

pFUSE-rIgG-Fc1

Plasmid designed for the construction of Fc-Fusion proteins

Catalog # pfuse-rfc1

For research use only

Version 20K04-MM

PRODUCT INFORMATION

Content:

- 20 µg of pFUSE-rIgG-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 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.
5' - Age I, Eco RI, Eco RV, Xho I, Bgl II - 3'
- **rIgG Fc (rabbit):** 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.

- **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.

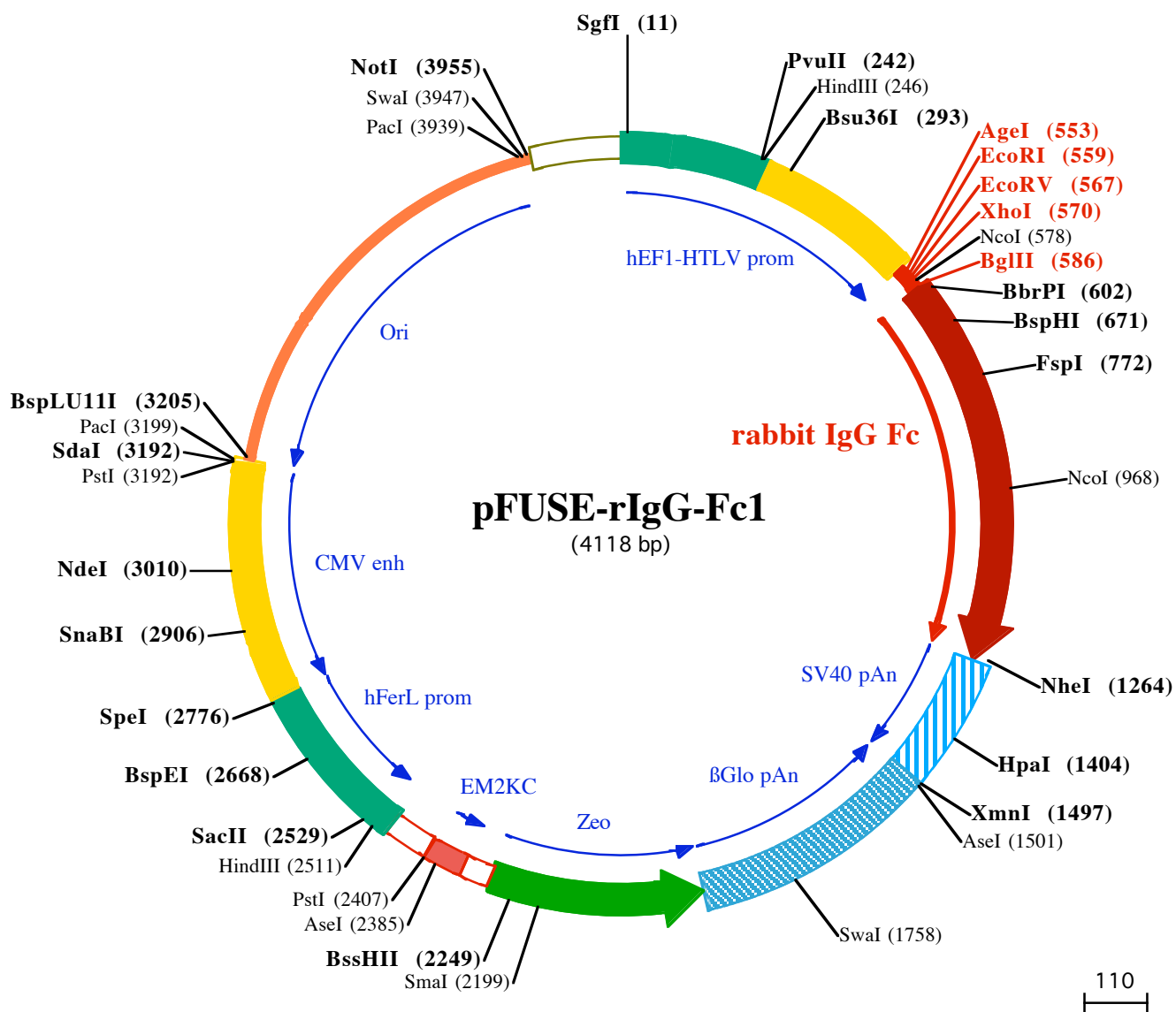
References:

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

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SgfI (11)
1 GGATCTCGATCGCTCCGGTCCCGTCAGTGGCAGAGCGCACATCGCCACAGTCCCGAGAAGTTGGGGGAGGGTTCGCAATTGAACGGGTGCCTA
101 GAGAAGGTGGCGGGGTAACCTGGGAAAGTGATGTCGTGTACTGGCTCCGCTTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

HindIII (246)
201 GTGAACGTTCTTTTTTCGAACGGGTTTGGCCGCAGAACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCCTTACGCGCCCGCCCTACCTGAGGCC
PvuII (242) Bsu36I (293)
301 GCCATCCACGCGGTTGAGTGCCTCTGCCCTCCCGCTGTGGTGCCTCTGAACTGCGTCCGCGTCTAGGTAAGTTAAAGCTCAGGTCGAGACC
401 GGGCTTTGTCCGGCTCCCTTGGAGCTACCTAGACTCAGCGGCTCTCCACGCTTTCCTGACCTGCTTGTCTCAACTCTACGCTTTGTTTCGTTT

EcoRI (559) XhoI (570)
501 TCTGTTCTGCGCGTTACAGATCCAAGCTGTGACCGGCGCTACCTGAGATCACCGTGAATTCGATATCTCGAGCACCATGGTTAGATCTAGCAAGCCC
AgeI (553) EcoRV (567) NcoI (578) BglII (586) BbrPI (602)
1 Ser Lys Pro

BspHI (671)
601 ACGTGCCACCCCTGAACTCCTGGGGGACCGTCTGTCTTTCATCTTCCCCCAAAACCAAGGACCCCTCATGATCTCACGCACCCCGAGGTCACAT
4 Thr Cys Pro Pro Glu Leu Leu Glu Gly Pro Ser Val Phe I Le Phe Pro Pro Lys Pro Lys Asp Thr Leu Met I Le Ser Arg Thr Pro Glu Val Thr C

FspI (772)
701 GCGTGGTGGTGGACGTGAGCCAGGATGACCCGAGGTGCGATTCACATGGTACATAAACACAGCAGGTGCGCACCGCCCGGCCGCTACGGGAGCA
37 ysVal Val Val AspVal Ser Gl nAspAspProGluVal Gl nPheThr TrpTyr I LeAsnAsnGlu Gl nVal ArgThr Al aArgProProLeuArgGlu Gl
801 GCAGTTCAACAGCACGATCCGCTGGT CAGCACCTCCCATCGCGCACAGGACTGGCTGAGGGGCAAGGAGTTCAAGTGCAAAGTCCACAAACAGGCA
70 nGl nPheAsnSer Thr I LeArgVal Val Ser Thr LeuPro I LeAl aHi sGl nAspTrpLeuArgGluLysGluPheLysCysLysVal Hi sAsnLysAl a

NcoI (968)
901 CTCCCGCCCCATCGAATAACATCTCAAAGCCAGAGGGCAGCCCTGGAGCCGAAGGTCTACACCATGGGCCCTCCCGGGAGGAGCTGAGCAGCA
104 LeuProAl aPro I LeGluLysThr I LeSerLysAl aArgGluGlu nProLeuGlu uProLysVal TyrThr MetGlyProProArgGluGluLeuSer Ser A
1001 GGTGCGTCACTGACCTGCATGATCAACGGCTTCTACCTTCCGACATCTCGTGGAGTGGGAGAAGACGGGAAGGCAGAGGACAACACTACAAGACCAC
137 rGSer Val Ser LeuThr CysMet I LeAsnGlu yPheTyrProSerAsp I LeSer Val Gl uTrpGluLysAsnGluLysAl aGluAspAsnTyrLysThr Th
1101 GCCGCGCTGCTGACAGCAGCGCTCTACTTCTCTACAGCAAGCTCTCAGTCCACGAGTGGTGGCAGCGGGCCGACGCTTCCACTGCTCCGTTG
170 rProAl aVal LeuAspSerAspGlySer TyrPheLeuTyrSer LysLeuSer Val ProThr Ser Gl uTrpGlu nArgGlu yAspVal PheThr CysSer Val

NheI (1264)
1201 ATGCACGAGCCCTGCAACAACACTACACGAGAAGTCCATCTCCCGCTCTCCGGTAAATGAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGT
204 MetHi sGluAl aLeuHi sAsnHi sTyrThr Gl nLysSer I LeSerArgSerProGlyLys ●●●
1301 TTGGACAAACCACAACCTAGAATGCAGTGAATAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGAATAAACA

HpaI (1404) XmnI (1497) AseI (1501)
1401 AGTTAAACAACAACAAATTGCATTCAATTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAA
1501 TTAATTTCAAATACAGCATAGCAAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTG
1601 TTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTTAAAGATATAGTGATTTTCCCAAGGTTTGAAGTACGCTTTCATTTCTTTAT

SwaI (1758)
1701 GTTTTAAATGCACTGACCTCCACATTCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTATTAGGCA
1801 GAATCCAGATGCTCAAGGCCCTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGAACCTTTAATAGAAATGGACAGCAAGAAAGCGA

125 ●●● AspGlu nGlu uGluAl aVal PheHi sVal CysAsnGlu yAl aProAspArgLeuAl aPheGlu uArgGly yTrpProGlu nGlu uGly I
2001 ATCTCGGTCATGGCCGGCCGGAGGCTCCCGAAGTTCGTGGACAGCCTCCGACACTCGGCGTACAGCTCTCCAGGCGCGCACCCACACCCAGG
96 I LeGlu uThr MetAl aProGlySerAl aAspArgPheAsnThr Ser Val Val Gl uSer TrpGlu uAl aTyrLeuGlu uAspLeuGlu yArgVal TrpVal TrpAl

SmaI (2199)
2101 CCAGGGTGTGTCGGCACACCTGGTCTGGACCGCGCTGATGAACAGGGTTCACGTCGTCGGGACACACCGCGGAAGTCTCCACGAAGTCCCG
63 aLeuThrAsnAspProVal Val Gl nAspGlu nValAl aSer I LePheLeuThr Val AspAspArgVal Val Gl yAl aPheAspAspGlu uVal PheAspArg

BssHII (2249)
2201 GGAGAACCCGAGCGGTCGGTCCAGAACTCGACCGCTCCGGCAGCTCGCGCGGTTGAGCACCAGGAAACGGCACTGGTCAACTGGCCATGATGGCTCCT
30 Ser PheGlu yLeuArgAspThr TrpPheGlu uValAl aGlyAl aVal AspArgAl aThrLeuVal ProValAl aSer ThrLeuLysAl aMet

AseI (2385)
2301 CctgtcaggagaggaaagagaagaaggttagtacaattgCTATAGTGAGTTGATTATACTATGCAGATATACTATGCCAATGATTAATTGTCAAACCTAG

PstI (2407)
2401 GGCTGCAGggttcatagtgccacttttctgtcactgccccctctcctgccaccctttccaggcatagacagctcagtgacttacCAAACCTCACAGGAGG

HindIII (2511) SacII (2529)
2501 GAGAAGGCAGAAGCTTGAGACAGACCCGCGGACCGCCGAACCTGCGAGGGGACGTGGCTAGGGCGCTTCTTTATGGTGCAGCGCCCTCGGAGCGAGG

BspEI (2668)
2601 GCGCTCGGGGAGGCTAGCGCCAATCTGCGGTGGCAGGAGCGGGGCGGAAGGCGGTGCTGACCAATCCGGAGCACATAGGAGTCTCAGCCCCCGCC

SpeI (2776)
2701 CCAAAGCAAGGGGAAGTACGCGCCTGTAGCGCCAGCGTGTGTGAAATGGGGCTTGGGGGGTGGGGCCCTGACTAGTCAAACAACAACTCCCAATTGA
2801 CGTCAATGGGGTGGAGACTTGGAAATCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTAAGTCCAAAACCGCATCATCATGGTAATAGCGATGAC

SnaBI (2906)
2901 TAATACGTAGATGTAAGTCCCAAGTAGGAAAGTCCATAAGGTGATGTAAGTGGGCATAATGCCAGGCGGGCCATTTACCCTGTCATTGACGTCATAGGGGGC

NdeI (3010)
3001 GTACTTGGCATATGATACACTTGTACTGCAAGTGGGCGAGTTACCGTAAATACTCCACCATTGACGTCATGGAAAGTCCCTATTGGCGTTACTA

PacI (3199)
PstI (3192)
SdaI (3192)

3101 TGGGAACATACGTCATTATTGACGTCAATGGGCGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCTGCAGGTTAATT

BspLU11I (3205)

3201 AAGAACATGTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAAAGGCCGCTGTTGCGCTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACA
←

3301 AAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCTGGAAAGCTCCCTCGTGCCTCTCCTGTTCCGAC

3401 CCTGCCGCTTACCGGATACCTGTCGCCCTTCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCAGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTT

3501 CGCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTACGCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACT

3601 TATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCTACAC

3701 TAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTTTGATCCGGCAAACAAACCACCGTGGTAGC

3801 GGTGGTTTTTTTGTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGAACG

PacI (3939) SwaI (3947) **NotI (3955)**

3901 AAAACTCAGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGT

4001 TTTTTGTGTAATCGTAACTAACATACGCTCTCCATCAAACAAAACGAAACAAAACAACTAGCAAAATAGGCTGTCCCAAGTGAAGTGCAGGTGCCA

4101 GAACATTTCTCTATCGAA