP** also can be used for cloning of open reading frames (ORF). Both reporter genes are flanked by unique sites (BspH I/Avr II for GFP and Nco I/Nhe I for SEAP) that allow for convenient cloning of ORF's.

## 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.

## 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 protein<sup>1</sup>. 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<sup>2</sup>. The enhancement varies from 2-fold in non-permissive cells to 20-fold in permissive cells.

- **CMV enhancer:** The major immediate early enhancer of the human cytomegalovirus (HCMV), located between nucleotides -118 and -524, 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>3</sup>.

- **pMB1 ori:** a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.

- **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).

- **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>4</sup>.

- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.

- **Blasti:** Resistance to blasticidin is conferred by the *bsr* gene from *Bacillus cereus*. In bacteria, *bsr* is expressed from the constitutive *E. coli* EM7 promoter. In mammalian cells, *bsr* is transcribed from the hFerH/mEF1α 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.

- **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™.

- **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>

1. Eisenstein RS. & Munro HN. 1990. Translational regulation of ferritin synthesis by iron. *Enzyme* 44(1-4):42-58.
2. Dean D.A. *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* 141(2):521-30.
4. Ramesh N. *et al.*, 1996. High-titer bicistronic retroviral vectors employing foot-and-mouth disease virus internal ribosome entry site. *Nucleic Acids Res.* 24(14):2697-700.
5. Carswell S. & Alwine J.C. 1989. Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. *Mol. Cell Biol.* 10:4248-58.

## TECHNICAL SUPPORT

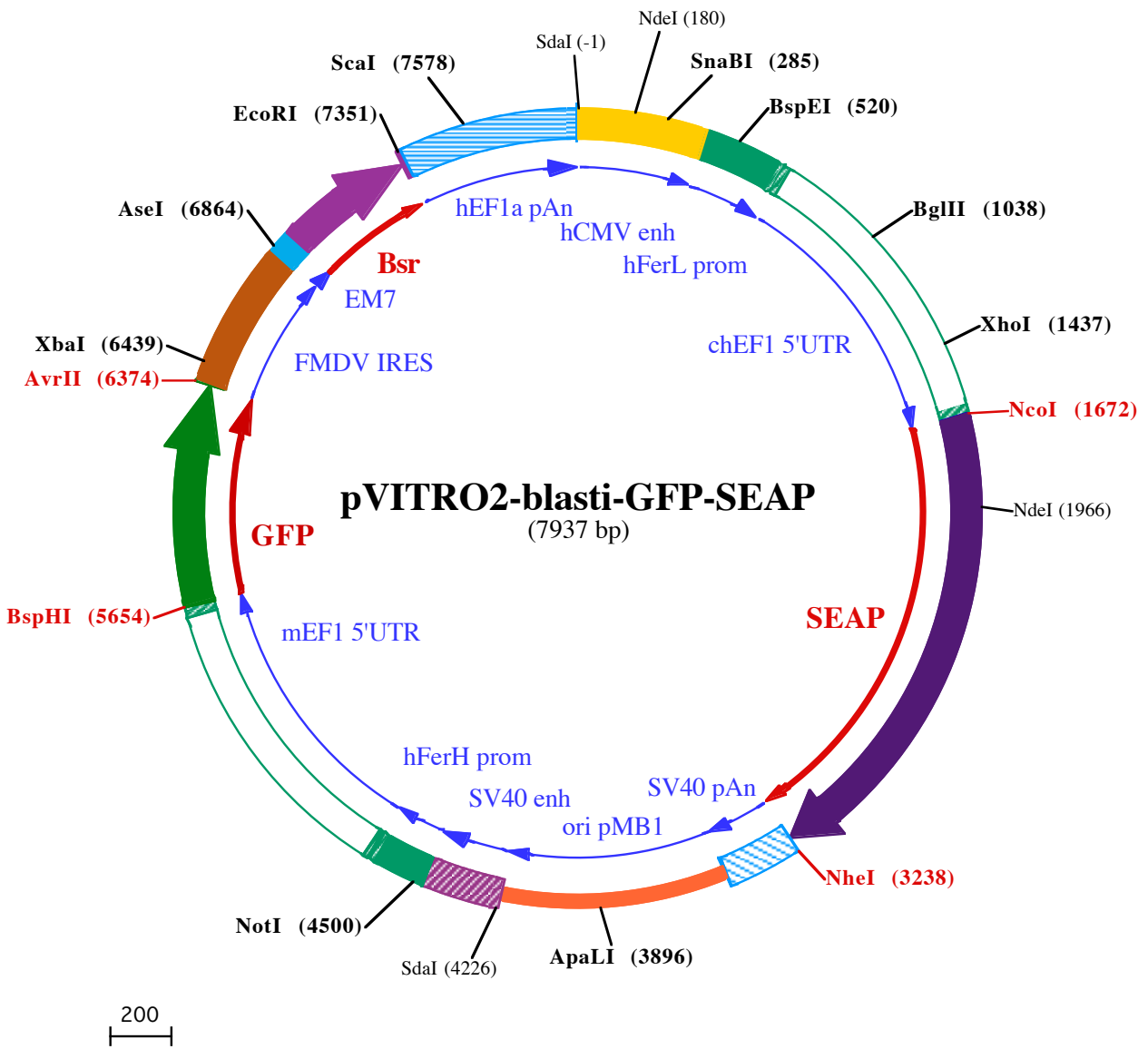
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SdaI (-1)  
1 CCTGCAGGCGTTACATACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA

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101 CGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTA  
NdeI (180)

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203 TTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTA  
SnaBI (285)

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305 TTACCATGATGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTACTCACGGGGATTTCCAAGTCTCCACCCCAATTGACGTCAATGGGAGT

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406 TTGTTTTGACTAGTCAGGGCCCAACCCCCCAAGCCCCATTTCACAACACGCTGGCGCTACAGGCGGTGACTTCCCTTGTCTTGGGCGGGGGGCTG  
BspEI (520)

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608 GCCGGCGCACATAAAGAAGCCGCCCTAGCCACGTCCCTCGCAGTTCGGCGTCCCGGGTCTGTCTCA AGCTTGCCGCAGAACAAGGtaagtgcc  
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1015 agatagtcttgaatatgcccgaagatctgcacactggtatctcggtttttggggcgcggggcggcgacggggcccgtgcgtcccagcgcacatggtcgg  
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1422 ggacacctgattagttctcgagcttttggagtagctgctctttaggttggggggaggggttttatgcatggagttccccacactgagtggtgggagact  
XhoI (1437)

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1625 gttttttcttccatttcagGTGTCGTGAAAAC TACCCCTAAAAGCCACCATGTTCTGGGGCCCTGCATGCTGCTGCTGCTGCTGCTGGCCTGAGGC  
NeoI (1672)

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1 M V L G P C M L L L L L L L L G L R

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1829 CACAGACAGCCCAAGAACCTCATCATCTTCTGGCGATGGGATGGGGGTGCTACGGTGACAGCTGCCAGGATCCTAAAAGGCAGAAGAAGGACAAC  
18 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 Q P

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52 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 D K

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1931 TGGGGCCTGAGATACCCCTGGCTATGGACCGCTTCCCATATGTGGCTCTGTCCAAGACATACAATGTAGACAAACATGTGCCAGACAGTGGAGCCACAGCCA  
NdeI (1966)

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2033 CGGCCTACCTGTGCGGGTCAAGGGCAACTTCCAGACCATTGGCTTGTGAGTGCAGCCGCCGCTTAACCAAGTGAACACGACACGCGGCAACGAGGTCATCT  
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2135 CCGTGATGAATCGGGCAAGAAGCAGGGAAGTCACTGGGAGTGGTAACACCACACGAGTGCAGCAGCCTCGCCAGCCGGCACCTACGCCACACGGTGA  
120 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 E V I

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2237 ACCGCAACTGGTACTCGGACCGGACGTGCCTGCCTCGGCCCGCAGGAGGGTGCAGGACATCGCTACGCAGTCTCATCTCCAACATGGACATTGATGTGA  
154 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 H T V

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2339 TCCTGGGTGAGGCGAAAAGTACATGTTTCGCATGGGAACCCAGACCTGAGTACCCAGATGACTACAGCCAAGTGGGACCAGGCTGGACGGGAAGAATC  
188 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 D I D V

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2441 TGGTGCAGGAATGGCTGGCGAAGCGCCAGGGTGGCCGGTATGTGTGGAACCGCACTGAGTCTATGCAGGCTTCCCTGGACCCGCTGTGACCCATCTCATGG  
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2543 GTCTCTTTGAGCCTGGAGACATGAAATACGAGATCCACCGAGACTCCACACTGGACCCCTCCCTGATGGAGATGACAGAGGCTGCCTGCGCCTGTGAGCA  
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