

pFUSE-hIgG1-Fc1

Plasmid designed for the construction of Fc-Fusion proteins

Catalog # pfuse-hg1fc1

For research use only

Version 20K04-MM

PRODUCT INFORMATION

Content:

- 20 µg of pFUSE-hIgG1-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. 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

- **hIgG1-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 IgG1 displays high ADCC and CDC, and is the most suitable for therapeutic use against pathogens and cancer cells.
- **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.

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

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

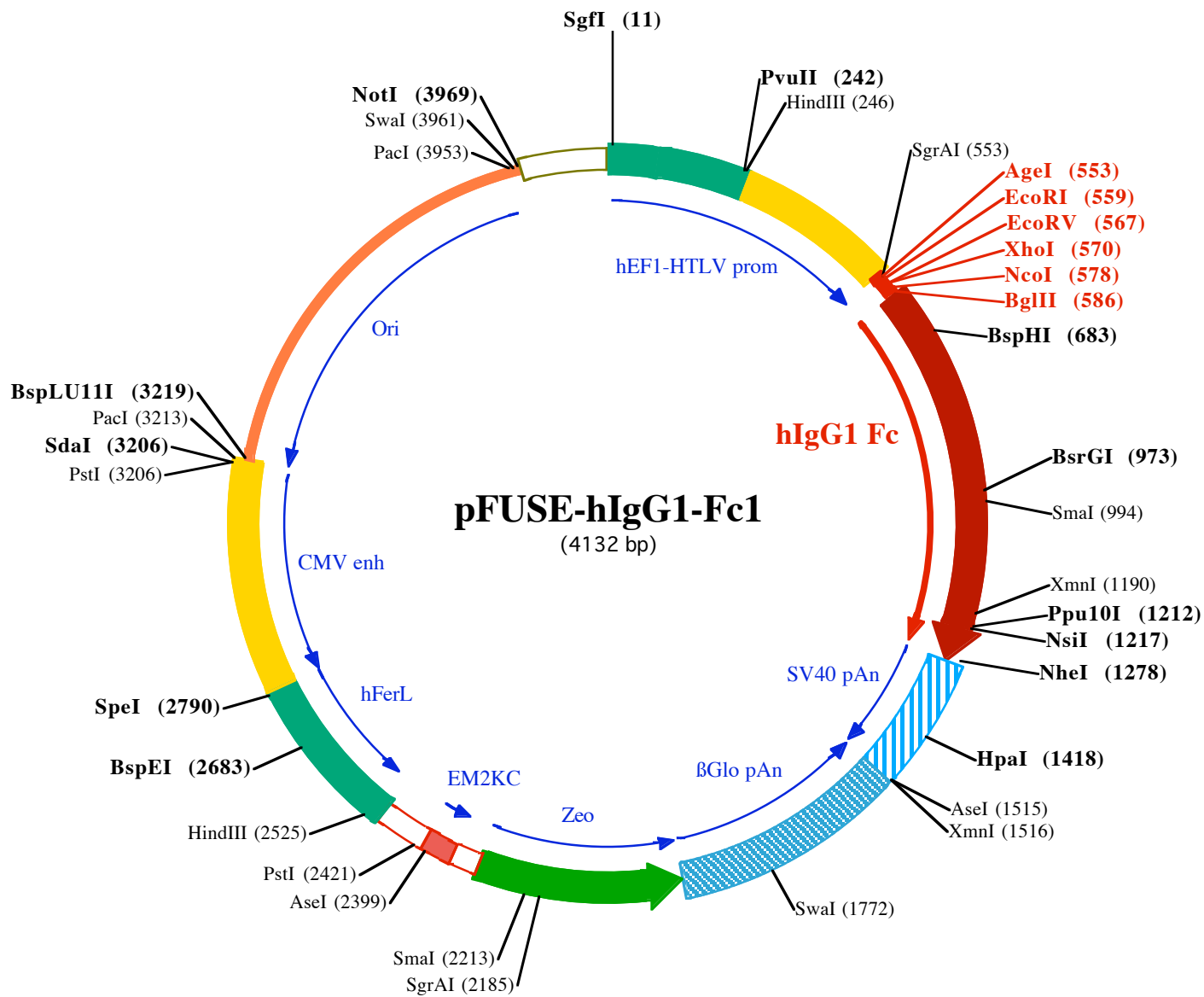
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SgfI (11)
1 GGATCTGCGATCGTCCGGTGCCTGAGTGGGCGAGCGCACATCGCCACAGTCCCGAGAAGTTGGGGGAGGGTTCGCAATTGAACGGGTGCCTA
101 GAGAAGGTGGCGGGGTAACCTGGGAAAGTGTGCTGTACTGGCTCCGCTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGGC

HindIII (246)
PvuII (242)
201 GTGAACGTTCTTTTTCGAACGGGTTTGCCTGCAGAACACAGCTGAAGCTTCAGAGGGCTCGCATCTCTCTTCCACGCGCCCGCCCTACCTGAGGCC
301 GCCATCCACGCGGGTGTGAGTCGCGTTCTGCCGCTCCCGCTGTGGTGCCTCTGAACCTGCTCCGCGTCTAGGTAAGTTAAAGCTCAGGTCGAGACC
401 GGGCTTTGTCCGCGCTCCCTTGAGCCTACCTAGACTCAGCGGCTCTCCACGCTTTCCTGACCTGCTTGTCTCAACTCTACGCTTTTGTTCGTTT

EcoRI (559) XhoI (570) BglII (586)
AgeI (553) EcoRV (567) NcoI (578)
501 TCTGTTCTGCGCGTTACAGATCCAAGCTGTGACCGGCGCTACCTGAGATCACCGTGAATTCGATATCTCGAGCACCATGGTTAGATCTGACAAAAC
1AspLysThr
601 CACACATGCCACCGTCCAGCACCTGAACCTCTGGGGGACCGTCACTTCTCTTCCCCAAAACCAAGGACACCCCTCATGATCTCCCGGACCC
4HisThrCysPProCysProAlaProGluLeuLeuGlyGlyProSerValPheLeuPheProProLysProLysAspThrLeuMetIleSerArgThrP
701 CTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGCAAAA
37ProGluValThrCysValValValAspValSerHisGluAspProGluValLysPheAsnTrpTyrValAspGlyValGluValHisAsnAlaLysThrLys
801 GCCCGGGAGGACAGTACAACAGCAGCTACCGTGTGGTCAAGCTCCTCACGCTCTGACCCAGGACTGGCTGAATGGCAAGGAGTACAAGTCAAGGTC
70sProArgGluGluGlnTyrAsnSerThrTyrArgValValSerValLeuThrValLeuHisGluAspTrpLeuAsnGlyLysGluTyrLysCysLysVal
901 TCCAACAAGCCCTCCAGCCCCATCGAAGAACATCTCCAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCATCCCGGAGG
104SerAsnLysAlaLeuProAlaProIleGluLysThrIleSerLysAlaLysGlyGlnProArgGluProGluNValTyrThrLeuProProSerArgGluG
1001 AGATGACCAAGAACCGTCAAGCTGACCTGCCTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGCGAGCCGGAGAACAA
137IleMetThrLysAsnGluNValSerLeuThrCysLeuValLysGlyPheTyrProSerAspIleAlaValGluTrpGluSerAsnGlyGluProGluAsnAs
1101 CTACAAGACCACGCTCCCGTGTGGACTCCGACGGCTCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCGGGGAACTCTTTC
170nTyrLysThrThrProProValLeuAspSerAspGlySerPhePheLeuTyrSerLysLeuThrValAspLysSerArgTrpGluNglNglYAsnValPhe
1201 TCATGCTCCGTGATGCACGAGGCTCTGCACAACCTACACGAGAAGGCTCTCCCTGTCTCCGGTAAATGAGTGTAGCTGGCCAGACATGATAAG
204SerCysSerValMetHisGluAlaLeuHisAsnHisTyrThrGlnLysSerLeuSerLeuSerProGlyLys
1301 ATACATTGATGAGTTTGGACAAACCACTAGAATGCAAGTAAAAAATGCTTTATTTGTGAAATTTGTATGCTATTGCTTTTAAACATTATA

HpaI (1418)
1401 AGCTGCAATAAACAAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTTCAGGGGAGGTGTGGAGGTTTTTAAAGCAAGTAAACCTCTACA

AseI (1515)
XmnI (1516)
1501 AATGTGGTATGGAATTAATCTAAAATACAGCATAGCAAACTTTAACTCCAATCAAGCCTCTACTTGAATCTTTTCTGAGGGATGAATAAGCATA
1601 GGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTCATGGAGTTTAAGATATAGTGTATTTTCCCAAGTTTGAACTAGCT

SwaI (1772)
1701 CTTCAATTTCTTTATGTTTTAAATGCACCTGACCTCCACATTCCTTTTATGATAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAAATAAATG
1801 TTTTTATTAGGCAAGATCCAGATGCTCAAGGCCCTCATAATATCCCCAGTTTAGTGTGGACTTAGGGAACAAGGAACCTTTAATAGAAATGGA
1901 CAGCAAGAAAGCGAGCTTCTAGCTTATCTCAGTCTGCTCTGCCACAAGTGCACGAGTTGCCGGCCGGTTCGCGAGGGCGAACTCCCGCCCC
125AspGlnGluGluAlaValPheHisValCysAsnGlyAlaProAspArgLeuAlaPheGluuArgGlyYTr
2001 ACGGCTGCTCGCCGATCTCGGTCTATGGCCGCGCCGAGGCGTCCCGAAGTTTCGTGGACAGCCTCCGACCACTCGCGTACAGCTCGTCCAGCCGCG
101pProGlnGluGlyIleGluThrMetAlaProGlySerAlaAspArgPheAsnThrSerValValGluSerTrpGluAlaTyrLeuGluAspLeuGlyYArg
2101 CACCACACCCAGGCGAGGGTGTGTCCGGCACCACTGGTCTGGACCGCGTGTGAAACAGGGTCAAGTCTCCGGACACACCCGGCGAAAGTCTGTC
68ValTrpValTrpAlaLeuThrAsnAspProValValGluAspGlnValAlaSerIlePheLeuThrValAspAspArgValValGlyAlaPheAspAspG
2201 TCCACGAAGTCCCGGGAAGACCCGAGCCGGTCCGAGAACTCGACCGCTCCGCGCAGCTCGCGCGGTGAGCACCGGAACCGCACTGGTCAACTTGG
34IleValPheAspArgSerPheGlyLeuArgAspThrTrpPheGluValAlaGlyAlaValAspArgAlaThrLeuValProValAlaSerThrLeuLysAla
2301 CCATGATGGCTCTCctgtcaggagaggaagagagaagggttagtacaattgCTATAGTGAGTTGTATTATACTATGCAGATATACTATGCCAATGATT
1aMet AseI (2399)

PstI (2421)
2401 AATTGTCAAATAGGGCTGCAggggttcattagtgccacttttctgcactgcccattctctgcccaccctttccaggcatagacagtcagtgacttacC

HindIII (2525)
2501 AAACCTCACAGGAGGAGAAGGCAGAAGCTTGAGACAGACCCCGGGACCCGCAACTGCGAGGGACGTGGCTAGGGCGGCTCTTTTATGGTGCGCCGG

BspEI (2683)
2601 CCCTCGGAGGCAGGGCGCTCGGGAGGCCTAGCGCCAATCTGCGGTGGCAGGAGCGGGCCGAAGGCCGTGCCTGACCAATCCGGAGCACATAGGAGT

SpeI (2790)
2701 CTCAGCCCCCGCCCCAAAGCAAGGGGAAGTCAACGCGCTGTAGCCACGCGTGTGTGAAATGGGGCTTGGGGGGTGGGGCCCTGACTAGTCAAAA
2801 CAAACTCCATTGACGTCATAGGGTGGAGACTTGAAGTCCCGTGGTCAAAACCGCTATCCACGCCATTGATGTACTGCCAAAACCGCATCATCATG
2901 GTAATAGCGATGACTAATACGTAGTGTACTGCCAAGTAGGAAAGTCCCATAAAGTGTACTGGGCATAATGCCAGGCGGGCATTACCGTCAATTGA
3001 CGTCAATAGGGGGCTACTTGGCATATGATACACTTGTGACTGCCAAGTGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGAAAAGTCCC
3101 TATTGGCTTACTATGGGAACATACGTCATTATTGACGTCAATGGCGGGGGTCTGGCGGTGAGCCAGGCGGGCCATTTACCGTAAAGTATGTAACG

PacI (3213)
PstI (3206) **SdaI (3206)** **BspLU11I (3219)**

3201 CCTGCAGGTTAATTAAAGACATGTGAGCAAAAGGCCAGCAAAGGCCAGGAACCGTAAAAAGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCC
3301 TGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCTGGAAGTCCCTCGTGCGC
3401 TCTCCTGTCCGACCTGCCGCTTACCGGATACCTGTCCGCCTTCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTATCTCAGTT
3501 CGGTGTAGGTCGTTGCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTACGCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCC
3601 GGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCT
3701 AACTACGGCTACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACAAA
3801 CCACCGCTGGTAGCGGTGGTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGGTCTGA

PacI (3953) SmaI (3961) **NotI (3969)**

3901 CGCTCAGTGGAAACGAAAACCTACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAAATATCTTTATTTTCATTAC
4001 ATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACAACTAGCAAAAATAGGCTGTCCCGAGTGC
4101 AAGTCAGGTGCCAGAACATTTCTCTATCGAA