

pINFUSE-hIgG2-Fc2

Plasmid designed for the construction of Fc-Fusion proteins

Catalog # pfc2-hgin2

For research use only

Version 20K06-MM

PRODUCT INFORMATION

Content:

- 20 µg of pINFUSE-hIgG2-Fc2 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 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.

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-1α (EF-1α) 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-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.
- **IL2 ss:** The IL2 signal sequence contains 20 amino acids and share common characteristics with signal peptides of other secretory proteins. The intracellular cleavage of the IL2 signal peptide occurs after Ser20 and leads to the secretion of the antigenic protein.
- **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 *Streptolothichus hindustanus*. The same resistance gene confers selection in both mammalian cells and *E. coli*.
- **βGlo 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:

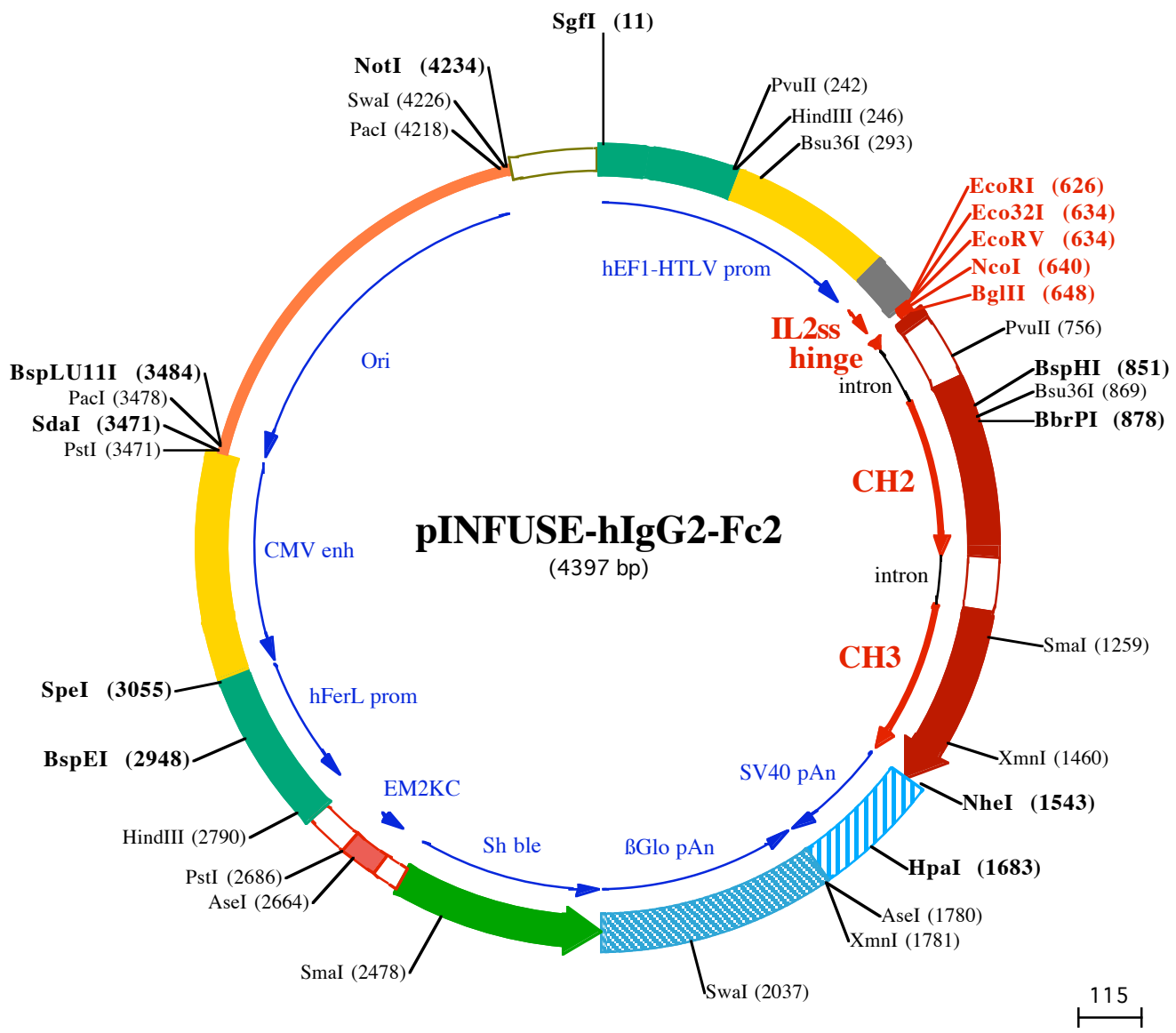
1. Nott A, et al. 2003. A quantitative analysis of intron effects on mammalian gene expression. *RNA*. 9(5):607-17.
2. Kim DW *et al.* 1990. Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. *91(2):217-23*.
3. Takebe Y. *et al.* 1988. SR alpha promoter: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

101 GAGAAGGTGGCGGGGTAACCTGGGAAAGTGATGCTGTACTGGCTCCGCTTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

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

201 GTGAACGTTCTTTTTCGCAACGGGTTTGGCGCCAGAACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCTTACGCGCCCGCCCTACCTGAGGCC

301 GCCATCCACCGCGTTGAGTGCCTCTGCGCCCTCCCGCTGTGGTGCCTCTGAACTGCGTCCGCGTCTAGTTAAGTTTAAAGCTCAGGTCGAGACC

401 GGGCTTTGTCGGCGCTCCCTTGAGCCTACCTAGACTCAGCGGCTCTCCACGCTTTCCTGACCCTGCTTGTCAACTCTACGTCTTTGTTTCGTTT

501 TCTGTTCTGCGCGTTACAGATCCAAGCTGTGACCGCCCTACCTGAGATCAcggcGAAGGAGGCCACCATGTACAGGATGCAACTCCTGTCTTGCAC

1MetTyrArgMetGlnLeuLeuSer CysI

EcoRV (634) BglII (648)

Eco32I (634) NcoI (640)

601 TTGCACTAAGTCTTGCACCTTGTACGAATTCGATATCGGCCATGGTTAGATCTGTGAGTGCACCCGTCGCCAGGtaagccagccaggcctcgccctc

101leAlaLeuSerLeuAlaLeuValThrAsnSer 1ValIgluCysProProCysProA

PvuII (756)

701 cagctcaaggcgggacaggtgcctagatagcctgcatccagggacagggccagcgggtgctgacacgtccacctcctctctctcagCACACC

laProPr

BspHI (851) BbrPI (878) Bsu36I (869)

801 TGTGGCAGGACCGTCACTTCTCTTCCCGAAAACCAAGGACACCTCATGATCTCCCGACCCCTGAGGTCACTGCGTGGTGGTGGACGTGAGC

2oValAlaGlyProSerValPheLeuPheProProLysProLysAspThrLeuMetIleSerArgThrProGluValValAspValSer

901 CACGAAGACCCGAGGTCAGTTCACTGGTACGTGGACGGCGTGGAGTGCATAATGCCAAGACAAAGCCAGGGAGGAGCAGTTCAACAGCACGTTCC

36HisGluAspProGluValGlnPheAsnTrpTyrValAspGlyValGluValHisAsnAlaLysThrLysProArgGluGluGlnPheAsnSerThrPheA

1001 GTGTGGTCAGCGTCTCACCCTGTGCACCAAGGACTGGCTGAACGGCAAGGAGTACAAGTCAAGTCAAGTCTCAACAAAGGCCAGCCAGCCCATCGAGAA

69rgValValSerValLeuThrValValHisGluAspTrpLeuAsnGlyLysGluTyrLysCysLysValSerAsnLysGlyLeuProAlaProIleGluLys

1101 AACCATCTCAAAACAAAGtggggaccgcggggtatgagggccacatggacagagggcggctcgggccaccctctgcccctgggagtgaccgctgtgcc

102sThrIleSerLysThrLysG

SmaI (1259)

1201 aacctctgtccctacagGCGAGCCCGAGAACCACAGGTGTACACCTGCCCCATCCCGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTG

IyGlnProArgGluProGlnValTyrThrLeuProProSerArgGluGluMetThrLysAsnGlnValSerLeuThrCysLeu

1301 GTCAAAGGCTTCTACCCAGCGACATCGCGTGGAGTGGGAGAGCAATGGGACCGGAGAACAACTACAAGACCACACTCCCATGCTGACTCCGAGC

28ValLysGlyPheTyrProSerAspIleAlaValGluTyrGluSerAsnGlyGluProGluAsnAsnTyrLysThrThrProProMetLeuAspSerAspG

XmnI (1460)

1401 GCTCTTCTTCTCTACAGCAAGCTCACCGTGACAGAGCAGGTGGCAGCAGGGAACTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCA

61IySerPhePheLeuTyrSerLysLeuThrValAspLysSerArgTrpGlnGluYAsnValPheSerCysSerValMetHisGluAlaLeuHisAsnHi

NheI (1543)

1501 CTACACGAGAAGGCCTCTCCCTGTCTCCGGTAAATGAGTGTAGTGGCCAGACATGATAAGATACATTGATGAGTTTGACAAACCACAACCTAGAA

94sTyrThrGlnLysSerLeuSerLeuSerProGlyLys

HpaI (1683)

1601 TGCAGTAAAAAATGCTTTATTTGTAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTCAATAAACAAGTTAACAACAACAAATTGCAT

AseI (1780) XmnI (1781)

1701 TCATTTTATGTTTCAGGTTTCAGGGGGAGGTGTGGAGGTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAATTAATCTAAAATACAGCATA

1801 GCAAACTTTAACTCCAAATCAAGCCTCTACTTGAATCTTTTCTGAGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCAATGTGCATTAGCTGT

1901 TTGCAGCCTACCTTCTTTCATGGAGTTTAAAGATATAGTGTATTTCCCAAGGTTTGAACCTAGCTCTTCAATTTCTTTATGTTTTAAATGCACTGACCTCC

Swal (2037)

2001 CACATTCCTTTTTAGTAAATATTAGAAATAATTTAAATACATCATTGCAATGAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCC

2101 TTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTC

125Asp

2201 CTGCTCTCTGCCACAAAGTGCACGAGTTGCCGGCGGGTCCGCGAGGGCACTCCGCCCCACGGCTGCTCGCGATCTCGGTCATGGCCGGCCCG

123GlnGluGluAlaValPheHisValCysAsnGlyAlaProProArgLeuAlaPheGluArgGlyTrpProGlnGluGlyIleGluThrMetAlaProGlySer

2301 GAGGCGTCCCGAAAGTTCGTGGACACGACTCCGACACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACCCAGGCGGGTGTGTTCCGGCACC

89IleAlaAspArgPheAsnThrSerValValGluSerTrpGluAlaTyrLeuGluAspLeuGlyArgValTrpValTrpAlaLeuThrAsnAspProValVal

SmaI (2478)

2401 CCTGCTCTGGACCGCTGATGAACAGGGTCACTGCTCCCGACACACCGCGAAGTCTGCTCCACGAAGTCCCGGAGAACCAGCGGTCGGT

56IleGlnAspGlnValAlaSerIlePheLeuThrValAspAspArgValValGlyAlaPheAspAspGluValPheAspArgSerPheGlyLeuArgAspThr

2501 CCAGAACTCGACCGCTCCGGCGAGCTGCGCGCGGTGAGCACCAGGCACTGGTCAACTGGCCATGATGCTCTCctgtcaggagaggaagaga

23TrpPheGluValAlaGlyAlaValAspArgAlaThrLeuValProValAlaSerThrLeuLysAlaMet

AseI (2664) PstI (2686)

2601 agaaggttagtacaattgCTATAGTGAAGTGTATTATACTATGCAGATATACTATGCCAATGATTAATTGTCAAACCTAGGGCTGCAGgggttcatagtgcc

HindIII (2790)

2701 acttttctgcactgccccatctcctgcccaccctttccaggcatagacagtcagtgacttacAAACTCACAGGAGGAGAAGGCAGAAGCTTGAGAC

2801 AGACCCGGGACCGCCGAACCTGCGAGGGACGTGGCTAGGGCGGCTTTTATGTTGCGCCGCCCTCGGAGGCAGGGGCTCGGGAGGCCTAGCGG

BspEI (2948)

2901 CCAATCTCGGTGGCAGGAGGGGGCCGAAGGCCGTGCCTGACCAATCCGGAGCACATAGGAGTCTCAGCCCCCGCCCAAGCAAGGGGAAGTCACG

SpeI (3055)

3001 CGCCTGTAGCGCCAGCGTGTGTGAAATGGGGCTTGGGGGGTGGGGCCCTGACTAGTCAAAACAACTCCATTGACGTCAATGGGGTGGAGACTTG

3101 GAAATCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCA
3201 AGTAGGAAAGTCCCATAAAGTCATGTACTGGGCATAATGCCAGGCGGGCATTACCGTCAATGACGTCAATAGGGGCGTACTTGGCATATGATACACT
3301 TGATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCAATTATTG

PacI (3478)
PstI (3471)
SdaI (3471) **BspLU11I (3484)**

3401 ACGTCAATGGGCGGGGTCGTTGGCGGTCAGCCAGGCGGGCATTACCGTAAGTTATGTAACGCCTGCAGGTTAATTAAGAACAATGTGAGCAAAGGC
3501 CAGCAAAAGGCCAGGAACCGTAAAAAGCGCGTTTCTGCTGGCGTTTTTCATAGGCTCCGCCCCCTGACGAGCATCAAAAATCGACGCTCAAGTCAGA
3601 GGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTTCCCCTGGAAGCTCCCTCGTGCGCTCTCTGTTCCGACCCTGCCGTTACCGGATACCT
3701 GTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGTTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCCAAGCTGGGCTGTGTG
3801 CACGAACCCCGTTTCAGCCGACCGCTGCGCCTTATCCGGTAACATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATGCCACTGGCAGCAGCCA
3901 CTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTCTACAGGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTAT
4001 CTGCGCTCTGTGAAGCCAGTTACCTTCGGAAAAAGATTGGTAGCTTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGGTTGCAAG
4101 CAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACACGTTAAGGGATT

PacI (4218) SnaI (4226) **NotI (4234)**

4201 TGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGAATAAAATATCTTTATTTTCATTACATCTGTGTGGTTTTTGTGTGAATCGTAACTA
4301 ACATACGCTCTCCATCAAAAACAAACGAAACAAACAAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA