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 dispays 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 activity[1]. 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

• **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 promoter[4] and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat[5]. 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 mRNA[6].

• **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*.

• **ßGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription[7].

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 Fcgamma 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 H_2O. Store resuspended plasmid at -20 °C.

Plasmid amplification and cloning

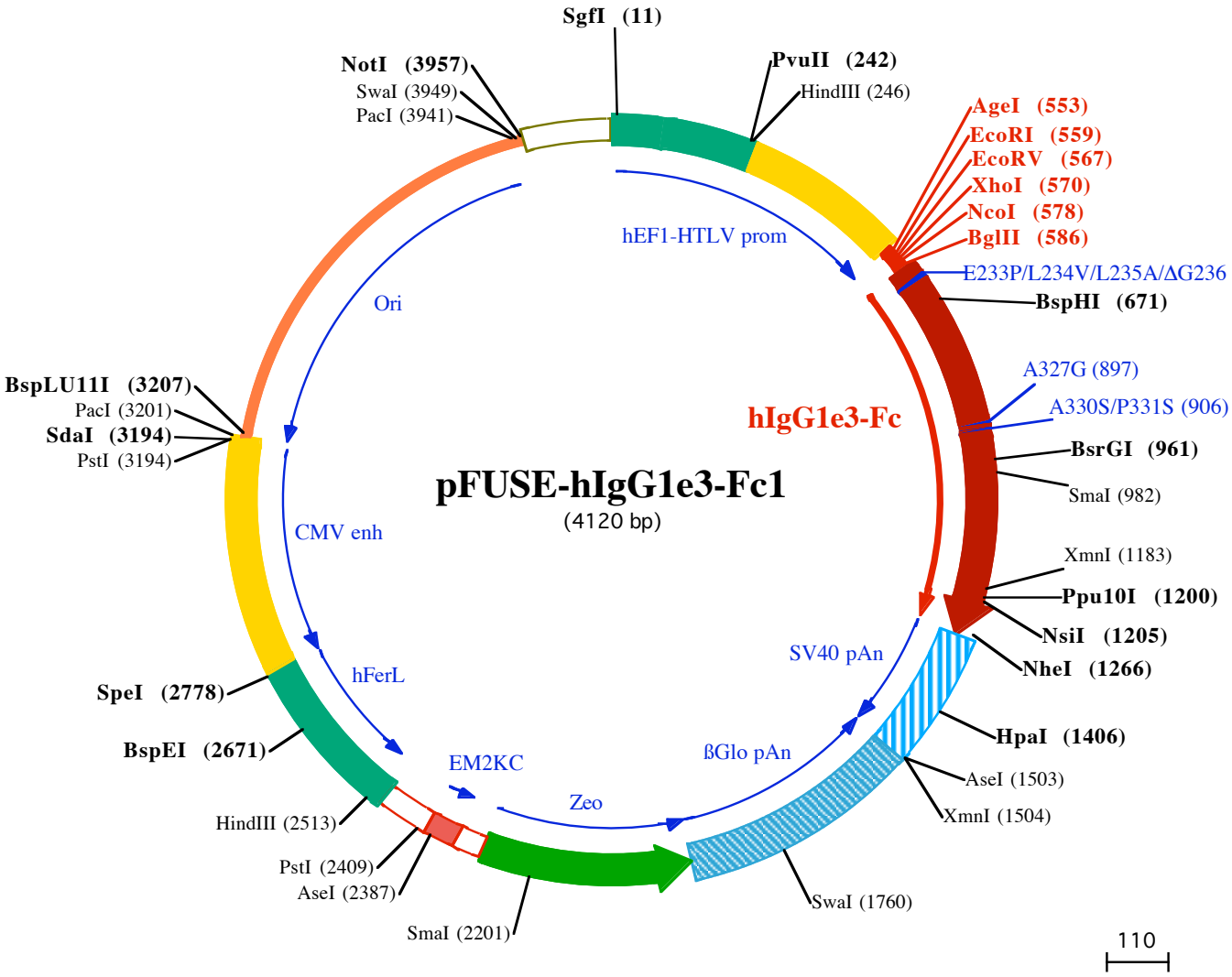
Plasmid amplification and cloning can be performed in *E. coli* GT116 or in other commonly used laboratory *E. coli* strains, such as DH5α.

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


pFUSE-hIgG1e3-Fc1
(4120 bp)
hIgG1e3-Fc
SgfI (11)
PvuII (242)
HindIII (246)
AgeI (553)
EcoRI (559)
EcoRV (567)
XhoI (570)
NcoI (578)
BglII (586)
E233P/L234V/L235A/ΔG236
BspHI (671)
A327G (897)
A330S/P331S (906)
BsrGI (961)
SmaI (982)
XmnI (1183)
Ppu10I (1200)
NsiI (1205)
NheI (1266)
HpaI (1406)
AseI (1503)
XmnI (1504)
SwaI (1760)
SV40 pAn
ßGlo pAn
Zeo
EM2KC
hFerL
hEF1-HTLV prom
Ori
CMV enh
NotI (3957)
SwaI (3949)
PacI (3941)
BspLU11I (3207)
PacI (3201)
SdaI (3194)
PstI (3194)
SpeI (2778)
BspEI (2671)
HindIII (2513)
PstI (2409)
AseI (2387)
SmaI (2201)
110


SgfI (11)
HindIII (246)
PvuII (242)
EcoRI (559) XhoI (570) BglII (586)
AgeI (553) EcoRV (567) NcoI (578)
E233P/L234V/L235A/ΔG236 (618)
BspHI (671)
A327G (897)
A330S/P331S (906)
BsrGI (961)
SmaI (982)
XmnI (1183)
Ppu10I (1200)
NsiI (1205)
NheI (1266)
HpaI (1406)
XmnI (1504)
AseI (1503)
SwaI (1760)
SmaI (2201)
AseI (2387)
PstI (2409)
HindIII (2513)
BspEI (2671)


SpeI (2778)
2701 CCCCAAAGCAAGGGGAAGTCACGCGCCTGTAGCGCCAGCGTGTTGTGAAATGGGGGCTTGGGGGGGTTGGGGCCCTGACTAGTCAAAACAAACTCCCATT
2801 GACGTCAATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAACCGCTATCCACGCCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATG
2901 ACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGG
3001 GCGTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTAC
PacI (3201)
PstI (3194)
SdaI (3194)
3101 TATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGCAGGTTAA
BspLU11I (3207)
3201 TTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCA
3301 CAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCG
3401 ACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCG
3501 TTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGA
3601 CTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTAC
3701 ACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTA
3801 GCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAA
PacI (3941) SwaI (3949) NotI (3957)
3901 CGAAAACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTG
4001 GTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACAAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGC
4101 CAGAACATTTCTCTATCGAA