

pFUSE-SEAP-hG2-Fc

Plasmid designed for the expression of a SEAP-Fc Fusion protein

Catalog # pfuse-hsp

For research use only

Version 22H30-MM

PRODUCT INFORMATION

Content:

- 20 µg of pFUSE-SEAP-hG2-Fc plasmid provided as lyophilized DNA
- 1 ml of Zeocin® (100 mg/ml)

Storage and Stability:

- Product is shipped at room temperature.
- Lyophilized DNA should be stored at -20°C and is stable 3 months.
- Resuspended DNA should be stored at -20°C and is stable up to 1 year.
- Store Zeocin® at 4°C or at -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

pFUSE-Fc is a family of plasmid developed to facilitate the construction of Fc-Fusion proteins by fusing a sequence encoding a given protein to the Fc region of an immunoglobulin.

pFUSE-Fc plasmids yield high levels of Fc-Fusion proteins. The level of expression is usually in the µg/mL range. They can be transfected in a variety of mammalian cells, including myeloma cell lines, CHO cells, monkey COS cells and human embryonic kidney (HEK)293 cells. These cells are commonly used in protein purification systems.

pFUSE-SEAP-hFc plasmids allow the production of SEAP-Fc fusion proteins. This plasmid can be used to make recombinant SEAP-Fc fusion proteins or can be used as a transfection control in experiments with other pFUSE-hFc constructs. Quantification of SEAP-Fc expression can be determined using QUANTI-Blue™ Solution (cat. code: rep-qbs).

PLASMID FEATURES

- **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter¹ and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat². The EF-1α promoter exhibits a strong activity and yields long lasting expression of a transgene *in vivo*. The R-U5' has been coupled to the EF-1α core promoter to enhance stability of RNA.
- **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.
- **hIgG2 Fc (human)**: The Fc region of human IgG2 comprises the CH2 and CH3 domains of the IgG heavy chain and the hinge region. The hinge serves as a flexible spacer between the two parts of the Fc-Fusion protein, allowing each part of the molecule to function independently.

- **SV40 pAn**: the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA³.
- **ori**: a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.
- **CMV enh / hFerL prom**: This composite promoter combines the human cytomegalovirus immediate-early gene 1 enhancer and the core promoter of the human ferritin light chain gene. This ubiquitous promoter drives the expression of the Zeocin®-resistance gene in mammalian cells.
- **EM2KC** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*. EM2KC is located within an intron and is spliced out in mammalian cells.
- **Zeo**: Resistance to Zeocin® is conferred by the *Sh ble* gene from *Streptoalloteichus hindustanus*. The same resistance gene confers selection in both mammalian cells and *E. coli*.
- **βGlo pAn**: The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription⁴.

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 H₂O. Store resuspended plasmid at -20 °C.

Plasmid amplification and cloning

Plasmid amplification and cloning can be performed in *E. coli* GT116 other commonly used laboratory *E. coli* strains, such as DH5α.

Zeocin® usage

This antibiotic can be used for *E. coli* at 25 µg/ml in liquid or solid media and at 50-200 µg/ml to select Zeocin®-resistant mammalian cells.

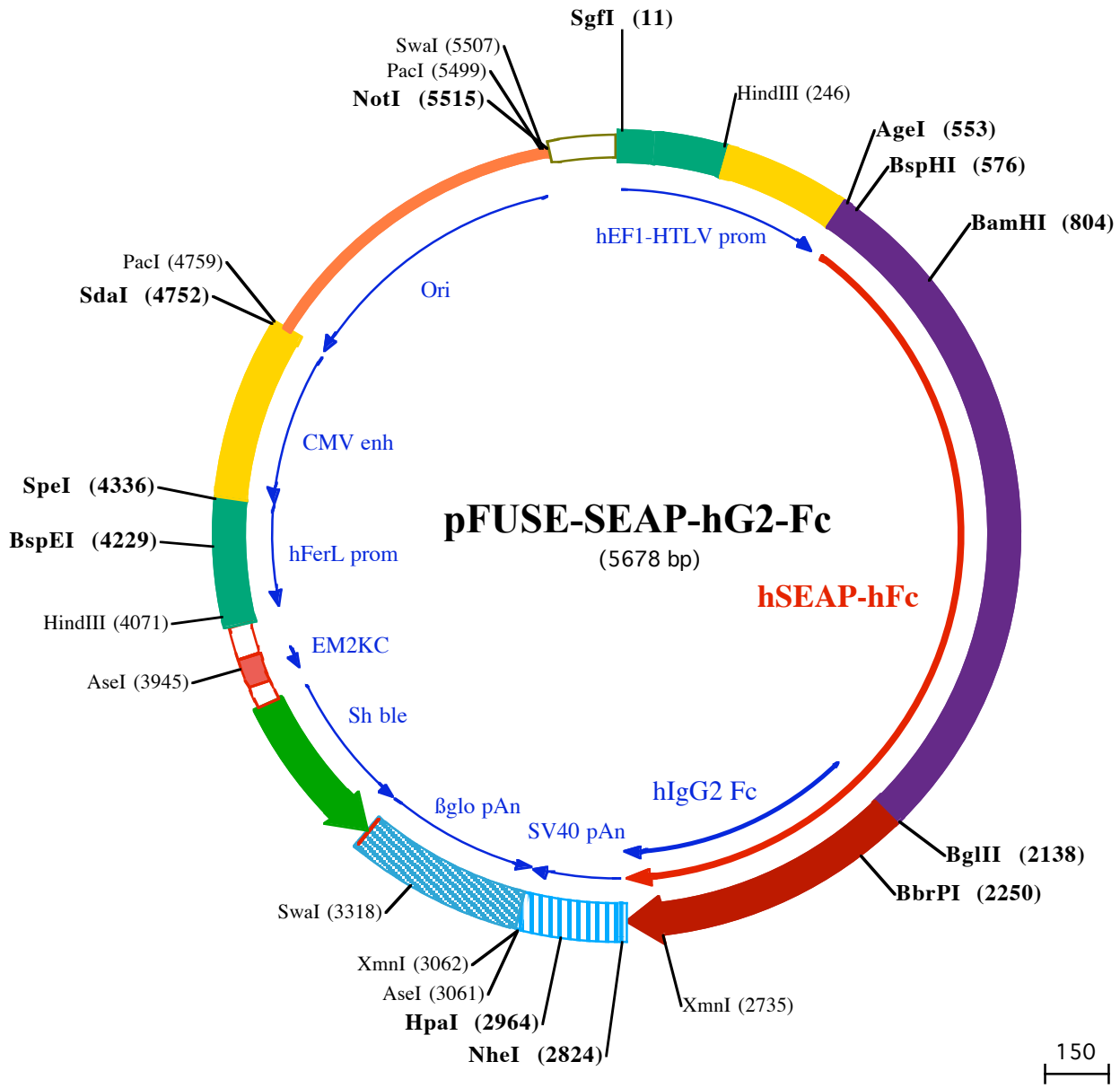
References:

1. Kim, D.W. *et al.* (1990). *Gene* 2: 217-223.
2. Takebe, Y. *et al.* (1988). *Mol. Cell Biol.* 1: 466-472.
3. Carswell, S., and Alwine, J.C. (1989). *Mol. Cell Biol.* 10: 4248-4258.
4. Yu J & Russell JE. (2001). *Mol Cell Biol*, 21(17):5879-88.

TECHNICAL SUPPORT

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SgfI (11)
1 GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGTTCGCAATTGAACGGGTGCCTA
101 GAGAAAGTGGCGCGGGGTAACCTGGGAAAGTGATGCTGTACTGGCTCCGCCTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

HindIII (246)
201 GTGAACGTTCTTTTTCGCAACGGGTTTCCGCCAGAACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCTTACCGCGCCGCCCTACTCTGAGGCC
301 GCCATCCACGCGGGTGTAGTGCCTTCTGCGCCCTCCCGCTGTGGTGCCTCCTGAAGTGCCTCCGCGTCTAGGTAAGTTAAAGCTCAGGTCGAGACC
401 GGGCCTTTGTCCGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTCTCAACTCTACGTCTTTGTTTCGTTT

AgeI (553) **BspHI (576)**
501 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCCCTACCTGAGATCACCGGTTTCAGCTGAGGAGGCACATCATGATTCTGGGGCCCTGCATGCT
601 GCTGCTGCTGCTGCTGCTGGCCCTGAGGCTACAGCTCTCCCTGGGCATCATCCAGTTGAGGAGGAGAACCAGGCTTCTGGAAACCGCGAGGCGCCGAG
801 CAGACGCTCCGACGCGCCACCTACGCCACAGCGTGAACCGCAACTGGTACTCGGACGCGGACGCTGCTGCTCCGCTCCGCGCCGAGGAGGCGCCAGGA
101 AGACAAACTGTGCCAGACAGTGGAGCCACAGCCACGCGCTACCTGTGGGGGTCAAGGGCACTTCCAGACCACTTGGCTTGTAGTGCAGCCGCGCCGCTT
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1601 AGCAGCCTCCGACGCGCCACCTACGCCACAGCGTGAACCGCAACTGGTACTCGGACGCGGACGCTGCTGCTCCGCTCCGCGCCGAGGAGGCGCCAGGA
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3601 CATCACAAAGCAGGGCTTACCAGGCTGACTGAGACGATCATGTTGACGACGCCATTGAGAGGGCGGCCAGCTCACCAGCGAGGAGGACAGCTGA
3801 HisHisSerArgAlaTyrArgAlaLeuThrGluThrIleMetPheAspAlaAlaIleGluuArgAlaGlyGlnLeuThrSerGluuAspThrLeuSer
4001 GCCTGCTACTCGCAGCCTCCGACCTCCACGCTTCTCCCTCCGAGGCTACCCCTCGGAGGAGCTCCATCTTCCGGCTGGCCCTCCGAGGCGCCGAGCAG
4201 erLeuValThrAlaAspHisSerHisValPheSerPheGlyGlyTyrProLeuArgGlySerSerIlePheGlyLeuAlaProGlyLysAlaArgAspAr
4401 GAAGGCTACAGGCTCCCTATACGAAACGGTCCAGGCTATGTGCTCAAGGACGCGCCGCGCGGATGTTACGAGAGCGAGGCGGAGCCCGGAG
4601 gLysAlaTyrThrValLeuLeuTyrGlyAsnGlyProGlyTyrValLeuLysAspGlyAlaArgProAspValThrGluSerGluSerGlySerProGlu
4801 TATCGGACGAGTCAAGCAGTGCCTGGACGAAAGAGACCCAGCAGGCGAGGACTGGCGGTGTTCCGCGCGCCCGCAGGCGCACCTGGTTCACGGCG
5001 TyrArgGlnGlnSerAlaValProLeuAspGluGlyThrHisAlaGlyGlyValAlaValPheAlaArgGlyProGlnAlaHisLeuValHisGlyY
5201 TGCAGGACAGACTTCATAGCGCAGCTCATGGCTTCCGCGCTGCTGAGGACCTACACCCCTGCGAGCTGGCAGCCCGCCGCGCCAGCAGCAGC
5401 aGlnGluGlnThrPheIleAlaHisValMetAlaPheAlaAlaCysLeuGluProTyrThrAlaCysAspLeuAlaProProAlaGlyThrThrAspAl

BamHI (804)
801 CCAGGATCCTAAAAGGGCAGAAGAAGGACAACTGGGGCTGAGATACCCCTGGCTATGGACCGCTTCCCATATGTGGCTGTGCCAAGCATAACAATGT
750 IlaArgIleLeuLysGlyGlnLysLysAspLysLeuGlyProGluIleProLeuAlaMetAspArgPheProTyrValAlaLeuSerLysThrTyrAsnVa
901 AGACAAACTGTGCCAGACAGTGGAGCCACAGCCACGCGCTACCTGTGGGGGTCAAGGGCACTTCCAGACCACTTGGCTTGTAGTGCAGCCGCGCCGCTT
1050 IAspLysHisValProAspSerGlyAlaThrAlaThrAlaTyrLeuCysGlyValLysGlyAsnPheGlnThrIleGlyuAsnProAspPheTrpAsnArgGluAlaAlaGlu
1200 AACCAGTGCACACGACGCGCGCAACAGAGTTCATCTCCGTGATGAATCGGGCCAGAAAGCAGGGAAGTCACTGGGAGTGGTAACCCACACGAGGATGC
1420 AsnGlnCysAsnThr Thr ArgGlnAsnGluVal IleSerValMetAsnArgAlaLysLysAlaGlyLysSerValGlyValValThrThrArgValG
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5080 aAlaHisProGlyYArgSerArgSerLysArgLeuAspArgSerValGluCysProProCysProAlaProProValAlaGlyProSerValPheLeuPhe
1000 ValGluCysProProCysProAlaProProValAlaGlyProSerValPheLeuPhe

BbrPI (2250)
2201 CCCCCAAAACCAAGGACACCTGATGATCTCCAGAACCCCTGAGGTCAGCTGGTGGTGGAGCTGAGCCACGAAAGACCCGAGGTTCACTCAACT
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2000 ProProLysProLysAspThrLeuMetIleSerArgThrProGluValThrCysValValValAspValSerHisGluAspProGluValGlnPheAsnT
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8600 sGlnAspTrpLeuAsnGlyLysGluTyrLysCysLysValSerAsnLysGlyLeuProAlaProIleGluLysThrIleSerLysThrLysGlyGlnPro
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6750 IleAlaValGluTrpGluSerAsnGlyGlnProGluuAsnAsnTyrLysThrThrProProMetLeuAspSerAspGlySerPhePheLeuTyrSerLysLe
1530 IleAlaValGluTrpGluSerAsnGlyGlnProGluuAsnAsnTyrLysThrThrProProMetLeuAspSerAspGlySerPhePheLeuTyrSerLysLe

XmnI (2735)
2701 CACCGTGACAAGAGCAGGTGGCAGCAGGGGAACGCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCTACACACAGAAGAGCTCTCCCTG
7080 uThrValAspLysSerArgTrpGlnGlnGlyAsnValPheSerCysSerValMetHisGluAlaLeuHisAsnHisTyrThrGlnLysSerLeuSerLeu
1860 uThrValAspLysSerArgTrpGlnGlnGlyAsnValPheSerCysSerValMetHisGluAlaLeuHisAsnHisTyrThrGlnLysSerLeuSerLeu

NheI (2824)
2801 TCTCCGGTAAATGAgtcacagcGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTGGACAACCACTAGAATGCAGTGAATAAATGCTT
7420 SerProGlyLys
2200 SerProGlyLys

HpaI (2964)
2901 TATTTGTAAATTTGTGATGCTATTGCTTTATTTGTAACCATTAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGT

AseI (3061) **XmnI (3062)**
3001 CAGGGGAGGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAATGTGGTATGGAATTAATCTAAAATACAGCATAGCAAACCTTAACTCCAA
3101 ATCAAGCTCTACTTGAATCCTTTCTGAGGATGAATAAGGCATAGGCATCAGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTT

3201 CATGAGTTTAAGATATAGTGTATTTTCCAAGGTTGAAGTAGCTCTTCATTTCTTTATGTTTAAATGCACTGACCTCCACATTCCCTTTTATAGTAA

Swal (3318)

3301 AATATTAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTT

3401 TAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCTGCTCTGCCACAAAG
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3601 TGGACACGACTCCGACACTCGGGCTACAGCTCGTCCAGGCCGCGACCCACACAGCCAGGCGGTGTGTCCGGCACACTGGTCTGACCGCGCT
83◀rSerValValGluSerTrpGluAlaTyrLeuGluAspLeuGlyArgValTrpValTrpAlaLeuThrAsnAspProValValGlnAspGlnValAlaSer

3701 GATGAACAGGGTCACGTCGTCGCCGACACCGGCGAAGTCGTCTCCACGAAGTCCCGGAGAACCCGAGCCGGTCCGAGACTCGACCGCTCCG
50◀llePheLeuThrValAspAspArgValValGlyAlaPheAspAspGluValPheAspArgSerPheGlyLeuArgAspThrTrpPheGluValAlaGlyA

3801 GCGACGTCGCGCGGTGAGCACCGAAGCGGACTGGTCAACTTGGCCATGATGGCTCTCctgtcaggagagaaagagagagaggttagtacaattgC
16◀laValAspArgAlaThrLeuValProValAlaSerThrLeuLysAlaMet

AseI (3945)

3901 TATAGTGAGTTGTATTACTATGCAGATATACTATGCCAATGATTAATTGTCAAAGTAGGGCTGCAGgggttcatagtgccacttttctgctcactgcccc

HindIII (4071)

4001 atctcctgccccacctttccaggcatagacagtcagtgacttaCAAAGTACAGAGGGGAGAAGGCAGAAGCTTGAGACAGACCCGCGGGACCGCCGA
←

4101 ACTGCGAGGGGACGTGGCTAGGGCGGCTTTTATGTTGCGCCGCCCTCGGAGGCAAGGGCGCTCGGGAGGCCATAGCGGCCAATCTGCGGTGGCAGGA

BspEI (4229)

4201 GCGGGGCGAAGGCCGTGCTGACCAATCCGGAGCACATAGGAGTCTAGCCCCCGCCCAAAGCAAGGGGAAGTCACGCGCTGTAGCGCCAGCGTG

SpeI (4336)

4301 TTGTGAAATGGGGCTTGGGGGGTTGGGGCCCTGACTAGTCAAAACAAACTCCCATTGACGTCATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAA
←

4401 CCGCTATCCACGCCATTGATGTAAGTCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGACTGCCAAGTAGGAAAGTCCATAAG

4501 GTCATGTAAGTGGGCATAATGCCAGCGGGCCATTTACCGTCAATTGACGTCATAGGGGGCGTACTTGGCATATGATACACTTGTGTAAGTGGG

4601 CAGTTTACCGTAAATACTCCACCATTGACGTCATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCATGGCGGGGGTCT

PaeI (4759)

SdaI (4752)

4701 GTTGGGCGGTACGCCAGGCGGGCCATTTACCGTAAGTTATGTAACCGCTGCAAGTTAATTAAGAACATGTGAGCAAAAGCCAGCAAAAGCCAGGAACC
←

4801 GTAAAAAGGCGCGTGTGGCGTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAATAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGG

4901 ACTATAAGATACAGGCGTTTTCCCTGGAAGTCCCTCGTGGCTCTCTGTCCGACCTGCCGTTACCGGATACCTGTCCGCCTTCTCCCTTCG

5001 GGAAGCGTGGCGTTTTCTATAGCTCACGCTGTAGGTATCTCAGTTCGGGTAGGTGCTTCCGCTCAAGTGGGCTGTGTGACGAAACCCCGTTACG

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5301 GTTACCTTCGGAAGAGTGGTAGCTTTGATCCGCAAAACACCAGCGTGGTAGCGGTGTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAA

PaeI (5499)

5401 AAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGCTGACGCTCAGTGAACGAAAACCTCACGTTAAGGATTTTGGTCATGGCTAGTTAATT

Swal (5507) NotI (5515)

5501 AACATTTAAATCAGCGGCCCAATAAAATATCTTTATTTTATTACATCTGTGTGTTGGTTTTTTGTGTAATCGTAACATAACGCTCTCCATCAA
5601 ACAAACGAAACAAACAAACTAGCAAAATAGGCTGTCCCAAGTCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA