

pFUSE-hIgG1e4-Fc2

Plasmid containing a human engineered IgG1 Fc region

Catalog # pfc2-hg1e4

For research use only

Version 20K05-MM

PRODUCT INFORMATION

Content:

- 20 µg of pFUSE-hIgG1e4-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 acid substitutions have been made in the human IgG1 Fc region in order to increase or reduce its ADCC and CDC.

PLASMID FEATURES

• **hIgG1e4-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 the activating Fc receptor FcγRIII and the first component of the complement C1q through the CH2 domain. Substitution of residue E333 with alanine in human IgG1 was shown to increase both ADCC and CDC *in vitro*^{1,2}.

• **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 mRNA⁶.

• **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 transcription⁷.

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

2. Shields RL. et al., 2001. High resolution mapping of the binding site on human IgG1 for Fc gamma RI, Fc gamma RII, Fc gamma RIII, and FcRn and design of IgG1 variants with improved binding to the Fc gamma R. J Biol Chem. 276(9):6591-604.

3. Idusogie EE. et al., 2001. Engineered antibodies with increased activity to recruit complement. J Immunol. 166(4):2571-5.

4. 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.

5. 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.

6. 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.

7. 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

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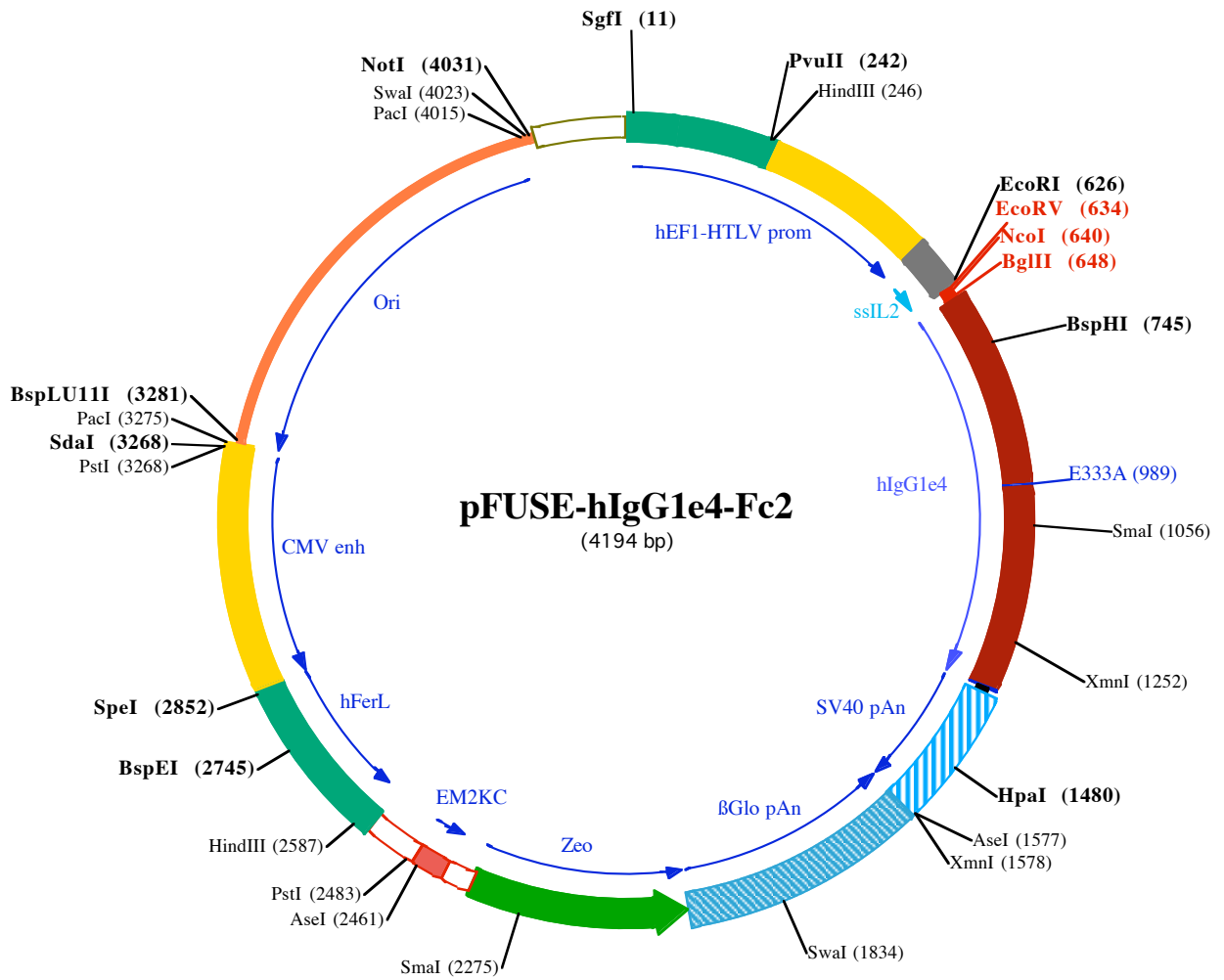
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100



SgfI (11)
1 GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGGTCGGCAATTGAACGGGTGCCTA
101 GAGAAGGTGGCGGGGTAAACTGGAAAGTGATGCTGTACTGGCTCCGCTTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

HindIII (246)
201 GTGAACGTTCTTTTTCGCAACGGGTTTCCGCCAGAACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCTTACCGCGCCCGCCCTACCTGAGGCC

PvuII (242)
301 GCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC
401 GGGCCTTTGTCCGGCGCTCCCTTGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGTCTCAACTCTACGCTTTTGTTCGTTT
501 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCTACTCGATCAccggcGAAGGAGGGCCACCATGTACAGGATGCAACTCCTGTCTTGCA

EcoRV (634) **BglII (648)**
EcoRI (626) **NcoI (640)**
601 TTGCACTAAGTCTTGCACTTGTCACGAATTCGATATCGGCCATGGTTAGATCTGACAAAACCTCACACATGCCACCCTGCCAGCACCTGAACTCCTGGG
1 AspLysThr His Thr CysP roP roCysP roAl aP roGl uLeuLeuGl

BspHI (745)
701 GGGACCGTCAGTCTTCTCTTCCCCAAAACCAAGGACACCTCATGATCTCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAA
16 yGl yProSer Val PheLeuPheP roP roLysP roLysAspThr LeuMet I eSer ArgThr ProGl uVal Thr CysVal Val Val AspVal Ser His Gl u
801 GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGG
50 AspP roGl uVal LysPheAsnTrpTyrVal AspGl yVal Gl uVal His AsnAl aLysThr LysP roArgGl uGl uGlnTyrAsnSer Thr TyrArgVal V
901 TCAGCGTCTCACCGTCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGAAGGTCTCCAAAGCCCTCCAGCCCCATCGCGAAAACCAT
83 aL Ser Val LeuThr Val LeuHis Gl nAspTrpLeuAsnGl yLysGl uTyrLysCysLysVal SerAsnLysAl aLeuProAl aProI l eAl aLysThr I l
E333A (989)

SmaI (1056)
1001 CTCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTC
116 eSer LysAl aLysGl yGl nProArgGl uP roGl nVal TyrThr LeuProP roSer ArgGl uGl uMetThr LysAsnGl nVal Ser LeuThr CysLeuVal
1101 AAAGGCTTCTATCCAGGCATCGCGTGGAGTGGAGAGCAATGGGACGCCGAGAACAACTACAAGACCAGCCCTCCCGTGTGGACTCCGACGGCT
150 LysGl yPheTyrProSerAspI l eAl aVal Gl uTrpGl uSerAsnGl yGl nProGl uAsnAsnTyrLysThr Thr ProP roVal LeuAspSerAspGl yS

XmnI (1252)
1201 CTTTCTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAAACGCTTCTCATGCTCCGTGATGCATGAGGC TCTGCACAACCACTA
183 er PhePheLeuTyrSer LysLeuThr Val AspLysSer ArgTrpGl nGl nGl yAsnVal PheSer CysSer Val lMetHis sGluAlaLeuHisAsnHisTy
1301 CACGCAGAAGGCCTCCCTGTCTCCGGTAAATGAGTGCAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAAACCAACTAGAATGC
216 r Thr Gl nLysSer LeuSer LeuSer ProGl yLys•••

HpaI (1480)
1401 AGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAAACAACAACAAATTGCATTCA

AseI (1577) **XmnI (1578)**
1501 TTTTATGTTTCAGGTTCAAGGGGAGGTGTGGAGGTTTTTAAAGCAAGTAAAACCTCTACAAATGGTATGGAATTAATTTCTAAAATACAGCATAGCA
1601 AAACTTTAACTCCAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTT
1701 CAGCCTCACCTTCTTTCATGGAGTTTAAGATATAGTATTTTCCAAAGGTTGAAGTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCCAC

Swal (1834)
1801 ATTCCCTTTTATGAAAAATTCAGAAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTC
1901 ATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCTCTG
125•••AspGl n
2001 CTCTCTGCCCAAAGTGCACGCAGTTGCCGCGGGTTCGCGCAGGGCGAACTCCCGCCCCACGGCTGCTCGCGATCTCGGTATGGCCGGCCGGAG
122 Gl uGl uAl aVal PheHis sVal CysAsnGl yAl aP roAspArgLeuAl aPheGl uArgGl yTrpP roGl nGl uGl yI l eGl uThr MetAl aP roGl ySer A
2101 CGTCCCGGAAGTTTCGTGGACACGACTCCGACCCTCGCGTACAGCTGTCAGGCGCGCACCCACCCACCCAGCCAGGGTGTTCGCGGACCCACT
88 l aAspArgPheAsnThr Ser Val Val Gl uSer TrpGl uAl aTyrLeuGl uAspLeuGl yA rgVal TrpVal TrpAl aLeuThrAsnAspP roVal Val Gl
2201 GGTCTGACCGCGCTGATGAACAGGGTACGTCGTCGCGGACACACCGCGGAAGTCTCTCCACGAAGTCCCGGGAACCCGAGCCGGTCTGGTCCA
55 nAspGl nValAl aSer I l ePheLeuThr Val AspAspArgVal Val Gl yAl aPheAspAspGl uVal PheAspArgSer PheGl yLeuArgAspThr Trp
2301 GAACTCGACCGCTCCGCGCAGCTCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTGGCCATGATGGCTCTCctgtcaggagaggaagagaga
22 PheGl uValAl aGl yAl aVal AspArgAl aThr LeuVal P roValAl aSer Thr LeuLysAl aMet

AseI (2461) **PstI (2483)**
2401 aggttagtacaattgCTATAGTGAGTTGATTATACATGAGATATACTATGCCAATGATTAATTGTCAAACCTAGGGCTGCAgggttcatagtgcact

HindIII (2587)
2501 tttcctgactgcccactctcctgcccacccttccaggcatagacagtcagtgacttacCAAACCTACAGGAGGAGAAGGCAGAAAGCTTGAGACAGA
2601 CCCGCGGACCGCCAACTGCGAGGGGAGCTGGCTAGGGCGGCTTTTTATGGTGCCTCGGCCCTCGGAGGCAGGGCGCTCGGGAGGCCTAGCGCCA

BspEI (2745)
2701 ATCTGCGGTGGCAGGAGCGGGGCCGAAGGCCGTGCTGACCAATCCGGAGCACATAGGAGTCTCAGCCCCCGCCCAAGCAAGGGGAAGTACGCGCC

SpeI (2852)
2801 CTGTAGCGCCAGCGTGTGTGAAATGGGGCTTGGGGGGTGGGGCCCTGACTAGTCAAACAACCTCCATTGACGTCAATGGGGTGGAGACTTGAA
2901 ATCCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTAAGTCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTAAGTCCAAAGT

