

pFUSE-hIgG3-Fc2

Plasmid designed for the construction of Fc-Fusion proteins

Catalog # pfuse-hg3fc2

For research use only

Version # 06G10-MT

PRODUCT INFORMATION

Content:

- 20 μg of pFUSE-hIgG3-Fc2(IL2ss) plasmid provided as lyophilized DNA
- 4 pouches of *E. coli* Fast-Media® Zeo (2 TB and 2 Agar)

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 *E. coli* Fast-Media® Zeo at room temperature. Fast-Media® pouches are stable 18 months when stored properly.

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 the effector region of a protein to the Fc region of an immunoglobulin G (IgG).

pFUSE-Fc plasmids yield high levels of Fc-fusion proteins. The level of expression is usually in the $\mu\text{g}/\text{mL}$ 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.

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.

InvivoGen provides pFUSE-Fc vectors featuring Fc regions 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

- **hIgG3-Fc (human):** 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. Human IgG3 displays high ADCC and CDC.
- **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1 α (EF-1 α) 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-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.
- **IL2 ss:** The IL2 signal sequence contains 21 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 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*.
- **BGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription⁴.

1. 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.

2. 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.

3. 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.

4. 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.

TECHNICAL SUPPORT

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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 $\mu\text{g}/\mu\text{l}$, resuspend the DNA in 20 μl of sterile H₂O. Store resuspended plasmid at -20°C.

Selection of bacteria with *E. coli* Fast-Media®

Fast-Media® is a **fast and convenient** way to prepare liquid and solid media for bacterial culture by using only a microwave. Fast-Media® is a TB (liquid) or LB (solid) based medium that already contains the antibiotic. Fast-Media® Zeo is available separately: #fas-zn-1 (liquid), #fas-zn-s (agar).

1- Pour the contents of a Fast-Media® pouch into a clean borosilicate glass bottle or flask.

2- Add 200 ml of distilled water to the flask

3- Heat in a microwave on MEDIUM power setting (about 400Watts), until bubbles start appearing (approximately 3 minutes). **Do not heat a closed container. Do not autoclave Fast-Media®.**

4- Swirl gently to mix the preparation. **Be careful, the bottle and media are hot, use heatproof pads or gloves and care when handling.**

5- Reheat the media for 30 seconds and gently swirl again. Repeat as necessary to completely dissolve the powder into solution. But be careful to avoid overboiling and volume loss.

6- Let agar medium cool to 45°C before pouring plates. Let liquid media cool to 37°C before seeding bacteria.

Note: Do not reheat solidified Fast-Media® as the antibiotic will be permanently destroyed by the procedure.

RELATED PRODUCTS

| Product | Catalog Code |
|----------------------|--------------|
| Zeocin™ | ant-zn-1 |
| Fast-Media® Zeo TB | fas-zn-1 |
| Fast-Media® Zeo Agar | fas-zn-s |

TECHNICAL SUPPORT

Toll free (US): 888-457-5873

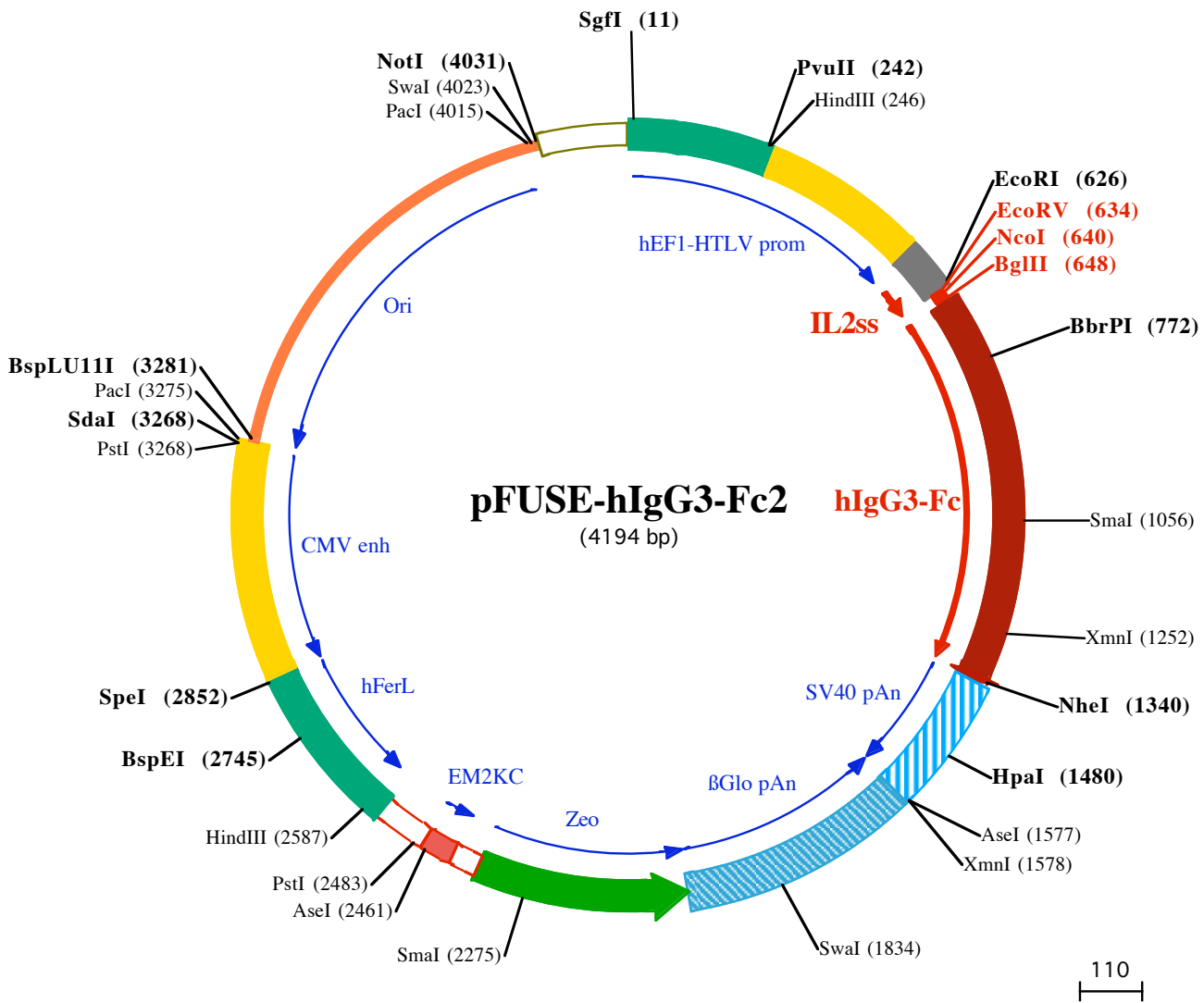
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SgfI (11)

1 GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGGTGGCAATTGAACGGGTGCCTA

101 GAGAAGGTGGCGGGGTAAACTGGAAAGTGATGCTGTACTGGCTCCGCTTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

HindIII (246)
PvuII (242)

201 GTGAACGTTCTTTTTCGCAACGGGTTTCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCTTACCGCGCCCGCCCTACCTGAGGCC

301 GCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC

401 GGGCCTTTGTCCGGCGCTCCCTTGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTCTCAACTCTACGCTTTTGTTCGTTT

501 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCTACTCGATCAccggcGAAGGAGGGCCACCATGTACAGGATGCAACTCCTGTCTTGCA

1►MetTyrArgMetGlnLeuLeuSerCysI

EcoRV (634) BglII (648)
EcoRI (626) NcoI (640)

601 TTGCACTAAGTCTTGCACTTGTCACGAATTCGATATCGGCCATGGTTAGATCTGACACACCTCCCCGTGCCAAGGTGCCAGCACCTGAACTCCTGGG

1►IeAlaLeuSerLeuAlaLeuValThrAsnSer 1►AspThrProProP roCysP roArgCysP roAlaP roGluLeuLeuGlu

BbrPI (772)

701 AGGACCGTCAGTCTTCTCTTCCCCAAAACCAAGGATACCTTATGATTTCCCGGACCCTGAGGTACGTGCGTGGTGGTGGACGTGAGCCACGAA

16►yGlyProSerValPheLeuPheProProLysProLysAspThrLeuMetIleSerArgThrProGluValThrCysValValValAspValSerHisGlu

801 GACCCGAGGTCCAGTTCAGTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAGCCGCGGGAGGAGCAGTTCAACAGCACGTTCCGTGTGG

50►AspProGluValGlnPheLysTrpTyrValIAspGlyValGluValHisAsnAlaLysThrLysProArgGluGluGlnPheAsnSerThrPheArgValI

901 TCAGCGTCTCACCGTCTGCACAGGACTGGCTGAACGGCAAGGAGTACAAGTCAAGGTCTCCAACAAAGCCCTCCAGCCCCATCGAGAAAACCAT

83►alSerValLeuThrValLeuHisGlnAspTrpLeuAsnGlyLysGlyTyrLysCysLysValSerAsnLysAlaLeuProAlaProIleGluLysThrIle

SmaI (1056)

1001 CTCCAAAACCAAGGACAGCCCCGAGAACCACAGGTGTACACCCTGCCCCATCCCGGAGGAGATGACCAAGAACCAGGTACGCTGACCTGCCTGGTC

116►eSerLysThrLysGlyGlnProArgGluProGluNValTyrThrLeuProProSerArgGluGluMetThrLysAsnGlnValSerLeuThrCysLeuVal

1101 AAAGGCTTCTACCCAGCGACATCGCGTGGAGTGGGAGAGCAGCGGGCAGCCGAGAACTACAACACCACGCTCCCATGCTGGACTCCGACGGCT

150►LysGlyPheTyrProSerAspIleAlaValGluTrpGluSerSerGlyGlnProGluAsnAsnTyrAsnThrThrProProMetLeuAspSerAspGlyS

XmnI (1252)

1201 CTTCTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACATCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCGCTT

183►erPhePheLeuTyrSerLysLeuThrValAspLysSerArgTrpGluNglNglYasnIlePheSerCysSerValMetHisGluAlaLeuHisAsnArgPh

NheI (1340)

1301 CACGAGAAGAGCTCTCCGTCTCCGGTAAATGAGTGTCTGCGCAGACATGATAAGATACATTGATGAGTTTGGACAACCACAAGTGAATGC

216►eThrGlnLysSerLeuSerLeuSerProGlyLys•••

HpaI (1480)

1401 AGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAAACAACAATTCATTCA

AseI (1577)
XmnI (1578)

1501 TTTTATGTTTCAGGTTCAAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAAACCTCTACAAATGGTATGGAATTAATCTAAAATACAGCATAGCA

1601 AAATTTAACCTCCAATCAAGCCTCTACTTGAATCCTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTG

1701 CAGCCTCACCTTCTTCATGGAGTTAAGATATAGTATTTTCCAAGGTTGAACTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCCAC

SwaI (1834)

1801 ATTCCCTTTTATGAAAAATTCAGAAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTC

1901 ATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCTCTG

125►•••AspGln

2001 CTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGTTCGCGCAGGGCGAACTCCCGCCCCACGGCTGCTCGCGATCTCGGTATGGCCGGCCGGAG

122►GluGluAlaValPheHisValCysAsnGlyAlaProAspArgLeuAlaPheGluArgGlyTrpProGluNglGlyIleGluThrMetAlaProGlySerA

2101 GCGTCCCGAAGTTCGTGGACACGACTCCGACCCTCGCGTACAGCTGCCAGGCCGCGCACCCACCCAGCCAGGCTGTGTTCGGCAGCCACT

88►IAspArgPheAsnThrSerValValGluSerTrpGluAlaTyrLeuGluAspLeuGlyArgValTrpValTrpAlaLeuThrAsnAspProValValGlu

SmaI (2275)

2201 GGTCTGGACCGGCTGATGAACAGGGTACGTCGTCGCGGACCACCCGGCGAAGTCTCCTCACGAAGTCCCGGAGAACCCGAGCCGGTCCGGTCCA

55►nAspGlnValAlaSerIlePheLeuThrValAspAspArgValValGlyAlaPheAspAspGluValPheAspArgSerPheGlyLeuArgAspThrTrp

2301 GAACTCGACCGTCCGGCGAGCTCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTGGCCATGATGGCTCTCctgtcaggagaggaagagaga

22►PheGluValAlaGlyAlaValAspArgAlaThrLeuValProValAlaSerThrLeuLysAlaMet

AseI (2461) PstI (2483)

2401 aggttagtacaattgCTATAGTGAAGTTGATTATACATATGCAGATATACTATGCCAATGATTAATTGTCAAACCTAGGGCTGCAgggttcagtgcacct

HindIII (2587)

2501 tttcctgactgcccactctcctgcccaccctttccaggcatagacagtcagtacttacCAAACCTACAGGAGGGAGAAGGCAGAGCTTGAGACAGA

2601 CCCGCGGACCGCGAACTGCGAGGGGAGCTGGCTAGGGCGGCTTTTATGTTGCGCCGGCCCTCGGAGGCAGGGCGCTCGGGAGGCCTAGCGGCCA

BspEI (2745)

2701 ATCTGCGGTGGCAGGAGCGGGGCCGAAGCCGTGCCTGACCAATCCGGAGCACATAGGAGTCTCAGCCCCCGCCCAAGCAAGGGGAAGTACGCGCC

SpeI (2852)

2801 CTGTAGCGCCAGCGTGTGTGAAATGGGGCTTGGGGGGTGGGGCCCTGACTAGTCAAAAACAACTCCCATGACGTCAAATGGGGTGGAGACTTGGAA

2901 ATCCCCGTGAGTCAAACCGTATCCACGCCATTGATGTAAGTCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTAAGTCCCAAGT

