

pFUSE-rtIgG-Fc2 (IL2ss)

Plasmid designed for the construction of rat IgG2B Fc-Fusion proteins

Catalog # pfuse-rtg2bfc2

For research use only

Version 20K04-MM

PRODUCT INFORMATION

Content:

- 20 µg of pFUSE-rtIgG2B-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

pFUSE-Fc is a family of plasmid developed to facilitate the construction of Fc-Fusion proteins by fusing a sequence encoding a given protein to the Fc region of an immunoglobulin.

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. These cells are commonly used in protein purification systems.

pFUSE-Fc2 (IL2ss) plasmids allow the secretion of Fc-Fusion proteins. They contain the IL2 signal sequence (IL2ss) for the generation of Fc-Fusion proteins derived from proteins that are not naturally secreted. 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.

PLASMID FEATURES

- **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.
- **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.
- **rtIgG2B Fc (rat):** The Fc region comprises the CH2 and CH3 domains of the IgG2B 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.

- **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*.
- **βGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription⁴.

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

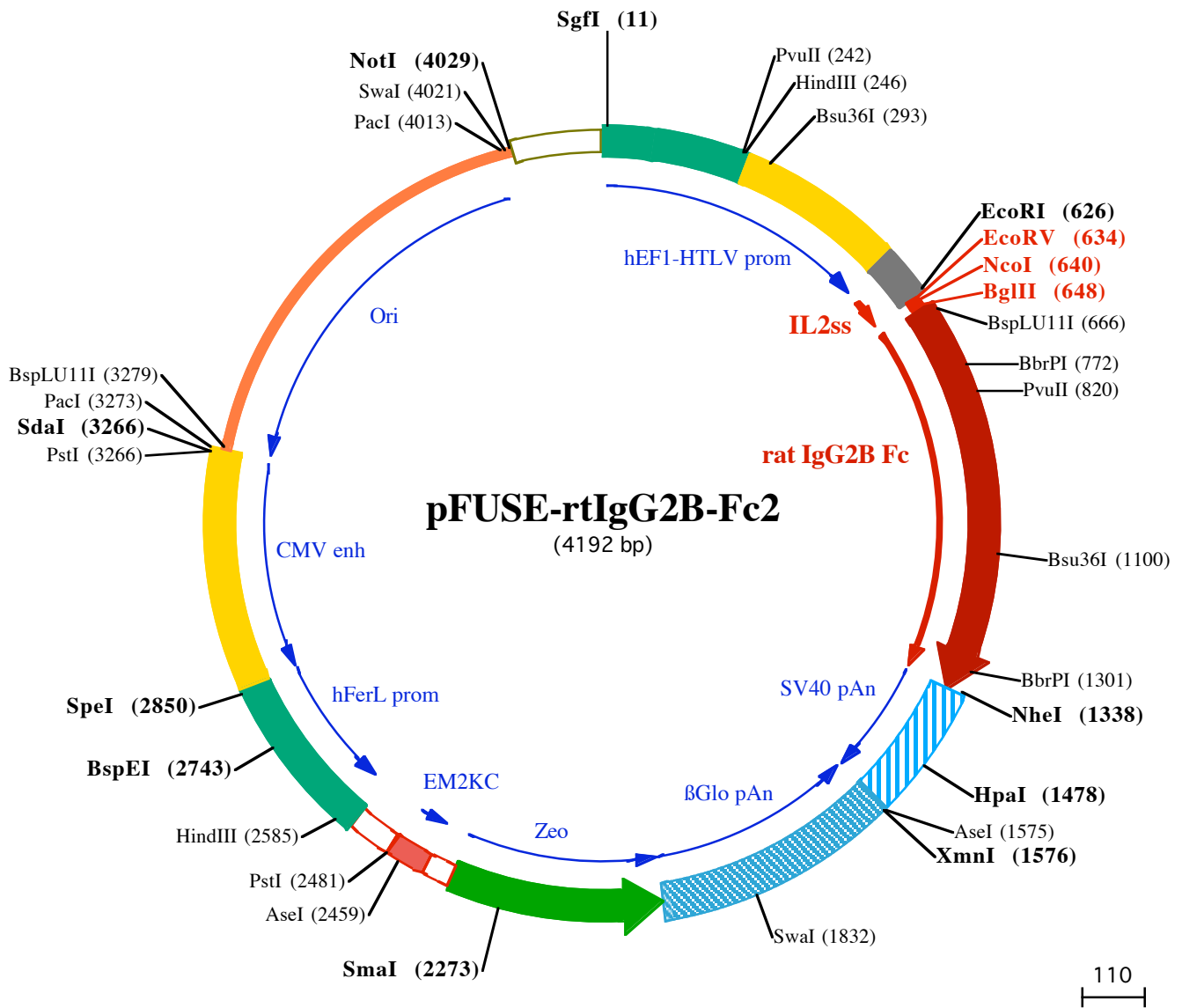
This antibiotic can be used for *E. coli* at 25 µg/ml in liquid or solid media and at 50-200 µg/ml to select Zeocin™-resistant mammalian cells.

References:

1. Kim, D.W. *et al.* (1990). *Gene* 2: 217-223.
2. Takebe, Y. *et al.* (1988). *Mol. Cell Biol.* 1: 466-472.
3. Carswell, S., and Alwine, J.C. (1989). *Mol. Cell Biol.* 10: 4248-4258.
4. Yu J & Russell JE. (2001). *Mol Cell Biol*, 21(17):5879-88.

TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3622-3480
E-mail: info@invivogen.com



SgfI (11)

1 GGATCTGCGATCGCTCCGGTCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCGGAGAAGTTGGGGGAGGGTTCGCAATTGAACGGGTGCCTA

101 GAGAAGGTGGCGGGGTAACCTGGGAAAGTGATGCTGTACTGGCTCCGCTTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

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

201 GTGAACGTTCTTTTTGCAACGGGTTTGGCCGAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTACGCGCCCGCCCTACCTGAGGCC

301 GCCATCCACGCGGGTTGAGTCGCGTTCTGCCGCTCCCGCTGTGGTGCCTCTGAAGCTCGCTCCGCGCTTAGGTAAGTTAAAGCTCAGGTCGAGACC

401 GGGCTTTGTCCGGCGCTCCCTTGAGCCTACCTAGACTCAGCGGCTCCACGCTTTCCTGACCTGCTTGTCTCAACTCTACGCTTTGTTTCGTTT

501 TCTGTTCTGCGCGCTTACAGATCCAAGCTGTGACCGGCGCTACCTGAGATCAcggcGAAGGAGGGCCACCATGTACAGGATGCAACTCCTGTCTTGC

1►MetTyrArgMetGlnLeuLeuSer CysI

EcoRV (634) BglIII (648) EcoRI (626) NcoI (640) BspLU11I (666)

601 TTGCACTAAGTCTTGCACCTGTGCACGAATTCGATATCGGCCATGGTTAGACTCTCTACATGCCCTACATGTACAAATGCCAGTTCCTGAAGCTTTGGG

10►IeAlaLeuSerLeuAlaLeuValThrAsnSer 1►ProThrCysProThrCysHisLysCysProValProGluLeuLeuGlu

701 TGGACCATCTGTCTTACCTTCCCGCAAAGCCAAAGGACATCCTCTTGTATCTCCAGAACGCCAAGGTACAGTGTGTGGTGGTGGATGTGAGCGAGGAG

16►yGluProSerValPheIlePheProProLysProLysAspIleLeuLeuIleSerGlnAsnAlaLysValThrCysValValValAspValSerGluGlu

BbrPI (772) PvuII (820)

801 GAGCCGGACGTCCAGTTCAGCTGGTTGTGAACAACGTAGAAGTACACAGCTCAGACACAACCCCGTGGAGGAGCAGTACAACAGCACCTTCAGAGTGG

50►GluProAspValGlnPheSerTrpPheValAsnAsnValGluValHisThrAlaGlnThrGlnProArgGluGluGlnTyrAsnSerThrPheArgValVal

901 TCAGTGCCTCCCATCCAGCACCAGGACTGGATGAGCGGCAAGGATCAAATGCAAGGTCAACAACAAGCCCTCCAAGCCCATCGAGAAAACCAT

83►alSerAlaLeuProIleGlnHisGlnAspTrpMetSerGlyLysGluPheLysCysLysValAsnAsnLysAlaLeuProSerProIleGluLysThrIle

Bsu36I (1100)

1001 CTCAAAACCCAAAGGGCTAGTCAGAAAACACAGGTATACGTCATGGTCCACCGACAGAGCAGTTGACTGAGCAAACGGTCAGTTTGACCTGCTTGACC

116►eSerLysProLysGluLeuValArgLysProGlnValTyrValMetGluProProThrGluGlnLeuThrGluGlnThrValSerLeuThrCysLeuThr

1101 TCAGGTTCTCCCTAACGACATCGGTGTGGAGTGGACGAGCAACGGGCATATAGAAAAGAACTACAAGAACCCGAGCAGTGTGACTCTGACGGTT

150►SerGluPheLeuProAsnAspIleGluValGluTrpThrSerAsnGluHisIleGluLysAsnTyrLysAsnThrGluProValMetAspSerAspGluSer

BbrPI (1301)

1201 CTTTCTTCATGTACAGCAAGCTCAATGTGGAAGGAGCAGTGGATAGCAGAGCGCCCTTCGTCTGCTCCGTGGTCCACGAGGGTCTGCACAATCACCA

183►erPhePheMetTyrSerLysLeuAsnValGluArgSerArgTrpAspSerArgAlaProPheValCysSerValValHisGluGluLeuHisAsnHisHis

NheI (1338)

1301 CGTGGAGAAGGACATCTCCCGCCTCCGGTAATGAGTAGCTGCCAGACATGATAAGATACATTGATGAGTTTGGCAAACCAACTAGAATGCA

216►sValGluLysSerIleSerArgProProGluLys•••

HpaI (1478)

1401 TGAATAAATGCTTTATTTGTGAATTTGTGATGCTATTGCTTTATTTGTAACCATATAAGTGAATAAACAAGTTAACAACAACAATTGCATTCAAT

AseI (1575) XmnI (1576)

1501 TTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAAACCTCTACAATGTGGTATGGAATTAATTTCAAATAACAGCATAGCAA

1601 ACTTTAACCTCAAATCAAGCCTCTACTTGAATCCTTTCTGAGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGA

1701 GCCTCACCTCTTTTCATGGAGTTAAGATATAGTGTATTTTCCCAAGGTTTGAAGTCTCTCATTTCTTTATGTTTTAAATGCACTGACCTCCACAT

Swal (1832)

1801 TCCCTTTTGTAGTAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAATAAATGTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCA

1901 AATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCTCCAGTCTGCT

125►•••AspGlnGlu

2001 CCTGTGCCACAAGTGCACGAGTTGCCGGCGGGTCCGCGAGGGCGAACTCCGCCCCACGGCTGCTCGCGATCTCGGTATGGCCGGCCGGGAGGC

121►uGluAlaValPheHisValCysAsnGluAlaProAspArgLeuAlaPheGluArgGluYrTrpProGluGluGluIleGluThrMetAlaProGluSerAla

2101 GTCCCGAAGTTCGTGGACACGACCTCCGACACTCGCGCTACAGCTCTCCAGCGCCGACCCACACCCAGGCCAGGGTGTGTCCGGCACCTGG

88►AspArgPheAsnThrSerValValGluSerTrpGluAlaTyrLeuGluAspLeuGluYrValTrpValTrpAlaLeuThrAsnAspProValValGlnA

SmaI (2273)

2201 TCCTGGACCGCTGATGAACAGGTCACGTCGTCGCGACACACCGGCGAAGTCGCTCCACGAAGTCCCGGAGAACCCGAGCCGGTCCGTCAGAG

54►spGlnValAlaSerIlePheLeuThrValAspAspArgValValGluAlaPheAspAspGluValPheAspArgSerPheGluLeuArgAspThrTrpPh

2301 ACTCGACCGCTCCGGCGAGCTCGCGCGGGTGAACCGGACCTGGTCAACTTGGCCATGATGGCTCCTCctgtcaggagaggaagagaaga

21►eGluValAlaGluAlaValAspArgAlaThrLeuValProValAlaSerThrLeuLysAlaMet

AseI (2459) PstI (2481)

2401 gttagtacaattgCTATAGTGAGTTGATTATACTATGCGATATACTATGCAATGATTAATTGTCAAAGTGGGCTGAggggttcacatgcccattt

HindIII (2585)

2501 tctgctactgccccatctctgcccaccccttccaggcatagacagtcagtgacttacAAACTCACAGGAGGAGAAGGCAGAAGCTTGAGACAGACC

2601 CGCGGACCGCAACTCGAGGGGACGTGGCTAGGGCGGCTTCTTTATGGTGCAGCCGCTCGGAGGAGGGCTCGGGAGGCTAGCGGCCAAT

BspEI (2743)

2701 CTGCGGTGGCAGGAGCGGGGCCAAGGCCGTGCCTGACCAATCCGGAGCACATAGGAGTCTCAGCCCCCGCCCAAAGCAAGGGGAAGTCACGCGCCT

SpeI (2850)

2801 GTAGCGCCAGCGTGTGTGAATGGGGCTTGGGGGTTGGGGCCCTGACTAGTCAAAACAACCTCCATTGACGTCAATGGGGTGGAGACTTGGAAAT

2901 CCCCCTGAGTCAAACCGCTATCCACGCCATTGATGACTGCCAAAACCGCATCATGGTAATAGCGATGACTAATACGTAGATGACTGCCAAGTAG

3001 GAAAGTCCATAAGGTCATGACTGGGCATAATGCCAGCGGGCCATTACCGTCATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGATG

3101 TACTGCCAAGTGGGCGTTTACCATAACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTACTATGGAAACATACGTCAATTATTGACGTC

PacI (3273)
PstI (3266)
SdaI (3266) BspLU11I (3279)

3201 AATGGGCGGGGGTCTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCA
3301 AAAGGCCAGGAACCGTAAAAAGGCGCGTTGCTGGCGTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGG
3401 CGAAACCCGACAGGACTATAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCTGTTCGACCCCTGCCGTTACCGGATACCTGTCCG
3501 CCTTTCTCCCTTCGGGAAGCGTGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCCAAGCTGGGCTGTGTGCACGA
3601 ACCCCCGTTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGT
3701 AACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCCTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCG
3801 CTCTGCTGAAGCCAGTTACCTTCGGAAAAAGATTGGTAGCTCTTGATCCGGCAAACAACCACCGCTGGTAGCGGTGGTTTTTTTGGTTGCAAGCAGCA
3901 GATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGCTGTGACGCTCAGTGGAAACGAAAACTCACGTTAAGGGATTTTGGTC

PacI (4013) SmaI (4021) **NotI (4029)**
4001 ATGGCTAGTTAATTAACATTTAAATCAGCGGCCCAATAAAATATCTTTATTTTCATTACATCTGTGTGGTTTTTTGTGTGAATCGTAACTAACATA
4101 CGCTTCCATCAAAACAAAACGAAACAAAACAAACTAGCAAAATAGGCTGTCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA