

# pFUSE-hIgG4-Fc1

Plasmid designed for the construction of Fc-Fusion proteins

Catalog # pfuse-hg4fc1

For research use only

Version # 06G07-MT

## PRODUCT INFORMATION

### Content:

- 20 µg of pFUSE-hIgG4-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

- **hIgG4-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 IgG4 displays low ADCC and no CDC, and therefore is the most suitable for diagnostic imaging or blocking molecular interactions<sup>1</sup>.
- **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter<sup>2</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>3</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>4</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*.
- **BGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription<sup>5</sup>.

1. Kim SJ. et al., 2005. Antibody engineering for the development of therapeutic antibodies. *Mol. Cells*. 20(1):17-29.

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

Toll free (US): 888-457-5873  
Outside US: (+1) 858-457-5873  
E-mail: [info@invivogen.com](mailto:info@invivogen.com)  
Website: [www.invivogen.com](http://www.invivogen.com)



3950 Sorrento Valley Blvd. Suite 100  
San Diego, CA 92121 - USA

## 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  $\mu\text{l}$  of sterile H<sub>2</sub>O. 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

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### TECHNICAL SUPPORT

Toll free (US): 888-457-5873

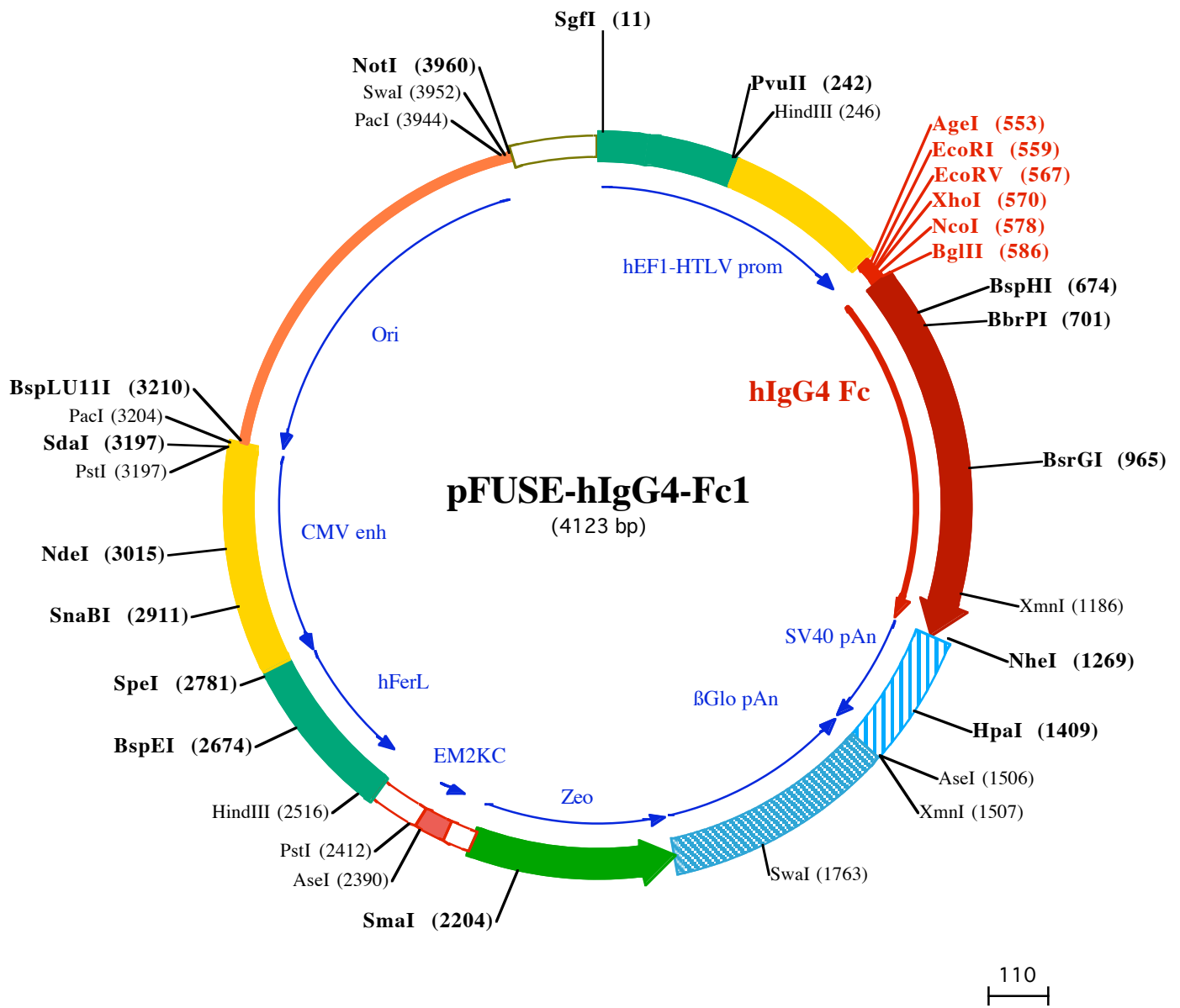
Outside US: (+1) 858-457-5873

E-mail: [info@invivogen.com](mailto:info@invivogen.com)

Website: [www.invivogen.com](http://www.invivogen.com)



3950 Sorrento Valley Blvd. Suite 100  
San Diego, CA 92121 - USA



SgfI (11)  
1 GGATCTGCGATCGCTCCGGTCCCGTCAGTGGCAGAGCGCACATCGCCACAGTCCCGAGAAGTTGGGGGAGGGTTCGCAATTGAACGGTGCCTA  
101 GAGAAGGTGGCGGGGTAAACTGGGAAAGTATGTCGTGTACTGGCTCCGCCTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

HindIII (246)  
PvuII (242)  
201 GTGAACGTTCTTTTTCGCAACGGGTTTCCCGCCAGAACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCCTTACGCGCCCGCCGCCCTACCTGAGGCC  
301 GCCATCCACGCGGGTTGAGTCGCGTTCTGCCGCTCCCGCTGTGGTGCCTCTGAATCGCTCCGCGCTAGGTAAGTTAAAGCTCAGGTCGAGACC  
401 GGGCCTTTGTCGGCGCTCCCTTGAGCCTACCTAGACTCAGCGGGCTCCACGCTTTCCTGACCTGCTTGCCTCAACTCTACGCTTTGTTTCGTTT

EcoRI (559) XhoI (570) BglII (586)  
AgeI (553) EcoRV (567) NcoI (578)  
501 TCTGTTCTGCGCGTTACAGATCCAAGCTGTGACCGGGCCTACCTGAGATCACCGTGAATTCGATATCTCGAGCACCATGGTTAGATCTCCCCCATGC  
1 ProProCys

BspHI (674) BbrPI (701)  
601 CCATCATGCCAGCACCTGAGTTCCTGGGGGACCATAGTCTTCTGTTCCCCCAAACCAAGGACACTCTCATGATCTCCGGACCCCTGAGGTCA  
4 ProSer CysProAl aProGluPheLeuGlyGlyProSer Val PheLeuPheProProLysP roLysAspThr LeuMetI leSer ArgThr ProGluValT  
701 CGTGCCTGGTGGAGCTGAGCCAGGAAGACCCCGAGGTCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGA  
37 hr CysVal Val Val AspVal Ser Gl nGluAspProGluVal Gl nPheAsnTrpTyrVal AspGlyVal Gl uVal Hi sAsnAl aLysThr LysProArgGly  
801 GGAGCAGTTCAACAGCACGTACCGTGTGTCAGCTCCTCACCTGCACAGGACTGGTGAACGGCAAGGAGTACAAGTCAAGGTCCTCAACAAA  
70 uGluGlu nPheAsnSer Thr TyrArgVal Val Ser Val LeuThr Val LeuHi sGlu nAspTrpLeuAsnGlyLysGly uTyrLysCysLysVal SerAsnLys

BsrGI (965)  
901 GGCCTCCGTCCTCCATCGAGAAAACCTCTCAAAGCCAAAGGCGAGCCCGAGAGCCACAGGTGTACACCCTGCCCCATCCAGGAGGAGATGACCA  
104 Gl yLeuProSer Ser I leGluLysThr I leSer LysAl aLysGlyGly nProArgGlu uProGlu nVal TyrThr LeuProProSer Gl nGlu uGluMetThr L  
1001 AGAACCAAGTTCAGCTGACCTGCCTGGTCAAAGGCTTCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGCGACCCGGAGACAACCTACAAGAC  
137 ysAsnGlu nVal Ser LeuThr CysLeuVal LysGlyPheTyrProSerAspI leAl aVal Gl uTrpGlu uSerAsnGlyGly nProGlu uAsnAsnTyrLysTh  
XmnI (1186)  
1101 CACGCTCCCGTGTGACTCCGACGCTCCTTCTTCTACAGCAGGTAACCGTGGACAAGAGCAGGTGGCAGGAGGGGAATGCTTCTCATGCTCC  
170 r Thr ProProVal LeuAspSerAspGlySer PhePheLeuTyrSer ArgLeuThr Val AspLysSer ArgTrpGlu nGlu yAsnVal PheSer CysSer

NheI (1269)  
1201 GTGATGCATGAGGCTGCAACAACCTACACAGAGAGCCTCCTCTGCTCCGGTAAATGAGTGTAGCTGCCAGACATGATAAGATACATTGA  
204 ValMetHisGluAl aLeuHisAsnHisTyrThr Gl nLysSer LeuSer LeuSerProGlyLys ●●●  
1301 TAGTTTGGACAACCACTAGAATGCAAGTGAATAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTTGTAAACATTATAAGCTGCAAT

HpaI (1409)  
1401 AAACAAGTTAAACAACAATTGCATTCATTTTATGTTTCAGGTTCCAGGGGAGGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAATGTGGTA

AseI (1506)  
XmnI (1507)  
1501 TGGAAATTAATCTAAAATACAGCATAGCAAACTTAACTCCAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGG  
1601 GGCTGTTGCCAATTGTGCTTAGCTGTTTGCAGCCTCACCTTCTTCATGAGGTTAAGATATAGTGTATTTTCCCAAGGTTTGAACGTAGCTCTTCAATTC

Swal (1763)  
1701 TTTATGTTTTAAATGCACTGACCTCCACATTCCTTTTATGATAAATATTAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTATT  
1801 AGGCAGAATCCAGATGCTCAAGGCCCTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAGGAACCTTAAATAGAATTTGGACAGCAAGAA  
1901 AGCGAGCTTCTAGCTTATCTCAGTCTGCTCCTCTGCCACAAAGTGCACGAGTTGCGCGCGGGTTCGCGCAGGGCGAACTCCCGCCCCACGGCTGCT  
125 ●●●AspGlu nGlu uGluAl aVal PheHisVal CysAsnGlyAl aProAspArgLeuAl aPheGlu uArgGly yTrpProGlu nGly  
2001 CGCCGATCTCGGTCTAGCCGCGCCGAGGCGTCCCGAAGTTCGTGGACACGACCTCCGACCACTCGCGGTACAGCTCTCCAGGCGCGCACCCACAC  
98 uGlyI leGlu uThr MetAl aProGlySerAl aAspArgPheAsnThr Ser Val Val Gl uSer TrpGluAl aTyrLeuGlu uAspLeuGly yArgVal TrpVal  
2101 CCAGGCCAGGGTGTGTCGGCACCACTGCTGGACCGCGCTGATGAACAGGGTTCAGCTGCTCCCGACCAACCGCGGAAGTCTCTCCACGAAAG  
65 TrpAl aLeuThrAsnAspProVal Val Gl nAspGlu nValAl aSer I lePheLeuThrVal AspAspArgVal Val Gl yAl aPheAspAspGluVal PheA  
SmaI (2204)  
2201 TCCCGGAGAACCAGCGGTCGGTCCAGAAGTCCAGCCGCTCCGGCAGCTCGCGCGGGTGAACCGGAACCGCACTGGTCAACTGGCCATGATGG  
31 spArgSer PheGlyLeuArgAspThr TrpPheGlu uValAl aGlyAl aVal AspArgAl aThr LeuValProValAl aSer ThrLeuLysAl aMet  
AseI (2390)  
2301 CTCCTCctgtcaggagaggaagagaagaaggttagtacaattgCTATAGTGAGTTGATTATCTACTATGCAGATATACTATGCCAATGATTAATTGTCAA

PstI (2412)  
HindIII (2516)  
2401 ACTAGGGCTGCAgggttcattagtgccacttttctgactgcccactctctgcccacctttccaggcatagacagttagtgacttacCAAACCTACA  
2501 GGAGGGAGAAGGAGAAGCTTGAGACAGACCCGCGGACCGCCGAAGTTCGAGGGGACGTGGCTAGGGCGGCTCTTTTATGGTGGCCGCGCCCTCGGAG

BspEI (2674)  
2601 GCAGGGCTCGGGGAGGCTAGCGCAATCTCGGTGGCAGGAGGCGGGGCCAAGGCGCTGCTGACCAATCCGGAGCACATAGGAGTCTCAGCCCC

SpeI (2781)  
2701 CCGCCCCAAAGCAAGGGGAAGTACGCGCTGTAGCCAGCGTGTGTGAAATGGGGCTTGGGGGGTTGGGGCCCTGACTAGTCAAAACAACTCCC  
2801 ATTGACGTCAATGGGGTGGAGACTTGAAATCCCGTGAAGTCAAACCGCTATCCACGCCATTGATGACTGCCAAAACCGCATCATGTAATAGCG

SnaBI (2911)  
2901 ATGACTAATACGTAGTACTGCCAAGTAGGAAAGTCCATAAGGTACTGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCAATGACGTCAATAG

NdeI (3015)  
3001 GGGCGTACTTGGCATATGATACACTTGATGTACTGCAAGTGGCAGTTTACCCTAAATACTCCACCATTGACGTCAATGAAAGTCCCTATTGGCGT

PacI (3204)

PstI (3197)

**SdaI (3197)**

3101 TACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGCAGGT

**BspLU11I (3210)**

3201 TAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGAACCGTAAAAAGGCCGCTTGCTGGCGTTTTCCATAGGCTCCGCCCTGACGAGCA

3301 TCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCTGGAAGCTCCCTCGTGCCTCTCTGT

3401 CCGACCTGCCGTTACCGGATACCTGTCCGCTTCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGG

3501 TCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTACGCCGACCGCTGCGCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACA

3601 CGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCTAACTACGGC

3701 TACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACAAACACCGCTG

3801 GTAGCGGTGGTTTTTTGTTTGAAGCAGCAGATTACGCCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTG

PacI (3944) SmaI (3952) **NotI (3960)**

3901 GAACGAAACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCCAATAAAATATCTTTATTTTATTACATCTGTGTG

4001 TTGGTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAACAAAACGAAACAAAACAACTAGCAAATAGGCTGTCCCAAGTCAAGTGCAGG

4101 TGCCAGAACATTTCTATCGAA