

pFUSE-hIgG1e5-Fc1

Plasmid containing a human engineered IgG1 Fc region

Catalog # pfc1-hg1e5

For research use only

Version 20K05-MM

PRODUCT INFORMATION

Content:

- 20 µg of pFUSE-hIgG1e5-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. 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

- **hIgG1e5-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 triple Fc mutation S239D:A330L:I332E has been reported to enhance ADCC and FcγRIIIA binding^{2,3}. pFUSE-hIgG1e5-Fc1 contains the triple Fc mutation S239D:A330L:I332E.
- **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter⁴ and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat⁵. 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 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. Lazar GA. et al., 2006. Engineered antibody Fc variants with enhanced effector function. PNAS 103(11): 4005–4010.
3. Ryan MC. et al., 2007. Antibody targeting of B-cell maturation antigen on malignant plasma cells. Mol. Cancer Ther., 6: 3009 - 3018.
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

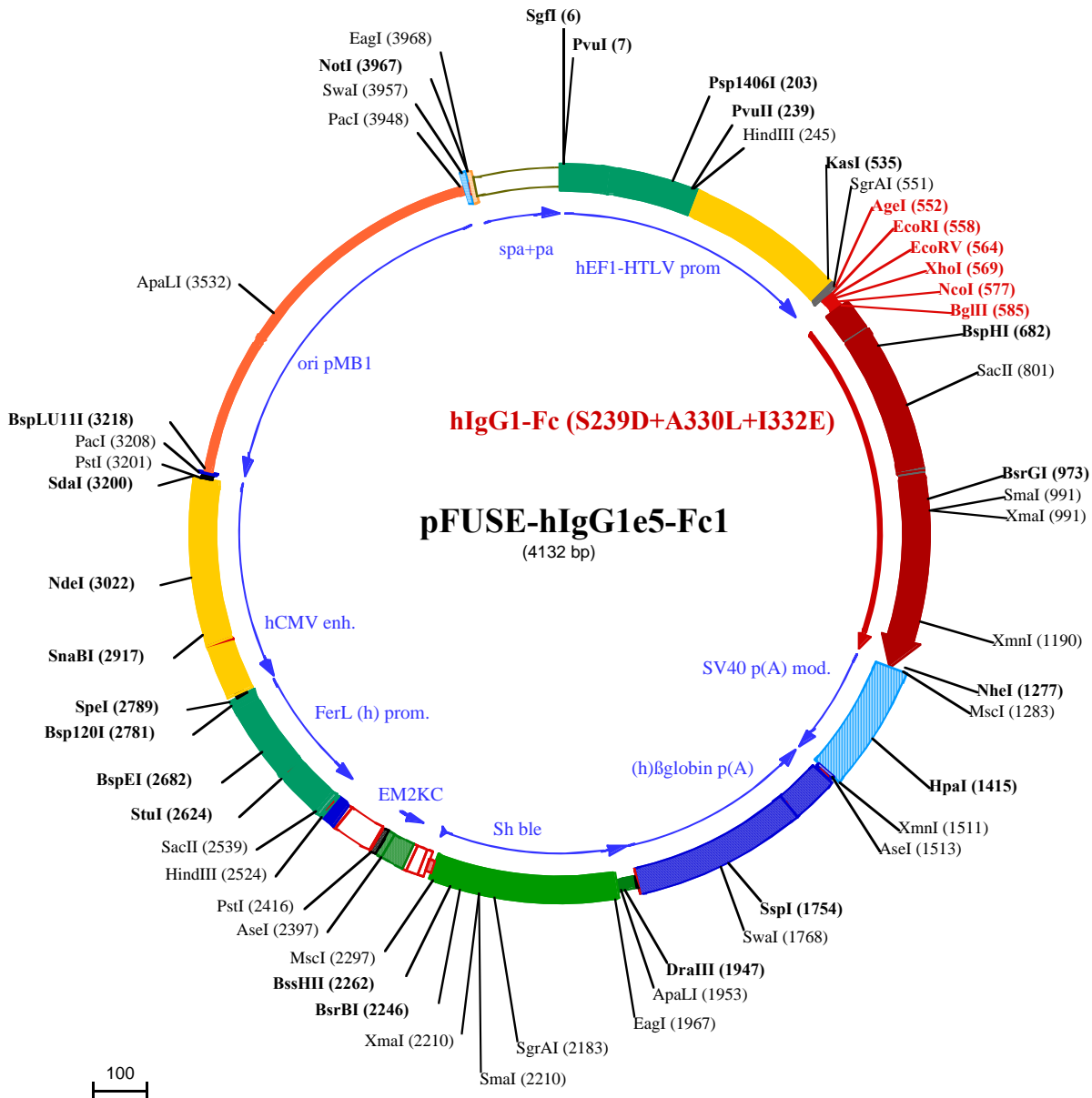
This antibiotic can be used for *E. coli* at 25 µg/ml in liquid or solid media and at 50-200 µg/ml to select Zeocin™-resistant mammalian cells.

RELATED PRODUCTS

Product	Catalog Code
Zeocin™	ant-zn-1

TECHNICAL SUPPORT

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PvuI (7)
SgfI (6)

1 GGATCTGCGATCGCTCCGGTGCCTCAGTGGGCGAGCGCACATCGCCACAGTCCCGGAGAAGTTGGGGGAGGGTTCGGCAATTGAACGGGTGCCTA

101 GAGAAGGTGGCGCGGGTAAACTGGGAAAGTGTGCTGACTGGCTCCGCCTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCC

HindIII (245)

Psp1406I (203) PvuII (239)

201 GTGAACGTTCTTTTCGCAACGGGTTTCCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCTTCACGGCCCGCCGCCCTACCTGAGGCC

301 GCCATCCACGCCGGTTGAGTTCGGCTTCTGCCGCTCCCGCCTGTGGTGCCTCTGAACTGCGTCCGCCCTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC

401 GGGCCTTTGTCGGGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCCTGCTTGCTCAACTCTACGCTTTGTTCGTTT

EcoRI (558) AgeI (552) XhoI (569) BglII (585)
KasI (535) SgrAI (551) EcoRV (564) NcoI (577)

501 TCTGTTCTGCGCGTTACAGATCCAAGCTGTGACCCGCGCTACCTGAGATCACCGGTGAATTCGATATCTCGAGCACCATGGTTAGATCTGACAAAACT

1 D K T

BspHI (682)

601 CACACATGCCCAACCGTGCAGCCTGAACTCCTGGGGGACCGGACCTCTTCTCTTCCCCCAAAAACCAAGGACACCCCTCATGATCTCCCGGACCC

4 H T C P P C P A P E L L G G P D V F L F P P K P K D T L M I S R T

701 CTGAGTCCACATCGTGGTGGTGGACCTGAGCCACGAAGACCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAA

37 P E V T C V V V D V S H E D P E V K F N W Y V D G V E V H N A K T K

SacII (801)

801 GCCCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCCAGCTCCTCACCCTGTCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGAACGGT

70 P R E E Q Y N S T Y R V V S V L T V L H Q D W L N G K E Y K C K V

XmaI (991) SmaI (991)

901 TCCAACAAGCCCTCCCACTCCCGAGGAGAAAACCATCTCCAAGCCAAAGGGCAGCCCGAGAACCACAGGTGTACACCTGCCCCATCCCGGGAGG

104 S N K A L P L P E E K T I S K A K G Q P R E P Q V Y T L P P S R E

1001 AGATGACCAAGAACCAGGTGACGCTGACCTGCCTGGTCAAAGGCTTCTATCCAGCAGACATCGCGTGGAGTGGGAGAGCAATGGGACCGGAGAACAA

137 E M T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N

XmnI (1190)

1101 CTACAAGACCACGCCTCCCGTGTGGACTCCGACGGCTCCTTCTCTCTACAGCAAGCTCACCCTGGACAAGAGCAGGTGCCAGCAGGGGAACGCTCTC

170 Y K T T P P V L D S D G S F F L Y S K L T V D K S R W Q Q G N V F

MscI (1283) NheI (1277)

1201 TCATGCTCCGTGATGCACGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGTAAATGAGTGTAGCTGGCCAGACATGATAAG

204 S C S V M H E A L H N H Y T Q K S L S L S P G K •

1301 ATACATGATGAGTTGGACAAACCACAACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATGTCTTTATTGTAAACCATTATA

HpaI (1415)

1401 AGCTGCAATAAACAAGTTAAACAACAATTCGATTCATTTTATGTTTCAGGTTTCAGGGGAGGTGTGGAGGTTTTTTAAAGCAAGTAAAACCTCTACA

AseI (1513) XmnI (1511)

1501 AATGTGGTATGGAAATTAATTCTAAAATACAGCATAGCAAACCTTTAACCTCCAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATA

1601 GGCATCAGGGGCTGTGGCAATGTGCATTAGCTGTGTTGAGCCTCACCTCTTTTTCATGGAGTTTAAAGATATAGTGTATTTTCCCAAGGTTTGAAGTGT

SspI (1754) SmaI (1768)

1701 CTTCAATTTCTTTATGTTTAAATGCAGTACCTCCACATTCCTTTTATGATAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATG

1801 TTTTTTATTAGGAGAAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTATGATGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTTGA

ApaLI (1953) DraIII (1947) EagI (1967)

1901 CAGCAAGAAAGCGAGCTTCTAGCTTATCCTTCAGTTCCTGCTCCTCTGCAAAAGTGCACGCAAGTTCGCGCGGGTTCGCGCAGGGCGAACTCCCGCCCC

125 D Q E E A V F H V C N G A P D R L A F E R G W

2001 ACGGCTGCTCGCGATCTCGGTTCATGGCCCGCGGAGGCTCCCGAAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCGCG

101 P Q E G I E T M A P G S A D R F N T S V V E S W E A Y L E D L G R

SgrAI (2183)

2101 CACCCACACCAGGCCAGGTTGTTCGCGCACCACTGGTCTGGACCGCGCTGATGAACAGGGTCACGTCGTCGCCGACCAACCGGCGAAGTGTCTC

68 V W V W A L T N D P V V Q D Q V A S I F L T V D D R V V G A F D D

XmaI (2210) SmaI (2210) BsrBI (2246) BssHIII (2262) MscI (2297)

2201 TCCACGAAGTCCCGGAGAACCCGAGCCGGTTCGATCCAGAACTCGACCGCTCCGGCAGCTCGCGCGCGGTGAGCACCGGAACCGCACTGGTCAACTTGG

34 E V F D R S F G L R D T W F E V A G A V D R A T L V P V A S T L K A

Asel (2397)

2301 CCATGATGGCTCCTCctgtcaggagaggaagaagaaggtagtacaattgCTATAGTGTGATTATACTATGCAGATATACTATGCCAATGATT

1 M

3 I A G

PstI (2416)

2401 AATGTCAAAGTGGCTGCAgggttcacagtgcacttttctgcaactgccccatctcctgcccccttccaggcatagacagtcagtgacttacC

HindIII (2524) SacII (2539)

2501 AAACCTCACAGGAGGGAGAAGGCAGAGCTTTCAGACAGACCCCGGGACCCCGCAACTGCGAGGGGACGTGGCTAGGGCGGCTCTTTTATGGTGCGCCGG

2601 **StuI (2624)** **BspEI (2682)**
 CCTCGGAGGCAGGGCGCTCGGGGAGGCTAGCGGCCAATCTGCGTGGCAGGAGCGGGCCGAAAGCCGTGCCTGACCAATCCGGAGCACATAGGAGT

2701 **SpeI (2789)**
 CTCAGCCCCCGCCCAAAGCAAGGGGAAGTCAAGCGCTGTAGCGCCAGCGTGTGTGAAATGGGGGCTTGGGGGGTGGGGCCCTGACTAGTCAAAA

2801 **Bsp120I (2781)**
 CAAACTCCCATTTGACGTCAATGGGGTGGAGACTTTGAAAATCCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTACTGCCAAAACCGCATCATCATG

2901 **SnaBI (2917)**
 GTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATTAAGTTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGA

3001 **NdeI (3022)**
 CGTCAATAGGGGCGTACTTTGGCATATGATACACTTGTAGTACTGCCAAGTGGGCGAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCC

3101 **NdeI (3022)**
 TATTGGCGTTACTATGGGAACATACGTCATTAATTGACGTCAATGGGCGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACG

3201 **PacI (3208)** **PstI (3201)** **SdaI (3200)** **BspLU11I (3218)**
 CCTGCAGGTTAATTAAGAACATGTGAGCAAAGGCCAGCAAAGGCCAGGAAACCGTAAAAAGCCGCTTGTGGCGTTTTTCCATAGGCTCCGCCCC

3301 **SdaI (3200)** **BspLU11I (3218)**
 TGACGAGCATCAAAAAATCGACGCTCAAGTCAAGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGC

3401 **SdaI (3200)** **BspLU11I (3218)**
 TCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTATCTCAGTT

3501 **ApaLI (3532)**
 CGGTGTAGGTGTTGCTCCAGCTGGGCTGTGTGCACGAAACCCCGTTTCAGCCCGACCGCTGCGCTTATCCGGTAACTATCGTCTTGAGTCCAACCC

3601 **ApaLI (3532)**
 GGTAAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGCCT

3701 **ApaLI (3532)**
 AACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAA

3801 **ApaLI (3532)**
 CCACCGCTGGTAGCGTGGTTTTTTTGTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGA

3901 **EagI (3968)** **PacI (3948)** **SwaI (3957)** **NotI (3967)**
 CGCTCAGTGGAAACGAAAACCTCACGTTAAGGGATTTGGTTCATGGCTAGTTAATTAAACATTTAAATCAGCGGCCGCAATAAAAATATCTTTATTTTCATTAC

4001 **EagI (3968)** **PacI (3948)** **SwaI (3957)** **NotI (3967)**
 ATCTGTGTGTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAAACAAAACGAAACAAAACAACTAGCAAAATAGGCTGTCCCCAGTGC

4101 **EagI (3968)** **PacI (3948)** **SwaI (3957)** **NotI (3967)**
 AAGTGCAGGTGCCAGAACATTTCTCTATCGAA