

pFUSE-hIgG1e3-Fc1

Plasmid containing a human engineered IgG1 Fc region

Catalog # pfc1-hg1e3

For research use only

Version 20K05-MM

PRODUCT INFORMATION

Content:

- 20 µg of pFUSE-hIgG1e3-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

• **hIgG1e3-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 FcγRI through two areas in the CH2 domain. IgG1 Fc binds with a high affinity to FcγRI while IgG2 and IgG4 bind with low affinity. Substitution into human IgG1 of IgG2 residues at positions 233-236 and IgG4 residues at positions 327, 330 and 331 greatly reduced ADCC and CDC^{2,3}. IgG1e3 contains the hinge and residues at positions 233-236 from IgG2. Substitutions at positions 327, 330 and 331 were performed by PCR.

• **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. Armour KL. et al., 1999. Recombinant human IgG molecules lacking Fcγ receptor I binding and monocyte triggering activities. Eur J Immunol. 29(8):2613-24.

3. 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.

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

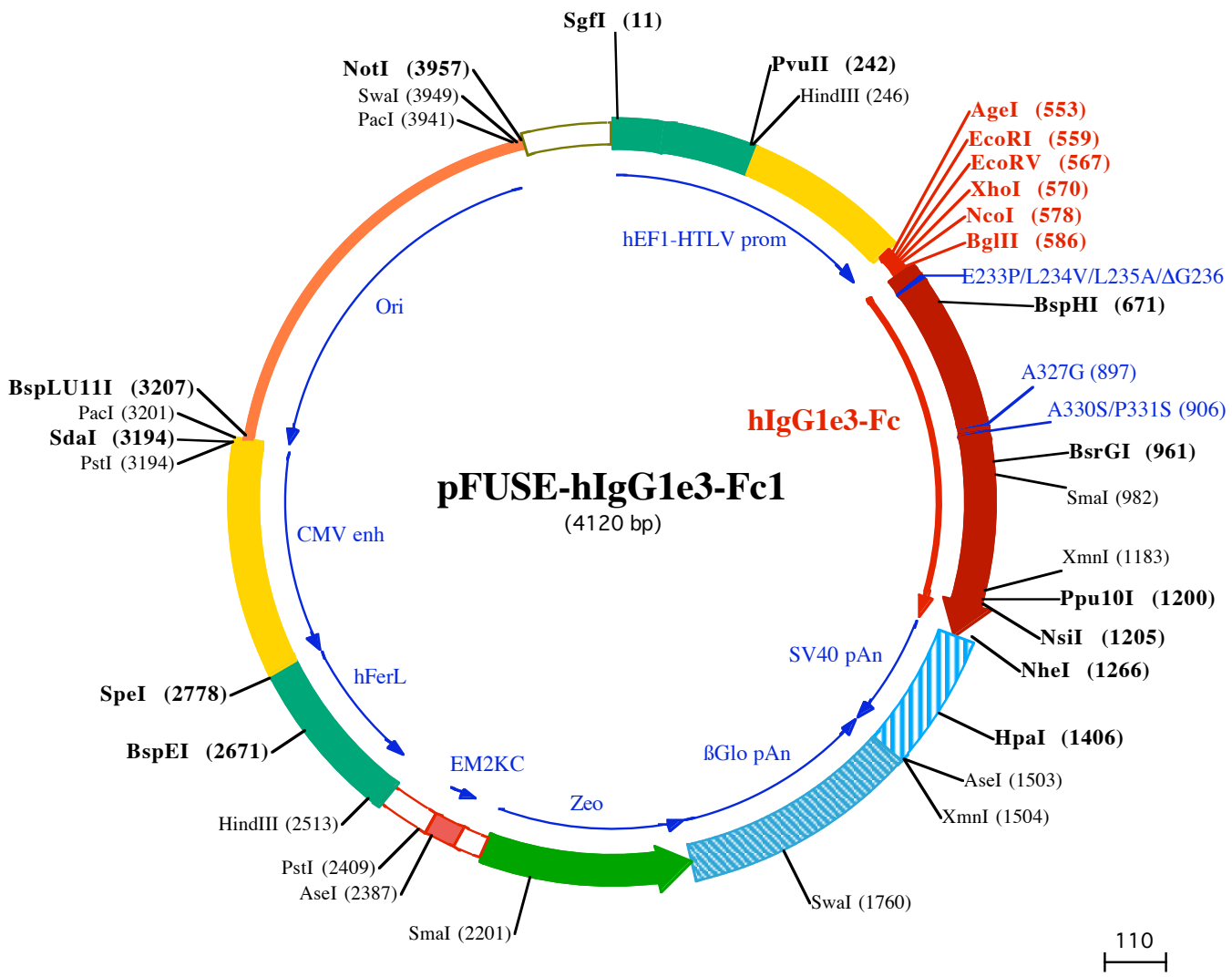
This antibiotic can be used for *E. coli* at 25 µg/ml in liquid or solid media and at 50-200 µg/ml to select Zeocin™-resistant mammalian cells.

RELATED PRODUCTS

Product	Catalog Code
Zeocin™	ant-zn-1

TECHNICAL SUPPORT

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1 GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGGTCGGCAATTGAACGGGTGCCTA
101 GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGACTGGCTCCGCCTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

HindIII (246)
201 GTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCTTCACGCGCCCGCCCTACCTGAGGCC
301 GCCATCCACGCGGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCTGAACTCGCTCCGCCGTCTAGGTAAGTTAAAGCTCAGGTCGAGACC
401 GGGCCTTTGTCGGCGCTCCCTTGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCTGACCTGCTTGTCTCAACTCTACGCTTTGTTTCGTTT

EcoRI (559) XhoI (570) BglIII (586)
AgeI (553) EcoRV (567) NcoI (578)
501 TCTGTTCTGCGCGTTACAGATCCAAGCTGTGACCGGCGCTACCTGAGATCACCGGTGAATTCGATATCTCGAGCACCATGGTTAGATCTGTGGAGTGC
1 Val I Gl u Cys

E233P/L234V/L235A/AG236 (618) BspHI (671)
601 CCACCTTGCCAGCACCACTGTGGCAGGACCTTCAGTCTTCTCTTCCCCAAAACCAAGGACACCTCATGATCTCCCGGACCCCTGAGGTACAT
4 P ro P ro Cys P ro A l a P ro P ro Va l A l a G l y P ro Ser Va l P he L eu P he P ro P ro Lys P ro Lys Asp Thr L eu M et I l e Ser Arg Thr P ro G l u Va l Thr C
701 GCGTGGTGGTGGAGCTGAGCCACGAAGACCTGAGGTCAAGTCAACTGGTACGTGGACGGCTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGA
37 y s Va l Va l Va l Asp Va l Ser H i s G l u Asp P ro G l u Va l L ys P he Asn T r p T yr Va l Asp G l y Va l G l u Va l H i s Asn A l a L ys Thr L ys P ro Arg G l u G l
801 GCAGTACAACAGCACGTACCGTGTGGTCAGCGTCTCACCGTCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTCAAGGTCTCCAACAAAGGC
70 u G l n Tyr Asn Ser Thr Tyr Arg Va l Va l Ser Va l L eu Thr Va l L eu H i s G l n Asp T r p L eu Asn G l y L ys G l u Tyr L ys Cys L ys Va l Ser Asn L ys G l y
A327G (897)

A330S/P331S (906) BsrGI (961) SmaI (982)
901 CTCCCATCCATCCATCGAGAAAACCATCTCCAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCATCCCGGAGGAGATGACCAAGA
104 L eu P ro Ser Ser I l e G l u L ys Thr I l e Ser L ys A l a L ys G l y G l n P ro Arg G l u P ro G l n Va l Tyr Thr L eu P ro P ro Ser Arg G l u G l u M et Thr L ys A
1001 ACCAGTCTAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCACGACATCGCCGTGGAGTGGGAGAGCAATGGGACGCGGAGAACAACTACAAGACCAC
137 s n G l n Va l Ser L eu Thr Cys L eu Va l L ys G l y P he Tyr P ro Ser Asp I l e A l a Va l G l u T r p G l u Ser Asn G l y G l n P ro G l u Asn Asn Tyr L ys Thr Th
XmnI (1183)
1101 GCCTCCCGTGTGGACTCCGACGGCTCTTCTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTG
170 r P ro P ro Va l L eu Asp Ser Asp G l y Ser P he P he L eu Tyr Ser L ys L eu Thr Va l Asp L ys Ser Arg T r p G l n G l n G l y Asn Va l P he Ser Cys Ser Va l
Ppu10I (1200)
NsiI (1205) NheI (1266)
1201 ATGCATGAGGCTCTGCACAACCACTACACGAGAAGAGCCTCTCCCTGTCTCCGGTAAATGAGTGCTAGCTGGCCAGACATGATAAGATACATTGATGA
204 M et H i s G l u A l a L eu H i s Asn H i s Tyr Thr G l n L ys Ser L eu Ser L eu Ser P ro G l y L ys
1301 GTTTGGACAACCACTAGAAATGCAGTGAATAATGCTTTATTTGTGAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAA

HpaI (1406) XmnI (1504)
1401 CAAGTTAAACAACAACAATTGCATTCAATTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGG

AseI (1503)
1501 AATTAATTCTAAAATACAGCATAGCAAACTTTAACCTCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGCC
1601 TGTTCGAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTAAGATATAGTGTATTTCCCAAGGTTTGAAGTCTTCTCATTTCCTT

SwaI (1760)
1701 ATGTTTTAAATGCACTGACCTCCACATTCCTTTTTAGTAAAATATTCAGAAAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTATTAGG
1801 CAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATGGACAGCAAGAAAGC
1901 GAGCTTCTAGCTTATCTCAGTCTGCTCTGCCACAAAGTGACAGCAGTTGCCGGCCGGTCCGCGAGGGCGAACTCCCGCCCCACGGCTGCTCGC
125 A s p G l n G l u G l u A l a Va l P he H i s Va l Cys Asn G l y A l a P ro Asp Arg L eu A l a P he G l u Arg G l y T r p P ro G l n G l u G l
2001 CGATCTCGGTCATGGCCGCGCCGAGGCGTCCCGAAAGTTCTGGACACGACCTCCGACACTCGGCGTACAGCTCGTCCAGGCGCGCACCCACACCCA
97 y l l e G l u Th r M et A l a P ro G l y Ser A l a Asp Arg P he Asn Thr Ser Va l Va l G l u Ser T r p G l u A l a Tyr L eu G l u Asp L eu G l y Arg Va l T r p Va l T r p
SmaI (2201)
2101 GGCCAGGGTGTGTCCGGCACCACTGGTCTGGACCGCTGATGAACAGGGTACGTCGTCGCCGACACACCGGCGAAGTCTCTCCACGAAGTCC
64 A l a L eu Thr Asn Asp P ro Va l Va l G l n Asp G l n Va l A l a Ser I l e P he L eu Thr Va l Asp Asp Arg Va l Va l G l y A l a P he Asp Asp G l u Va l P he Asp A
2201 CGGGAGAACCCGAGCCGGTCCGTCAGAACTCGACCGCTCCGGCAGCTCGCGCGGTGAGCACCGAAGCGCACTGGTCAACTTGGCCATGATGGCTC
30 r g Ser P he G l y L eu Arg Asp Thr T r p P he G l u Va l A l a G l y A l a Va l Asp Arg A l a Thr L eu Va l P ro Va l A l a Ser Thr L eu L ys A l a M et
AseI (2387)
2301 CTCTgtcaggagaggaagagaagaggttagtacaattgCTATAGTGAGTTGTATTACTATGCAGATATACTATGCCAATGATTAATTGTCAAAC

PstI (2409)
2401 AGGGCTGCAGggttcatagtgcacttttctgcaactgccccatctctgccccactttccagcatagacagtcagtgacttacCAAACCTCACAGGA
HindIII (2513)
2501 GGGAGAAGGCAGAAGCTTGAGACAGACCCCGCGGACCGCCAAGCTGCGAGGGGACGTGGCTAGGGCGGCTTTTTATGGTGCGCCGGCCCTCGGAGGCA

BspEI (2671)
2601 GGGCGCTCGGGGAGGCTAGCGGCAATCTCGGTGGCAGGAGGGGGCCGAAGCCGTGCCTGACCAATCCGGAGCACATAGGAGTCTCAGCCCCCG

2701 CCCAAAGCAAGGGGAAGTCACGCGCCTGTAGCGCCAGCGTGTGTGAAATGGGGCTTGGGGGGTTGGGGCCCTGACTAGTCAAAAACAAACTCCCATT
2801 GACGTCAATGGGGTGGAGACTTGAAATCCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATG
2901 ACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTACCCTGTCATTGACGTCAATAGGGG
3001 GCGTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCCTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTAC

SpeI (2778)

PacI (3201)

3101 TATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGTCGTTGGGCGGTGAGCCAGGCGGGCCATTTACCCTAAGTTATGTAACGCCTGCAGGTTAA

PstI (3194)

SdaI (3194)

BspLU11I (3207)

3201 TTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAGGCGCGTGTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCA
3301 CAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACAGGCGTTTTCCCTGGAAAGTCCCTCGTGCCTCTCCTGTTCCG
3401 ACCCTGCCGTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCCG
3501 TTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTAGCCCGACCGCTGCGCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGA
3601 CTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCTAC
3701 ACTAGAAGAACAGTATTTGGTATCTGCCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTA
3801 GCGGTGGTTTTTTTGTTCGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAA

PacI (3941) SmaI (3949) NotI (3957)

3901 CGAAAACACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTG
4001 GTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAACGAAACAAACAAACTAGCAAATAGGCTGTCCCAGTGCAAGTGCAGGTGC
4101 CAGAACATTTCTCTATCGAA