

# pFUSE-hIgG2-Fc2

Plasmid designed for the construction of Fc-Fusion proteins

Catalog # pfuse-hfc2

For research use only

Version 20K05-MM

## PRODUCT INFORMATION

### Content:

- 20  $\mu$ g of pFUSE-hIgG2-Fc2(IL2ss) 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  $\mu$ g/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-Fc2 (IL2ss) plasmids allow the secretion of Fc-Fusion proteins. They contain the IL2 signal sequence (IL2ss) for the generation of Fc-Fusion proteins derived from proteins that are not naturally secreted. 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-1 $\alpha$  (EF-1 $\alpha$ ) 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-1 $\alpha$  promoter exhibits a strong activity and yields long lasting expression of a transgene *in vivo*. The R-U5' has been coupled to the EF-1 $\alpha$  core promoter to enhance stability of RNA.
- **IL2 ss:** The IL2 signal sequence contains 22 amino acids and share common characteristics with signal peptides of other secretory proteins. The intracellular cleavage of the IL2 signal peptide occurs after Ser20 and leads to the secretion of the antigenic protein.
- **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. & 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.
4. Yu J. & Russell JE. 2001. Structural and functional analysis of an mRNA 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

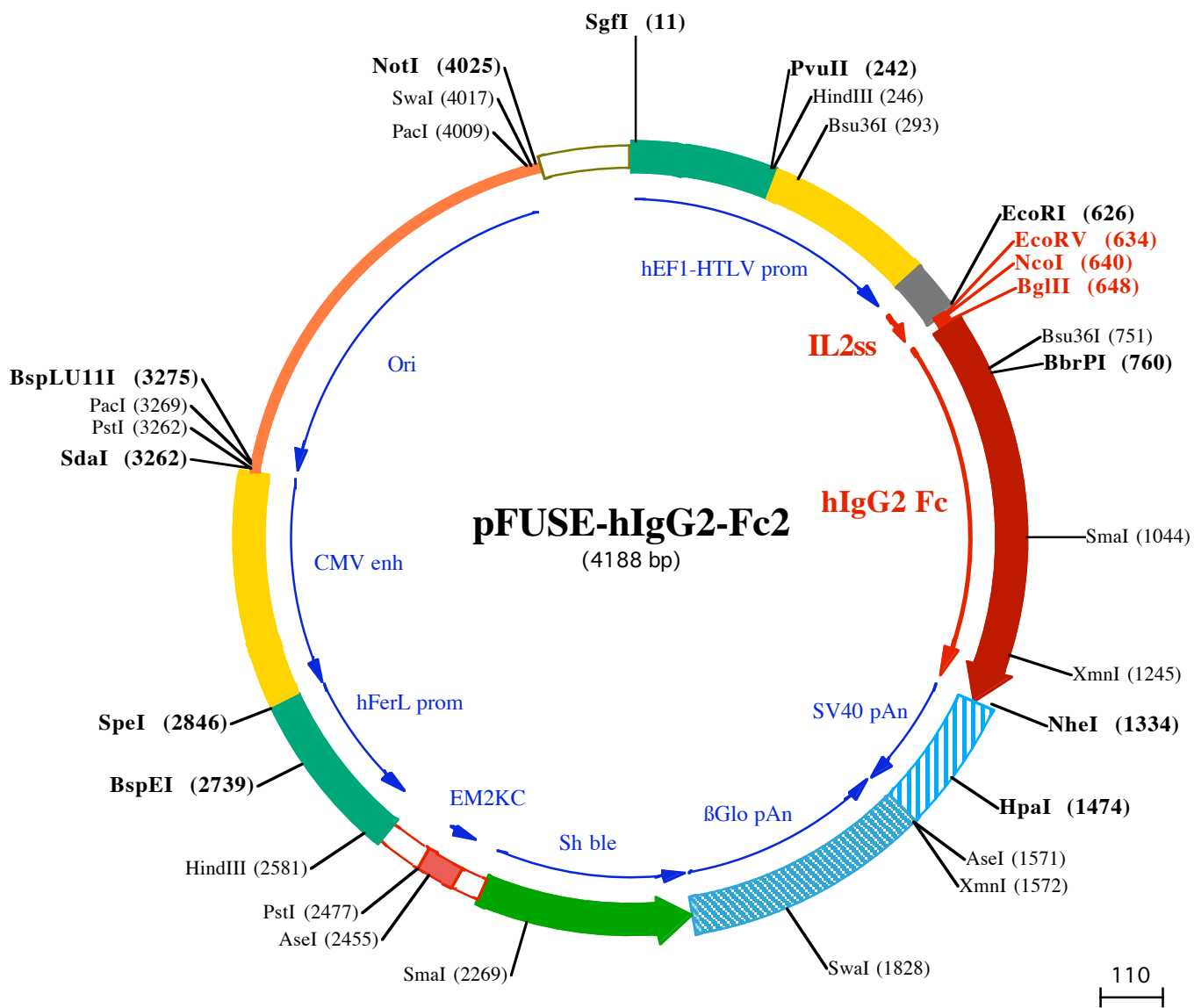
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SgfII (11)  
1 GGATCTGCGATCGCTCCGGTGCCTCGTGGGCGAGCGCACATCGCCACAGTCCCGAGAAGTTGGGGGAGGGTTCGCAATTGAACGGGTGCCTA  
101 GAGAAGGTGGCGCGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCTTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGGC

HindIII (246) Bsu36I (293)  
201 GTGAACGTTCTTTTTGCAACGGGTTTGC CGCAGAACACAGCTGAAGCTTCAGAGGGCTCGCATCTCTCTTCCACGCGCCCGCCCTACCTGAGGCC  
301 GCCATCCACGCGGGTTGAGTCGCGTTCTGCCGCTCCCGCTGTGGTGCCTCTGAATCGCTCCGCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC  
401 GGGCTTTGTCGGCGCTCCCTTGAGCCTACCTAGACTCAGCGGGCTCCACGCTTTCCTGACCCTGCTTGTCTCAACTCTACGCTTTTGTTCGTTT  
501 TCTGTTCTGCGCGTTACAGATCCAAGCTGTGACCGGGCCTACCTGAGATCAccggcGAAGGAGGGCCACCATGTACAGGATGCAACTCCTGCTTTCGA  
1 MetTyrArgMetGlnLeuLeuSer CysI

EcoRV (634) BglII (648)  
601 TTGCACTAAGTCTTGCACCTGTACGAATTCGATATCGGCCATGGTTAGATCTGTGGAGTGCCACCTTGCCAGCACCACCTGTGGCAGGACCTTCAGT  
10 MetI LeuSer LeuAlaLeuVal ThrAsnSer 1 ValI GluCysProProCysProAlaProProValAlaGluProSerVal

EcoRI (626) NcoI (640)  
701 CTTCTCTTCCCCAAAACCAAGGACACCTGATGATCTCCAGAACCCCTGAGGTCACGTGCGTGGTGGTGAGCAGGACACCAAGACCCCGAGGTC  
16 PheLeuPheProProLysProLysAspThrLeuMetIleSerArgThrProGluValThrCysValValValAspValSerHisGluAspProGluVal  
801 CAGTTCAACTGGTACGTGGACGGCATGGAGTGCATAATGCCAAGACAAAGCCACGGGAGGAGCAGTTCAACAGCACGTTCCGTGGTGCAGCGTCCCA  
50 GluPheAsnTrpTyrValAspGluMetGluValHisAsnAlaLysThrLysProArgGluGluGluPheAsnSerThrPheArgValValSerValLeuT  
901 CCGTGTGACACAGGACTGGCTAACGGCAAGGAGTACAAGTCAAGGTCTCCAAACAAAGGCTCCACGCCCCATCGAGAAAACCATCTCCAAAACCAA  
83 ThrValValHisGluAspTrpLeuAsnGluLysGluTyrLysCysLysValSerAsnLysGluLeuProAlaProIleGluLysThrIleSerLysThrLys  
SmaI (1044)  
1001 AGGGCAGCCCCGAGAACCACAGGTGTACACCTGCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTACGCTGACCTGCCTGGTCAAAGGCTTCTAC  
116 sGluGluProArgGluProGluValTyrThrLeuProProSerArgGluGluMetThrLysAsnGluValSerLeuThrCysLeuValLysGluPheTyr  
1101 CCCAGCGACATCGCGTGGAGTGGAGAGCAATGGGACGCCGGAGAACAACCTACAAGACCACACCTCCCATGCTGGACTCCGACGGCTCCTTCTCTCT  
150 ProSerAspIleAlaValGluTrpGluSerAsnGluGluProGluAsnAsnTyrLysThrThrProProMetLeuAspSerAspGluSerPhePheLeuT  
XmnI (1245)  
1201 ACAGCAAGCTCACCGTGACAGAGCAGGTGGCAGCGGGAAACGCTCTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACAGAAAGAG  
183 yrSerLysLeuThrValAspLysSerArgTrpGluGluGluValPheSerCysSerValMetHisGluAlaLeuHisAsnHisTyrThrGluLysSer  
NheI (1334)  
1301 CCTCTCCGTCTCCGGTAAATGAgTgcccagcGCTAGCTGGCAGACATGATAAGATACATTGATGAGTTTGACAAAACCACTAGAATGCAGTGAA  
216 rLeuSerLeuSerProGluLys•••

HpaI (1474)  
1401 AAAAAATGCTTATTTGTGAAATTTGTGATGCTATTGCTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTATTTAT

AseI (1571) XmnI (1572)  
1501 GTTTCAGGTTACAGGGGAGGTGTGGGAGTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAATTAATCTAAAATACAGCATAGCAAACTT  
1601 TAACCTCAAATCAAGCCTCTACTTGAATCCTTTCTGAGGATGAATAAGGCATAGGCATCAGGGGCTGTGCAATGTGCATTAGCTGTTTGCAGCCT  
1701 CACCTTCTTCATGAGTTAAGATATAGTATTTTCCAAAGTTTGAAGTACTCTTCTATTCTTTATGTTTTAAATGCAGTACCTCCACATTCCC

SwaI (1828)  
1801 TTTTATGATAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTCATAATA  
1901 TCCCCAGTTTAGTGGACTTAGGGAACAAGAACCTTTAATAGAAATGGACAGCAAGAAAGCGAGCTTCTAGCTTATCTCAGTCTGCTCCTC  
125•••AspGluGluGlu  
2001 TGCCACAAGTGACAGCAGTTGCCGGCGGGTCCGCGAGGGCAACTCCGCCCCACGGTGTCTGCCGATCTCGGTATGGCCGGCCGGAGGCGTCC  
120AlaValPheHisValCysAsnGluAlaProArgArgLeuAlaPheGluArgGluTyrProGluGluGluIleGluThrMetAlaProGluSerAlaAspA  
2101 CGGAAGTTCCGTGGACACCTCCGACCACTCGCGTACAGCTCTCCAGGCGCGCACCCACCCAGGCGGTTGTCCGGCACCACCTGGTCT  
86rGluPheAsnThrSerValValGluSerTrpGluAlaTyrLeuGluAspLeuGluArgValTrpValTrpAlaLeuThrAsnAspProValValGluAspGlu  
SmaI (2269)  
2201 GGACCGCGCTGATGAACAGGGTACGCTCGTCCGACACCGCGAAGTCTCTCCAGAAAGTCCCGGAGAACCCGAGCCGGTCCGAGAACTC  
53nValAlaSerIlePheLeuThrValAspAspArgValValGluAlaPheAspAspGluValPheAspArgSerPheGluLeuArgAspThrTrpPheGlu  
2301 GACCGCTCCGGCAGCTCGCGCGGTGAGCACCGGAACGGCACTGTTCACTTGGCCATGATGGCTCCTCctgtcaggagaggaagagaagaaggtta  
20ValAlaGluAlaValAspArgAlaThrLeuValProValAlaSerThrLeuLysAlaMet  
AseI (2455) PstI (2477)  
2401 gtacaattgCTATAGTGAGTTGATTATACTATGCAGATATACTATGCCAATGATTAATTGTCAAACCTAGGGCTGCAGgggttcattagtgccacttttctt  
HindIII (2581)  
2501 gcactgccccatctcctgccccacctttccaggcatagacagtcaacttacCAAACCTACAGGAGGAGAAGGCAGAAGCTTGAGACAGACCCGCG  
2601 GGACCGCAACTGCGAGGGACGTGGTAGGGGGCTTTTATGTTGCGCCGCCCTCGAGGCAAGGGCGCTCGGGAGGCTAGCGCCAATCTGC

BspEI (2739)  
2701 GGTGGCAGGAGCGGGCCGAAGCCGTGCTGACCAATCCGGAGCACATAGGAGTCTCAGCCCCCGCCCAAGCAAGGGGAAGTACGCGCTGTAG

SpeI (2846)  
2801 CGCCAGCTGTTGTGAAATGGGGCTTGGGGGGTGGGGCCCTGACTAGTCAAACAAACTCCCATTTGACGTCAATGGGGTGGAGACTTGGAAATCCCC  
2901 GTGAGTCAAACCGCTATCCACGCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGACTGCCAAGTAGGAAA  
3001 GTCCCATAAAGGTACTGACTGGGCATAATGCCAGCGGGCCATTACCGTCAATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGATGACT  
3101 GCCAAGTGGGCAAGTTACCGTAAATACTCCACCCATTGACGTCAATGAAAAGTCCCTATTGGCGTACTATGGGAACATACGTCATTATTGACGTCAATG

PacI (3269)  
 PstI (3262)  
**SdaI (3262)**                      **BspLU11I (3275)**

3201 GGCGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAG  
 3301 GCCAGGAACCGTAAAAAGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAA  
 3401 ACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCTGGAAGCTCCCTCGTGCCTCTCCTGTTCCGACCCTGCCGTTACCGGATACCTGTCCGCCTT  
 3501 TCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCCAAGCTGGGCTGTGTGCACGAACCC  
 3601 CCCGTTCAGCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACA  
 3701 GGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCT  
 3801 GCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGTTTTTTGTTTGCAAGCAGCAGATT  
 3901 ACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAAACGAAAACACGTTAAGGGATTTTGGTCATGG

PacI (4009)    SwaI (4017)    **NotI (4025)**

4001 CTAGTTAATTAACATTTAAATCAGCGGCCCAATAAAATATCTTTATTTTATTACATCTGTGTGGTTTTTTGTGTAATCGTAACTAACATACGCT  
 4101 CTCCATCAAAACAAAACGAAACAAAACAACTAGCAAATAGGCTGTCCCAAGTCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA