

pFUSE-hIgG2e1-Fc1

Plasmid containing a human engineered IgG2 Fc region

Catalog # pfc1-hg2e1

For research use only

Version 20K05-MM

PRODUCT INFORMATION

Content:

- 20 µg of pFUSE-hIgG2e1-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. 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

- **hIgG2e1-Fc (human IgG2 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 human IgG2 Fc mediates low CDC and very low ADCC. To further reduce its cytotoxicity, the Fc region was engineered to reduce its binding affinity to the first component of the complement C1q by substituting the lysine residue at position 322 by an alanine².
- **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.
- **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.

References:

1. Carter PJ., 2006. Potent antibody therapeutics by design. Nature Reviews Immunology. Advance online publication.
2. Idusogie EE. *et al.*, 2000. Mapping of the C1q binding site on rituxan, a chimeric antibody with a human IgG1 Fc. J Immunol. 164(8):4178-84. .
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

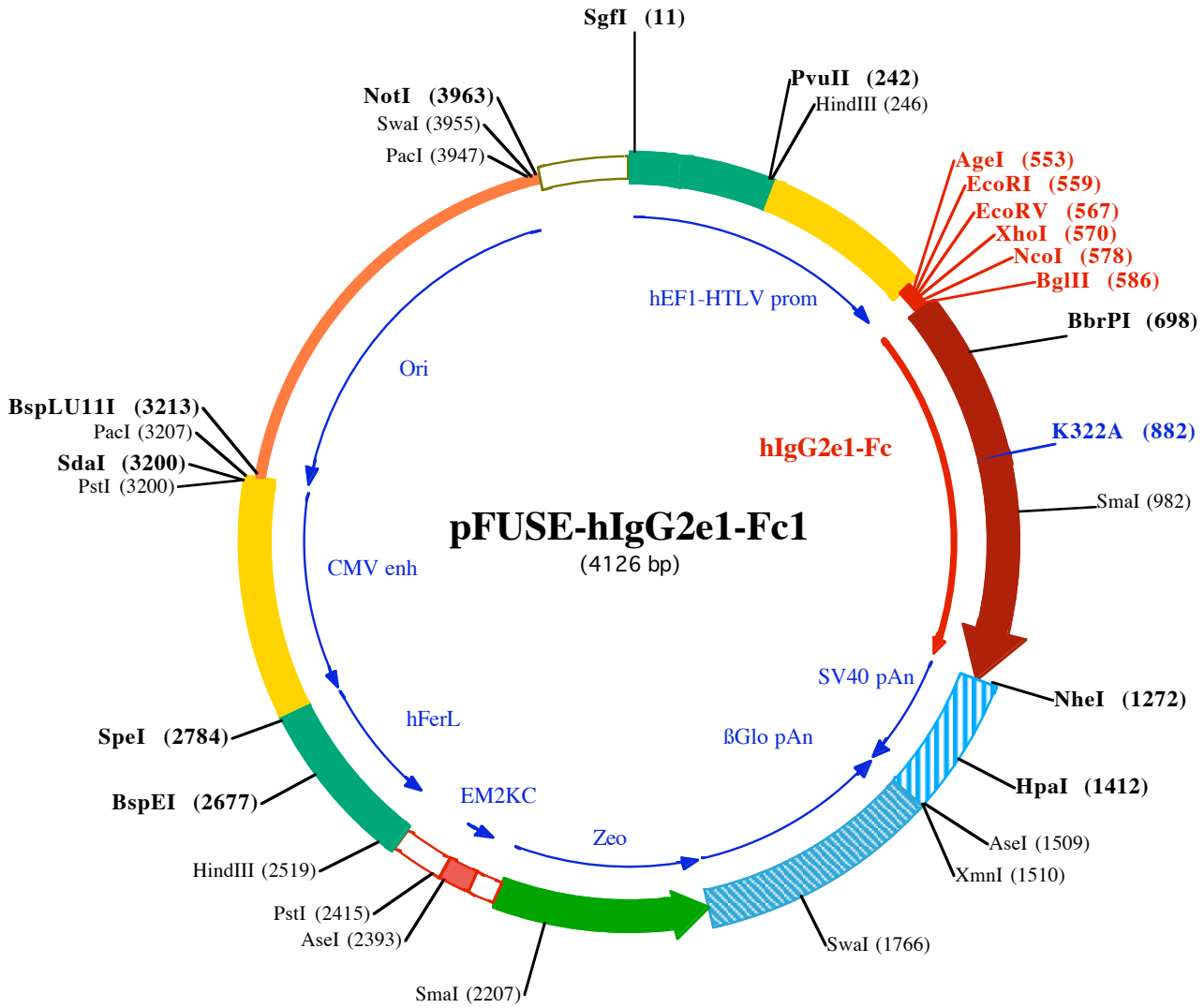
This antibiotic can be used for *E. coli* at 25 µg/ml in liquid or solid media and at 50-200 µg/ml to select Zeocin™-resistant mammalian cells.

RELATED PRODUCTS

Product	Catalog Code
Zeocin™	ant-zn-1

TECHNICAL SUPPORT

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SgfI (11)
1 GGATCTGCGATCGCTCCGGTGCCTGTCAGTGGGCGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGGTGGCAATTGAACGGTGCCTA
101 GAGAAGGTGGCGGGGTAACACTGGGAAAGTGATGTCGTGTAAGTGGCTCCGCTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCC

HindIII (246)
PvuII (242)
201 GTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCTACCTGAGGGC
301 GCCATCCACGCGGGTTGAGTGCAGTCTGCGCTCCCGCTGTGGTGCCTCCTGAAGTGCCTCCGCTCTAGGTAAGTTAAAGCTCAGGTCGAGACC
401 GGGCCTTTGTCCGGGCTCCCTTGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCTGACCCTGCTTGTCAACTCTACGTCTTTGTTTCGTTT

EcoRI (559) XhoI (570) BglII (586)
AgeI (553) EcoRV (567) NcoI (578)
501 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGGCTACCTGAGATCACCGTGAATTCGATATCTCGAGCACCATGGTTAGATCTGTGGAGTGC
1▶Val Gl uCys
BbrPI (698)
601 CCACCTTGCCAGCACCACTGTGGCAGGACCTTCAGTCTTCTCTTCCCCCAAACCAAGGACACCTGATGATCTCCAGAACCCCTGAGGTCACGT
4▶ProProCysProAlaProProValAlaGlyProSerValPheLeuPheProProLysProLysAspThrLeuMetIleSerArgThrProGluValThrC
701 GCGTGGTGGTGGAGCTGAGCCAGAACCCGAGGTCAGTTCAGTGTACGTGGACGGCATGGAGGTGCATAATGCCAAGACAAAGCCACGGGAGGA
37▶ysValValValAspValSerHisGluAspProGluValGlnPheAsnTrpTyrValAspGlyMetGluValHisAsnAlaLysThrLysProArgGluGlu
K322A (882)
801 GCAGTTCAACAGCACGTTCCGTGTGGTCAAGCTCCTACCGTCTGACAGGACTGGTGAACGGCAAGGAGTACAAGTGGCGGCTCCAAACAAAGGC
70▶uGlnPheAsnSerThrPheArgValValSerValLeuThrValValHisGlnAspTrpLeuAsnGlyLysGluTyrLysCysAlaValSerAsnLysGly
SmaI (982)
901 CTCCCAGCCCCATCGAGAAAACCATCTCCAAAACAAAGGGCAGCCCCGAGAACCACAGGTGTACACCTGCCCCATCCCGGAGGAGATGACCAAGA
104▶LeuProAlaProIleGluLysThrIleSerLysThrLysGlyGlnProArgGluProGluValTyrThrLeuProProSerArgGluGluMetThrLysA
1001 ACCAGTGCAGCTGACCTGCTGGTCAAAGGCTTCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGACGGGAGAACAACTACAAGACCAC
137▶snGlnValSerLeuThrCysLeuValLysGlyPheTyrProSerAspIleAlaValGluTrpGluSerAsnGlyGlnProGluAsnAsnTyrLysThrTh
1101 ACCTCCATGTGGACTCCGACGGCTCTTCTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGCTTCTCATGCTCCGTG
170▶rProProMetLeuAspSerAspGlySerPhePheLeuTyrSerLysLeuThrValAspLysSerArgTrpGluGlnGluAsnValPheSerCysSerVal
NheI (1272)
1201 ATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCGTCTCCGGTAAATGAgTgccagGCTAGCTGGCCAGACATGATAAGATACAT
204▶MetHisGluAlaLeuHisAsnHisTyrThrGlnLysSerLeuSerLeuSerProGlyLys●●●
1301 TGATGAGTTTGACAAACCACAAC TAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGC

HpaI (1412)
1401 AATAACAAGTTAACAAACAATTCATTTCATTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAAATGTG

AseI (1509)
XmnI (1510)
1501 GTATGGAATTAATTTAAAATACAGCATAGCAAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATC
1601 AGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTCATGGAGTTAAGATATAGTGTATTTCCCAAGGTTTGAAGTCTCTTCAT

SwaI (1766)
1701 TTCTTTATGTTTTAAATGCAGCTGACCTCCACATTCCCTTTTTAGTAAAATATTAGAAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTT
1801 ATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTAAATAGAAATTGGACAGCAA
1901 GAAAGCGAGCTTCTAGCTTATCTCAGTCTGCTCTGCCCACAAAGTGCACGAGTTGCCGGCCGGTCCGCGAGGGCGAACTCCCGCCCCACGGCT
125▶●●●AspGlnGluGluAlaValPheHisValCysAsnGlyAlaProAspArgLeuAlaPheGluArgGlyTrpProGlu
2001 GCTCGCGATCTCGGTTCATGGCCGGCCGGAGGCTCCCGAAAGTTCGTGGACACGACTCCGACCACTCGCGGTACAGCTCGTCCAGGCCCGCACCCCA
99▶nGluGlyIleGluThrMetAlaProGlySerAlaAspArgPheAsnThrSerValValGluSerTrpGluAlaTyrLeuGluAspLeuGlyArgValTrp
2101 CACCCAGGCCAGGGTGTTCGCGCACCACTGGTCTGGACCGCGCTGATGAACAGGGTACGTCGTCCCGACCACACCGGCAAGTCTCTCCACG
66▶ValTrpAlaLeuThrAsnAspProValValGlnAspGlnValAlaSerIlePheLeuThrValAspAspArgValValGlyAlaPheAspAspGluValP
SmaI (2207)
2201 AAGTCCCGGAGAACCCGAGCCGGTCCGTCAGAACTCGACCGCTCCGGCGACGTCGCGCGGGTGGACCCGGAACGGCACTGGTCAACTTGGCCATGA
32▶heAspArgSerPheGlyLeuArgAspThrTrpPheGluValAlaGlyAlaValAspArgAlaThrLeuValProValAlaSerThrLeuLysAlaMet
AseI (2393)
2301 TGGCTCCTCctgtcaggagaggaagagaagaaggttagtacaattgCTATAGTGAGTTGTATTACTATGCAGATATACTATGCCAATGATTAATTGT

PstI (2415)
2401 CAAACTAGGGTGCAGgggttcatagtgccacttttctgactgcccactctcctgcccacccttcccaggcatagacagtcagtgacttacCAAACCTC

HindIII (2519)
2501 ACAGGAGGGAGAAGGCAGAAGCTTGAGACAGACCCGCGGACCGCCAACTGCGAGGGACGTGGCTAGGGCGGCTTCTTTTATGGTGCGCCGCCCTCG

BspEI (2677)
2601 GAGGCAGGGCGCTCGGGGAGGCCTAGCGCCAATCTGCGGTGGCAGGAGGGCGGCGAAGGCCGTGCTGACCAATCCGGAGCACATAGGAGTCTCACG

SpeI (2784)
2701 CCCCCGCCAAAGCAAGGGGAAGTACGCGCTGTAGCGCCAGCGTGTGTGAAATGGGGCTTGGGGGGTTGGGGCCCTGACTAGTCAAAAACAACT

2801 CCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATA
2901 GCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCATAAGGTACGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAA
3001 TAGGGGGCGTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGG

PstI (3200)
SdaI (3200)

3101 CGTTACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGGTCTGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGCA

PacI (3207) BspLU111 (3213)

3201 GGTAAATTAAGAACATGTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAAAGCCGCTTGTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGA
3301 GCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAGATACCAGCGTTTTCCCTGGAAGCTCCCTCGTGGCTCTCCT
3401 GTTCCGACCCTGCCGTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGT
3501 AGGTCGTTGCTCCAAGCTGGGCTGTGTGCAGAACCCCGTTAGCCGACCGCTGCGCTTATCCGGTAACATCGTCTTGAGTCCAACCCGGTAAG
3601 ACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTAC
3701 GGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCG
3801 CTGGTAGCGGTGGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCA

PacI (3947) SmaI (3955) NotI (3963)

3901 GTGGAACGAAAACACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGAATAAAATATCTTTATTTTATTACATCTGT
4001 GTGTTGGTTTTTTGTGTAATCGTAACTAACATACGCTCTCCATCAAACAAAACGAAACAAAACAACTAGCAAATAGGCTGTCCCAGTGCAAGTGC
4101 AGGTGCCAGAACATTTCTCTATCGAA