

pFUSE-hIgG3-Fc1

Plasmid designed for the construction of Fc-Fusion proteins

Catalog # pfuse-hg3fc1

For research use only

Version # 06G10-MT

PRODUCT INFORMATION

Content:

- 20 µg of pFUSE-hIgG3-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.

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.

PLASMID FEATURES

• **hIgG3-Fc (human):** 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. Human IgG3 displays high ADCC and CDC.

• **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter¹ and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat². 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 mRNA³.

• **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 transcription⁴.

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. & Atwine 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 $\mu\text{g}/\mu\text{l}$, resuspend the DNA in 20 μl of sterile H_2O . 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-1 (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-1 |
| Fast-Media® Zeo TB | fas-zn-1 |
| Fast-Media® Zeo Agar | fas-zn-s |

TECHNICAL SUPPORT

Toll free (US): 888-457-5873

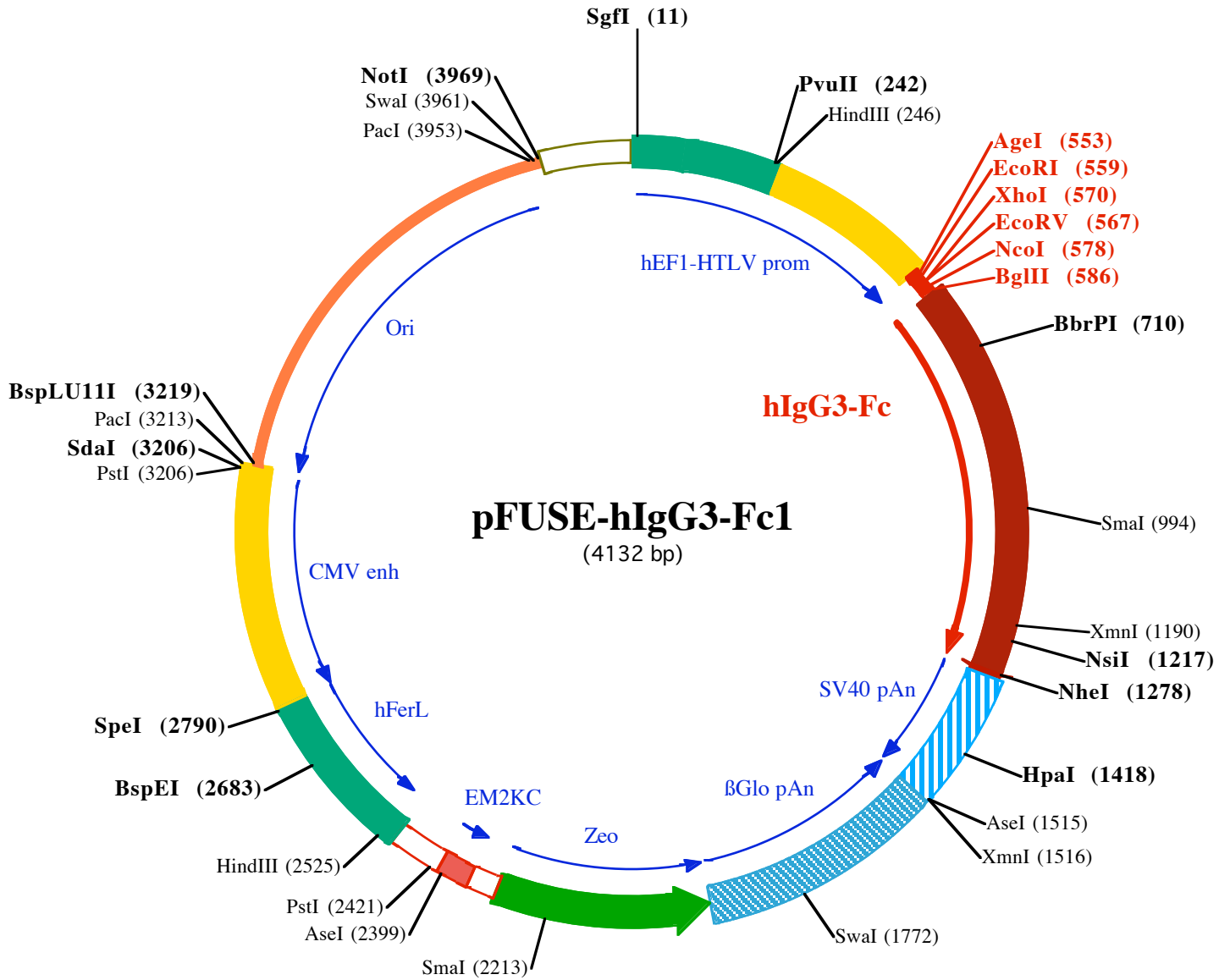
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SgfI (11)
1 GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGGTGGCAATTGAACGGGTGCCTA
101 GAGAAGGTGGCGGGGTAAACTGGAAAGTGATGCTGTACTGGCTCCGCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCC

HindIII (246)
PvuII (242)
201 GTGAACGTTCTTTTTCGCAACGGGTTTCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCTTACCGCGCCCGCCCTACTCTGAGGCC
301 GCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCTCCCGCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC
401 GGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTCTCAACTCTACGCTTTTGTTCGTTT

EcoRI (559) XhoI (570) BglIII (586)
AgeI (553) EcoRV (567) NcoI (578)
501 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGCGCTACCTGAGATCACCGTGAATTCGATATCTCGAGCACCATGGTTAGACTGTGACACACCT
1▶AspThrPro
601 CCCCCGTGCCAAGGTGCCAGCACCTGAACTCCTGGGAGGACCGTCAGTCTTCTCTTCCCCAAAACCAAGGATACCCCTTATGATTTCCCGGACCC
4▶ProProCysProArgCysProAlaProGluLeuLeuGluGlyProSerValPheLeuPheProProLysProLysAspThrLeuMetIleSerArgThrP

BbrPI (710)
701 CTGAGGTCACGTGCGTGGTGGTGGACGTGAGCCACGAAGACCCCGAGGTCAGTTCAAGTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAA
37▶roGluValThrCysValValValAspValSerHisGluAspProGluValGlnPheLysTrpTyrValAspGlyValGluValHisAsnAlaLysThrLy
801 GCCCGGGGAGGAGCAGTTCAACAGCACGTTCCGTGTGGTCAAGCTCCTACCCTGTCACAGGACTGGCTGAACGGCAAGGAGTACAAGTCAAAGGTC
70▶sProArgGluGluGlnPheAsnSerThrPheArgValValSerValLeuThrValLeuHisGlnAspTrpLeuAsnGlyLysGluTyrLysCysLysVal

SmaI (994)
901 TCCAACAAGCCCTCCAGCCCCATCGAGAAAACCATCTCAAACCAAGGACAGCCCCGAGAACCACAGGTGTACACCCTGCCCCATCCCGGGAGG
104▶SerAsnLysAlaLeuProAlaProIleGluLysThrIleSerLysThrLysGlyGluProArgGluProGluValTyrThrLeuProProSerArgGluG
1001 AGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAGCGGGCAGCCGGAGAACA
137▶IuMetThrLysAsnGluValSerLeuThrCysLeuValLysGlyPheTyrProSerAspIleAlaValGluTrpGluSerSerGlyGluProGluAsnAs

XmnI (1190)
1101 CTACAACACCACGCTCCATGCTGGACTCCGACGGCTCCTTCTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACATCTTC
170▶nTyrAsnThrThrProProMetLeuAspSerAspGlySerPhePheLeuTyrSerLysLeuThrValAspLysSerArgTrpGluGlnGlyAsnIlePhe

NsiI (1217) NheI (1278)
1201 TCATGCTCCGTGATGCATGAGGCTGACACAACCGCTTACGCGAAGAGCCTCCTCTGCTCCGGTAAATGAGTGCTAGCTGGCCAGACATGATAAG
204▶SerCysSerValMetHisGluAlaLeuHisAsnArgPheThrGlnLysSerLeuSerLeuSerProGlyLys●●●
1301 ATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATA

HpaI (1418)
1401 AGCTGCAATAAAAGTTAAACAACAACATTCATTTATGTTTCAGGTTACGGGGAGGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACA

AseI (1515) XmnI (1516)
1501 AATGTGGTATGGAATTAATCTAAAATACAGCATAGCAAACTTTAACCTCAAATCAAGCCTTACTTGAATCCTTTCTGAGGGATGAATAAGGCATA
1601 GGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTCTTTCATGGAGTTAAGATATAGTGATTTTTCCAAGGTTTGAAGTCTAGCT

SwaI (1772)
1701 CTTCAATTTCTTTATGTTTTAAATGCACTGACCTCCACATTCCCTTTTTAGTAAATATTAGAAATAATTTAAATACATCATTGCAATGAAAATAAATG
1801 TTTTTTATTAGGCAGAAATCCAGATGCTCAAGGCCCTTATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTAAATGAAATTTGGA
1901 CAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCTGCTCCTCTGCCACAAGTGACGAGTTGCCGGCCGGTTCGCGCAGGGCGAACTCCCGCCCC
125▶●●●AspGluGluGluAlaValPheHisValCysAsnGlyAlaProAspArgLeuAlaPheGluArgGlyTr
2001 ACGGCTGCTCGCGATCTCGGTCATGGCCGGCCGGAGGCGTCCCGAAAGTTCGTGGACACGACCTCCGACCCTCGGCTACAGCTCGTCCAGGCGCCG
101▶pProGluGluGlyIleGluThrMetAlaProGlySerAlaAspArgPheAsnThrSerValValGluSerTrpGluAlaTyrLeuGluAspLeuGlyArg
2101 CACCCACACCAAGCCAGGGTGTGTCCGGCACCACTGGTCTGGACCGCTGATGAACAGGTCACGTCGTCGGGACCAACCGGCAAGTCTGCTCC
68▶ValTrpValTrpAlaLeuThrAsnAspProValValGlnAspGluValAlaSerIlePheLeuThrValAspAspArgValValGlyAlaPheAspAspG
2201 TCCACGAAGTCCCGGAGAACCCGAGCCGTCGGTCCAGAAGTCCGACCGCTCCGGCAGCTCGCGCGGTGAGCACCGAACCGCACTGGTCAACTTGG
34▶IuValPheAspArgSerPheGlyLeuArgAspThrTrpPheGluValAlaGlyAlaValAspArgAlaThrLeuValProValAlaSerThrLeuLysAl

AseI (2395)
2301 CCATGATGGCTCCTCctgtcaggagaggaaagagaagggttagtacaattgCTATAGTGAGTTGATTATACTATGCAGATATACTATGCCAATGATT
1▶aMet

PstI (2421)
2401 AATTGTCAAACCTAGGCTGCAGgttcatagtccacttttctgcactgccccatctctgcccaccttccaggcatagacagtcagtgacttacC

HindIII (2525)
2501 AAATCACAGGAGGGAGAAGGCAGAAGCTTGAGACAGACCCGGGACCGCGAACTGCGAGGGGACGTGGCTAGGGCGGCTCTTTTATGGTGCGCCGG

BspEI (2683)
2601 CCCTCGAGGCAGGGCCTCGGGAGGCCTAGCGCAATCTCGGTGGCAGGAGGGGGCGGAAGCCGTGCTGACCAATCCGGAGCATAGGAGT

SpeI (2790)
2701 CTCAGCCCCCGCCCAAAGCAAGGGGAAGTCAAGCGCCTGTAGCGCCAGCGTGTGTGAAATGGGGCTTGGGGGGTGGGGCCCTGACTAGTCAAAA
2801 CAAACTCCCATTGACGTCAATGGGTGGAGACTTGGAAATCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTACTGCCAAAACCGCATCATGTG

2901 GTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGA
3001 CGTCAATAGGGGGCGTACTTGCCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCC
3101 TATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACG

PacI (3213)
PstI (3206)
SdaI (3206) **BspLU11I (3219)**
3201 CCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTCCATAGGCTCCGCCCCC
3301 TGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCTGGAAGCTCCCTCGTGCGC
3401 TCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCTTCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTATCTCAGTT
3501 CGGTGTAGGTCGTTGCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCC
3601 GGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCT
3701 AACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAA
3801 CCACCGCTGGTAGCGGTGGTTTTTTTGGTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGA

PacI (3953) SwaI (3961) **NotI (3969)**
3901 CGCTCAGTGAACGAAAACCTCACGTTAAGGGATTTTGGTTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAAATATCTTTATTTTCATTAC
4001 ATCTGTGTGTTGGTTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACAACTAGCAAAATAGGCTGTCCCAGTGC
4101 AAGTGCAGGTGCCAGAACATTTCTCTATCGAA