

pFUSE-hIgG1e1-Fc2

Plasmid containing a human engineered IgG1 Fc region

Catalog # pfc2-hg1e1

For research use only

Version 20K05-MM

PRODUCT INFORMATION

Content:

- 20 µg of pFUSE-hIgG1e1-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 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-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. Human IgG1 displays high ADCC and CDC, and is the most suitable for therapeutic use against pathogens and cancer cells.

Under certain circumstances, for example when depletion of the target cell is undesirable, abrogating effector functions is required. On the contrary, in the case of antibodies intended for oncology use, increasing effector functions may improve their therapeutic activity¹. Modifying effector functions can be achieved by engineering the Fc regions to either improve or reduce their binding to FcγRs or the complement factors. Amino acids substitutions have been made in the human IgG1 Fc region in order to increase or reduce its ADCC and CDC.

PLASMID FEATURES

• **hIgG1e1-Fc (human IgG1 engineered Fc):** 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. The Fc region binds to neonatal FcR (FcRn), a receptor expressed on the surface of endothelial cells. This interaction, which is pH-dependent, protects the IgG from lysosomal degradation thus mediating the serum persistence of IgG antibodies. The human IgG1 Fc domain was engineered by introducing mutations in the FcRn binding sites leading to higher FcRn binding affinity at pH 6.0². The engineered hIgG1e2 Fc contains two amino acid substitutions: T250Q and M428L.

• **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter³ and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat⁴. 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.

• **IL2 ss:** The IL2 signal sequence contains 20 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 mRNA⁵.

• **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 transcription⁶.

1. Carter PJ., 2006. Potent antibody therapeutics by design. Nature Reviews Immunology. Advance online publication.

2. Hinton PR. *et al.*, 2004. Engineered human IgG antibodies with longer serum half-lives in primates. J Biol Chem. 279(8):6213-6.

3. 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.

4. 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.

5. 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.

6. 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 H₂O. 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

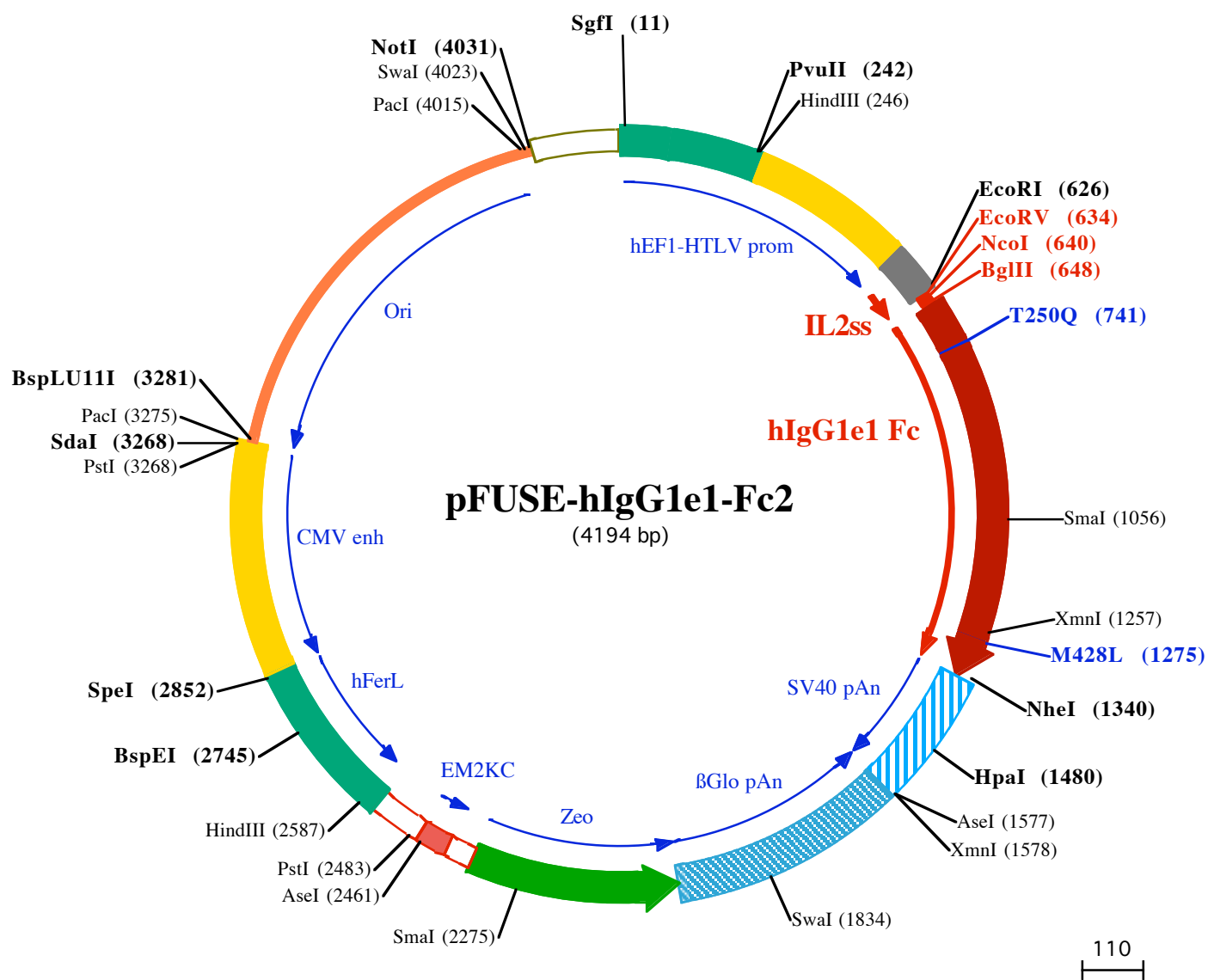
This antibiotic can be used for *E. coli* at 25 µg/ml in liquid or solid media and at 50-200 µg/ml to select Zeocin™-resistant mammalian cells.

RELATED PRODUCTS

Product	Catalog Code
Zeocin™	ant-zn-1

TECHNICAL SUPPORT

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SgfI (11)

1 GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGGTCGCAATTGAACGGGTGCCTA

101 GAGAAGGTGGCGGGGTAAACTGGGAAAGTGATGCTGTACTGGCTCCGCTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCC

HindIII (246)

PvuII (242)

201 GTGAACGTTCTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCTTCACGCGCCCGCCGCTACCTGAGGCC

301 GCCATCCACGCGGTTGAGTCGCGTTCTGCCGCTCCCGCCTGTGGTGCTCCTGAACTGCGTCCGCGCTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC

401 GGGCCTTTGTCCGGCGCTCCCTTGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCTGACCCTGCTTGTCTCAACTCTACGCTTTGTTTCGTTT

501 TCTGTTCTGCGCGGTTACAGATCCAAGCTGTGACCGGCGCTACCTGAGATCAccggcGAAGGAGGGCCACCATGTACAGGATGCAACTCCTGTCTTGCA

1 MetTyrArgMetGlnLeuLeuSerCysI

EcoRV (634) **BglII (648)**

EcoRI (626) **NcoI (640)**

601 TTGCACTAAGTCTTGCACTTGTCACGAATTCATATCGGCCATGGTTAGATCTGACAAAACCTCACACATGCCACCGTGCCAGCACCTGAACTCCTGGG

10 LeuAlaLeuSerLeuAlaLeuValThrAsnSer 1 AspLysThrHisThrCysProProCysProAlaProGluLeuLeuGlu

T250Q (741)

701 GGGACCGTCAGTCTTCTCTTCCCCAAAACCAAGGACCAACTGATGATCTCCCGGACCCTGAGGTCACATGCGTGGTGGACGTGAGCCACGAA

16 yGlyProSerValPheLeuPheProProLysProLysAspGlnLeuMetIleSerArgThrProGluValThrCysValValValAspValSerHisGlu

801 GACCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGAGGAGCAGTACAACAGCACGTACCGTGTGG

50 AspProGluuValLysPheAsnTrpTyrValAspGlyValGluValHisAsnAlaLysThrLysProArgGluGluGlnTyrAsnSerThrTyrArgValVal

901 TCAGCGTCTCACCCTCTGCACAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCAGCCCCCATCGAGAAAACCAT

83 alSerValLeuThrValLeuHisGlnAspTrpLeuAsnGlyLysGluTyrLysCysLysValSerAsnLysAlaLeuProAlaProIleGluLysThrIle

SmaI (1056)

1001 CTCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCATCCCGGAGGAGATGACCAAGAACCAGGTGACGCTGACCTGCTGGT

116 eSerLysAlaLysGlyGlnProArgGluProGluNValTyrThrLeuProProSerArgGluGluGluMetThrLysAsnGlnValSerLeuThrCysLeuVal

1101 AAAGGCTTCTATCCAGCGACATCGCGTGGAGTGGGAGAGCAATGGGCGAGCCGGAGAACAACTACAAGACCACGCTCCCGTGTGGACTCCGACGGCT

150 yLysGlyPheTyrProSerAspIleAlaValGluTrpGluSerAsnGlyGlnProGluAsnAsnTyrLysThrThrProProValLeuAspSerAspGlySer

XmnI (1257) **M428L (1275)**

1201 CTTCTTCTCTACAGCAAGCTCACCCTGGACAAGAGCAGGTGGCAGCAGGGGAACGCTTCTCATGCTCCGTGCTGCATGAGGCTCTGCACAACCACTA

183 erPhePheLeuTyrSerLysLeuThrValAspLysSerArgTrpGluGlnGlyAsnValPheSerCysSerValLeuHisGluAlaLeuHisAsnHisTyr

NheI (1340)

1301 CACGCAAGAGCCTCTCCCTGTCTCCGGTAAATGAGTGTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGC

216 rThrGlnLysSerLeuSerLeuSerProGluLys...

HpaI (1480)

1401 AGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAAACAACAATTGCATTCA

AseI (1577) **XmnI (1578)**

1501 TTTTATGTTTCAGGTTCAAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAATTAATTTCTAAATACAGCATAGCA

1601 AAACTTTAACTCCAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTG

1701 CAGCCTCACCTTCTTTCATGGAGTTTAAGATATAGTGATTTTCCCAAGGTTTGAACCTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCCAC

SwaI (1834)

1801 ATTCCCTTTTATGAAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAAATAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTC

1901 ATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCTCAGTCTCTG

125 AspGln

2001 CTCCTCTGCCACAAAGTGCACGCAAGTTGCCGGCCGGTTCGCGCAGGGCGAACTCCCGCCCCACGGCTGCTCGCGGATCTCGGTATGGCCGGCCCGAG

122 GluGluAlaValPheHisValCysAsnGlyAlaProAspArgLeuAlaPheGluArgGlyLysTrpProGluGluGlnIleGluThrMetAlaProGlySerAct

2101 GCGTCCCGGAAGTTTCGTGGACACGACTCCGACCATCGGCGTCAAGCTGCTCGCAGCGCGCACCCACCCAGCCAGGGGTGTGTTCCGGCACCACT

88 lAspArgPheAsnThrSerValValGluSerTrpGluAlaTyrLeuGluAspLeuGlyArgValTrpValTrpAlaLeuThrAsnAspProValValGlu

SmaI (2275)

2201 GGTCTGGACCGGCTGATGAACAGGTCACGTCGTCGCGGACCACACCGGCGAAGTCGCTCCACGAAGTCCCGGAGAACCCGAGCGGTGCGGTCCA

55 nAspGlnValAlaSerIlePheLeuThrValAspAspArgValValGlyAlaPheAspAspGluValPheAspArgSerPheGlyLeuArgAspThrTrp

2301 GAACTCGACCGCTCCGGCGAGTCGCGCGGTCGAGACCGGAACGGCACTGGTCAACTGGCCATGATGGCTCTCctgtcaggagaggagaagagaaga

22 PheGluValAlaGlyAlaValAspArgAlaThrLeuValProValAlaSerThrLeuLysAlaMet

AseI (2461) **PstI (2483)**

2401 aggttagtacaattgCTATAGTGAGTTGTATTATATCTATGCAGATATACATATGCCAATGATTAATTGTCAAACCTAGGCTGCAGgggttcagtgcacct

HindIII (2587)

2501 tttcctgcactgccccatctcctgccaccctttccaggcatagacagtcagtgacttacCAAACCTACAGGAGGGAGAAGGCAGAGCTTGAGACAGA

2601 CCCGCGGACCGCCGAACTGCGAGGGGACGTGGCTAGGGCGGCTCTTTTATGGTGCGCGGCCCTCGGAGGCAGGGCGCTCGGGAGGCTAGCGGCCA

BspEI (2745)

2701 ATCTGCGGTGGCAGGAGGCGGGCCGAAGCCGTGCCTGACCAATCCGGAGCACATAGGAGTCTCAGCCCCCGCCCCAAAGCAAGGGGAAGTCACGCGC

SpeI (2852)

2801 CTGTAGCGCCAGCGTGTGTGAAATGGGGCTTGGGGGGTGGGGCCCTGACTAGTCAAAACAAACTCCCATTTGACGTCAATGGGGTGGAGACTTGGAA

2901 ATCCCGGTGAGTCAACCGCTATCCACGCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGT

3001 AGGAAAGTCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGA
 3101 TGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCAATTATTGACG

PacI (3275)
 PstI (3268)
SdaI (3268) **BspLU11I (3281)**

3201 TCAATGGGCGGGGTCGTTGGGCGGTGAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGCAAGGTTAATTAAGAACATGTGAGCAAAAGGCCAG
 3301 CAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGT
 3401 GCGGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTC
 3501 CGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTGCTTCGCTCCAAGCTGGGCTGTGTGCAC
 3601 GAACCCCCCGTTAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTG
 3701 GTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTG
 3801 CGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAACCACCGCTGGTAGCGGTGGTTTTTTTGTGTGCAAGCAG
 3901 CAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACCTCACGTTAAGGGATTTTGG

PacI (4015) SwaI (4023) **NotI (4031)**

4001 TCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTGTGTGAATCGTAACTAACA
 4101 TACGCTCTCCATCAAAACAAAACGAAACAAAACAACTAGCAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA