

pFUSE-hIgG1e4-Fc1

Plasmid containing a human engineered IgG1 Fc region

Catalog # pfc1-hg1e4

For research use only

Version 20K05-MM

PRODUCT INFORMATION

Content:

- 20 µg of pFUSE-hIgG1e4-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 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 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.

References:

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

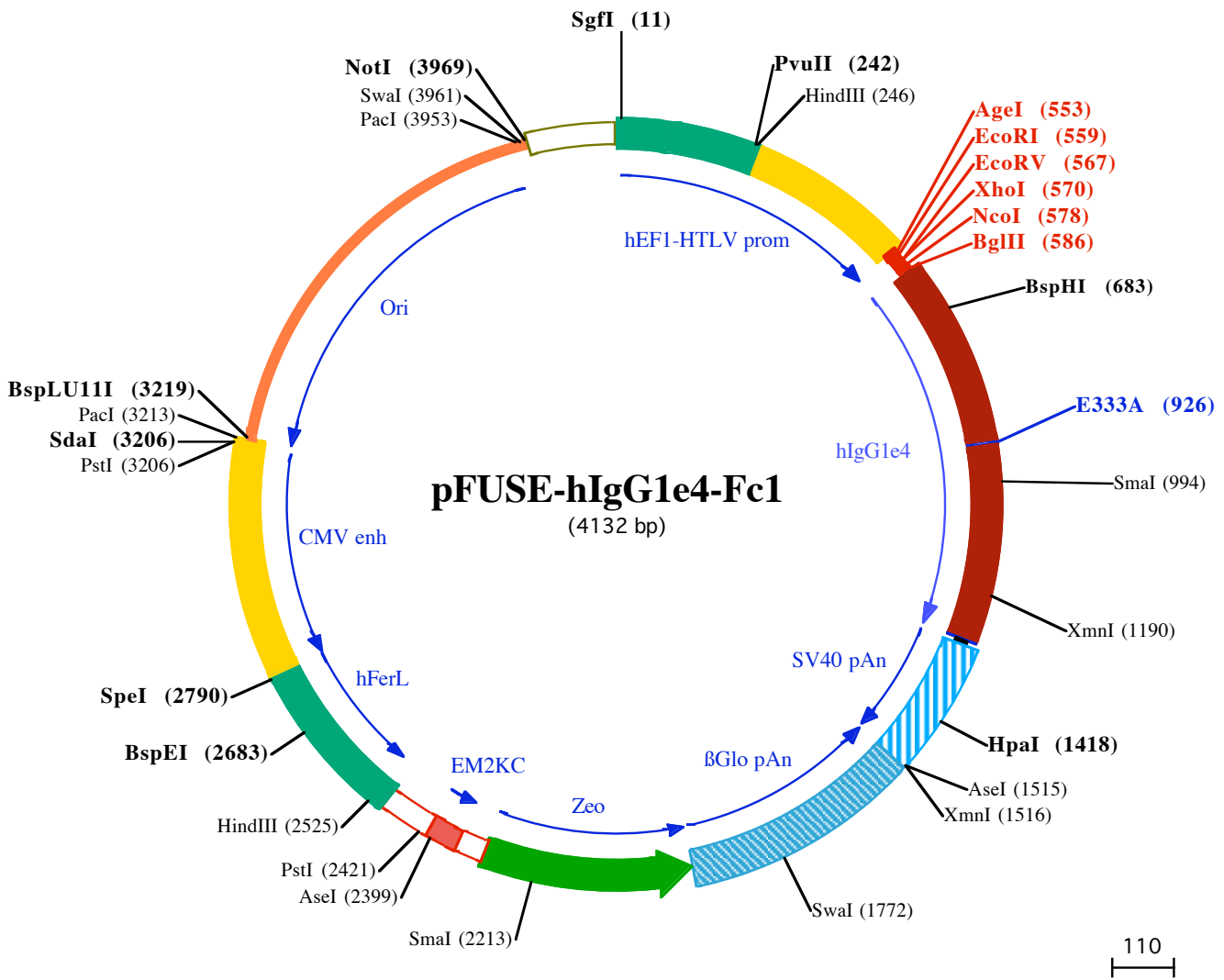
This antibiotic can be used for *E. coli* at 25 µg/ml in liquid or solid media and at 50-200 µg/ml to select Zeocin™-resistant mammalian cells.

RELATED PRODUCTS

Product	Catalog Code
Zeocin™	ant-zn-1

TECHNICAL SUPPORT

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1 GGATCTGC GATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGGTGGCAATTGAACGGGTGCCTA

101 GAGAAGGTGGCGCGGGTAAACTGGGAAAGTATGTCGTACTGGCTCCGCCTTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

201 GTGAACGTTCTTTTTCGCAACGGGTTTGCCGCGAGAACAACAGCTGAAGCTTCGAGGGCTCGCATCTCTCTTACACGCCGCCGCCCTACCTGAGGCC

301 GCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCTCCCGCCTGTGGTGCCTCTGAACTGCGTCCGCCGTCTAGTTAAGTTTAAAGCTCAGGTCGAGACC

401 GGGCCTTTGTCCGGCGCTCCCTTGAGGCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCTGACCTGCTTGTCAACTCTACGCTTTTGTTCGTTT

501 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCCCTACCTGAGATCACCGTGAATTCGATATCTCGAGCACCATGGTTAGATCTGACAAAACT

601 CACACATGCCACCGTCCAGCACCTGAACTCCTGGGGGACCGTCAGTCTTCTCTTCCCCCAAACCAAGGACACCCTCATGATCTCCCGGACCC

701 CTGAGGTCACATGCGTGGTGGACGTGAGCCACGAAGACCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAA

801 GCGCGGGAGGAGCAGTACAACAGCAGTACCGTGTGGTCAAGCTCCTACCGTCTGACCCAGGACTGGCTGAATGGCAAGGAGTACAAGTGAAGGTC

901 TCCAACAAAGCCCTCCAGCCCATCGGAAAACCTCCAAAGCCAAAGGGCAGCCCGAGAACCACAGGTGTACACCCTGCCCCATCCCCGGAGG

1001 AGATGACCAAGAACCAGGTCAGCTGACCTGCCTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCGCCGGAGAACAA

1101 CTACAAGACCAGCCTCCCGTGTGGACTCCGACGGCTCTTCTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGAACTCTTC

1201 TCATGCTCCGTGATGCATGAGGC TCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGTAAATGAGTGTAGCTGGCCAGACATGATAAG

1301 ATACATTGATGAGTTTGACAAAACCAACTAGAATGCAGTGAAAAAATGCTTTATTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATA

1401 AGCTGCAATAAACAAAGTTAACAAACAACAAATTGCATTCAATTTATGTTTCAGGTTACAGGGGAGGTGTGGGAGTTTTTAAAGCAAGTAAACCTCTACA

1501 AATGTGGTATGGAATTAATTTCTAAAAACAGCATAGCAAACTTTAACTCCAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATA

1601 GGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTCTTTTCATGGAGTTAAGATATAGTGTATTTTCCAAGGTTTGAAGTAGCT

1701 CTTCAATTTCTTTATGTTTTAAATGCACTGACCTCCACATTCCTTTTTAGTAAATATTTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATG

1801 TTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGAACCTTTAATAGAAATTTGGA

1901 CAGCAAGAAAGCGAGCTTCTAGCTTATCTCAGTCTGCTCTGCCCACAAAGTGCACGAGTTGCGGCGGGTTCGCGCAGGGGCAACTCCCGCCCC

2001 ACGGCTGCTCGCCGATCTCGGTATGGCCGGCCGGAGGCGTCCCGAAGTTCGTGGACAGACTCCGACCACTCGGCGTACAGCTGCTCCAGGCCGCG

2101 CACCACACCCAGGCCAGGGTGTGTCGGCACCACTGCTGACCGCGCTGATGAACAGGGTACGTCGTCGGACACACCGCGGAAGTCTGTC

2201 TCCACGAAGTCCCGGGAGAACCCGAGCCGGTTCGTCAGAACTCGACCGCTCCGCGCAGCTCGCGCGCGGTGAGCACCGGAACGGCCTGTTCACTTGG

2301 CCATGATGGCTCCTcgtcaggagaggaagagaagaaggttagtacaattgCTATAGTGAGTTGTATTATACTATGCAGATATACTATGCCAATGATT

2401 AATTGTCAAAC TAGGGCTGCAGggttcatagtgcacttttctgcactgccccatctctgcccaccctttccaggcatagacagtcagtgacttacC

2501 AAACACAGGAGGGAGAAGGCAGAAGCTTGAGACAGACCCCGGGACCGCCGAACCTGCGAGGGGACGTGGCTAGGGCGGCTCTTTTATGGTGCGCCGG

2601 CCCTCGGAGGAGGGCGCTCGGGGAGGCTAGCGCCAATCTGCGGTGGCAGGAGGGGGCCGAAGCCGTGCTGACCAATCCGGAGCACATAGGAGT

2701 CTCAGCCCCCGCCCAAAGCAAGGGAGTACGCGCCTGTAGCGCCAGCGTGTGTGAAATGGGGCTTGGGGGGTGGGGCCCTGACTAGTCAAAA

2801 CAAACTCCCATTGACGTCAATGGGGTGGAGACTTGAAAATCCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTACTGCCAAAACCGCATCATCATG

SgfI (11)

HindIII (246)

PvuII (242)

EcoRI (559) XhoI (570) BglIII (586)

AgeI (553) EcoRV (567) NcoI (578)

BspHI (683)

SmaI (994)

XmnI (1190)

HpaI (1418)

AseI (1515)

XmnI (1516)

SwaI (1772)

SmaI (2213)

AseI (2399)

PstI (2421)

HindIII (2525)

BspEI (2683)

SpeI (2790)

2901 GTAATAGCGATGACTAATACGTAGATGTAAGTACTGCAAGTAGGAAAGTCCATAAAGGTCATGTAAGTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGA
3001 CGTCAATAGGGGGCGTACTTGGCATATGATACACTTGATGTAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCC
3101 TATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACG

PacI (3213)

PstI (3206)

SdaI (3206)

BspLU11I (3219)

3201 CCTGCAGGTTAATTAAGAACATGTGAGCAAAAGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTCCATAGGCTCCGCCCCC
3301 TGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTTCCCTGGAAGTCCCTCGTGCGC
3401 TCTCCTGTTCCGACCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTATCTCAGTT
3501 CGGTGTAGGTCGTTGCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTAGCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCC
3601 GGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCT
3701 AACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAA
3801 CCACCGCTGGTAGCGGTGGTTTTTTTTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGTCTGA

PacI (3953) SwaI (3961) **NotI (3969)**

3901 CGCTCAGTGAACGAAACTCACGTTAAGGGATTTGGTTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGAATAAAATATCTTTATTTTCATTAC
4001 ATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACAACTAGCAAATAGGCTGTCCCCAGTGC
4101 AAGTGCAGGTGCCAGAACATTTCTCTATCGAA