

pSELECT-mHMGB1-Lucia

A mammalian expression plasmid encoding a murine HMGB1::Lucia fusion protein

Catalog code: psetz-mhmgbluc

<http://www.invivogen.com/pselect-hmgb1>

For research use only

Version 20K30-MM

PRODUCT INFORMATION

Contents

- 20 µg of pSELECT-mHMGB1-Lucia 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.
- Resuspended DNA should be stored at -20°C and is stable for 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 has been purified by ion exchange chromatography.

GENERAL PRODUCT USE

pSELECT plasmids are specifically designed for strong and constitutive expression of a gene of interest in a wide variety of cell lines. They allow variation in selection markers for obtaining stable transfectants. pSELECT plasmids contain two expression cassettes: the first one drives the expression of the gene of interest, and the second one drives the expression of a large choice of dominant selection markers for both *E. coli* and mammalian cells. Each cassette terminates with a strong polyadenylation signal (polyA) thus preventing any transcription interference. The late SV40 polyA terminates the transcription of the gene of interest while the human β-globin polyA terminates the transcription of the selection marker.

pSELECT-mHMGB1-Lucia is a mammalian expression vector containing the murine HMGB1 gene fused at its 3' end to the Lucia luciferase gene. This plasmid is selectable in bacteria and mammalian cells with Zeocin™. Expression of HMGB1::Lucia in cells equipped for inflammasome-mediated-pyroptosis and/or necroptosis allows monitoring of these forms of necrotic cell death. The release of the HMGB1::Lucia protein in the extracellular milieu upon pyroptosis or necroptosis can be readily measured using QUANTI-Luc™, a coelenterazine-based luminescence assay reagent.

PLASMID FEATURES

First expression cassette

• **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter 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. 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.

- **mHMGB1::Lucia** encodes a 46.5 kDa fusion protein in which the C-terminus of murine HMGB1 is fused to Lucia luciferase, a secreted coelenterazine-utilizing luciferase reporter protein. Levels of HMGB1::Lucia in the cell culture supernatant can be monitored by measuring the light signal produced after addition of QUANTI-Luc™.
- **SV40 pAn:** the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA.
- **ori:** a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer *Ori*.

Second expression cassette

- **CMV enh/prom:** The human cytomegalovirus immediate-early gene 1 promoter/enhancer was originally isolated from the Towne strain and was found to be stronger than any other viral promoters.
- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- **Zeocin™** Resistance to Zeocin™ is conferred by the *Sh ble* gene from *Streptoalloteichus hindustanus*. The *Sh ble* gene is driven by the CMV enhancer/promoter in tandem with the bacterial EM7 promoter allowing 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 transcription¹.

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

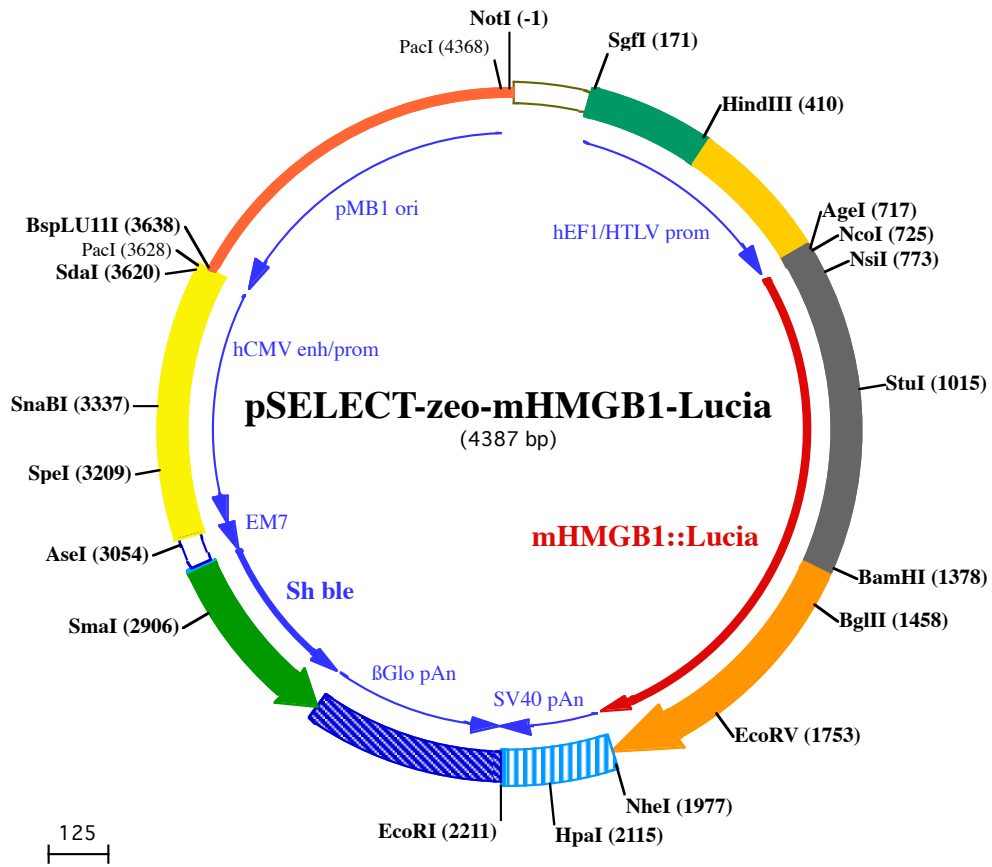
Plasmid amplification and cloning can be performed in *E. coli* GT116 other commonly used laboratory *E. coli* strains, such as DH5α.

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.

TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873
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NotI (-1)

1 **GCGGCCGCA**ATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAACAAAACGAAACA
101 AAACAACTAGCAAATAGGCTGTCCCCAGTGAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCA
201 **GAGCGCACATCGCCACAGTCCCGGAGAAGTTGGGGGAGGGGTGCGCAATTGAACGGGTGCCTAGAGAAGGTGGCGCGGGTAAACTGGGAAAGTATG**
301 **TCGTGTACTGGCTCCGCCTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAG**

SgfI (171)

HindIII (410)

401 **AACACAGTGAAGCTTCGAGGGCTCGCATCTCTCCTTACGCGCCCGCCCTACCTGAGGCCCATCCACGCCGTTGAGTCGCGTTCTGCCGCT**
501 **CCCGCTGTGGTGCCTCTGAAGTGCCTCCGCGTCTAGGTAAGTTTAAAGCTCAGGTGAGACCGGCTTTGTCCGGCGCTCCCTTGAGCCTACCTA**
601 **GACTCAGCCGGCTCTCCACGCTTTCCTGACCTGCTTGTCTCAACTCTACGCTTTTGTTCGTTTTCTGTTCTGCGCGTTACAGATCCAAGCTGTGACC**

AgeI (717) NcoI (725)

NsiI (773)

701 **GGCGCTAC**CTGAGATCACCGTCCACATGGGCAAAGGAGATCCTAAGAAGCCGAGAGCAAATGTCCTCATATGCATTCTTTGTGCAAACCTGCCGGG
801 AGGAGCACAAAGAAGAAGCACCCGGATGTTCTGTCAACTTCTCAGAGTTTTCCAAGAAGTGCAGAGAGGTGGAAGACCATGTCTGTAAAGAAAAGGG
25▶ **E E H K K K H P D A S V N F S E F S K K C S E R W K T M S A K E K G**
901 GAAATTTGAAGATATGGCAAAGGCTGACAAGGCTCGTTATGAAAGAGAATGAAAACCTACATCCCCCAAAGGGGAGACAAAAAAGATTCAAGGAC
58▶ **K F E D M A K A D K A R Y E R E M K T Y I P P K G E T K K K F K D**

StuI (1015)

1001 CCCAATGCACCCAAGAGGCTCTTCGGCTTCTTCTGTTCTGTTCTGAGTACCGCCCCAAATCAAAGGCGAGCATCTGGCTTATCCATTGGTGTATG
92▶ **P N A P K R P P S A F F L F C S E Y R P K I K G E H P G L S I G D**
1101 TTGCAAAGAACTAGGAGAGATGTGGAACAACACTGCAGCAGATGACAAGCAGCCCTATGAGAAGAAAGTGCCTAAGTGAAGGAGAAGTATGAGAAGGA
125▶ **V A K K L G E M W N N T A A D D K Q P Y E K K A A K L K E K Y E K D**
1201 TATTGCTCCTACAGAGCTAAAGGAAAACCTGATGCAGCGAAAAGGGGTGGTCAAGGCTGAAAAGGCAAGAAAAAAGGAAAGGAAAGATGATGAG
158▶ **I A A Y R A K G K P D A A K K G V V K A E K S K K K K E E E D D E**

BamHI (1378)

1301 GAGGATGAAGAGGATGAGGAAGAGGAGGAAGAAGAGGAAGACGAAGATGAAGAAGAAGATGATGATGATGAAGGAGGTGGATCCAGCGCAGCT**GCAAAAC**
192▶ **E D E E D E E E E E E D E D E E D D D D E G G G S S A A A K**

BglII (1458)

1401 **CCACTGAAATCAATGAAGACCTCAATATAGCTGCTGTGGCCTCAACTTTGCCACCACAGATCTTGAGACTGACCTGTTCACTGGGAGACCATGAA**
225▶ **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 N W E T M N**
1501 **TGTGATTAGCACTGACACAGAGCAGGTGAACACAGATGCTGACAGGGGCAAGCTGCCTGGCAAAAACTCCCCCAGATGTCTGAGGGAGCTGGAGGCC**
258▶ **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 L R E L E A**
1601 **AATGCCAGAAGGGCTGGTTGCACAAGAGGCTGCCTCATTGGCTCTCCACATTAAGTGCACCCTAAGATGAAGAAATTTATCCCTGGCAGGTGCCACA**
292▶ **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 P G R C H**

EcoRV (1753)

1701 **CTTATGAAGGTGAAAAGGAGTCTGCTCAGGGAGGGATTGGAGAGGCAATTGTTGATATCCCAGAGATTCTGGCTTCAAGGATAAGGAGCCACTGGACCA**
325▶ **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 K E P L D Q**
1801 **GTTTATTGCTCAAGTGGACTCTGTGCTGATTGCACCACTGGCTGTCTGAAGGGCCTTGCCAAATGTCCAGTGTCTGACCTCCTGAAGAAAGTGGCTCCC**
358▶ **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 L K K W L P**

NheI (1977)

1901 **CAGAGGTGACCCTTTTCCAGCAAGATTGAGGGTAGGGTGGACAAAATCAAGGGTCTGGCTGGGGACAGATGATAGCTAGCTGGCCAGACATGATAAG**
392▶ **Q R C T T F A S K I Q G R V D K I K G L A G D R •**
2001 **ATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATA**

HpaI (2115)

2101 **AGCTGCAATAAACAAAGTTAACACAACAATTGCATTCATTTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAACCTCTACA**

EcoRI (2211)

2201 **AATGTGGTATGGAATTCTAAAATACAGCATAGCAAACCTTAACTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCA**
2301 **TCAGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTCATGGAGTTAAGATATAGTGTATTTTCCCAAGGTTTGAAGTACTGCTCTC**
2401 **ATTTCTTATGTTTTAAATGCACTGACCTCCACATTCCTTTTTAGTAAAATATTAGAAAATATTAAATACATCATTGCAATGAAAATAAATGTTTT**
2501 **TTATTAGCGAATCCAGATGCTCAAGGCCCTCATAATATCCCCAGTTTAGTAGTTGACTTAGGGAACAAAGGAACCTTAAATAGAAATTGGACAGC**
2601 **AAGAAAGCGAGCTTCTAGCTTATCTCAGTCTGCTCCTGTGCCACAAAGTGCACGCAAGTGGCCGCCGGTTCGCGCAGGGCGAACTCCCGCCCCACGG**
125▶ **D Q E E A V F H V C N G A P D R L A F E R G W P**
2701 **CTGCTCGCCGATCTCGGTGATGCGCCGGCCGGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCAC**
100▶ **Q E G I E T M A P G S A D R F N T S V V E S W E A Y L E D L G R V**
2801 **CACACCAGGCCAGGGTGTGTCGGCACCACTGGTCTGGACCGGCTGATGAACAGGGTACGTCGTCCCGACCACACCGGCGAAGTCGTCTCCA**
66▶ **W V W A L T N D P V V Q D Q V A S I F L T V D D R V V G A F D D E V**

SmaI (2906)
2901 CGAAGTCCCGGGAGAACCCGAGCCGGTCGGTCCAGAACTCGACCGCTCCGGCGACGTGCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTGGCCAT
33 F D R S F G L R D T W F E V A G A V D R A T L V P V A S T L K A M

AseI (3054)
3001 GATGGCCCTCCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAAACACGCGTGGATGGCGTCTCCAGCTTATCTGACG
3101 GTTCACTAAACGAGCTCTGCTTATATAGACCTCCACCGTACACGCTACCGCCATTGCGTCAATGGGGCGGAGTTGTTACGACATTTGGAAAGTCC

SpeI (3209)
3201 CGTTGATTTACTAGTCAAACAAACTCCATTGACGTCAATGGGGTGGAGACTTGAAATCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTACT

SnaBI (3337)
3301 GCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCATAAGGTCACTGACTGGGCATAATGCCAGGC
3401 GGGCCATTTACCGTCATTGACGTCAATAGGGGGCTACTTGGCATATGATACACTTGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCAT
3501 TGACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCAATTATTGACGTCAATGGGGCGGGTTCGTTGGGCGGTGAGCCAGGCGGGCCAT

SdaI (3620) **PacI (3628)** **BspLU11I (3638)**
3601 TTACCGTAAGTTATGTAACGCCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGCGCGTTGCTGGCGTTT
3701 TTCCATAGGCTCCGCCCCCTGACGAGCATCACA AAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAGATACCAGGCGTTTCCCC
3801 CTGGAAGCTCCCTCGTGCCTCTCCTGTCCGACCCTGCCGTTACCGGATACCTGTCCGCTTTCTCCCTTCGGAAGCGTGGCGCTTTCTCATAGCTC
3901 ACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTGCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTGAGCCGACCGTGGCCTTATCCGGTAAC
4001 TATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGTAACAGGATTAGCAGAGCGAGGTATGTAGCGGTGCTACAG
4101 AGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAG
4201 CTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTG

PacI (4368)
4301 ATCTTTTCTACGGGTCTGACGCTCAGTGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCA