

STOP

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TECHNICAL SUPPORT

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pFUSE-Lucia-hG1-Fc

Plasmid designed for the expression of a Lucia-Fc Fusion protein

Catalog # pfuse-hg1lc

For research use only

Version 22H31-MM

PRODUCT INFORMATION

Content:

- 20 µg of pFUSE-Lucia-hG1-Fc 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 plasmids 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 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, cells that are commonly used in protein purification systems.

pFUSE-Lucia-hG1-Fc plasmids allow the production of Lucia-Fc fusion proteins. This plasmid can be used to make recombinant Lucia-Fc fusion proteins or can be used as a transfection control in experiments with other pFUSE-hFc constructs. Quantification of Lucia-Fc expression can be determined utilizing InvivoGen's QUANTI-Luc™ (rep-qlc1 or rep-qlc2).

PLASMID FEATURES

- **Lucia luciferase** is a secreted coelenterazine-utilizing luciferase reporter protein with advantageous characteristics when associated with Fc-fusion proteins. It possesses superior carrier ability for excellent secretion of the chimeric protein. It provides a simple means to screen for recombinant clones and it minimally affects the activity of the protein of interest.

- **hlgG1 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 IgG1 displays high ADCC and CDC, and is the most suitable for therapeutic use against pathogens and cancer cells.

- **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.

- **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.

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 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 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.

RELATED PRODUCTS

Product	Catalog Code
Zeocin®	ant-zn-1
QUANTI-Luc™	rep-qlc1
ChemiComp GT116	gt116-11

TECHNICAL SUPPORT

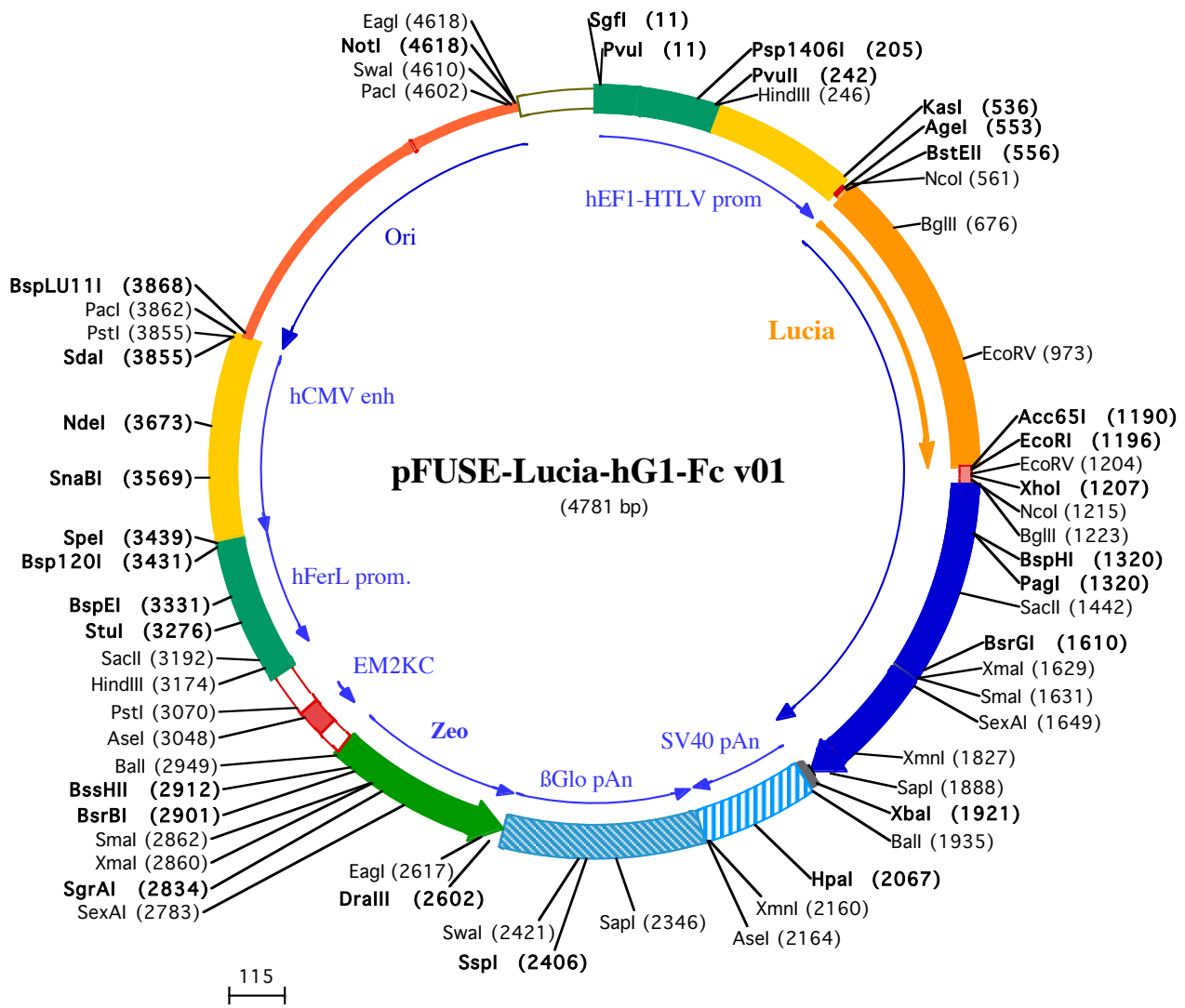
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PvuI (11)
SgfI (11)
 1 GGATCTGCGATCGCTCCGGTCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCGGAGAAGTTGGGGGAGGGGTCGGCAATTGAACGGTGCCTA
 101 GAGAAGTGGCGCGGGTAAACTGGGAAAGTATGTCGTGACTGGCTCCGCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTCAGTAGTCGCC

Psp1406I (205) **HindIII (246)**
PvuII (242)
 201 GTGAACGTTCTTTTTTCGAACGGGTTTCCGCCAGAACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCCTTACGCGCCCGCCCTACCTGAGGCC
 301 GCCATCCACGCGGGTTGAGTCGCGTTCGCCGCCCTCCCGCCTGTGGTGCCTCTGAACTGCGTCCGCCGCTAGGTAAGTTTAAAGCTCAGGTCGAGACC
 401 GGGCCTTTGTCCGGCGCTCCCTTGGAGCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCTGACCCTGCTTGCTCAACTCTACGCTTTGTTTCGTTT

NcoI (561)
BstEII (556)
AgeI (553)
KasI (536)
 501 TCTGTTCTGCGCGTTACAGATCCAAGCTGTGACCGCGCCCTACCTGAGATCACCGTcaCATGGAATCAAGGTGCTGTTTGCCTCATCTGTATTGC
 1 M E I K V L F A L I C I A
BglII (676)
 601 TGTTGCTGAGGCAAAACCACTGAAATCAATGAAGACCTCAATATAGCTGCTGTGGCCTCCAACTTTGCCACCACAGATCTTGAGACTGACCTGTTCAACC
 13 V A E A K P T E I N E D L N I A A V A S N F A T T D L E T D L F T
 701 AACTGGGAGACCATGAATGTGATTAGCACTGACACAGAGCAGGTGAACACAGATGCTGACAGGGGCAAGCTGCCTGGCAAAAACTCCCCCAGATGTCC
 47 N W E T M N V I S T D T E Q V N T D A D R G K L P G K K L P P D V
 801 TGAGGGAGCTGGAGGCAATGCCAGAAGGGCTGGTGCACAAGAGGCTGCCTCATTGGCTCTCCACATTAAGTGACCCCTAAGATGAAGAAATTTAT
 80 L R E L E A N A R R A G C T R G C L I C L S H I K C T P K M K K F I
EcoRV (973)
 901 CCCTGGCAGGTGCCACACTTATGAAGTGAAAAGGAGTCTGCTCAGGAGGGATTGGAGAGGCAATTGTTGATATCCAGAGATTCTGGCTCAAGGAT
 113 P G R C H T Y E G E K E S A Q G G I G E A I V D I P E I P G F K D
 1001 AAGGAGCCACTGGACCAAGTTATTGCTCAAGTGGACCTCTGTGCTGATTGCACCACTGGCTGTCTGAAGGGCCTTGCCAATGTCAGTGTCTGACCTCC
 147 K E P L D Q F I A Q V D L C A D C T T G C L K G L A N V Q C S D L
EcoRI (1196)
Acc65I (1190)
 1101 TGAAGAAGTGGCTTCCCAGAGGTGTACCACTTTTCCAGCAAGATTGAGGGTAGGGTGGCAAAATCAAGGGTCTGGCTGGGGACAGAGGTACCGAATT
 180 L K K W L P Q R C T T F A S K I Q G R V D K I K G L A G D R G T E F
XhoI (1207) **BglII (1223)**
EcoRV (1204) **NcoI (1215)**
 1201 CGATATCTCGAGCACCATGGTTAGATCTGACAAAACCTCACACATGCCACCCTGCCAGCACCTGAACTCCTGGGGGACCGTCAGTCTTCTCTTCCCC
 213 D I S S T M V R S D K T H T C P P C P A P E L L G G P S V F L F P
PagI (1320)
BspHI (1320)
 1301 CAAAAACCAAGGACACCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCTGAGGTCAAGTTCAACTGGT
 247 P K P K D T L M I S R T P E V T C V V V D V S H E D P E V K F N W
SacII (1442)
 1401 ACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCTCACCGTCTGCACCA
 280 Y V D G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H Q
 1501 GGACTGGCTGAATGGCAAGGAGTACAAGTCAAGGTCTCCAACAAAGCCCTCCAGCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA
 313 D W L N G K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R
BsrGI (1610) **XmaI (1629)** **SexAI (1649)**
SmaI (1631)
 1601 GAACCACAGGTGTACACCCCTGCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTTCAGCCTGACCTGCTGGTCAAAGGCTTCTATCCAGCGACATCG
 347 E P Q V Y T L P P S R E E M T K N Q V S L T C L V K G F Y P S D I
 1701 CCGTGGAGTGGGAGAGCAATGGGAGCGGAGAACAACTACAAGACCACGCTCCCGTGTGGACTCCGACGGCTCCTTCTCTACAGCAAGCTCAC
 380 A V E W E S N G Q P E N N Y K T T P P V L D S D G S F F L Y S K L T
XmnI (1827) **SapI (1888)**
 1801 CGTGGAACAGGACAGGTGGCAGCAGGGGAACGCTTCTCATGCTCCGTGATGCAAGAGGCTCTGCACAACCACTACACGAGAAGGACCTCTCCCTGTCT
 413 V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L S
XbaI (1921) **Ball (1935)**
 1901 CCGGTAATAGTGTAGTCTAGACCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTGGACAAACCACTAGAATGCAGTGAATAAATG
 447 P G K •
HpaI (2067)
 2001 CTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAAACAACAATTGCATTCAATTTATGTTTCAG
AseI (2164)
XmnI (2160)
 2101 GTTCAGGGGGAGGTGTGGGAGTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAATTAATTTAAATAACAGCATAGCAAACTTTAACCTC
 2201 CAAATCAAGCCTCTACTTGAATCCTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTC
SapI (2346)
 2301 TTTCTATGAGTTTAAAGATATAGTGTATTTTCCCAAGTTTGAAGTACTCTTCTTCTTTATGTTTTAAATGCACTGACCTCCCACTTCCCTTTTATG
SspI (2406) **Swal (2421)**
 2401 TAAAATATTGAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAAATCCAGATGCTCAAGGCCCTTCATAATATCCCCCA

2501 GTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCTGCTCTGCCCACA **DrallI** (2602)
125 • D Q E E A V

2601 AAGTGCACGCAGTTGCCGGCCGGTTCGCGCAGGGCGAACTCCCGCCCCACGGCTGCTCGCCGATCTCGGTCATGGCCGGCCGGAGGCGTCCCGGAAGT
1174 F H V C N G A P D R L A F E R G W P Q E G I E T M A P G S A D R F N

2701 TCGTGGACACGACCTCCGACCCTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACCCAGGCCAGGGTGTGTCCGGCACCACCTGGTCTGGACCGC
84 T S V V E S W E A Y L E D L G R V W V W A L T N D P V V Q D Q V A

2801 GCTGATGAACAGGGTCACGTCGTCGCCGACACACCGGCGAAGTCGTCCTCCAGAACTCCCGGAGAACCAGCCGGTCCGAGAACTCGACCGCT
514 S I F L T V D D R V V G A F D D E V F D R S F G L R D T W F E V A

2901 CCGGCAGCTCGCGCGCGTGGAGCACCAGGAACTGGTCAACTGGCCATGATGGCTCCTCctgtcaggagaggaagagaagaaggttagtacaat
174 G A V D R A T L V P V A S T L K A M

3001 tgCTATAGTGAGTTGTATTATACTATGCAGATATACTATGCCAATGATTAATTGTCAAACCTAGGGCTGCAggggttcatagtgccacttttctgcactgc
Asel (3048) PstI (3070)

3101 cccatctcctgccaccctttccaggcatagacagtcaagtactacCAAACCTCACAGGAGGAGAAGGCAGAAGCTTGAGACAGACCCCGGGACCGC
HindIII (3174) SacI (3192)

3201 CGAACTGCGAGGGGACGTGGCTAGGGGGCTTCTTTATGGTGCGCCGCCCTCGGAGGCAGGGCGCTCGGGAGGCCTAGCGGCAATCTGCGGTGGCA
Stul (3276)

3301 GGAGGCGGGCCGAAGCCGTGCCTGACCAATCCGGAGCAGATAGGAGTCTCAGCCCCGCCCAAGCAAGGGGAAGTCACGCGCTGTAGCGCCAGC
BspEI (3331)

3401 GTGTTGTGAAATGGGGCTTGGGGGGTGGGGCCCTGACTAGTCAAAACAAACTCCCAATTGACGTCATGGGGTGGAGACTTGGAAATCCCCGTGAGTC
SpeI (3439)
Bsp120I (3431)

3501 AAACCGCTATCCAGCCCATTTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCAT
SnaBI (3569)

3601 AAGGTCACTGACTGGGCATAATGCCAGGGGGCCATTTACCCTGATTGACGTCATAGGGGGCTACTTGGCATATGATACACTTGTACTGCCAAGT
NdeI (3673)

3701 GGCAGTTTACCCTAAATACTCCACCATTGACGTCATGGAAAGTCCCTATTGGCGTTACTATGGAAACATACGTCATTATTGACGTCATGGCGGGG

3801 GTCGTTGGGGGTCAGCCAGGGGGCCATTTACCCTAAGTTATGTAACGCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGAAAAGGCCAGGA
Pacl (3862)
PstI (3855)
Sdal (3855) **BspLU111** (3868)

3901 ACCGTAAAAAGGCCGCTTGTGCGTTTTTCCATAGGCTCCGCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGTGGCGAAACCCGAC

4001 AGGACTATAAAGTACCAGCGTTTTCCCCGGAAGCTCCCTCGTGCCTCTCTGTTCCGACCTGCCGTTACCGGATACCTGTCCGCTTTCTCCCT

4101 TCGGGAAGCGTGGCGTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTGTTGCTCCAAAGCTGGGCTGTGTGCACGAACCCCGTTC

4201 AGCCGACCGCTGCGCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAG

4301 CAGAGCGAGGTATGTAGCGGTGTACAGAGTTCTTGAAGTGGTGCCTAACTACGGTACACTAGAAGAACAGTATTTGGTATCTGCGCTtgattgcAG

4401 CCAGTTACCTTCGAAAAAGATTGGTAGCTTTGATCCGGCAAACAAACCACCCTGGTAGCGGTGTTTTTTTGTGCAAGCAGCAGATTACGCGCA

4501 GAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTACGGGGTCTGACGCTCAGTGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGGCTAGTTA
Pacl (4602)

4601 ATTAACATTTAAATCAGCGCCCAATAAATACTTTATTTTATTACATCTGTGTGTTGGTTTTTGTGTAATCGTAACTAACATACGCTCTCCATC
EagI (4618)
Swal (4610) **NotI** (4618)

4701 AAAACAAACGAAACAAACAACTAGCAAATAGGCTGTCCCCAGTGAAGTGCAGGTGCCAGAACATTTCTCTATCGAA