

pFUSE-mIgG1-Fc1

Plasmid designed for the construction of Fc-Fusion proteins

Catalog # pfuse-mg1fc1

For research use only

Version # 10E18-MMv10

PRODUCT INFORMATION

Content:

- 20 µg of pFUSE-mIgG1-Fc1 plasmid provided as lyophilized DNA
- 4 pouches of *E. coli* Fast-Media® Zeo (2 TB and 2 Agar)

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 *E. coli* Fast-Media® Zeo at room temperature. Fast-Media® pouches are stable 18 months when stored properly.

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.

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.

Warning: If you are considering using protein A for purification of mouse IgG1 Fc-fusion proteins, you may have to optimize purification condition as affinity is weak. High salt concentration and alkaline pH are expected to increase affinity¹. Alternatively you can use protein G, that has a stronger affinity for mouse IgG1 but your fraction must be free from high affinity antibodies such as bovine IgG (fetal calf serum).

PLASMID FEATURES

- **mIgG1 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 IgG1 mediates low CDC and no ADCC².
- **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.

1. Nagaoka M. & Akaike T., 2003 Single amino acid substitution in the mouse IgG1 Fc region induces drastic enhancement of the affinity to protein A. *Protein Eng.* 16(4):243-245.

2. Kipps TJ. *et al.*, 1985. Importance of immunoglobulin isotype in human antibody-dependent, cell-mediated cytotoxicity directed by murine monoclonal antibodies. *J Exp Med.* 161(1):1-17.

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

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.

Selection of bacteria with *E. coli* Fast-Media®

Fast-Media® is a **fast and convenient** way to prepare liquid and solid media for bacterial culture by using only a microwave. Fast-Media® is a TB (liquid) or LB (solid) based medium that already contains the antibiotic. Fast-Media® Zeo is available separately: #fas-zn-l (liquid), #fas-zn-s (agar).

- 1- Pour the contents of a Fast-Media® pouch into a clean borosilicate glass bottle or flask.
- 2- Add 200 ml of distilled water to the flask
- 3- Heat in a microwave on MEDIUM power setting (about 400Watts), until bubbles start appearing (approximately 3 minutes). **Do not heat a closed container. Do not autoclave Fast-Media®.**
- 4- Swirl gently to mix the preparation. **Be careful, the bottle and media are hot, use heatproof pads or gloves and care when handling.**
- 5- Reheat the media for 30 seconds and gently swirl again. Repeat as necessary to completely dissolve the powder into solution. But be careful to avoid overboiling and volume loss.
- 6- Let agar medium cool to 45°C before pouring plates. Let liquid media cool to 37°C before seeding bacteria.

Note: Do not reheat solidified Fast-Media® as the antibiotic will be permanently destroyed by the procedure.

RELATED PRODUCTS

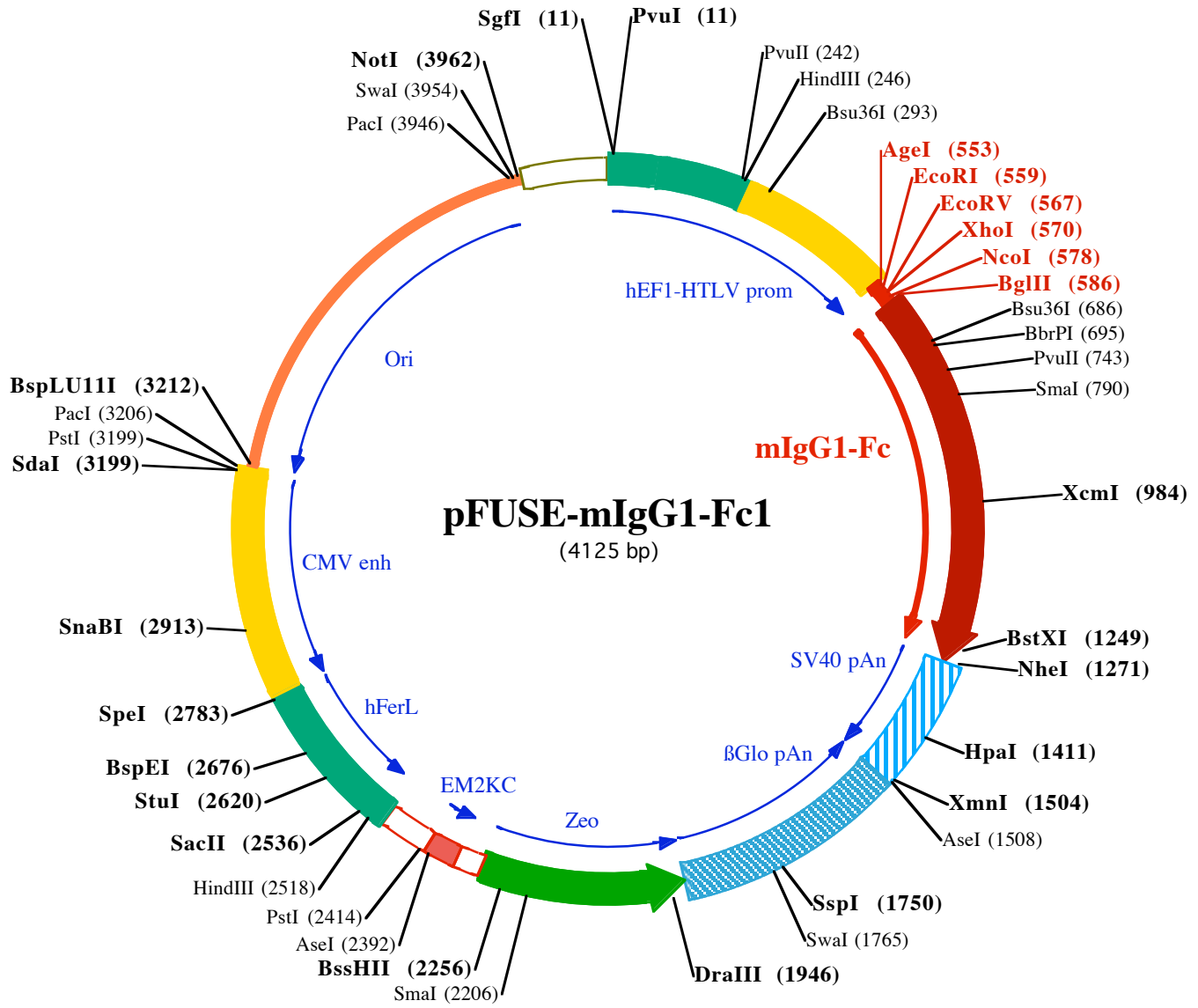
Product	Catalog Code
Zeocin™	ant-zn-l
Fast-Media® Zeo TB	fas-zn-l
Fast-Media® Zeo Agar	fas-zn-s

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PvuI (11)
SgfI (11)
1 GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCCACATCGCCACAGTCCCGAGAAGTTGGGGGAGGGTTCGCAATTGAACGGGTGCCTA
101 GAGAAGTGGCGCGGGTAAACTGGAAAGTGTGCTGTACTGGCTCCGCCCTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

HindIII (246)
201 GTGAACGTTCTTTTTCGCAACGGGTTTGGCCGCAGAACACAGCTGAAGCTTCAGAGGGCTCGCATCTCTCCTTACCGCGCCGCCCTACCTGAGGGC
301 GCCATCCACGCCGGTTGAGTGCCTTCTGCCCTCCCGCTGTGGTGCTCCTGAACTGCGTCCGCCGTCTAGTTAAGTTTAAAGCTCAGTTCGAGACC
401 GGGCCTTTGTCCGGCGCTCCCTTGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTCCTGACCTGCTTGTCTCAACTCTACGCTTTTGTTCGTTT

EcoRI (559) **XhoI (570)** **BglII (586)**
AgeI (553) **EcoRV (567)** **NcoI (578)**
501 TCTGTTCTGCGCGTTACAGATCCAAGCTGTGACCGCGCCTACCTGAGATCACCGTGAATTCGATATCTCGAGCACCATGGTTAGATCTGGTTGTAAG
1► Gl yCysLys
BbrPI (695)
601 CCTTCATATGTACAGTCCAGAAAGTATCATCTGTCTTACTTCCCCCAAAGCCAAAGGATGTGCTCACCATTACTCTGACTCCTAAGGTCACGTGTG
4► ProCysI l eCysThr Val P roGl uVal l Ser Ser Val Phe l l ePheP roP roLysP roLysAspVal l LeuThr l l eThr LeuThr P roLysVal l Thr CysV

PvuII (743) **SmaI (790)**
701 TTGTGGTAGACATCAGCAAGGATGATCCCGAGGTCAGTTCAGCTGGTTGTAGATGATGTGGAGTGCACACAGCTCAGACGCAACCCCGGAGGAGCA
37► a l Val Val Asp l l eSer LysAspAspP roGl uVal l Gl nPheSer T rpPheVal l AspAspVal l Gl uVal l Hi sThr Al aGl nThr Gl nP roArgGl uGl uGl
801 GTTCAACAGCACTTCCGCTCAGTCACTGACTTCCATCATGACCCAGGACTGGCTCAATGGCAAGGAGTTCAAATGCAAGGTCACAGGTCACAGTGCAGCTTTC
70► nPheAsnSer Thr PheArgSer Val l Ser Gl uLeuP ro l l eMetHi sGl nAspT rpLeuAsnGl yLysGl uPheLysCysArgVal l AsnSer Al aAl aPhe

XcmI (984)
901 CCTGCCCCATCGAAAAACCATCTCCAAAACCAAAGGCAGACCGAAGGCTCCACAGGTGTACACCATTCCACCTCCCAAGGAGCAGATGGCAAGGATA
104► P roAl aP ro l l eGl uLysThr l l eSer LysThr LysGl yArgP roLysAl aP roGl nVal l TyrThr l l eP roP roP roLysGl uGl nMe tAl aLysAspL
1001 AAGTCAGTCTGACCTGCATGATAACAGACTTCTCCCTGAAGACATTACTGTGGAGTGGCAGTGAATGGCAGCCAGCGGAGAAGTACAAGAACACTCA
137► ysVal l Ser LeuThr CysMe t l l eThr AspPhePheP roGl uAsp l l eThr Val l Gl uT rpGl nT rpAsnGl yGl nP roAl aGl uAsnTyrLysAsnThr Gl
1101 GCCATCATGGACACAGATGGCTTACTTCTGCTACAGCAAGCTCAATGTGCAGAAGAGCAACTGGGAGGAGGAAATACCTTACCTGCTGTGTATA
170► nP ro l l eMetAspThr AspGl ySer TyrPheVal l TyrSer LysLeuAsnVal l Gl nLysSerAsnT rpGl uAl aGl yAsnThr PheThr CysSer Val l Leu

BstXI (1249) **NheI (1271)**
1201 CATGAGGCGTGCACAACCACACTAGAGAAGAGCCTCCCACTCTCCTGGTAAATGATCCAGTGTGCTAGCTAGCTGGCCAGACATGATAAGATACATT
204► Hi sGl uGl yLeuHi sAsnHi sHi sThr Gl uLysSer LeuSer Hi sSer P roGl yLys •••
1301 GATGAGTTGGACAACCACAACCTAGAATGCAGTGAATAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCA

HpaI (1411)
1401 ATAAACAAGTTAAACAACAACATTCGATTCATTTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAAACCTCTACAATGTGG

AseI (1508) **XmnI (1504)**
1501 TATGGAATTAATCTAAAATACAGCATAGCAAAAATTTAACCTCCTCAAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCA
1601 GGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTTAAAGATATAGTGTATTTTCCAAGGTTTGAACCTAGCTCTTCATT

SspI (1750) **SwaI (1765)**
1701 TCTTTATGTTTTAAATGCACTGACCTCCACATTCCTTTTTAGTAAAATATTAGAAAATTAATTAATATACATTCATTGCAATGAAAATAAATGTTTTTTA
1801 TTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTGTAGTGTGGACTTAGGGAACAAAGGAACCTTAAATAGAAATTGGACAGCAAG

DraIII (1946)
1901 AAAGCGAGTCTTCTAGCTTATCTCAGTCTGCTCTGCTGCCACAAGTGCACGAGTTCGCGCGGGTTCGCGAGGCGAAGTCCCGCCCCACGGCTG
125► •••AspGl nGl uGl uAl aVal l PheHi sVal l CysAsnGl yAl aP roAspArgLeuAl aPheGl uArgGl yT rpP roGl n
2001 CTGCGCATCTCGGTTCATGGCCGGCCGGAGGCTCCCGAAGTTCGTGGACACGACTCCGACACTCGGCGTACAGCTCGTCCAGCGCCGCCACCCAC
99► Gl uGl y l l eGl uThr Me tAl aP roGl ySer Al aAspArgPheAsnThr Ser Val l Val l Gl uSer T rpGl uAl aT yrLeuGl uAspLeuGl yA rgVal l T rpV
2101 ACCAGGCCAGGGTGTGTCCGGCACCTGGCTGACCGCGCTGATGAACAGGGTCACTGCTCCCGACACACCGCGAAGTCTGCTCCACGCA
65► Al l T rpAl aLeuThrAsnAspP roVal l Val l Gl nAspGl nVal l Al aSer l l ePheLeuThr Val l AspAspArgVal l Val l Gl yAl aPheAspAspGl uVal l Ph
2201 AGTCCCGGAGAACCCGAGCCGGTCCGTCAGAACTCGACCGCTCCGGCGAGCTGCGCGCGGTGAGCACCAGGACGGCAGTGGTCAACTGGCCATGAT
32► eAspArgSer PheGl yLeuArgAspThr T rpPheGl uVal l Al aGl yAl aVal l AspArgAl aThr LeuVal l P roVal l Al aSer Thr LeuLysAl aMe t
2301 GGCTCCTCgtgcaggagagaagaagaaggttagtacaattgCTATAGTGAAGTATTACTATGCAGATATACTATGCCAATGATTAATTGTC
AseI (2392)

PstI (2414)
2401 AAAGTAGGCTGCAGgttcatagtccacttttctgactgccccatctcctgccaccctttccaggcatagacagtcagtgacttacCAAACCTCA

HindIII (2518) **SacII (2536)**
2501 CAGGAGGAGAAGGCAGAAGCTTGAGACAGACCCCGGGACCGCCAACTGCGAGGGGACGTGGCTAGGGCGCTCTTTTATGTTGCGCCGCCCTCGG

StuI (2620) **BspEI (2676)**
2601 AGGACGGGCGCTCGGGAGGCTAGCGGCCAATCTGCGGTGGCAGGAGCGGGCCGAAGGCGGTGCTGACCAATCCGGAGCACATAGGAGTCTCAGCC

SpeI (2783)
2701 CCCC GCCCAAAGCAAGGGGAAGTCACCGCCTGTAGCGCCAGCGTGTGTGAAATGGGGCTTGGGGGTTGGGGCCCTGACTAGTCAAACCAAACCTC
2801 CCATTGACGTCATGGGGTGGAGACTTGGAAATCCCGTGAAGTCAAACCGCTATCCACGCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAG

SnaBI (2913)
2901 CGATGACTAATACGTAGATGACTGCCAAGTAGGAAAGTCCATAAGGTTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAAT

3001 AGGGGGCGTACTTGCCATATGATACACTTGATGTAAGTGGCAAGTGGGCGTTTACCGTAAATACTCCACCATTGACGTCAATGAAAAGTCCCTATTGGC

PstI (3199)

SdaI (3199)

3101 GTTACTATGGGAACATACGTATTATTGACGTCAATGGGCGGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAAGTTATGTAACGCCCTGCAG

PacI (3206) **BspLU11I (3212)**

3201 GTTAATTAAGAACATGTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAAAGGCCGCTTGTGGCGTTTTTCCATAGGCTCCGCCCTGACGAG

3301 CATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCTGGAAGCTCCCTCGTGGCTCTCTG

3401 TTCCGACCCTGCCGTTACCGGATACCTGTCCGCCTTCTCCCTTCGGGAAGCGTGGCGCTTTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTA

3501 GGTCGTTTCGCTCCAAGCTGGGCTGTGTGCAGAACCCCGTTACGCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGA

3601 CACGACTTATGCCACTGGCAGCAGCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGATTCTTGAAGTGGTGGCCTAACTACG

3701 GCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAGAGTTGGTAGCTTTGATCCGGCAAACAACCACGCG

3801 TGGTAGCGGTGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGGTCTGACGCTCAG

PacI (3946) SmaI (3954) **NotI (3962)**

3901 TGGAACGAAAACACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCCAATAAAATATCTTTATTTTATTACATCTGTG

4001 TGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAACAAAACGAAACAAAACAACTAGCAAATAGGCTGTCCCAAGTCAAGTGCA

4101 GGTGCCAGAACATTTCTATCGAA