pFUSE-rtIgG2B-Fc1

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

Catalog # pfuse-rtg2bfc1

For research use only

Version 20K05-MM

PRODUCT INFORMATION

Content:

- 20 μg of **pFUSE-rtIgG2B-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

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 promoter[1] and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat[2]. 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.

5' - Age I, Eco RI, Eco RV, Xho I, Nco I, Bgl II - 3'

• **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 mRNA[3].

• **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 transcription[4].

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 H_2O. 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
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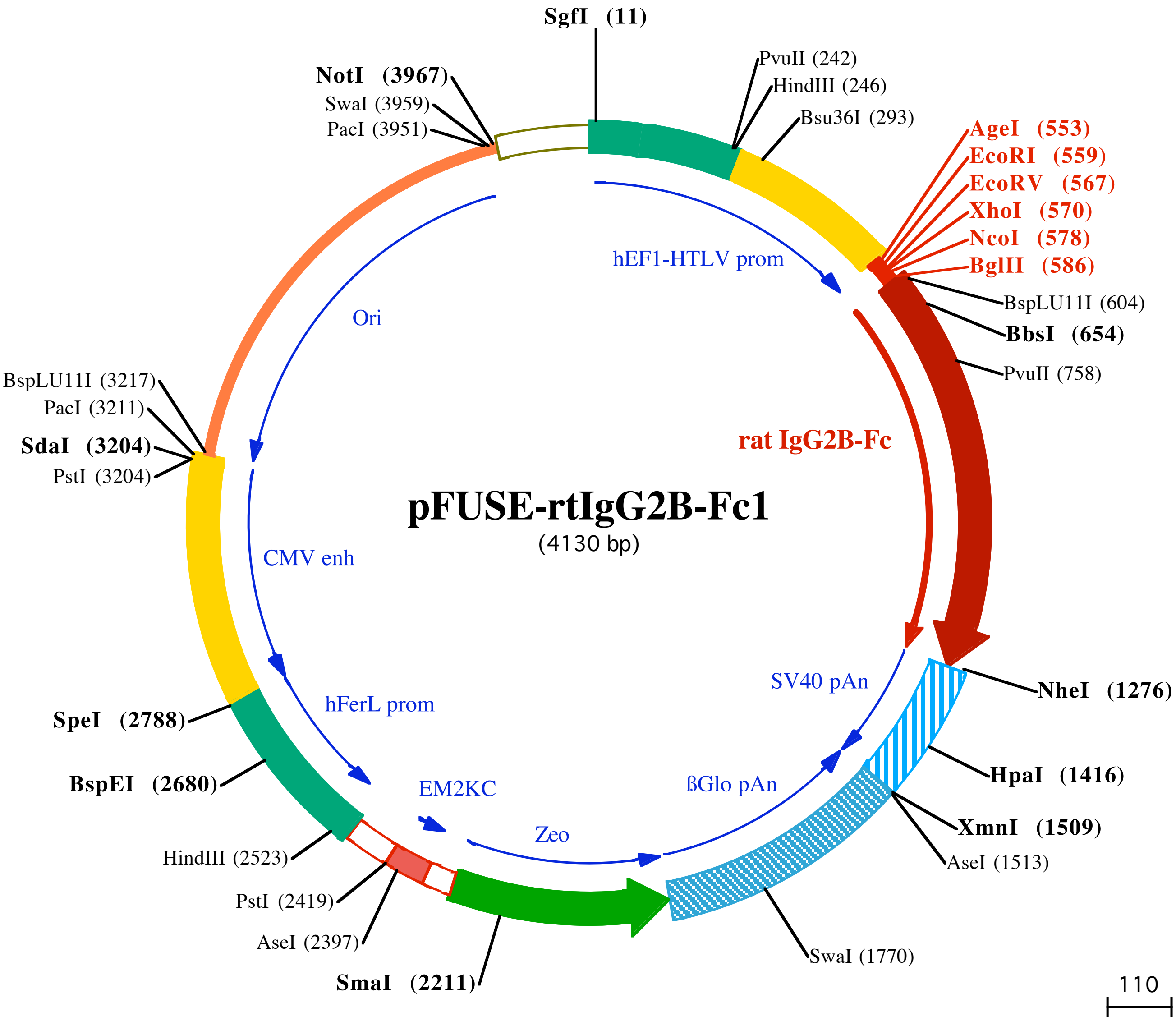
pFUSE-rtIgG2B-Fc1
(4130 bp)
SgfI (11)
PvuII (242)
HindIII (246)
Bsu36I (293)
AgeI (553)
EcoRI (559)
EcoRV (567)
XhoI (570)
NcoI (578)
BglII (586)
BspLU11I (604)
BbsI (654)
PvuII (758)
rat IgG2B-Fc
NheI (1276)
HpaI (1416)
XmnI (1509)
AseI (1513)
SwaI (1770)
SmaI (2211)
AseI (2397)
PstI (2419)
HindIII (2523)
BspEI (2680)
SpeI (2788)
PstI (3204)
SdaI (3204)
PacI (3211)
BspLU11I (3217)
PacI (3951)
SwaI (3959)
NotI (3967)
hEF1-HTLV prom
Ori
CMV enh
hFerL prom
EM2KC
Zeo
ßGlo pAn
SV40 pAn
110

SgfI (11)

1 GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACGGGTGCCTA

101 GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCC

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

201 GTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCC

301 GCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC

401 GGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTT

EcoRI (559) XhoI (570) BglII (586)
AgeI (553) EcoRV (567) NcoI (578)

501 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCACCGGTGAATTCGATATCTCGAGCACCATGGTTAGATCTCCTACATGC
1▶ ProThrCys

BspLU11I (604)
BbsI (654)

601 CCTACATGTCACAAATGCCCAGTTCCTGAACTCTTGGGTGGACCATCTGTCTTCATCTTCCCGCCAAAGCCCAAGGACATCCTCTTGATCTCCCAGAACG
4▶ ProThrCysHisLysCysProValProGluLeuLeuGlyGlyProSerValPheIlePheProProLysProLysAspIleLeuLeuIleSerGlnAsnA

PvuII (758)

701 CCAAGGTCACGTGTGTGGTGGTGGATGTGAGCGAGGAGGAGCCGGACGTCCAGTTCAGCTGGTTTGTGAACAACGTAGAAGTACACACAGCTCAGACACA
37▶ laLysValThrCysValValValAspValSerGluGluGluProAspValGlnPheSerTrpPheValAsnAsnValGluValHisThrAlaGlnThrGl

801 ACCCCGTGAGGAGCAGTACAACAGCACCTTCAGAGTGGTCAGTGCCCTCCCCATCCAGCACCAGGACTGGATGAGCGGCAAGGAGTTCAAATGCAAGGTC
70▶ nProArgGluGluGlnTyrAsnSerThrPheArgValValSerAlaLeuProIleGlnHisGlnAspTrpMetSerGlyLysGluPheLysCysLysVal

901 AACAACAAAGCCCTCCCAAGCCCCATCGAGAAAACCATCTCAAAACCCAAAGGGCTAGTCAGAAAACCACAGGTATACGTCATGGGTCCACCGACAGAGC
104▶ AsnAsnLysAlaLeuProSerProIleGluLysThrIleSerLysProLysGlyLeuValArgLysProGlnValTyrValMetGlyProProThrGluG

1001 AGTTGACTGAGCAAACGGTCAGTTTGACCTGCTTGACCTCAGGCTTCCTCCCTAACGACATCGGTGTGGAGTGGACCAGCAACGGGCATATAGAAAAGAA
137▶ lnLeuThrGluGlnThrValSerLeuThrCysLeuThrSerGlyPheLeuProAsnAspIleGlyValGluTrpThrSerAsnGlyHisIleGluLysAs

1101 CTACAAGAACACCGAGCCAGTGATGGACTCTGACGGTTCTTTCTTCATGTACAGCAAGCTCAATGTGGAAAGGAGCAGGTGGGATAGCAGAGCGCCCTTC
170▶ nTyrLysAsnThrGluProValMetAspSerAspGlySerPhePheMetTyrSerLysLeuAsnValGluArgSerArgTrpAspSerArgAlaProPhe

NheI (1276)

1201 GTCTGCTCCGTGGTCCACGAGGGTCTGCACAATCACCACGTGGAGAAGAGCATCTCCCGGCCTCCGGGTAAATGAGCTAGCTGGCCAGACATGATAAGAT
204▶ ValCysSerValValHisGluGlyLeuHisAsnHisHisValGluLysSerIleSerArgProProGlyLys•••

1301 ACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAG

HpaI (1416)

1401 CTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAA

AseI (1513)
XmnI (1509)

1501 TGTGGTATGGAATTAATTCTAAAATACAGCATAGCAAAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGG

1601 CATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTTAAGATATAGTGTATTTTCCCAAGGTTTGAACTAGCTCT

SwaI (1770)

1701 TCATTTCTTTATGTTTTAAATGCACTGACCTCCCACATTCCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTT

1801 TTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACA

1901 GCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGGTCGCGCAGGGCGAACTCCCGCCCCCAC
125◀ •••AspGlnGluGluAlaValPheHisValCysAsnGlyAlaProAspArgLeuAlaPheGluArgGlyTrpP

2001 GGCTGCTCGCCGATCTCGGTCATGGCCGGCCCGGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCA
100◀ roGlnGluGlyIleGluThrMetAlaProGlySerAlaAspArgPheAsnThrSerValValGluSerTrpGluAlaTyrLeuGluAspLeuGlyArgVa

2101 CCCACACCCAGGCCAGGGTGTTGTCCGGCACCACCTGGTCCTGGACCGCGCTGATGAACAGGGTCACGTCGTCCCGGACCACACCGGCGAAGTCGTCCTC
67◀ lTrpValTrpAlaLeuThrAsnAspProValValGlnAspGlnValAlaSerIlePheLeuThrValAspAspArgValValGlyAlaPheAspAspGlu

SmaI (2211)

2201 CACGAAGTCCCGGGAGAACCCGAGCCGGTCGGTCCAGAACTCGACCGCTCCGGCGACGTCGCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTTGGCC
34◀ ValPheAspArgSerPheGlyLeuArgAspThrTrpPheGluValAlaGlyAlaValAspArgAlaThrLeuValProValAlaSerThrLeuLysAlaM

AseI (2397)

2301 ATGATGGCTCCTCctgtcaggagaggaaagagaagaaggttagtacaattgCTATAGTGAGTTGTATTATACTATGCAGATATACTATGCCAATGATTAA
0◀ et

PstI (2419)

2401 TTGTCAAACTAGGGCTGCAgggttcatagtgccacttttcctgcactgccccatctcctgcccaccctttcccaggcatagacagtcagtgacttacCAA

HindIII (2523)

2501 ACTCACAGGAGGGAGAAGGCAGAAGCTTGAGACAGACCCGCGGGACCGCCGAACTGCGAGGGGACGTGGCTAGGGCGGCTTCTTTTATGGTGCGCCGGCC

BspEI (2680)

2601 CTCGGAGGCAGGGCGCTCGGGGAGGCCTAGCGGCCAATCTGCGGTGGCAGGAGGCGGGGCCGAAGGCCGTGCCTGACCAATCCGGAGCACATAGGAGTCT

SpeI (2788)

2701 CAGCCCCCCGCCCCAAAGCAAGGGGAAGTCACGCGCCTGTAGCGCCAGCGTGTTGTGAAATGGGGGCTTGGGGGGGTTGGGGCCCTGACTAGTCAAAACA

2801 AACTCCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAACCGCTATCCACGCCCATTGATGTACTGCCAAAACCGCATCATCATGGT

2901 AATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACG

3001 TCAATAGGGGGCGTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTA

PstI (3204)
SdaI (3204)

3101 TTGGCGTTACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCC

PacI (3211) BspLU11I (3217)

3201 TGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTG

3301 ACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTC

3401 TCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCG

3501 GTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGG

3601 TAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAA

3701 CTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACC

3801 ACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACG

PacI (3951) SwaI (3959) **NotI (3967)**

3901 CTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAATATCTTTATTTTCATTACAT

4001 CTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACAAACTAGCAAAATAGGCTGTCCCCAGTGCAA
4101 GTGCAGGTGCCAGAACATTTCTCTATCGAA