

pVITRO1-hygro-GFP/SEAP

An expression plasmid coding for the GFP and SEAP reporter genes

Catalog code: pvitro1-gfpsep

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

For research use only

Version 18J06-MM

PRODUCT INFORMATION

Contents:

- 20 µg of pVITRO1-hygro-GFP/SEAP provided as lyophilized DNA
- 1 ml 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

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.

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

• rEF1 and mEF1 prom: pVITRO1-hygro-GFP/SEAP 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*.

• hph gene confers resistance to Hygromycin B both in *E. coli* and mammalian cells. In bacteria, *hph* is expressed from the constitutive *E. coli* EM7 promoter. In mammalian cells, *hph* 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-iter bicistronic retroviral vectors employing foot-and-mouth disease virus internal ribosome entry site. Nucleic Acids Res. 24(14):2697-700.

TECHNICAL SUPPORT

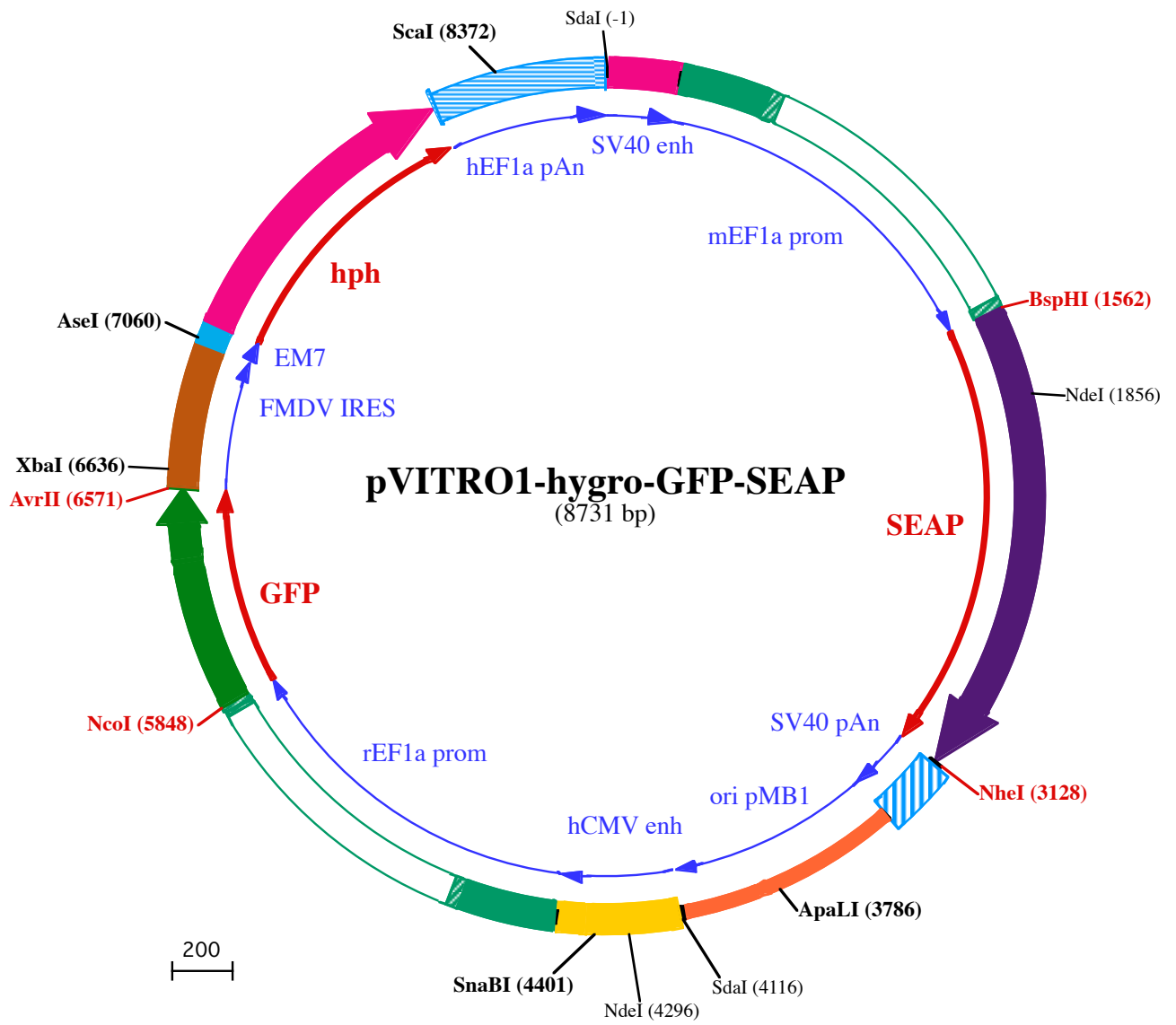
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SdaI (-1)
1 CCTGCAGGGCCTGAAATAACCTCTGAAAGGAACTTGGTTAGTACCTTCTGAGGCGAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGGAA

101 AGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTACAGCAACCAGGTGTGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAG

201 TATGCAAAGCATGCATCTCAATTAGTACAGCAACCATAGTCCACTAGTGGAGCCGAGAGTAATTCATACAAAAGGAGGGATCGCCTTCGCAAGGGGAGAG

301 CCCAGGGACCGTCCCTAAATTCTCACAGACCCAAATCCCTGTAGCCGCCCCACGACAGCGGAGGAGCATGCGCTCAGGGCTGAGCGCGGGGAGAGCAGA

401 GCACACAAGCTCATAGACCTGGTCTGGGGGGAGGACCGGGGAGCTGGCGGGGCAAACGGAAAGCGGTGCTGTGTGCTGGCTCCGCCTCTTCC

501 CGAGGGTGGGGAGACGGTATATAAGTGGCAGTCGCTTGGACGTTCTTTTTCGCAACGGTTTGGCGTCAGAACGCAGGTGAGGGGCGGTGTGGC

601 TTCCGCGGCGCCGAGCTGGAGTCTGCTCCGAGCGGGCCGGCCCGCTGCTGCGGGGGATTAGTGCAGCATTCCCGCTTCGAGTTGCGGGC

701 GGGCGGGAGGCAGAGTGCAGGCTAGCGGCAACCCGTAGCCTCGCTGCTGCGGCTTGGGCTAGCGTGGTGTCCGCGCCGCCCGCGTGTCTA

801 CTCCGGCCGACTCTGGTCTTTTTTTTTTTTGTGTTGTTGCCCTGCTGCTTCGATTGCCGTTACGAATAGGGGTAAACAAAGGAGGGTGCAGGGCT

901 TGCTCGCCCGAGCCCGAGAGTGTGTTGGGAGGAATGGAGGGACAGGAGTGGCGGCTGGGGCCCGCCCGCTTCGGAGCACATGTCGACGCCAC

1001 CTGGATGGGGCAGGCTGGGGTTTTTCCGGAAGCAACCAGGCTGGGGTTAGCGTCCGAGGCCATGTGGCCCGCAGCACCCGGCACGATCTGGCTTGGCG

1101 GCGCCGCTTGCCTGCTCCCTAACTAGGGTGGGCGATCCCGTCCGGCACCAGTTGCGTGGTGGAAAGATGGCCGCTCCCGGGCCCTGTTGCAAGGA

1201 GCTCAAATGGAGACCGCGCAGCCGGTGGAGCGGGCGGGTGGTACCACACAAAGGAAGAGGGCTGGTCCCTACCGGCTGCTGCTTCTGTGAC

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1501 TTCAAAGGTATCTTTTAAACCTTTTTTAGGTGTTGTGAAAACCACCGCTAATTCAAAGCAATCATGATTCTGGGGCCCTGCATGCTGCTGCTGCTGCTG

BspHI (1562)

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1601 CTGCTGGGCTGAGGCTACAGCTCTCCCTGGGCATCATCCAGTTGAGGAGGAGAACCAGGACTTCTGGAACCGCAGGACCGGAGCCCTGGTGGCC

13▶ 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 CCAAGAAGCTGCAGCTGCACAGACAGCCGCAAGAACCTCATCATCTTCTGGCGATGGATGGGGGTGTCTACGGTGACAGCTGCCAGGATCCTAAA

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 AGGGCAGAAGAAGACAACTGGGGCTGAGATACCCCTGGCTATGGACCGTTCATATGTGGCTGTCCAAAGACATACATGTAGACAAACATGTG

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 CCAGACAGTGGAGCCACAGCCAGCCCTACCTGTGCGGGTCAAGGGCAACTCCAGACATTGGCTTGGTGCAGCCGCCCTTAAACAGTGAACA

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 CGACACGCGCAACGAGGTCTCCGTGATGAATCGGGCAAGAAAGCAGGGAAAGTCAAGTGGAGTGGTAACCAACACAGAGTGCAGCACGCTCGCC

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 AGCCGGACCTACGCCACACGGTGAACCGCAACTGTTACTCGGACCGCAGCTGCTGCTCGCCGCCAGGAGGGGTGCCAGGACATCGCTACGACG

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 CTCATCTCCAACATGGACATTGATGTGATCCTGGTGGAGGCCAAAAGTACATGTTTCGATGGGAACCCAGACCTGAGTACCCAGATGACTACAGCC

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 AAGTGGGACAGGCTGGACGGGAAGAACTGGTGCAGGAATGGCTGGGAAGCGCAGGGTGCCCGTATGTGTGAACCGCACTGAGCTCATGCAGGC

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

2401 TTCCCTGGACCGTCTGTGACCATCTCATGGTCTCTTTGAGCTGGAGACATGAAATACGAGATCCACCAGACTCCACTGGACCCCTCCCTGATG

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 GAGATGACAGAGGCTGCCCTGCGCTGCTGAGCAGGAACCCCGCGGCTTCTCCTTCGTGGAGGGTGGTGCATCGACCAGGTCATCACGAAAGCA

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 GGGCTTACCGGGCACTGACTGAGACGATCATGTTGACGACGCCATTGAGAGGGCGGGCAGCTACCAGCGAGGAGGACAGCTGAGCCTGCTACTGCT

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 CGACCACTCCACGCTTCTCCTTCGGAGGCTACCCCTGCGAGGAGCTCATCTTCGGGCTGGCCCTGGCAAGGCCGGGACAGGAAGCCCTACACG

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 GTCCTCTATACGGAAACGGTCCAGGCTATGTGCTCAAGGACGGCGCCCGCGGATGTTACCGAGAGCGAGGCGGGAGCCCGAGTATCGGCAGCAGT

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 CAGCAGTGGCCCTGGACGAAGAGACCCACGAGGCGAGGACGTGGGGTGTTCGCGCGGGCCCGAGGCGCACCTGGTTCACGGCTGCAGGAGCAGAC

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 CTTCATAGCGCACGTCATGGCCTTCGCCCTGCCTGGAGCCCTACACCGCTGCGACCTGGCGCCCCCGCCGGCACCACCGACGCCGCGCACCCGGGG
479▶ F I A H V M A F 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
NheI (3128)
3101 CGGTCCCGTCCAAGCGTCTGGATTGAAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCAACTAGAATGCAGTGAAAAAA
513▶ R S R S K R L D •
3201 TGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAAGTTAACACAACAATTGCATTCATTTTATGTTT
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ApaLI (3786)
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SdaI (4116)
4101 ACATGTTCTTAATTAACCTGCAGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCAAAGACCCCGCCATTGACGTCAATAATGACG
NdeI (4296)
4201 TATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGATCATA
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NcoI (5848)
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AvrII (6571)
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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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AseI (7060)
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7201 GCTGCTGAAGGTGAAGAAAGCAGAGCCTTTCTTTTGTGTTGGAGGAAGAGGTTATGTTCTGAGGGTCAATCTTGTGCTGATGGTTTTTACAAGAC
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327▶ S G N R R P S T R P R A K E •
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ScaI (8372)
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▶