

# pFUSE-hIgG2-Fc1

Plasmid designed for the construction of Fc-Fusion proteins

Catalog # pfuse-hfc1

For research use only

Version 20K04-MM

## PRODUCT INFORMATION

### Content:

- 20 µg of pFUSE-hIgG2-Fc1 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 the effector region of a protein to the Fc region of an immunoglobulin G (IgG).

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, cells that are commonly used in protein purification systems.

pFUSE-Fc plasmids allow the secretion of Fc-Fusion proteins. As Fc-Fusion proteins are secreted, they can be easily detected in the supernatant of pFUSE-Fc-transfected cells by SDS-PAGE. Furthermore, functional domains can be identified by immunoblotting and ligand blotting.

Fc-Fusion proteins can be easily purified by single-step protein A or protein G affinity chromatography.

InvivoGen provides pFUSE-Fc vectors featuring Fc regions from different species and isotypes. In humans, there are four isotypes: IgG1, IgG2, IgG3 and IgG4. The Fc region mediates effector functions, such as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). IgG isoforms exert different levels of effector functions increasing in the order of IgG4<IgG2<IgG1≤IgG3.

## PLASMID FEATURES

- **hIgG2-Fc (human):** The Fc region 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. Human IgG2 displays low ADCC and CDC.
- **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter<sup>1</sup> and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat<sup>2</sup>. 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.
- **MCS:** The multiple cloning site contains several restriction sites that are compatible with many other enzymes, thus facilitating cloning.
- **SV40 pAn:** the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA<sup>3</sup>.
- **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*.
- **BGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription<sup>4</sup>.

### References:

1. Kim DW *et al.* 1990. Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. 91(2):217-23.
2. Takebe Y. *et al.* 1988. SR alpha promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. Mol Cell Biol. 8(1):466-72.
3. Carswell S. & Atwine JC. 1989. Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. Mol Cell Biol. 9(10):4248-58.
4. Yu J. & Russell JE. 2001. Structural and functional analysis of an mRNP complex that mediates the high stability of human beta-globin mRNA. Mol Cell Biol. 21(17):5879-88.

### TECHNICAL SUPPORT

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## 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 H<sub>2</sub>O. Store resuspended plasmid at -20 °C.

### Plasmid amplification and cloning

Plasmid amplification and cloning can be performed in *E. coli* GT116 or in 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.

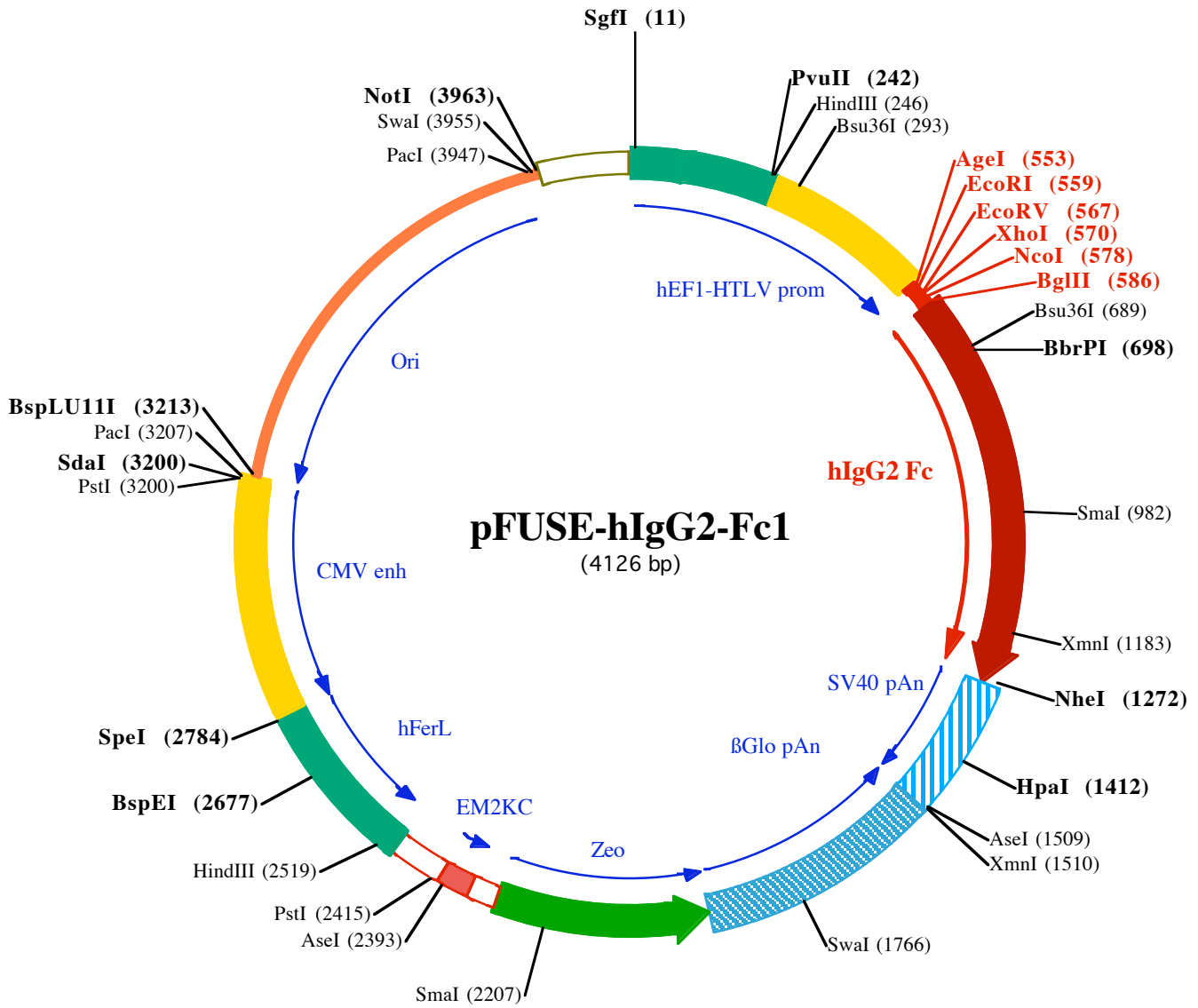
## RELATED PRODUCTS

Product	Catalog Code
Zeocin™	ant-zn-1

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### TECHNICAL SUPPORT

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**SgfI (11)**  
1 GGATCTGCGATCGCTCCGGTGCCCGTCACTGGGAGAGCGCACATCGCCACAGTCCCGGAGAAGTTGGGGGAGGGTTCGCAATTGAACGGTGCCTA  
101 GAGAAGTGGCGCGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

**HindIII (246)**  
**PvuII (242)**  
201 GTGAACGTTCTTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCCTACCTGAGGCC  
301 GCCATCCACGCGGTTGAGTCGCGTTCTGCCGCCTCCCGCTGTGGTGCCTCCTGAATCGCTCCGCGCTAGGTAAGTTTAAAGCTCAGGTCGAGACC  
401 GGGCCTTTGTCGGCGCTCCCTTGAGGCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGCTTTGTTTCGTTT

**EcoRI (559) XhoI (570) BglII (586)**  
**AgeI (553) EcoRV (567) NcoI (578)**  
501 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACC GGCGCTACCTGAGATCACCGTGAATTCGATATCTCGAGCACCATGGTTAGATCTGTGGAGTGC  
1 Val Gl uCys  
**BbrPI (698)**  
601 CCACCTTGCCAGCACACCTGTGGCAGGACCTTCACTCTCTCTCCCCCAAACCAAGGACACCCTGATGATCTCCAGAACCCTGAGGTACAGT  
4 P roP roCysP roAl aP roP roValAl aGl yP roSer Val lPheLeuPheP roP roLysP roLysAspThr LeuMe t l eSer ArgThr P roGl uVal Thr C  
701 GCGTGGTGGTGGAGTGAGCCACGAAGACCCGAGGTCCAGTTCAACTGGTACGTGGACGGCATGGAGTGCATAATGCCAAGACAAAGCCCGGGAGGA  
37 y sVal Val Val AspVal Ser Hi sGl uAspP roGl uVal Gl nPheAsnTrpTyrVal AspGl yMe tGl uVal l Hi sAsnAl aLysThr LysP roArgGl uGl  
801 GCAGTTCAACAGCAGCTTCCGTGTGGTCAAGCTCTCACCGTCTGACCAAGGACTGGCTGAACGGCAAGGAGTACAAGTCAAGGTCTCCAACAAAGGC  
70 uGl nPheAsnSer Thr PheArgVal Val Ser Val LeuThr Val Val Hi sGl nAspTrpLeuAsnGl yLysGl uTyrLysCysLysVal SerAsnLysGl y  
901 CTCCCAGCCCCATCGAGAAAACATCTCCAAAACAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCATCCCGGAGGAGATGACCAAGA  
104 P roP roAl aP ro l eGl uLysThr l eSer LysThr LysGl yGl nP roArgGl uP roGl nVal TyrThr LeuP roP roSer ArgGl uGl uMe tThr LysA  
1001 ACCAGGTGAGCTGACCTGCCTGGTCAAAGGCTTACCCACGCGACATCCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACCACTACAAGACCAC  
137 s nGl nVal Ser LeuThr CysLeuVal LysGl yPheTyrP roSer Asp l eAl aVal Gl uT rpGl uSerAsnGl yGl nP roGl uAsnAsnTyrLysThr Th  
1101 ACCTCCATGCTGGACTCCGACGGCTCTTCTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACTCTTCTCATGCTCCGTG  
170 r P roP roMe tLeuAspSer AspGl ySer PhePheLeuTyrSer LysLeuThr Val AspLysSer ArgT rpGl nGl yAsnVal lPheSer CysSer Val  
**XmnI (1183)**  
**NheI (1272)**  
1201 ATGCATGAGGCTCTGCACAACCACTACACAGAAGAGCCTCTCCCTGTCTCCGGTAAATGAgTgcacgGCTAGCTGGCCAGACATGATAAGATACAT  
204 Me tHi sGl uAl aLeuHi sAsnHi sTyrThr Gl nLysSer LeuSer LeuSer P roGl yLys ●●●  
1301 TGATGAGTTTGACAAACCAACTAGAATGCAGTGAATAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGTGC

**HpaI (1412)**  
1401 AATAAACAAGTTAAACAACAATTGCATTCATTTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAAATGTG

**AseI (1509)**  
**XmnI (1510)**  
1501 GTATGGAATTAATTTCAAATACAGCATAGCAAACTTAACTCCAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATC  
1601 AGGGGCTGTTGCAATGTGCATTAGCTGTTTGACGCTCACCTCTTCATGAGTAAAGATATAGTGTATTTTCCAAGGTTGAACTAGCTCTTCAT

**SwaI (1766)**  
1701 TTCTTTATGTTTTAAATGCAGTACCTCCACATTCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTT  
1801 ATTAGGCAGAATCCAGATGCTCAAGGCCCTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTAAATAGAAATGGACAGCAA  
1901 GAAAGCGAGCTTCTAGTTATCTCAGTCTGCTCTGCCACAAGTGACAGCAGTTGCCGGCCGGTCCGCGAGGGCGAACTCCCGCCCCACGGCT  
125 ●●●AspGl nGl uGl uAl aVal lPheHi sVal CysAsnGl yAl aP roAspArgLeuAl aPheGl uArgGl yTrpP roGl  
2001 GCTCGCCGATCTCGGTATGGCCGGCCGGAGGCGTCCCGGAAGTTCTGGACACGACCTCCGACACTCGGCTACAGCTCGTCCAGGCGCGCACCA  
99 nGl uGl y l eGl uThr Me tAl aP roGl ySer Al aAspArgPheAsnThr Ser Val Val Gl uSer TrpGl uAl aTyrLeuGl uAspLeuGl yArgVal lTrp  
2101 CACCCAGGCCAGGTTGTGTCCGGCACCTGCTGACCGCGCTGATGAACAGGGTACGCTCGTCCCGACCACACCGCGAAAGTCTCTCCACG  
66 Val lTrpAl aLeuThrAsnAspP roVal Val Gl nAspGl nValAl aSer l ePheLeuThr Val lAspAspArgVal Val Gl yAl aPheAspAspGl uVal lP  
2201 AAGTCCCGGAGAACCAGCCGGTCCGACTCGACCGCTCCGGCAGCTCGCGCGGGTGAACCCGAAACGGCACTGGTCAACTTGGCCATGA  
32 heAspArgSer PheGl yLeuArgAspThr TrpPheGl uValAl aGl yAl aVal lAspArgAl aThr LeuVal lP roValAl aSer Thr LeuLysAl aMe t  
2301 TGGCTCTCctgtcaggagaggaagagaagaaggttagtacaattgCTATAGTGAGTTGATTATACTATGCAGATATACTATGCCAATGATTAATTGT  
**AseI (2393)**

**PstI (2415)**  
2401 CAAACTAGGGTGCAGgttcatagtgccacttttctgactgcccactctctgcccacctttccaggcatagacagtcagtgacttacCAAATC

**HindIII (2519)**  
2501 ACAGGAGGGAGAAGCGAAGCTTGAGACAGACCCGCGGACCGCCAACTGCGAGGGACGTGGCTAGGGCGGCTTCTTTTATGGTGCGCCGCCCTCG

**BspEI (2677)**  
2601 GAGGAGGGCGCTCGGGAGGCTAGCGCCAATCTGCGGTGGCAGGAGGCGGGCCGAAGCCGTGCCTGACCAATCCGGAGCACATAGGAGTCTCAGC

**SpeI (2784)**  
2701 CCCCCGCCAAAGCAAGGGAAGTACGCGCCTGTAGCGCCAGCGTGTGTGAAATGGGGCTTGGGGGTTGGGGCCCTGACTAGTCAAACAAACT  
2801 CCCATTGACGTCAATGGGTGGAGACTTGGAAATCCCGTGAGTCAAACCCTATCCACGCCATTGATGTACTGCCAAACCCGATCATCATGGTAATA

2901 GCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTACCCTCATTGACGTCAA  
3001 TAGGGGGCGTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCCTAAATACTCCACCATTGACGTCAATGGAAAGTCCCTATTGG

PstI (3200)  
**SdaI (3200)**

3101 CGTTACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGGTCTGTTGGGCGGTGAGCCAGGCGGGCCATTACCCTAAGTTATGTAACGCTGCA

PacI (3207) **BspLU11I (3213)**

3201 GGTTAATTAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGA

3301 GCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTTCCCTGGAAGCTCCCTCGTGCGCTCTCCT

3401 GTTCGACCCCTGCCGTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCAGCTGTAGGTATCTCAGTTCGGTGT

3501 AGGTCGTTGCTCCAAGCTGGCTGTGTGCACGAACCCCGTTAGCCCGACCGCTGCGCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAG

3601 ACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAACTAC

3701 GGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACAAACCACCG

3801 CTGGTAGCGGTGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCA

PacI (3947) SmaI (3955) **NotI (3963)**

3901 GTGGAACGAAAACACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCCAATAAAATATCTTTATTTTCATTACATCTGT

4001 GTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAACAAAACGAAACAAAACAACTAGCAAATAGGCTGTCCCCAGTGAAGTGC

4101 AGGTGCCAGAACATTTCTCTATCGAA