

pFUSE-mIgG2Ae1-Fc2

Plasmid containing a mouse engineered IgG2A Fc region

Catalog # pfc2-mg2ae1

For research use only

Version 20K05-MM

PRODUCT INFORMATION

Content:

- 20 µg of pFUSE-mIgG2Ae1-Fc2 (IL2ss) 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-Fc2 (IL2ss) plasmids allow the secretion of Fc-Fusion proteins. They contain the IL2 signal sequence (IL2ss) for the generation of Fc-Fusion proteins derived from proteins that are not naturally secreted. 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). IgG isoforms exert different levels of effector functions increasing in the order of mIgG1<mIgG3<mIgG2a.

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 mouse IgG2a Fc region in order to reduce its ADCC and CDC.

PLASMID FEATURES

• **mIgG2Ae1 Fc (mouse IgG2A 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 of mouse IgG2a mediates high ADCC and CDC. To reduce its cytotoxicity, mIgG2a Fc was engineered by mutating the amino acids that are critical for FcγRs and C1q binding. The engineered form mIgG2ae1 contains the following mutations: L235E and E318A/K320A/K322A².

• **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.

• **IL2 ss:** The IL2 signal sequence contains 20 amino acids and share common characteristics with signal peptides of other secretory proteins. The intracellular cleavage of the IL2 signal peptide occurs after Ser20 and leads to the secretion of the antigenic protein.

• **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. Carter PJ., 2006. Potent antibody therapeutics by design. Nature Reviews Immunology. Advance online publication.

2. Steurer W. *et al.*, 1995. Ex vivo coating of islet cell allografts with murine CTLA4/Fc promotes graft tolerance. J Immunol. 155(3):1165-74.

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

InvivoGen USA (Toll-Free): 888-457-5873

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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

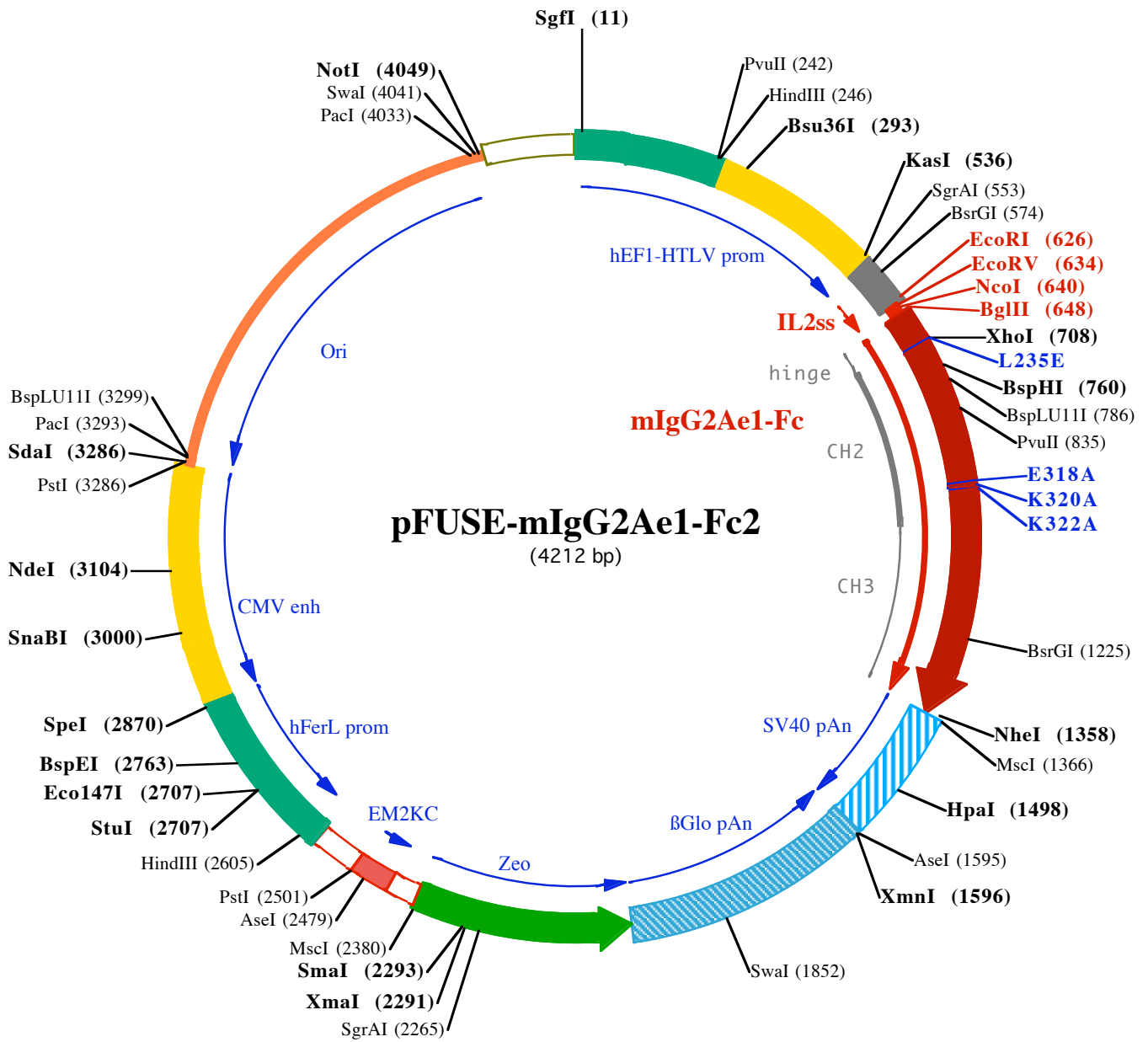
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100

SgfI (11)

1 GGATCTGCGATCGCTCCGGTCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCGAGAAAGTTGGGGGAGGGTTCGCAATTGAACGGGTGCCTA

101 GAGAAGGTGGCGGGGTAACCTGGGAAAGTGATGCTGTACTGGCTCCGCTTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGGC

HindIII (246) **Bsu36I (293)**

201 GTGAACGTTCTTTTTGCAACGGGTTTGGCGCCAGAAACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCCTTACGCGCCCCGCCCTACCTGAGGCC

301 GCCATCCACGCGGGTTGAGTGCCTCTGCGCCCTCCCGCTGTGGTGCCTCTGAAGCTCGCTCCGCGCTTAGGTAAGTTAAAGCTCAGGTCGAGACC

401 GGGCTTTGTCCGGCTCCCTTGAGCCTACCTAGACTCAGCGGGCTCCACGCTTTGCTGACCTGCTTGTCTCAACTCTACGCTTTTGTTCGTTT

KasI (536) **SgrAI (553)** **BsrGI (574)**

501 TCTGTTCTGCGCGTTACAGATCCAAGCTGTGACCGGGCTACTCTGAGATCACGGCGAAGGAGGCCACCATGTACAGGATGCAACTCCTGTCTTGGCA
 1►MetTyrArgMetGlnLeuLeuSer CysI

EcoRV (634) **BglII (648)**

601 TTGCACTAAGTCTTGCCTTGTACGAATTCGATATCGGCCATGTTAGATCTCCAGAGGGCCCAATCAAGCCTGTCTCCATGCAATGCCAGC
 10►IeAlaLeuSerLeuAlaLeuValThrAsnSer 1►ProArgGlyProThrIleLysProCysProProCysLysCysProAl

EcoRI (626) **NcoI (640)**

L235E

701 ACCTAACCTCGAGGGTGGACCATCCGCTTTCATCTTCCCTCCAAAGATCAAGGATGTAAGTCTGACTCATGATCTCCCTGAGCCCATAGTCACATGTGTGGTGGT
 16►aProAsnLeuGluGlyGlyProSerValPheIlePheProProLysIleLysAspValLeuMetIleSerLeuSerProIleValThrCysValValVal

XhoI (708) **BspHI (760)** **BspLU111 (786)**

801 GATGTGAGCGAGGATGACCCAGATGTCCAGATCAGCTGGTTTGTGAACAAGTGGAGTACACAGCTCAGACACAAACCCATAGAGAGGATTACAACA
 50►AspValSerGluAspAspProAspValGlnIleSerTrpPheValAsnAsnValGluValHisThrAlaGlnThrGlnThrHisArgGluAspTyrAsnS

PvuII (835)

901 GACTCTCCGGTGGTCACTGCCCTCCCATCCAGCACCAGGACTGGATGAGTGGCAAGGCTTTCGATCGCGGGTCAACAACAAGACCTCCAGCGCC
 83►erThrLeuArgValValSerAlaLeuProIleGlnHisGlnAspTrpMetSerGlyLysAlaPheAlaCysAlaValAsnAsnLysAspLeuProAlaPr

E318A **K322A** **K320A**

1001 CATCGAGAGAACCATCTCAAACCCAAAGGGTCAAGAGCTCCACAGGATATGTCTTGCCTCCACAGAAGAAGAGATGACTAAGAAACAGGCTCACT
 116►oIleGluArgThrIleSerLysProLysGlySerValArgAlaProGlnValTyrValLeuProProProGluGluGluMetThrLysLysGlnValThr

1101 CTGACCTGCATGGTCAAGACTTTCATGCTGAAGACATTTACTGGAGTGGACCAACACGGGAAAACAGAGCTAACTACAAGAACACTGAACAGTCC
 150►LeuThrCysMetValThrAspPheMetProGluAspIleTyrValGluTrpThrAsnAsnGlyLysThrGluLeuAsnTyrLysAsnThrGluProValL

BsrGI (1225)

1201 TGGACTCTGATGTTTCTTACTTTCATGTACAGCAAGCTGAGAGTGGAAAAGAAGAACTGGGTGGAAAAGAACTACTCTCTTCTGAGTGGTCCACGAGGG
 183►euAspSerAspGlySerTyrPheMetTyrSerLysLeuArgValGluLysLysAsnTrpValGluArgAsnSerTyrSerCysSerValValHisGluGlu

MseI (1366) **NheI (1358)**

1301 TCTGCACAATCACACAGCTAAGAGCTTCTCCCGACTCCGGGTAAGTGGAGCTAGCTAGCTGCGCCAGACATGATAAGATACATTGATGAGTTGGAC
 216►yLeuHisAsnHisHisThrThrLysSerPheSerArgThrProGlyLys•••

HpaI (1498)

1401 AAACCACAACCTAGATGAGTGAAGAAATGCTTTATTTGTGAATTTGTGATGCTATTGCTTTATTTGTAACATTATAAGCTGCAATAAACAAGTTAA

AseI (1595) **XmnI (1596)**

1501 CAACAACAATTGCATTCAATTTATGTTTCAGGTTCAAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAATTAAT
 1601 CTAATAACAGCATAGCAAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGCTGTGGCA
 1701 ATGTGCATTAGCTTTGCGACCTCACCTTCTTTCATGGAGTTAAGATATAGTGTATTTCCCAAGGTTTGAAGTCTTCTTCTTATGTTTTA

Swal (1852)

1801 AATGCACTGACCTCCACATTCCCTTTTAGTAAAATATTCAGAAATATTTAAATACATCATTGCAATGAAAATAAATGTTTTTATTAGGCAGAATCC
 1901 AGATGCTCAAGGCCCTTCATAATATCCCCAGTTTGTAGTGGACTTAGGGAACAAGGAACCTTTAATAGAAATGGACAGCAAGAAAGCGAGCTTCT
 2001 AGCTTATCCTCAGTCTGCTCTGCTGCTGCCAAGTGCACGAGTTCGCCGGCGGGTCCGCGAGGGCAACTCCCGCCCCACGGCTGCTGCCGATCTCG
 2101 GTCATGGCCGGCCCGAGGCGTCCGGAAGTTCGTGGACACGACTCCGACACTCGCGGTACAGCTCGTCCAGGCGCGCACCCACACCCAGGCCAGGG
 94►hrMetAlaProGlySerAlaAspArgPheAsnThrSerValValGluSerTrpGluAlaTyrLeuGluAspLeuGlyArgValTrpValTrpAlaLeuTh

SgrAI (2265) **XmaI (2291)** **SmaI (2293)**

2201 TGTGTCCGGCACCTGGTCTGACCGCTGATGAACAGGGTTCAGCTGCTCCCGGACACACCGCGCAAGTCTGCTCCAGGAAGTCCCGGGAGAA
 61►rAsnAspProValValGlnAspGlnValAlaSerIlePheLeuThrValAspAspArgValValGlyAlaPheAspAspGluValPheAspArgSerPhe

MseI (2380)

2301 CCCGAGCGGTCGGTCCAGAACTGACCGCTCCGGCGAGCTCGCGCGGGTGAAGCAGCGGCAAGGCACTGGTCAACTTGGCCATGATGGCTCTCctgtc
 28►GlyLeuArgAspThrTrpPheGluValAlaGlyAlaValAspArgAlaThrLeuValProValAlaSerThrLeuLysAlaMet

AseI (2479) **PstI (2501)**

2401 aggagaggaaagagaagaaggttagtacaattgCTATAGTGAAGTGTATTATACTATGAGATATACTATGCAATGATTAATTGCAAACTAGGGCTGC
 2501 AgggttcatagtgccacttttctgactgccccactctctgccacccttccaggcatagacagtcagtacttacCAAACCTCACAGGAGGGAGAA
 2601 GCAGAAGCTTGAGACAGACCCCGGGACCGGCAACTGCGAGGGGACGTGGCTAGGGCGGCTTCTTTATGTTGCGCCGGCCCTGGAGGCAGGGCGCTC

StuI (2707)
Eco147I (2707)

2701 GGGGAGGCCTAGCGCCAATCTGCGGTGGCAGGAGCGGGGCCGAAGCCGTGCCTGACCAATCCGGAGCACATAGGAGTCTCAGCCCCGCCCAAAG

BspEI (2763)

2801 CAAGGGGAAGTCACGCGCCTGTAGCGCCAGCGTGTGTGAAATGGGGCTTGGGGGGTTGGGGCCCTGACTAGTCAAAACAAACTCCCAATTGACGTCAA

SpeI (2870)

2901 TGGGGTGAGACTTGGAAATCCCGTGAGTCAAACCGTATCCACGCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATAC

SnaBI (3000)

3001 GTAGATGTACTGCCAAGTAGGAAAGTCCATAAGGTCAATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGCGTACTT

NdeI (3104)

3101 GGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAA

PacI (3293)
PstI (3286)
SdaI (3286)

3201 CATACTGATTATTGACGTCAATGGCGGGGGTCTGTTGGGGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGCAGGTTAATTAAGAAC

BspLU11I (3299)

3301 ATGTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAAAGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAATC

3401 GACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCTCGGAAGCTCCCTCGTGCCTCTCCTGTTCCGACCTGCC

3501 GCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTGCTCGCTCC

3601 AAGCTGGGCTGTGTGCACGAACCCCGTTCCAGCCGACCGCTGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGC

3701 CACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGCGGTGTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAG

3801 AACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTTTGATCCGGCAAACAACCCAGCTGGTAGCGGTGGT

3901 TTTTTTGTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGTCTGACGCTCAGTGAACGAAAAC

PacI (4033) **Swal (4041)** **NotI (4049)**

4001 CACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGTTTTTG

4101 TGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACAACTAGCAAAATAGGCTGTCCCAGTGAAGTGCAGGTGCCAGAACAT

4201 TTCTCTATCGAA