

pFUSE-hIgG1e9-Fc2

Plasmid containing a human engineered IgG1 Fc region

Catalog # pfc2-hg1e9

For research use only

Version 20K05-MM

PRODUCT INFORMATION

Content:

- 20 µg of pFUSE-hIgG1e9-Fc2 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 plasmids 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. 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

• **hIgG1e9-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 mutations S239D/I332E/G236A has been reported to increase FcγRIIa/FcγRIIb ratio and increase macrophage phagocytosis². pFUSE-hIgG1e9-Fc2 contains the S239D/I332E/G236A mutation.

• **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*.

• **βGlo 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. *Nat Rev Immunol.* 6(5):343-57.

2. Richards JO. *et al.*, 2008. Optimization of antibody binding to FcγRIIIa enhances macrophage phagocytosis of tumor cells. *Mol Cancer Ther.* 7(8):2517-27.

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

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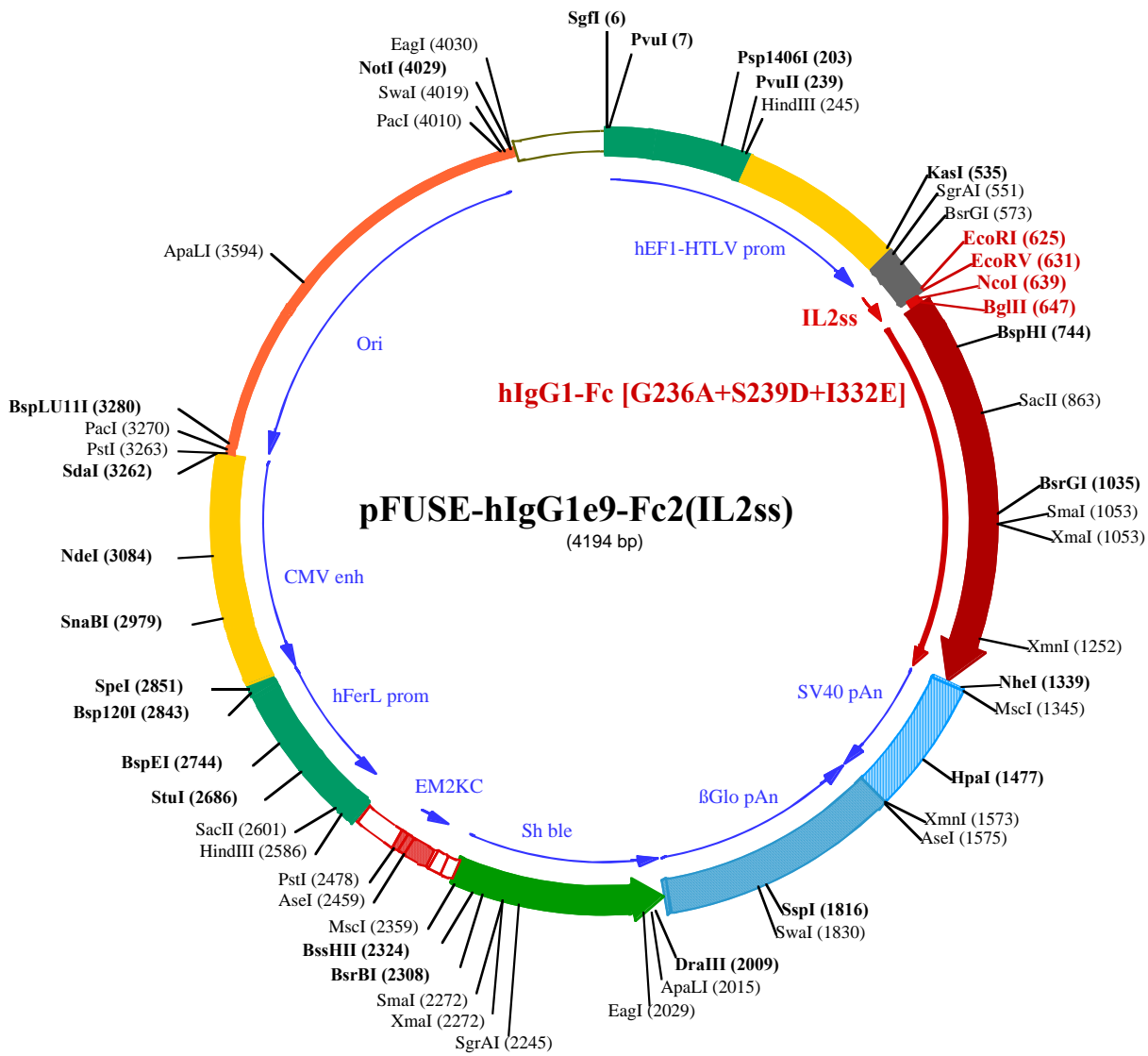
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PvuI (7)
SgfI (6)
 1 GGATCTGCGATCGCTCCGGTCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGGTCGGCAATTGAACGGGTGCCTA
 101 GAGAAGGTGGCGGGGTAACCTGGGAAAGTGTATGTCGTGTAAGTCTGGCTCCGCCTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

HindIII (245)
Psp1406I (203)

201 GTGAACGTTCTTTTCGCAACGGGTTTGGCCGCAGAACACACAGCTGAAGCTTCGAGG**GGCTCGCATCTCTCCTTTCAGCGCCCGCCCTACTCGAGGCC**
 301 GCCATCCACGCGGTTGAGTTCGCGTTCTGCCGCCCTCCCGCCTGTGGTGCCTCTGAAGTGCCTCGCGCTTAGGTAAGTTTAAAGCTCAGGTCGAGACC
 401 GGGCCTTTGTCCGGCGTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGTCTCAACTCTACGTCCTTTGTTTCTTT

KasI (535) **SgrAI (551)** **BsrGI (573)**
 501 TCTGTCTCGCGGTTACAGATCCAAGCTGTGACCGCGCC**TAC**CTGAGATCACCCGCGAAGGAGGGCCACCATGTACAGGATGCAACTCCTGTCTTGCA
 1▶ M Y R M Q L L S C

EcoRV (631) **BglII (647)**
EcoRI (625) **NcoI (639)**
 601 TTGCACTAAGTCTTGCACTTGTACGAAATTCGATATCGGCCATGGTGTAGATCTGACAAAACACACATGCCACCCTGCCAGCACCTGAACTCCTGGC
 10▶ I A L S L A L V T N S 1▶ D K T H T C P P C P A P E L L A

BspHI (744)
 701 GGGACCGGAGCTCTTCTCTTCCCCCAAACCAAGGACACCCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCAGAA
 16▶ G P D V F L F P P K P K D T L M I S R T P E V T C V V V D V S H E

SacII (863)
 801 GACCTGAGGTCAAGTTCAACTGGTACGTGGACGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGAGGAGCAGTACAAAGCACGTACCGTGTGG
 50▶ D P E V K F N W Y V D G V E V H N A K T K P R E E Q Y N S T Y R V
 901 TCAGCGTCTCACCGTCTCGACCAAGGACTGGCTGAATGGCAAGGATCAAGTGAAGGTCTCCAACAAAGCCCTCCAGCCCGGAGGAGAAACCAT
 83▶ V S V L T V L H Q D W L N G K E Y K C K V S N K A L P A P E E K T I

XmaI (1053) **SmaI (1053)**
BsrGI (1035) **SmaI (1053)**
 1001 CTCCAAGCCAAAGGGCAGCCCGAGAACCACAGGTGTACACCTGCCCCATCCCGGAGGAGATGACCAAGAACAGGTCAGCCTGACCTGCCTGGTC
 116▶ S K A K G Q P R E P Q V Y T L P P S R E E M T K N Q V S L T C L V
 1101 AAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGCAATGGGACCGGAGAACAACTACAAGACCAGCCTCCCGTGTGACTCCGACCGCT
 150▶ K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S D G

XmnI (1252)
 1201 CCTTCTCTCTACAGCAAGCTCACCGTGACCAAGAGCAGTGGCAGCAGGGGAACGCTTCTCATGCTCCGTGATGCACGAGGCTCTGCACAACCACTA
 183▶ S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y

MscI (1345)
NheI (1339)
 1301 CACGCAAGAGCCTCTCCCTGTCTCCGGTAAATGAGTGCCTAGCTGGCCAGACATGATAAGATACATGTAGATTGGACAAACCAACTAGAAATGC
 216▶ T Q K S L S L S P G K •

HpaI (1477)
 1401 AGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCAATTATAAGCTGCAATAAACCAAGTTAACAAACCAATTCATTCA

AseI (1575) **XmnI (1573)**
 1501 TTTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAAATGGGTATGGAATTAATTTCTAAAATACAGCATAGCA
 1601 AAACCTTAACTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTGGCAATGTGCATTAGCTGTTTG
 1701 CAGCCTCACCTTCTTTCATGAGGTTTAAAGATATAGTGTATTTTCCCAAGGTTTGAAGTACTCTTTCATTTCTTTATGTTTAAATGCACCTGACCTCCAC

SspI (1816) **SwaI (1830)**
 1801 ATTCCCTTTTGTAGTAAATATTTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAAATCCAGATGCTCAAGGCCCTTC
 1901 ATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCTCTG

ApaLI (2015)
DraIII (2009) **EagI (2029)**
 2001 CTCTCTGCGCAAAAGTGCACGCAAGTTCGGCCCGGTTCGCGCAGGGCGAACTCCCGCCCCACGGCTGCTCGCCGATCTCGGTCATGGCCGCGCCGAG
 122▶ E E A V F H V C N G A P D R L A F E R G W P Q E G I E T M A P G S
 2101 GCGTCCCGAAGTTCTGTGGACACGACCTCCGACCACTCGCGTACAGCTGTCCAGCCGCGCACCCACACCCAGGCGAGGTTGTCTCGGCAGCACCAT
 88▶ A D R F N T S V V E S W E A Y L E D L G R V W V W A L T N D P V V Q

XmaI (2272) **SmaI (2272)**
SgrAI (2245)
 2201 GGTCTGGACCGCGTGTGAACAGGGTACGTCGTCCCGGACACACCGCGGAAGTCTCTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCCGTTCCA
 55▶ D Q V A S I F L T V D D R V V G A F D D E V F D R S F G L R D T W

BsrBI (2308) **BssHIII (2324)** **MscI (2359)**
 2301 GAACTCGACCGCTCCGCGCAGCTCGCGCGGTGAGCACCAGGAAAGGCACTGGTCAACTTGGCCATGATGGCTCCTCctgtcaggagaggaagagaga
 22▶ F E V A G A V D R A T L V P V A S T L K A M

AseI (2459) **PstI (2478)**
 2401 aggttagtacaattgCTATAGTGTGATTATATACTATGCAGATATACTATGCCAATGATTAATTGTCAAACCTAGGGCTGCAgggttcatagtgccact

HindIII (2586)
 2501 tttctgcaactgccccatctcctgccccaccttccaggcatagacagtcagtgacttacCAAACCTCACAGGAGGAGAAGGCAGAACTTGAGACAGA

SacII (2601) StuI (2686)
 2601 CCCGCGGGACCGCCGAACCTGCGAGGGGACGTGGCTAGGGCGGCTTCTTTTATGGTGCGCCGCCCTCGGAGGCAGGGCGCTCGGGGAGGCCTAGCGGCCA

BspEI (2744)
 2701 ATCTGCGGTGGCAGGAGGCGGGCCGAAGGCCGTGCCTGACCAATCCGGAGCACATAGGAGTCTCAGCCCCCGCCCCAAAGCAAGGGGAAGTCA CGCGC

SpeI (2851)
Bsp120I (2843)
 2801 CTGTAGCGCCAGCGTGTGTGAAATGGGGCTTGGGGGGTTGGGGCCCTGACTAGTCAAAACAAACTCCCAATTGACGTC AATGGGGTGGAGACTTGGAA

SnaBI (2979)
 2901 ATCCCCGTGAGTCAAACCGCTATCCACGCCCATTTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGT

NdeI (3084)
 3001 AGGAAAGTCCCATTAAGTTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTC AATAGGGGCGTACTTGGCATATGATACACTTGA
 3101 TGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTC AATGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACG

PacI (3270)
PstI (3263)
SdaI (3262) **BspLU11I (3280)**
 3201 TCAATGGGCGGGGTCGTTGGGCGGTGAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGCAGGTTAATTAAAGAACATGTGAGCAAAGGCCAG

 3301 CAAAAGGCCAGGAACCGTAAAAAGGCCGCGTGTGCGGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATC AAAAAATCGACGCTCAAGTCAGAGGT

 3401 GCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCTGCGCTTACCGGATACCTGTG

ApaLI (3594)
 3501 CGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTTCGGTGTAGGTCGTTTCGCTCCAAGCTGGGCTGTGTGCAC

 3601 GAACCCCGGTTGAGCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCAAACCCGGTAAGACACGACTTATCGCACTGGCAGCAGCCACTG

 3701 GTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTG

 3801 CGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTGTTGCAAGCAG

 3901 CAGATTACCGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTGGAACGAAAACCTCACGTTAAGGGATTTTGG

EagI (4030)
PacI (4010) SmaI (4019) NotI (4029)
 4001 TCATGGCTAGTTAATTAACATTTAAATCAGCGGCCCAATAAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAAC TAACA

 4101 TACGCTCTCCATCAAAACAAAACGAAACAAAACAAACTAGCAAATAGGCTGTCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA