

pINFUSE-hIgG1-Fc1

Plasmid designed for the construction of Fc-Fusion proteins

Catalog # pfc1-hgin1

For research use only

Version 20K06-MM

PRODUCT INFORMATION

Content:

- 20 μ g of pINFUSE-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

pINFUSE-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).

pINFUSE-Fc plasmids yield high levels of Fc-fusion proteins. The level of expression is usually in the μ g/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.

pINFUSE-Fc plasmids allow the secretion of Fc-Fusion proteins. As Fc-Fusion proteins are secreted, they can be easily detected in the supernatant of pINFUSE-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 pINFUSE-Fc vectors featuring Fc regions containing introns 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

- **human genomic IgG1-Fc (with introns):** 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. A short intron is present between each region (one intron between the hinge and CH2 and one intron between CH2 and CH3). The presence of introns is known to enhance the level of gene expression as splicing is known to promote rapid and efficient mRNA export¹. 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-1 α (EF-1 α) core promoter² and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat³. The EF-1 α promoter exhibits a strong activity and yields long lasting expression of a transgene *in vivo*. The R-U5' has been coupled to the EF-1 α 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 mRNA⁴.
- **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*.
- **β Glo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription⁵.

TECHNICAL SUPPORT

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

InvivoGen USA (International): +1 (858) 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.

References:

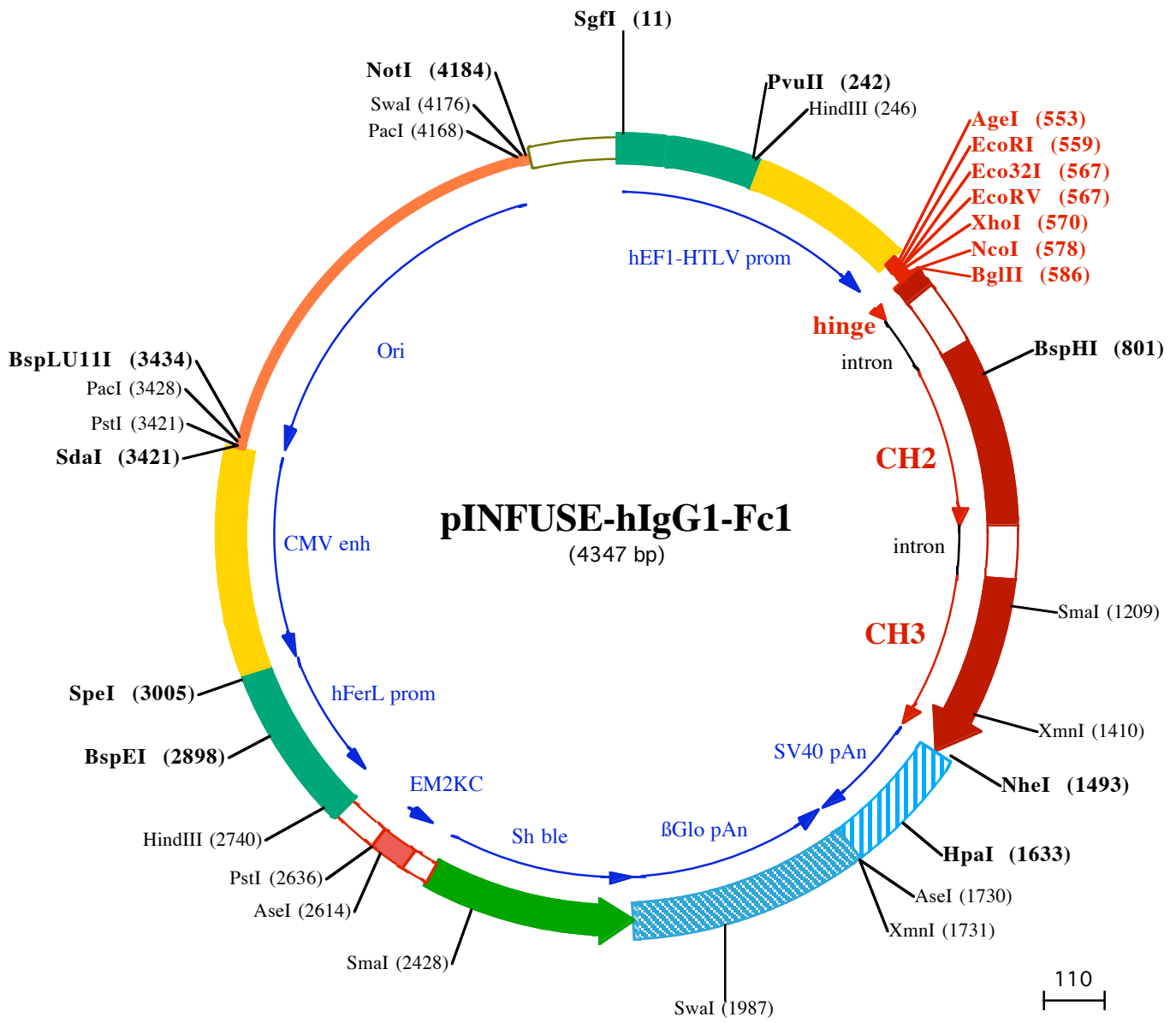
1. Nott A, et al. 2003. A quantitative analysis of intron effects on mammalian gene expression. *RNA*. 9(5):607-17.
2. Kim DW *et al.* 1990. Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. *91(2):217-23*.
3. Takebe Y. *et al.* 1988. SR alpha promoter: 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.

RELATED PRODUCTS

Product	Catalog Code
Zeocin™	ant-zn-1

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SgfI (11)
1 GGATCTGCGATCGCTCCGGTCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCGGAGAAGTTGGGGGAGGGTTCGCAATTGAACGGTGCCTA
101 GAGAAGGTGGCGGGGTAACCTGGGAAAGTGATGTCGTGACTGGCTCCGCCTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

HindIII (246)
PvuII (242)
201 GTGAACGTTCTTTTCGCAACGGTTTGGCCGACAGCAGCTGAAGCTTCGAGGGCTCGCATCTCTCTTACGCGCCCGCCCTACTCTGAGGCC
301 GCCATCCACCGCGGTTGAGTCCGCTTCTGCCCTCCCGCTGTGGTGCCTCTGAAGCTCCGCGCTTAGGTAAGTTAAAGCTCAGGTCGAGACC
401 GGGCTTTGTCCGGCTCCCTTGAGCCTACCTAGACTCAGCGGCTCTCCACGCTTTCCTGACCCTGCTTCTCAACTCTACGCTTTGTTCGTTT

EcoRV (567)
EcoRI (559) XhoI (570) BglII (586)
AgeI (553) Eco32I (567) NcoI (578)
501 TCTGTTCTGCGCGTTACAGATCCAAGCTGTGACCGCGCCTACCTGAGATCACCGTGAATTCGATATCTCGAGCACCATGGTGTAGATCTGACAAA
1▶AspLysThr
601 CACACATGCCACCGTCCCAAGtaagccagccagcctcgcctccagctcaaggcgggacaggtgcctagatagcctgcatccagggacagggcc
4▶HisThrCysProP roCysProA
701 cagccgggtgctgacagctccacctccatctctctcagCACCTGAAGCTCTGGGGGACCGTCACTTCTCTTCCCCCAAACCAAGGACACCC
laProGluLeuLeuGlyProSerValPheLeuPheProP roLysProLysAspThrL

BspHI (801)
801 TCATGATCTCCCGACCCCTGAGGTACATGCTGGTGGTGGACGTGAGCCACGAAGACCTGAGGTCAAGTTCAACTGGTACGTGGACGGCTGGAGGT
20▶euMetIleSerArgThrProGluValThrCysValValValAspValSerHisGluAspProGluValLysPheAsnTrpTyrValAspGlyValGluVal
901 GCATAATGCCAAGCAAAAGCCGCGGGAGGAGCAGTACAACAGCAGTACCGTGTGGTCAAGCTCTCACCGTCTGACCCAGGACTGGCTGAATGGCAAG
53▶IHisAsnAlaLysThrLysProArgGluGluGlnTyrAsnSerThrTyrArgValValSerValLeuThrValLeuHisGluAspTrpLeuAsnGlyLys
1001 GAGTCAAGTCAAGGCTCCAAACAAAGCCCTCCAGCCCATCGAGAAAACCATCTCCAAAGCCAAAGgtgggacccgtggggtgagggccacatg
87▶GluTyrLysCysLysValSerAsnLysAlaLeuProAlaP roIleGluLysThrIleSerLysAlaLysG
1101 gacagagggcggctcggccaccctctgacctgagagtgactgctgtaccaacctctgtcctacagGGCAGCCCGAGAACCACAGGTGTACACCCTGC
lyGlnProArgGluProGluValTyrThrLeuP

SmaI (1209)
1201 CCCATCCCGGATGAGCTGACCAAGAACCAGTCAAGCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGGCAATGG
11▶roProSerArgAspGluLeuThrLysAsnGluValSerLeuThrCysLeuValLysGlyPheTyrProSerAspIleAlaValGluTrpGluSerAsnGlu
1301 GCAGCCGGAGAACAACTACAAGACCAGCCTCCCGTGTGGACTCCGACGGCTCTTCTTCTCTACAGCAAGCTCACCGTGGACAAAGAGCAGGTGGCAG
44▶yGlnProGluAsnAsnTyrLysThrThrProProValLeuAspSerAspGlySerPhePheLeuTyrSerLysLeuThrValAspLysSerArgTrpGln

XmnI (1410)
NheI (1493)
1401 CAGGGGAAGCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGAGAAGAGCCTCTCCCTGTCTCCGGTAATGAGTGTAGCTG
78▶GlnGlyAsnValPheSerCysSerValMetHisGluAlaLeuHisAsnHisTyrThrGlnLysSerLeuSerLeuSerLeuSerProGlyLys●●●
1501 GCCAGACATGATAAGATACATTGATGAGTTGGACAAACCACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAATTTGTGATGCTATTGCTTTA

HpaI (1633)
1601 TTTGTAACCATTATAAGCTGCAATAAACAAGTTAACCAACAACAAATTGCATTATTTTATGTTTCAGGTTCAAGGGGAGGTGGGAGGTTTTTAAAGCA

AseI (1730)
XmnI (1731)
1701 AGTAAACCTCTACAAATGTGGTATGGAATTAATTCTAAATAACAGCATAGCAAACTTTAACTCCAAATCAAGCCTCTACTTGAATCCTTTCTGAGG
1801 GATGAATAAGGCATAGGCATCAGGGCTGTTGCCAATGTGCATTAGCTGTTGCAGCCTCACCTTCTTTCATGGAGTTAAGATATAGTATTTCCCA

SwaI (1987)
1901 AGGTTTGAAGTACTCTTCTATTCTTTATGTTTTAAATGACTGACCTCCACATTCCTTTTTAGTAAATATTAGAAATAATTTAAATACATCATTG
2001 CAATGAAAAATAATGTTTTTATTAGGCAGAAATCCAGATGCTCAAGGCCCTCATAATATCCCCAGTTTAGTAGTTGACTTAGGAACAAGGAACCT
2101 TTAATAGAAATTTGACAGCAAGAAAGCGAGCTTCTAGCTTATCTCAGTCTGCTCTGCCACAAAGTGCACGAGTTGCCGCGGGTTCGCGCAGGG
125▶●●●AspGlnGluGluAlaValPheHisValCysAsnGlyAlaP roAspArgLeuAl
2201 CGAACTCCCGCCCCACGGCTGCTCGCGATCTCGGTCAAGCCGCGGAGGCGTCCCGAAAGTTCTGGACAGCCTCCGACCACTCGGCGTACAG
106▶aPheGluArgGlyTrpProGluGluGlnIleGluThrMetAlaP roGlySerAlaAspArgPheAsnThrSerValValGluSerTrpGluAlaTyrLeu
2301 CTCGTCAGGCGCGCACCCACACCCAGGCCAGGTTGTGTCGGCACCACTGGTCTGGACCGGCTGATGAACAGGGTCACTGCTCCCGACCA
73▶GluAspLeuGlyArgValTrpValTrpAlaLeuThrAsnAspProValValGlnAspGluValAlaSerIlePheLeuThrValAspAspArgValValG

SmaI (2428)
2401 CCGCGAAGTCTCTCCACGAAGTCCCGGAGAACCCGAGCGGTCGGTCCAGAAGTCCGACCGTCCGCGAGCTCGCGCGGTGAGCACCGGAACGG
39▶lyAlaPheAspAspGluValPheAspArgSerPheGlyLeuArgAspThrTrpPheGluValAlaGlyAlaValAspArgAlaThrLeuValProValAla
2501 CACTGGTCAACTGGCCATGATGGCTCCTcgtgagagaggaagagaaggttagtacaattgCTATAGTGAAGTTGATTACTATGCAGATAT
6▶aSerThrLeuLysAlaMet

AseI (2614)
PstI (2636)
2601 ACTATGCCAATGATTAATTGTCAAAGTGGCTGCAgggttcatagtgccacttttctgactgccccatctcctgccccctttccaggcatagac

HindIII (2740)
2701 agtcagtgacttacAAAACCTCACAGGAGGAGAAGGCAGAAGCTTGAGACAGACCCCGGACCGCCGAAGTTCGAGGGGACGTGGCTAGGGCGGCTTCT

BspEI (2898)
2801 TTTATGGTGCGCCGCCCTCGGAGGCAAGGCGCTCGGGAGGCTAGCGGCAATCTGCGGTGGCAGGAGGCGGGCCGAAGGCGTGCCTGACCAATCC
2901 GGAGCACATAGGAGTCTCAGCCCCCGCCCAAGCAAGGGAAGTACGCGCCTGTAGCGCCAGCGTGTGTGAAATGGGGCTTGGGGGGTTGGGGC

SpeI (3005)
3001 CCTGACTAGTCAAACAACCTCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTAAGTCCAA
3101 AACCCGATCATCATGGTAATAGCGATGACTAATACGTAGATGACTGCAAGTAGGAAAGTCCATAAAGGTGATGACTGGGCATAATGCCAGGCGGGC
3201 ATTTACCGTCAATTGACGTCAATAGGGGGCTACTTGGCATATGATACACTTGTACTGCAAGTGGGAGTTTACCGTAAATACTCCACCCATTGACC

