

pINFUSE-mIgG2b-Fc1

Plasmid designed for the construction of Fc-Fusion proteins

Catalog # pfc1-mgin2b

For research use only

Version 20K06-MM

PRODUCT INFORMATION

Content:

- 20 μ g of pINFUSE-mIgG2b-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

- **murine genomic IgG2b-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. Murine IgG2b displays high 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 *Streptoalloteichus hindustanus*. The same resistance gene confers selection in both mammalian cells and *E. coli*.
- **β G10 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:

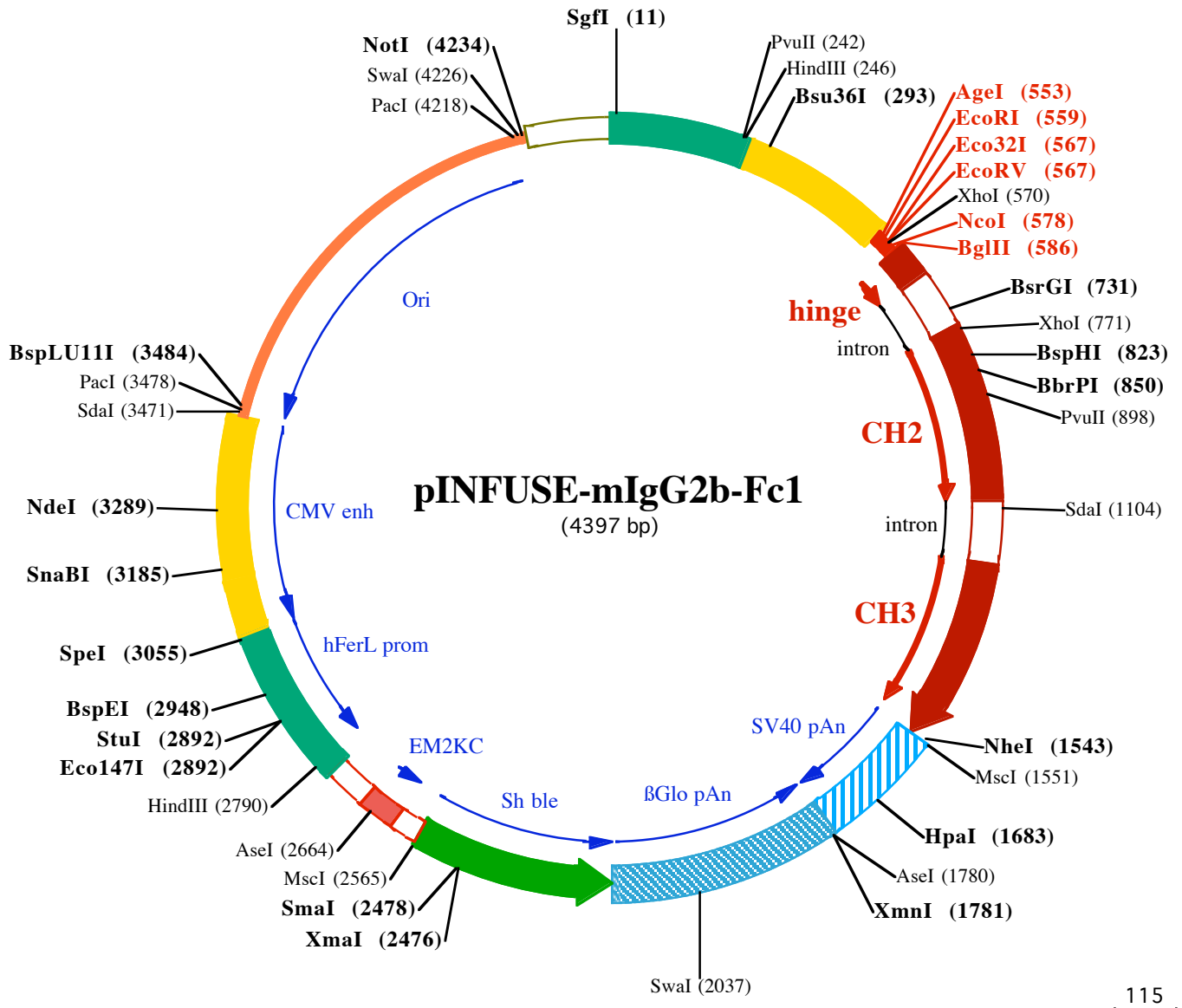
1. Nott A, et al. 2003. A quantitative analysis of intron effects on mammalian gene expression. *RNA*. 9(5):607-17.
2. Kim DW *et al.* 1990. Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. *91(2):217-23*.
3. Takebe Y, *et al.* 1988. SR alpha promoter: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

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SgfI (11)
1 GGATCTGCGATCGCTCCGGTGCCTGAGTGGGAGAGCGACATCGCCACAGTCCCGGAGAAGTTGGGGGAGGGGTCGGCAATTGAACGGGTGCCTA
101 GAGAAGGTGGCGGGGTAACCTGGGAAAGTGATGCTGTACTGGCTCCGCTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC
HindIII (246)
PvuII (242) Bsu36I (293)
201 GTGAACGTTCTTTTTCGCAACGGGTTTGCCTGACAGTGAAGCTTCAGGGGCTCGCATCTCTCTTACGCGCCCGCCCTACCTGAGGCC
301 GCCATCCACCGCGGTTGAGTGGCGTTCTGCCGCTCCCGCTGTGGTGCCTCCTGAACTCGCTCCGCGTCTAGGTAAGTTAAAGCTCAGGTGAGAC
401 GGGCTTTGTCCGCGCTCCCTTGGAGCTTACCTAGACTCAGCCGGCTCCACGCTTTGCTGACCTGCTTGTCTCAACTCTAGTCTTTTCTGTTT
EcoRV (567)
EcoRI (559) XhoI (570) BglII (586)
AgeI (553) Eco32I (567) NcoI (578)
501 TCTGTTCTGCGCGTTACAGATCCAAGCTGTGACCGCGCTACCTGAGATCACCGTGAATTCGATATCTCGAGCACCATGGTGTAGTCTCCAGCGGG
1P ProSer Gl y
601 CCCATTTCAACAATCAACCCTGTCTCCATGCAAGGAGTGTCAAAATGCCAGGtaagtactaccagagctcactcccaggagaatggaagtgc
4P ProI leSer Thr l leAsnP roCysP roP roCysLysGl uCysHi sLysCysP roA
BsrGI (731) XhoI (771)
701 gtaaaaatccctgtaatggaggataagccatgtacaaatccatttccatctctctcatcagCTCCTAACCTGAGGGTGGACCATCGTCTTTCATCTTC
la ProAsnLeuGl uGl yGl yProSer Val PheI lePhe
BspHI (823) BbrPI (850) PvuII (898)
801 CCTCAAATATCAAGGATGACTCATGATCTCCCTGACACCAAGTGTGTGGTGGATGTGAGCGAGGATGACCCAGACGCTCCAGATCAGCT
13P ProProAsnI leLysAspVal LeuMetI leSer LeuThr ProLysVal Thr CysVal Val Val AspVal Ser Gl uAspAspP roAspVal Gl nI leSer T
901 GGTGTGAAACAACGTTGAAAGTACACACAGCTCAGACACAAACCATAGAGAGGATTACAACGACTATCCGGTGGTCCAGCACCTCCCATCCAGCA
46P rPheVal AsnAsnVal Gl uVal Hi sThr Al aGl nThr Gl nThr Hi sArgGl uAspTyrAsnSer Thr l leArgVal Val Ser Thr LeuP roI leGl nHi
SdaI (1104)
1001 CCAGGACTGGATGAGTGGCAAGGAGTCAAATGCAAGGTCAACAACAAGACCTCCATCACCCATCGAGAGAACCATCTCAAAAATTAAGGtgggacc
79P sGl nAspTrpMetSer Gl yLysGl uPheLysCysLysVal AsnAsnLysAspLeuP roSer P roI leGl uArgThr l leSer LysI leLysG
1101 tgcaggacaactgcattggggctgggatgggcataagaataaattgtctatgtggacagccttccattcggccatgacctctatgtatttcttaacc
acagGGCTAGTCAGAGCTCCACAAGTATACATCTTCCGCCACCAGCAGAGCAGTTGTCAGGAAAGATGTCAGTCTCACTTGCCTGGTGGTGGCTTCA
lyLeuVal ArgAl aP roGl nVal TyrI leLeuP roP roP roAl aGl uGl nLeuSer ArgLysAspVal Ser LeuThr CysLeuVal Val Gl yPheA
1301 ACCCTGGAGACATCAGTGTGGATGGACAGCAATGGGCATACAGAGGAACTACAAGACACCGCACCAGTCTGGACTCTGACGGTCTTACTTCAT
32P snP roGl yAspI leSer Val Gl uTrpThr SerAsnGl yHi sThr Gl uGl uAsnTyrLysAspThr Al aP roVal l leuAspSerAspGl ySer TyrPheI l
1401 ATATAGCAAGCTCAATATGAAAACAAGCAAGTGGAGAAAACAGATTCTCTCATGCAACGTGAGACACGAGGGTCTGAAAAATTACTACCTGAAGAAG
65P eTyrSer LysLeuAsnMetLysThr Ser LysTrpGl uLysThr AspSer PheSer CysAsnVal ArgHi sGl uGl yLeuLysAsnTyrTyrLeuLysLys
MscI (1551)
NheI (1543)
1501 ACCATCTCCCGGTCTCCGGTAAATGAGCTCGCACCCACAAAGTACTGGCAGACATGATAAGATACATTGATGAGTTTGACAAACCACAAC TAGAA
99P Thr l leSer ArgSer ProGl yLys...
HpaI (1683)
1601 TGCAGTAAAAAATGCTTTATTGTGAAATTTGTGATGCTATTGCTTTATTGTAAACATTATAAGCTCAATAAACAAGTTAAACAACAACAAATTGCAT
AseI (1780)
XmnI (1781)
1701 TCATTTTATGTTTCAGGTTCAAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAAACCTACAAATGTGGTATGGAATTAATCTAAAAACAGCATA
1801 GCAAAACTTTAACTCCAAATCAAGCCTCTACTTGAATCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGT
1901 TTGCAGCCTCACCTTTTTCATGGAGTTTAAAGATATAGTATTTTTCCCAAGTTTGAACAGCTCTTCAATTTCTTTATGTTTTAAATGCACTGACCTCC
SwaI (2037)
2001 CACATTCCTTTTTAGTAAATATTAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTATTAGGCAGAATCCAGATGCTCAAGGCC
2101 TTCATAATATCCCCAGTTTGTAGTGGACTTAGGGAACAAGGAACCTTAAATAGAAATGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTC
125P...Asp
2201 CTGCTCTCTGCCACAAAGTGCACGAGTTGCCGGCGGGTCCGCGAGGGCAACTCCGCCCCACGGCTGCTCGCGATCTCGGTATGGCGGCGCCG
123P Gl nGl uGl uAl aVal l PheHi sVal CysAsnGl yAl aP roAspArgLeuAl aPheGl uArgGl yTrpP roGl nGl uGl yI leGl uThr MetAl aP roGl yS
2301 GAGGCGTCCCGAAAGTTCTGGACAGCCTCCGACACTCGGCGTACAGCTCTGTCAGGCCGCGCACCCACCCAGCCAGGCTGTGTTCGGCAGCA
89P er Al aAspArgPheAsnThr Ser Val Val Gl uSer TrpGl uAl aTyrLeuGl uAspLeuGl yArgVal l TrpVal l TrpAl aLeuThrAsnAspP roVal Val
XmaI (2476)
SmaI (2478)
2401 CCTGGTCTGGACCGCTGATGAACAGGTCACGTCGTCGCCGACACCCGCGAAGTCTGCTCCACGAAGTCCCGGAGAACCCGAGCCGCTCGGT
56P l Gl nAspGl nVal Al aSer l lePheLeuThr Val l AspAspArgVal Val Gl yAl aPheAspAspGl uVal l PheAspArgSer PheGl yLeuArgAspThr
MscI (2565)
2501 CCAGAACTCGACCGCTCCGCGCAGCTGCGCGCGGTGAGCACCGAACGGCAGCTGTTCAACTTGGCCATGATGGCTCCTcgtcaggagaggaagaga
23P TrpPheGl uVal Al aGl yAl aVal l AspArgAl aThr LeuVal l ProVal l Al aSer Thr LeuLysAl aMet
AseI (2664)
2601 agaaggttagtacaattgCTATAGTGAGTTGTATTATACTATGCAGATATACTATGCCAATGATTAATTGTCAAAGTGGCTGCAGggttcatatgctc
HindIII (2790)
2701 acttttctgcactgccccatctcctgccacccttccaggcatagacagtcagtacttacCAAACACAGGAGGGAGAAGCAGAAGCTTGAGAC
StuI (2892)
Eco147I (2892)
2801 AGACCCCGGGACCGCCGAACCTGCGAGGGAGCTGGCTAGGGCGGCTCTTTTATGGTGCCTGGCCCTCGGAGGCAGGGCGCTCGGGAGGCGCTAGCGG
BspEI (2948)
2901 CCAATCTGCGGTGGCAGGAGCGGGCCGAAGCCGTGCTGACCAATCCGGAGCACATAGGAGTCTCAGCCCCCGCCCAAGCAAGGGGAAGTCAGC
SpeI (3055)
3001 CGCCTGTAGCGCCAGCTGTTGTGAAATGGGGCTTGGGGGGTGGGGCTGACTAGTCAAAACAAACTCCCATTGACGTCATGGGGTGGAGACTTG
SnaBI (3185)
3101 GAAATCCCGGTGAGTCAAAACCGCTATCCACGCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCA

3201 AGTAGGAAAGTCCATAAAGGTCATGTA CTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACT **NdeI (3289)**

3301 TGATGTA CTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCATTGACGTC AATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTG

3401 ACGTCAATGGGCGGGGGTCTGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAAGTTATGTAACGCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGC SdaI (3471) PacI (3478) **BspLU11I (3484)**

3501 CAGCAAAAGGCCAGGAACCGTAAAAAGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGA

3601 GGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTTCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCT

3701 GTCCGCCTTCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTACGCTGTAGGTATCTCAGTTCGGGTAGGTCGTTGCTCCAAGCTGGGCTGTGTG

3801 CACGAACCCCGTT CAGCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATGCCACTGGCAGCAGCCA

3901 CTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTAT

4001 CTGCGCTCTGTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGTTTTTTTGTGTTGCAAG

4101 CAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGGTCTGACGCTCAGTGGAAACGAAAACTCACGTTAAGGGATTT

4201 TGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTAATCGTAACTA PacI (4218) SmaI (4226) **NotI (4234)**

4301 ACATACGCTCTCCATCAAAACAAAACGAAACAAAACAACTAGCAAATAGGCTGTCCCCAGTGAAGTGCAGGTGCCAGAACATTTCTCTATCGAA