

pFUSE-mIgG3-Fc1

Plasmid containing a mouse IgG3 Fc region

Catalog # pfuse-mg3fc1

For research use only

Version 20K04-MM

PRODUCT INFORMATION

Content:

- 20 µg of pFUSE-mIgG3-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. Three murine isotypes are available: IgG1, IgG2a and IgG3. The Fc region mediates effector functions, such as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). In ADCC, the Fc region of an antibody binds to Fc receptors (FcγRs) on the surface of immune effector cells such as natural killers and macrophages, leading to the phagocytosis or lysis of the targeted cells. In CDC, the antibodies kill the targeted cells by triggering the complement cascade at the cell surface. IgG isoforms exert different levels of effector functions increasing in the order of mIgG1<mIgG3<mIgG2a.

PLASMID FEATURES

- **mIgG3 Fc (mouse):** 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 of mouse IgG3 mediates high ADCC and low CDC¹.
- **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter² and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat³. 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 mRNA⁴.
- **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*.
- **BGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription⁵.

References:

1. Dangl JL. *et al.*, Segmental flexibility and complement fixation of genetically engineered chimeric human, rabbit and mouse antibodies. EMBO J. 7(7):1989-94.
2. 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.
3. 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.
4. 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.
5. 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

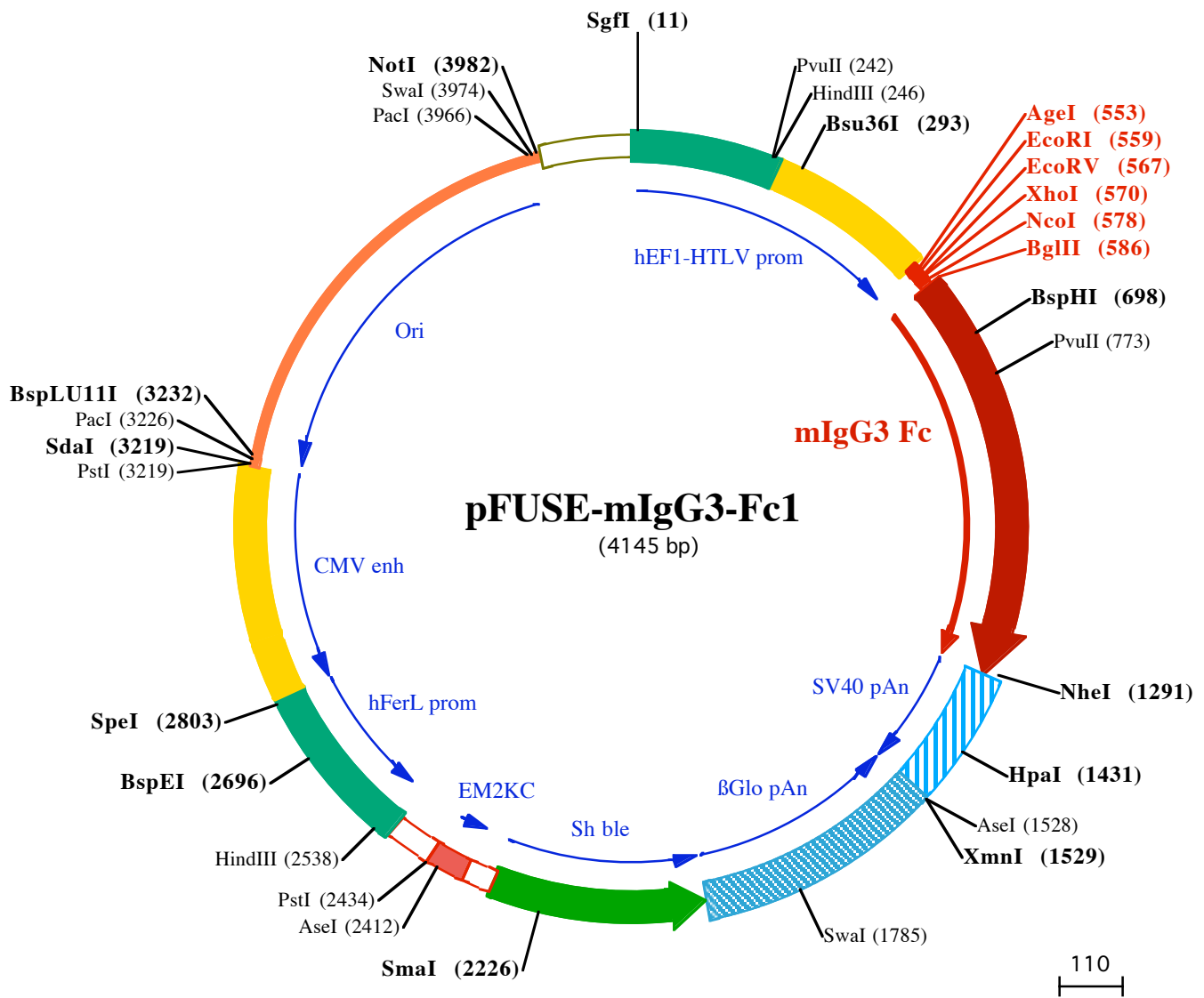
This antibiotic can be used for *E. coli* at 25 µg/ml in liquid or solid media and at 50-200 µg/ml to select Zeocin™-resistant mammalian cells.

RELATED PRODUCTS

Product	Catalog Code
Zeocin™	ant-zn-1

TECHNICAL SUPPORT

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SgfI (11)
1 GGATCTGCGATCGCTCCGGTGCCTGAGTGGCGAGCGCACATCGCCACAGTCCCGAGAAGTTGGGGGAGGGTTCGCAATTGAACGGTGCCTA
101 GAGAAGGTGGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGGC

HindIII (246) PvuII (242) Bsu36I (293)
201 GTGAACGTTCTTTTTGCAACGGGTTTCCGCGCAGAACACAGCTGAAGCTTCAGGGGCTCGCATCTCTCCTTACGCGCCCGCCGCCCTACCTGAGGCC
301 GCCATCCACGCGGGTTGAGTCCGCTTCTGCCGCTCCCGCTGTGGTGCCTCTGAAGTCCGCTCCGCGCTAGGTAAGTTTAAAGCTCAGGTCGAGACC
401 GGGCTTTGTCGGCGCTCCCTTGAGCCTACCTAGACTCAGCGGCTCTCCACGCTTTCCTGACCTGCTTGTCTCAACTCTACGCTTTTGTTCGTTT

EcoRI (559) XhoI (570) BglII (586)
AgeI (553) EcoRV (567) NcoI (578)
501 TCTGTTCTGCGCGTTACAGATCCAAGCTGTGACCGCGCTACCTGAGATCACCGTGAATTCGATATCTCGAGCACCATGGTTAGTCTCTCTAGAATA
1ProArgIle
BspHI (698)
601 CCCAAGCCAGTACCCCGAGTTCTTCATGCCACCTGGTAACATCTTGGGTGGACCATCCGCTCTCATCTTCCCCCAAGCCCAAGGATGCACCTCA
4ProLysProSerThrProProGlySerSerCysProProGlyAsnIleLeuGlyGlyProSerValPheIlePheProLysProLysAspAlaLeuM
PvuII (773)
701 TGATCTCCCTAACCCCAAGTTACGTGTGGTGGTGGATGTGAGCGAGGATGACCCAGATGTCCATGTGAGCTGGTTGTGGCAACAAAGAGTACA
37e t l eSerLeuThrProLysValThrCysValValValAspValSerGluAspAspProAspValHisValSerTrpPheValAspAsnLysGluValHis
801 CACAGCCTGGACACAGCCCGTGAAGCTCAGTACAACAGTACTTCCGAGTGGTTCAGTGCCTCCCATCCAGCACAGGACTGGATGAGGGGCAAGGAG
70sThrAlaTrpThrGlnProArgGluAlaGlnTyrAsnSerThrPheArgValValSerAlaLeuProIleGlnHisGlnAspTrpMetArgGlyLysGlu
901 TTCAAATGCAAGGTCAACAACAAGCCCTCCAGCCCCATCGAGAGAACCATCTCAAAACCCAAAGGAGAGCCAGACCTCAAGTATACACCATA
104PheLysCysLysValAsnAsnLysAlaLeuProAlaProIleGluArgThrIleSerLysProLysGlyArgAlaGlnThrProGluAlaTyrThrIleP
1001 CCCCACCTCGTGAACAAATGTCCAAGAAGAAGTTAGTCTGACCTGCCTGGTCAACCACTTCTTCTGAAAGCCATCAGTGTGGAGTGGGAAAGAACGG
137roProProArgGluGlnMetSerLysLysLysValSerLeuThrCysLeuValThrAsnPhePheSerGluAlaIleSerValGluTrpGluArgAsnG
1101 AGAAGTGGAGCAGGATTACAAGAACAACCTCCACCATCCTGGACTCAGATGGGACCTACTTCTCTACAGCAAGCTCACTGGGTGATACAGACAGTTGGTTG
170yGluLeuGluGlnAspTyrLysAsnThrProProIleLeuAspSerAspGlyThrTyrPheLeuTyrSerLysLeuThrValAspThrAspSerTrpLeu
NheI (1291)
1201 CAAGGAGAAATTTTACCTGCTCCGTGGTGCATGAGGCTCTCCATAACCACCACACAGAAGAACCCTGCTCGCTCCCTGGTAAATGAGCTAGCTGGC
204GlnGlyGluIlePheThrCysSerValValHisGluAlaLeuHisAsnHisHisThrGlnLysAsnLeuSerArgSerProGlyLys
1301 CAGACATGATAAGATACATTGATGAGTTGGACAAACCAACTAGAATGCAAGTGAAGAAATGCTTTATTTGTAAATTTGTGATGCTATTGCTTTATT

HpaI (1431)
1401 TGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTTCATTTTATGTTTCAGGTTTCAGGGGAGGTTGGGGAGTTTAAAAAGCAAG

AseI (1528) XmnI (1529)
1501 TAAAACCTCTACAATGTGGTATGGAATTAATTTCAAATAACAGCATAGCAAACTTTAACTCCAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGA
1601 TGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTGACGCTCACCTTCTTTCATGGAGTTAAGATATAGTGTATTTTCCCAAG

SwaI (1785)
1701 GTTTGAACTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCACATTCCTTTTTAGTAAATATTCAGAAATAATTTAAATACATCATTGCA
1801 ATGAAATAAATGTTTTTATTAGGCAGAATCCAGATGCTCAAGCCCTTCAATAATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGAACCTTT
1901 AATAGAAATGGACAGCAAGAAAGCGAGCTTAGCTTATCTCAGTCTGCTCTGCCACAAGTGCACGAGTTGCGCGCGGGTTCGCGCAGGGCG
125AspGlnGluGluAlaValPheHisValCysAsnGlyAlaProAspArgLeuAlaP
2001 AACTCCCGCCCGCGCTGCTCGCGATCTCGGTTCATGGCCGGCCGAGGCGCTCCGGAAAGTTCTGGACACGACTCCGACCTCGCGCTACAGCT
105heGluArgGlyTrpProGlnGluGlyIleGluThrMetAlaProGlySerAlaAspArgPheAsnThrSerValValGluSerTrpGluAlaTyrLeuG
2101 CGTCCAGGCGCGCACCCACACCAGGCCAGGGTGTTCGGCACCACTGGTCTGACCGCGCTGATGAACAGGGTACGTCGTCGGGACCAACC
72uAspLeuGlyArgValTrpValTrpAlaLeuThrAsnAspProValValGlnAspGlnValAlaSerIlePheLeuThrValAspAspArgValValGly
SmaI (2226)
2201 GCGAAGTCTCTCCACGAAGTCCCGGAGAACCCGAGCCGCTCGTCCAGAACTCGACCGCTCCGGCGACGTCGCGCGGGTGAACCCGGAACCGCA
39AlaPheAspAspGluValPheAspArgSerPheGlyLeuArgAspThrTrpPheGluValAlaGlyAlaValAspArgAlaThrLeuValProValAlaS
2301 CTGGTCAACTTGCCATGATGGCTCCTCctgtcaggagaggaagaagaaggttagtacaattgCTATAGTGAGTTGATTATACTATGCAGATATAC
5erThrLeuLysAlaMet
AseI (2412) PstI (2434)
2401 TATGCCAATGATTAATGTCAAACCTAGGCTGCAgggttcatagtgccacttttctgactgccccatctctgccccctttccaggcatagacag
HindIII (2538)
2501 tcagtacttacAAAACCTCACAGGGGAGAAGCGAAGCTTGAGACAGCCCGGGACCGCGAACTGCGAGGGGAGCTGGCTAGGCGGCTTCTTT

BspEI (2696)
2601 TATGGTCCCGCCCTCGGAGGCAGGGCGCTCGGGAGGCTAGCGCCAACTGCGGTGGCAGGAGCCGGGCGGAGGCGCTGACCAATCCGG
2701 AGCACATAGGAGTCTACGCCCCCGCCCAAGCAAGGGAAAGTACGCGCTGTAGCGCCAGCGTGTGTGAAATGGGGGCTTGGGGGGTGGGGCC

SpeI (2803)
2801 TGACTAGTCAAAACAACTCCCATGACGTCAATGGGTGGAGACTTGGAAATCCCGTGAGTCAAACCGCTATCCACGCCATTGATGACTGCCAAA
2901 CCGCATCATCTGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAAGTCACTGACTGGGCATAATGCCAGGCGGCCAT
3001 TTACCGTCATTGACGTCAATAGGGGGCTACTTGGCATATGATACACTTGTACTGCCAAGTGGGCGTTTACCCTAAATCTCCACCCATTGACGCT
3101 AATGGAAAGTCCCTATTGGCGTACTATGGAAACATACGTCTATTATGACGTCAATGGCGGGGCTGTTGGCGGTGACGAGCGGGCCATTTACCGT

PacI (3226)

PstI (3219)

SdaI (3219) **BspLU11I (3232)**

3201 AAGTTATGTAACGCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAGGCCAGGAACCGTAAAAAGGCCGTTGCTGGCGTTTTCCATA
 3301 GGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAGATACCAGGCGTTTTCCCCCTGGAAG
 3401 CTCCCTCGTGCCTCTCCTGTTCCGACCCGCTTACCGGATACCTGTCGCGCTTTCTCCCTTCGGGAAGCGTGCGCTTTCTCATAGCTCACGCTGT
 3501 AGGTATCTCAGTTCGGTGTAGGTCGTTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTTCAGCCCAGCCGCTGCGCTTATCCGGTAACTATCGTC
 3601 TTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTT
 3701 GAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGA
 3801 TCCGCAAACAACACCGCTGGTAGCGGTGGTTTTTTTGTGGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTT

PacI (3966) SmaI (3974) **NotI (3982)**

3901 CTACGGGCTGACGCTCAGTGAACGAAACTCACGTTAAGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCCAATAAAATATCT
 4001 TTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACAACTAGCAAATAGG
 4101 CTGTCCCAGTGAAGTGCAGGTGCCAGAACATTTCTCTATCGAA