

pINFUSE-hIgG2-Fc1

Plasmid designed for the construction of Fc-Fusion proteins

Catalog # pfc1-hgin2

For research use only

Version 20K06-MM

PRODUCT INFORMATION

Content:

- 20 μ g of pINFUSE-hIgG2-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 IgG2-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 IgG2 displays low ADCC and CDC.
- **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 *Streptalloteichus 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

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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:

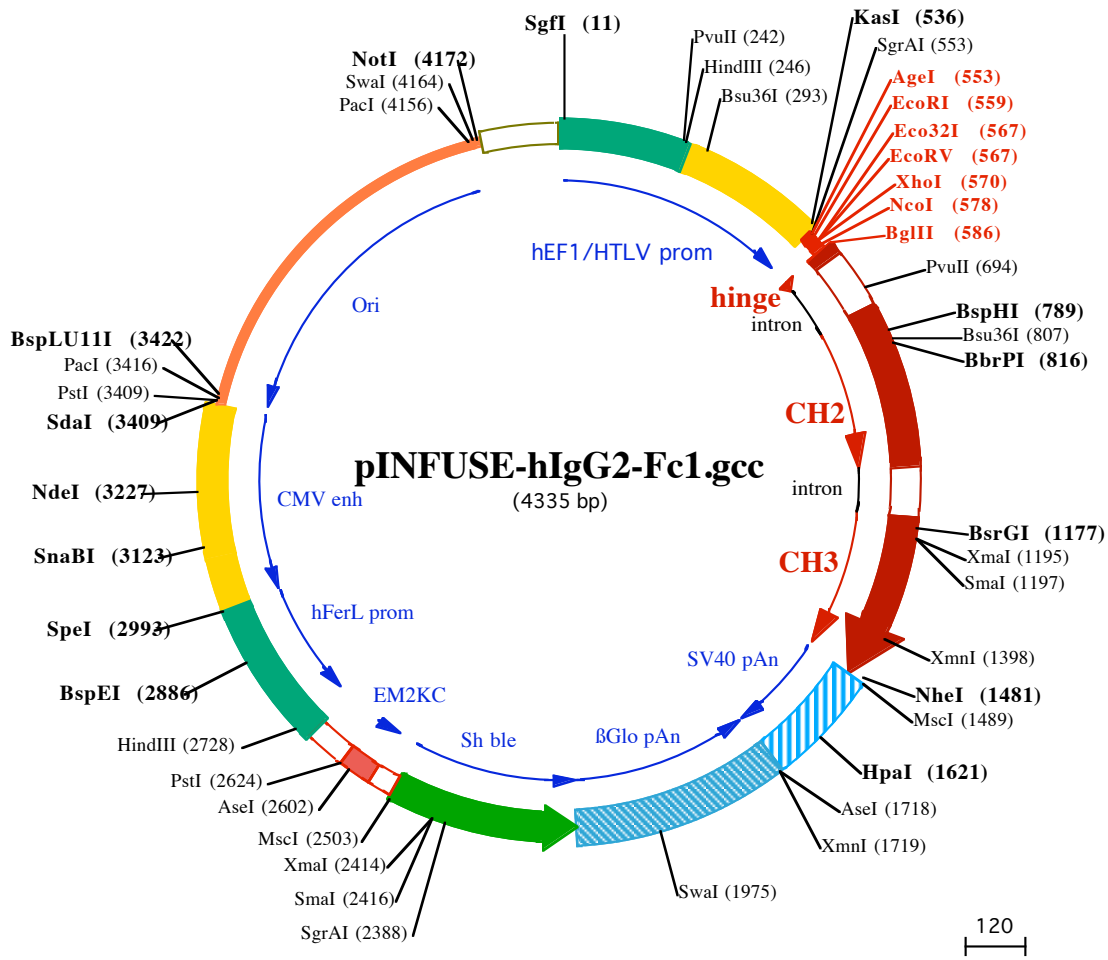
1. Nott A, et al. 2003. A quantitative analysis of intron effects on mammalian gene expression. *RNA*. 9(5):607-17.
2. Kim DW *et al.* 1990. Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. *91(2):217-23*.
3. Takebe Y. *et al.* 1988. SR alpha promoter: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

TECHNICAL SUPPORT

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SgfI (11)
1 GGATCTGCGATCGTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGTTCGGCAATTGAACGGGTGCTA

101 GAGAAGGTGGCGGGTAAACTGGGAAAGTGATGTCGTGACTGGCTCCGCTTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

HindIII (246) PvuII (242) Bsu36I (293)
201 GTGAACGTTCTTTTTCGCAACGGGTTTCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCTTCACGCGCCCGCCCTACCTGAGGGC

301 GCCATCCACGCCGGTTGAGTCGCGTTCTGCCCTCCCGCTGTGGTGCCTCTGAACTGCGTCCGCCCTTAGGTAAGTTTAAAGCTCAGGTCGAGACC

401 GGGCCTTTGTCCGGCGCTCCCTTGAGGCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGTCAACTCTACGTCTTTGTTTCGTTT

KasI (536) EcoRI (559) XhoI (570) AgeI (553) EcoRV (567) BglII (586) SgrAI (553) Eco32I (567) NcoI (578)
501 TCTGTTCTGCGCGTTACAGATCCAAGCTGTGACCGGCCCTACCTGAGATCACCGGTGAATTCGATATCTCGAGCACCATGGTTAGATCTGTGAGTGC
1▶ Val Gl uCys

601 CCACCGTCCCAgtaagccagccaggcctcgccctccagctcaaggcgggacaggtgcctagagtagcctgcatccagggacagggccagcctgggt
4▶ ProProCysPro PvuII (694)

701 gctgacacgtccacctccatctcttcctcagCACCACCTGTGGCAGGACCGTCACTTCTCTTCCCCCAAACCCAAGGACACCTCATGATCTCCC
1▶ ProP roVal Al aGl yProSer Val PheLeuPheP roP roLysP roLysAspThr LeuMet I l eSer A BspHI (789)

BbrPI (816) Bsu36I (807)
801 GGACCCCTGAGGTCACGTCGCTGGTGGTGACGTCAGCCACGAAGACCCGAGGTCAGTTCACCTGCTGACGTCGGACGGCGTGGAGGTGCATAATGCCAA
23▶ r gThr P roGl uVal Thr CysVal Val Val AspVal Ser Hi sGl uAspP roGl uVal Gl nPheAsnTrpTyrVal AspGl yVal Gl uVal Hi sAsnAl aLy

901 GACAAAGCCAAGGAGGAGCAGTTCACAGCAGCTTCCGTGGTGCAGCTCTCACCGTTGTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGC
56▶ s Thr LysP roArgGl uGl nPheAsnSer Thr PheArgVal Val Ser Val LeuThr Val Val Hi sGl nAspTrpLeuAsnGl yLysGl uTyrLysCys

1001 AAGGTCTCCAACAAAGGCTCCAGCCCCATCGAGAAAACCATCTCCAAAACCAAGtggggaccgcggggtatgagggccacatggacagagggccgg
90▶ LysVal SerAsnLysGl yLeuP roAl aP roI l eGl uLysThr I l eSer LysThr Lys XmaI (1195) SmaI (1197)

1101 ctcgccaccctctgcccctgggagtgaccgctgtgccaacctctgcccctacagGGCAGCCCCGAGAACCACAGGTGTACACCTGCCCCATCCCGGG
1▶ Gl nP roArgGl uP roGl nVal TyrThr LeuP roP roSer ArgG BsrGI (1177)

1201 AGGAGATGACCAAGAACAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCAGCGACATCGCCGTGGAGTGGGAGGCAATGGGACGGCGAGAA
15▶ l uGl uMe tThr LysAsnGl nVal Ser LeuThr CysLeuVal LysGl yPheTyrP roSerAspI l eAl aVal Gl uTrpGl uSerAsnGl yGl nP roGl uAs XmnI (1398)

1301 CAACTACAAGACCACACCTCCCATGCTGGACTCCGACGGCTCTTCTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTC
48▶ nAsnTyrLysThr Thr P roP roMe tLeuAspSerAspGl ySer PhePheLeuTyrSer LysLeuThr Val AspLysSer ArgT rpGl nGl nGl yAsnVal MscI (1489)

1401 TTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGTAAATGAGTGCTAGCTGGCCAGACATGAT
82▶ PheSer CysSer Val l eMe tHi sGl uAl aLeuHi sAsnHi sTyrThr Gl nLysSer LeuSer LeuSer P roGl yLys••• NheI (1481)

1501 AAGATACATTGATGAGTTGGACAAACCAACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATT

HpaI (1621)
1601 ATAAGCTGCAATAAACAAGTTAAACAACAATTGCATTCAATTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCT

AseI (1718) XmnI (1719)
1701 ACAAATGTGGTATGGAATTAATTCTAAAAATACAGCATAGCAAACTTTAACCTCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGC

1801 ATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTTAAGATATAGTGATTTTCCCAAGGTTTGAACTA

Swal (1975)
1901 GCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCACATTCCTTTTTAGTAAAAATTCAGAAAATAATTTAAATACATCATTGCAATGAAAATAA

2001 ATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATT

2101 GGACAGCAAGAAAGCGAGCTTCTAGCTTATCTCAGTCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGTTCGCGCAGGGCGAACTCCCGCC
125▶ •••AspGl nGl uGl uAl aVal PheHi sVal CysAsnGl yAl aP roAspArgLeuAl aPheGl uArgGl

2201 CCCACGGCTGCTCGCGATCTCGGTATGGCCGGCCGGAGGCTCCGGAAGTTCTGTGGACACGACCTCCGACACTCGGCTACAGCTCGTCCAGGCC
102▶ yTrpP roGl nGl uGl yI l eGl uThr Me tAl aP roGl ySer Al aAspArgPheAsnThr Ser Val Val Gl uSer T rpGl uAl aTyrLeuGl uAspLeuGl y SgrAI (2388)

2301 GCGACCCACACCCAGGCCAGGGTGTGTCCGGCACCACTGGTCTGGACCGCGCTGATGAACAGGGTACGTCGTCCCGACACCCGGCGAAGTCCG
69▶ ArgVal TrpVal TrpAl aLeuThrAsnAspP roVal Val Gl nAspGl nVal Al aSer I l ePheLeuThr Val AspAspArgVal Val Gl yAl aPheAspA XmaI (2414) SmaI (2416)

2401 TCCTCCACGAAGTCCCGGAGAACCCGAGCCGGTCCGGTCCGAACTCGACCGCTCCGGCGACGTCGCGCGGGTGGACCCGGAACGGCACTGGTCAACT
35▶ spGl uVal PheAspArgSer PheGl yLeuArgAspThr TrpPheGl uVal Al aGl yAl aVal AspArgAl aThr LeuVal P roVal Al aSer Thr LeuLy MscI (2503)

2501 TGGCCATGATGGCTCCTcctgtcaggagaggaagagaagaaggttagtacaattgCTATAGTGAGTTGATTATACTATGCAGATATACTATGCCAATG
2▶ sAl aMe t AseI (2602) PstI (2624)

2601 ATTAATTGTCAAACCTAGGGCTGCAgggttcatagtgccacttttctgcactgccccatctcctgcccaccctttccagggcatagacagtcagtgactt

HindIII (2728)
2701 acCAAACCTCAGGAGGGAGAAGGAGGAGGCTTGGAGACAGACCCGGGACCCGGAACCTGCGAGGGGACGTGGCTAGGGCGGCTCTTTTATGGTGGC

2801 CGGCCCTCGGAGGCAGGGCGCTCGGGAGGCCTAGCGGCAATCTGCGGTGGCAGGAGCGGGGCCGAAGGCCGTGCCTGACCAATCCGGAGCACATAGG **BspEI (2886)**

2901 AGTCTCAGCCCCCGCCCAAAGCAAGGGGAAGTCACGCGCCTGTAGCGCCAGCGTGTGTGAAATGGGGGCTTGGGGGGTGGGGCCCTGACTAGTCA **SpeI (2993)**

3001 AAACAAACTCCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTACTGCCAAAACCGCATCATC

3101 ATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAAGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCAT **SnaBI (3123)**

3201 TGACGTCAATAGGGGGCTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGT **NdeI (3227)**

3301 CCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGTCGTTGGGCGGTGACCCAGGCGGGCCATTTACCGTAAGTTATGTA

3401 ^{PstI (3409)} **SdaI (3409)** ^{PacI (3416)} **BspLU11I (3422)** ACGCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGTTGCTGGCGTTTTTCCATAGGCTCCGCC

3501 CCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTG

3601 CGCTCTCTGTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTATCTCA

3701 GTTCGGTGTAGGTCGTTGCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTCAGCCCGACCGCTGCGCTTATCCGGTAACTATCGTCTTGAGTCCAA

3801 CCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTG

3901 CCTAACTACGGTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAC

4001 AAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTC

4101 TGACGCTCAGTGAACGAAAACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGAATAAAATATCTTTATTTTCAT ^{PacI (4156)} ^{SwaI (4164)} **NotI (4172)**

4201 TACATCTGTGTGTTGGTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAACAAAACGAAACAAAACAACTAGCAAATAGGCTGTCCCCAG

4301 TGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA