

# pFUSE-mIgG2A-Fc1

Plasmid containing a mouse IgG2A Fc region

Catalog # pfuse-mg2afc1

For research use only

Version 20K04-MM

## PRODUCT INFORMATION

### Content:

- 20 µg of pFUSE-mIgG2A-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 (when Fc portion is fused to a naturally secreted protein). 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

- **mIgG2A 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 IgG2A mediates high ADCC and CDC.
- **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter<sup>1</sup> 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<sup>2</sup>. 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<sup>3</sup>.
- **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 transcription<sup>4</sup>.

### References:

1. 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.
2. 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.
3. 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.
4. 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<sub>2</sub>O. 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

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### TECHNICAL SUPPORT

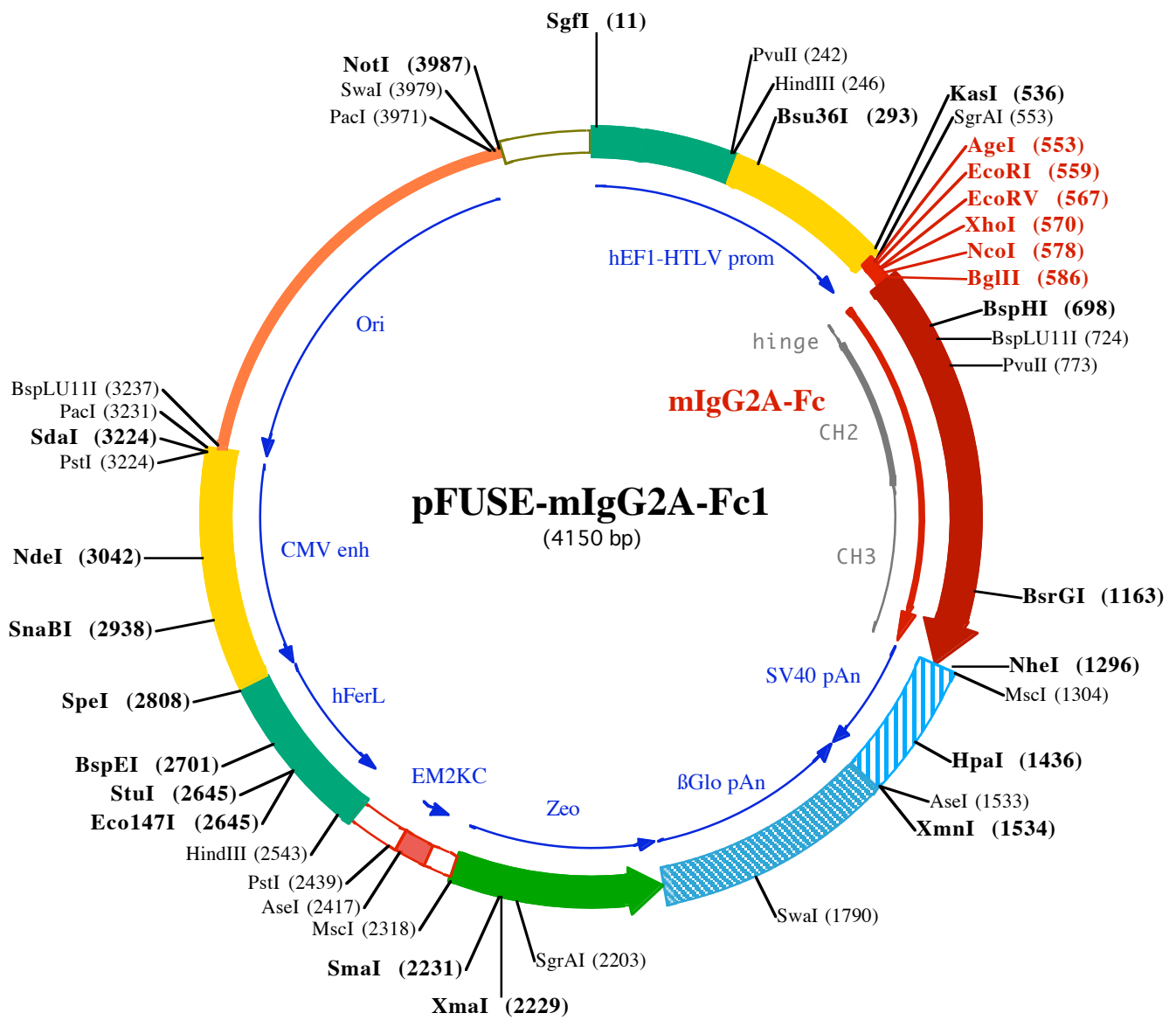
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**SgfI (11)**  
1 GGATCTGCGATCGTCCGGTCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCGGAGAAGTTGGGGGAGGGTTCGCAATTGAACGGTGCCTA  
101 GAGAAGGTGGCGGGGTAACCTGGGAAAGTGATGCTGCTGACTGGCTCCGCTTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGGC

**HindIII (246)**  
**PvuII (242)**  
**Bsu36I (293)**  
201 GTGAACGTTCTTTTTCGCAACGGGTTTGGCCGAGAACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCCTTACGCGCCCGCCCTACCTGAGGCC  
301 GCCATCCACGCGGGTTGAGTGCCTCTGCCGCTCCCGCTGTGGTGCCTCTGAACCTGCTCCGCGTCTAGGTAAGTTAAAGCTCAGGTCGAGACC  
401 GGGCTTTGTCCGGCGCTCCCTGGAGCTACCTAGACTCAGCGGCTCTCCACGCTTGGCTGACCTGCTTGTCTCAACTCTACGCTTTGTTCGTTT

**KasI (536)**  
**AgeI (553)**  
**EcoRI (559)**  
**XhoI (570)**  
**BglII (586)**  
**SgrAI (553)**  
**EcoRV (567)**  
**NcoI (578)**  
501 TCTGTTCTGGCGGTTACAGATCCAAGCTGTGACCGGGCCCTACCTGAGATCACCGGTGAATTCGATATCTCGAGCACCATGGTTAGACTCTCCAGAGGG  
1►ProArgGI y

**BspHI (698)**  
601 CCCACAATCAAGCCTGTCTCCATGCAATGCCAGCACCTAACCTTTGGGTGGACCTCCGCTTTCATCTTCCCTCAAAGATCAAGGATGTACTCA  
4►ProThr I l eLysP roCysP roP roCysLysCysP roAl aP roAsnLeuLeuGI yGI yP roSer Val P heI l eP heP roP roLys l l eLysAspVal l euM

**BspLU111 (724)**  
**PvuII (773)**  
701 TGATCTCCCTGAGCCCATAGTCACATGTGTGGTGGTGGATGTGAGCGAGGATGACCCAGATGTCCAGATCAGCTGGTTTGTGAACAACGTGGAAGTACA  
37►e t l l eSer LeuSer P ro l l eVal Thr CysVal l Val l AspVal l Ser GI uAspAspP roAspVal l GI n l l eSer T rpP heVal l AsnAsnVal l GI uVal l Hi  
801 CACAGCTCAGACAAAACCATAGAGAGGATTACAACAGTACTCTCCGGTGGTGCAGTGCCTCCCATCCAGCACCAGGACTGGATGAGTGGCAAGGAG  
70►sThr Al aGI nThr GI nThr Hi sArgGI uAspTyrAsnSer Thr LeuArgVal l Val l Ser Al aLeuP ro l l eGI nHi sGI nAspT rpMetSer GI yLysGI u  
901 TTCAAATGCAAGGTCAACAACAAGACCTCCAGCGCCATCGAGAGAACCATCTCAAAACCCAAAGGGTCAGTAAGAGCTCCACAGGTATATGTCTTGC  
104►P heLysCysLysVal l AsnAsnLysAspLeuP roAl aP ro l l eGI uArgThr l l eSer LysP roLysGI ySer Val l ArgAl aP roGI nVal l TyrVal l LeuP  
1001 CTCCACAGAAGAAGATGACTAAGAAACAGTCACTGACCTGCATGGTCACAGACTTCATGCCTGAAGACATTTACGTGGAGTGGACCAACAACGG  
137►r oP roP roGI uGI uGI uMe tThr LysLysGI nVal l Thr LeuThr CysMe tVal l ThrAspP heMe tP roGI uAsp l l eTyrVal l GI uT rpThrAsnAsnGI

**BsrGI (1163)**  
1101 GAAAACAGAGCTAACTACAAGAACTGAACAGTCTGGACTCTGATGGTTCTTACTTCATGTACAGCAAGCTGAGAGTGGAAAAGAAGAACTGGGTG  
170►yLysThr GI uLeuAsnTyrLysAsnThr GI uP roVal l LeuAspSer AspGI ySer TyrP heMe tTyrSer LysLeuArgVal l GI uLysLysAsnT rpVal l

**NheI (1296)**  
1201 GAAAGAAATAGTACTCTGTTCAGTGGTCCAGGGTCTGCACAATCACACAGACTAAGAGCTTCTCCCGACTCCGGTAATGAGCTCAGCTCAGCTAG  
204►GI uArgAsnSer TyrSer CysSer Val l Val l Hi sGI uGI yLeuHi sAsnHi sHi sThr Thr LysSer P heSer ArgThr P roGI yLys●●●

**MscI (1304)**  
1301 CTGGCAGACATGATAAGATACATTGATGAGTTGGACAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTGTGAAATTTGTGATGCTATTGCT

**HpaI (1436)**  
1401 TTATTTGAACATTATAAGCTGCAATAAACAAGTTAAACAACAACATTCATTCATTTTATGTTTCAGGTTACAGGGGAGGTGGGAGGTTTTTAA

**AseI (1533)**  
**XmnI (1534)**  
1501 GCAAGTAAACCTCTACAAATGTGGTATGGAATTAATCTAAAATACAGCATAGCAAACTTTAACCTCAAATCAAGCCTCTACTTGAATCCTTTTCTG  
1601 AGGGATGAATAAGGCATAGGCATCAGGGCTGTGCCAATGTGCATTAGCTGTTGCAGCCTCACCTTCTTTCATGGAGTTAAGATATAGTGATTTTTCT

**SwaI (1790)**  
1701 CCAAGTTTGAAGTACTCTTCTATTCTTTATGTTTTAAATGACTGACCTCCACATTCCTTTTTAGTAAATATTAGAAATAATTTAAATACATCA  
1801 TTGCAATGAAAATAAATGTTTTTATTAGCGAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGACTTAGGGAACAAGGAA  
1901 CCTTTAATAGAAATTTGGACAGCAAGAAGCGAGCTTCTAGCTTATCTCAGTCTGCTCTGCCACAAAGTGCACGAGTTGCCGCGCGGGTCCGCGCA  
125►●●●AspGI nGI uGI uAl aVal l P heHi sVal l CysAsnGI yAl aP roAspArgLe  
2001 GGGCAACTCCGCCCCACGGCTGCTCGCCGATCTCGGTATGGCGGCCGGAGGCGTCCCGAAAGTTCGTGGACACGACTCCGACACTCGGCGTA  
107►uAl aP heGI uArgGI yT rpP roGI nGI uGI y l l eGI uThr Me tAl aP roGI ySer Al aAspArgP heAsnThr Ser Val l Val l GI uSer T rpGI uAl aTyr  
2101 CAGCTCGTCCAGGCGCGCACCCACACCCAGGCCAGGCTGTGTCCGGCACCTGGCTGGACCGCGCTGATGAACAGGTCACGTCGTCGCGGACC  
74►LeuGI uAspLeuGI yArgVal l T rpVal l T rpAl aLeuThrAsnAspP roVal l Val l GI nAspGI nVal l Al aSer l l eP heLeuThr Val l AspAspArgVal l V

**XmaI (2229)**  
**SmaI (2231)**  
2201 ACACCGCGAAGTCTCTCCACGAAGTCCCGGAGAACCCGAGCGGTGCGTCCAGAATCGACCGCTCCGCGCAGCTCGCGCGCGGTGAGCACCGGAA  
40►a l GI yAl aP heAspAspGI uVal l P heAspArgSer P heGI yLeuArgAspThr T rpP heGI uVal l Al aGI yAl aVal l AspArgAl aThr LeuVal l P roVa  
2301 CGGCACTGGTCAACTGGCCATGATGGCTCTCctgtcaggagaggaaagagaagaaggttagtacaattgCTATAGTGAGTTGTATTACTATGCGAGA  
7►l Al aSer Thr LeuLysAl aMe t

**AseI (2417)**  
**PstI (2439)**  
2401 TATACTATGCCAATGATTAATTGTCAAAGTGGCTGCAgggttcatagtgcacttttctgcactgccccatctcctgccccctttccaggcata

**HindIII (2543)**  
2501 gacagtcagtgacttacCAAACCTACAGGAGGGAGAAGCGAAGCTTGAGACAGACCCCGGGACCGCCAAGTGCAGGGGACGTGGCTAGGGCGGT

**StuI (2645)**  
**Eco147I (2645)**  
2601 TCTTTTATGTTGCGCGGCCCTCGAGGCAGGGCGCTCGGGGAGGCTAGCGGCCAATCTGCGGTGGCAGGAGCGGGCCGAAGGCGGTGCTGACCAA

**BspEI (2701)**  
 2701 TCCGGAGCACATAGGAGTCTCAGCCCCCGCCCAAAGCAAGGGGAAGTCACGCGCTGTAGGCCAGCGTGTGTGAAATGGGGCTTGGGGGGTTGG

**SpeI (2808)**  
 2801 GGCCCTGACTAGTCAAACAACACTCCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTACTGC

**SnaBI (2938)**  
 2901 CAAAACCCATCATCATGGTAATAGCGATGACTAATACGTAGATGACTGCCAAGTAGGAAAGTCCATAAGGTCATGTACTGGGCATAATGCCAGGCGG

**NdeI (3042)**  
 3001 GCCATTTACCGTCATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCCTAAATACTCCACCCATTG

3101 ACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCAATTATTGACGTCAATGGCGGGGGTCTGTTGGCGGTCAGCCAGGCGGGCCATTT

Pacl (3231)  
 PstI (3224) **SdaI (3224)** BspLU11I (3237)  
 3201 ACCGTAAGTTATGTAACGCCTGCAGGTTAATTAAGAACATGTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAAAGGCCGCTTGTGGCGTTTTT

3301 CCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCT

3401 GGAAGCTCCCTCGTGGCTCTCCTGTTCCGACCTGCCGTTACCGGATACCTGTCCGCTTTTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGTCAC

3501 GCTGTAGGTATCTCAGTTCGGGTAGGTCGTTCCGCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTCCAGCCGACCGCTGCCTTATCCGGTAACCTA

3601 TCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGTACAGAG

3701 TTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCT

3801 CTTGATCCGGCAAACAACACCCTGAGCGGTGGTTTTTTTGTGCAAGCAGCAGATTACCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGAT

Pacl (3971) Swal (3979) **NotI (3987)**  
 3901 CTTTTCTACGGGGTCTGACGCTCAGTGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAA

4001 TATCTTTATTTTATTACATCTGTGTGGTTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAACAAAACGAAACAAAACAACTAGCAA

4101 ATAGGCTGTCCCCAGTGAAGTGCAGGTGCCAGAACATTTCTCTATCGAA