

pSELECT-mASC-GFP

A mammalian expression plasmid encoding a murine ASC::GFP fusion protein

Catalog code: psetz-mascgfp

<http://www.invivogen.com/gfp-inflammasome-genes>

For research use only

Version 20K30-MM

PRODUCT INFORMATION

Contents

- 20 µg of pSELECT-mASC-GFP 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 selectable 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-mASC-GFP is a mammalian expression vector containing the murine ASC gene fused at its 3' end to the green fluorescent protein (GFP) gene. This plasmid is selectable in bacteria and mammalian cells with Zeocin™. Expression of ASC::GFP in cells equipped with inflammasome components allows the visual monitoring of ASC specks, a hallmark of inflammasome activation. During inflammasome formation, ASC::GFP polymerizes to form a macromolecular complex that can be imaged using time-lapse confocal or high-resolution fluorescence microscopy. In most cells, only one speck forms upon inflammasome activation.

The same plasmid is available with the GFP gene alone as a control. This control plasmid is called pSELECT-CGFP-zeo (cat. code: psetz-cgfp).

PLASMID FEATURES

First expression cassette

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

• **mASC::GFP** encodes a 48.8 kDa fusion protein in which GFP is fused via a six-amino-acid linker at the C terminus of the murine ASC protein to avoid interfering with ASC functionality. The N-terminal pyrin domain of ASC is important for self-dimerization of the protein and for its recruitment by upstream molecules such as pyrin or cryopyrin. This fusion protein absorbs blue light (major peak at 480 nm) and emits green light (major peak at 505 nm).

• **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*.

• **Zeo:** 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*.

• **β Glo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription[†].

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 DH5 α .

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

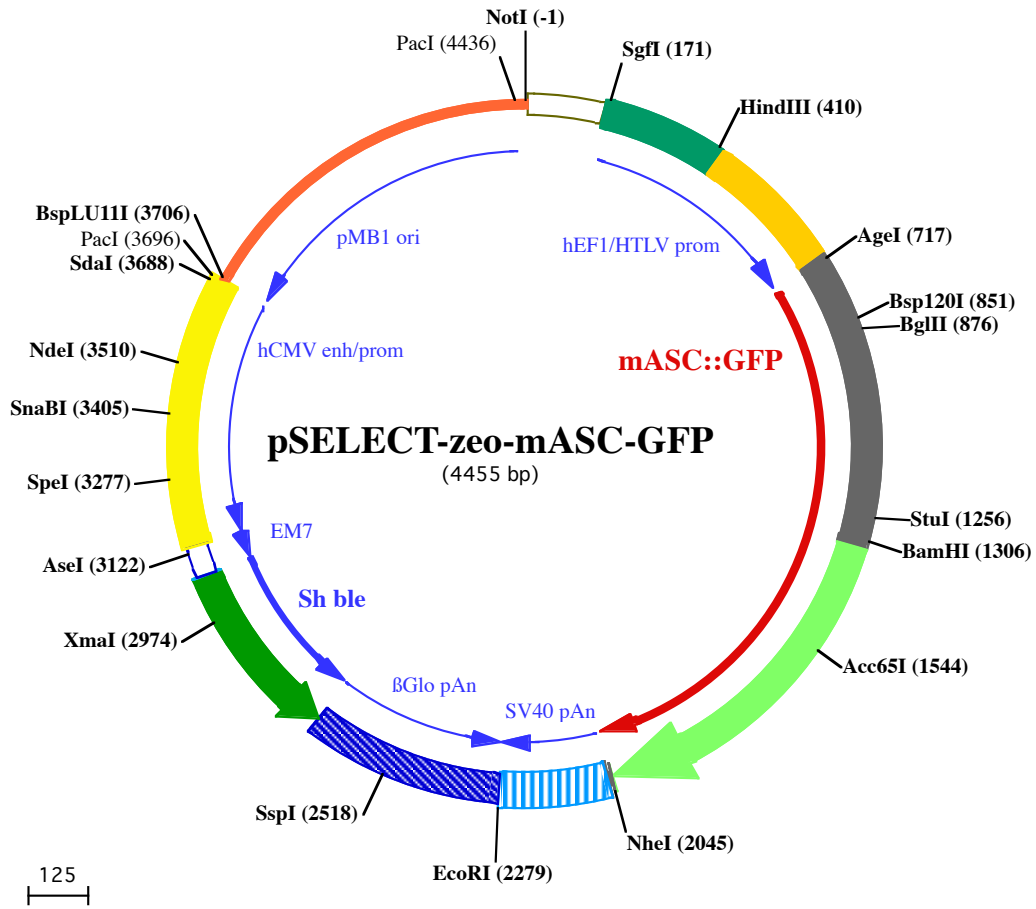
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NotI (-1)

1 GCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGTTTTTGTGTGAATCGTAACATAACGCTCTCCATCAAAACAAAACGAAACA
101 AAACAACTAGCAAATAGGCTGTCCCCAGTGAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCA
201 GAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGGTCGGCAATTGAACGGGTGCCTAGAGAAGGTGGCGCGGGTAAACTGGAAAGTGATG
301 TCGTGTACTGGCTCCGCCTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAG

SgfI (171)

HindIII (410)

401 AACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCCTTACCGCCCGCCCTACCTGAGGCCCATCCACGCCGTTGAGTCGCGTTCTGCCGCT
501 CCCGCTGTGGTCCCTCTGAAGTGCCTCCGCCGTCTAGTAAAGTTAAAGCTCAGGTCGAGACCGGCCCTTTGTCCGGCGCTCCCTTGAGCCTACCTA
601 GACTCAGCCGGCTCTCCACGCTTTCCTGACCTGCTTGTCTCAACTTACGCTTTTGTTCGTTTTCTGTTCTGCGCGTTACAGATCCAAGTGTGACC

AgeI (717)

701 GCGCCTACCTGAGATCACCGGTCAACATGGGGCGGGCAGAGATGCCATCCTGGACGCTCTTAAAACTTGTGAGGGATGAACTCAAAAAGTTCAAGA
1 M G R A R D A I L D A L E N L S G D E L K K F K

Bsp120I (851)

BglII (876)

801 TGAAGCTGCTGACAGTGAAGTGCAGAGGCTATGGGCGCATCCCACGCGGGCCCTGCTGCAGATGGACGCCATAGATCTCACTGACAACTTGTGAG
25 M K L L T V Q L R E G Y G R I P R G A L L Q M D A I D L T D K L V S
901 C TACTATCTGAGTCTGATGGCTTGGAGCTCACAATGACTGTCTTAGAGACATGGGCTTACAGGAGCTGGCTGAGCAGCTGCAAACGACTAAAGAAGAG
58 Y Y L E S Y G L E L T M T V L R D M G L Q E L A E Q L Q T T K E E
1001 TCTGGAGCTGTGCAGTGCAGCCAGTCCCTGCTCAGAGTACAGCAGAACAGGACACTTTGTGGACCAGCACAGGCAAGCACTATTGCCAGGGTCA
92 S G A V A A A A S V P A Q S T A R T G H F V D Q H R Q A L I A R V
1101 CAGAAGTGGACGGAGTGTGGATGCTTTCATGGCAGTGTGCTGACTGAAGGACAGTACCAGGCGAGTTCGTGCAGAGACCACCAGCCAAGACAAGATGAG
125 T E V D G V L D A L H G S V L T E G Q Y Q A V R A E T T S Q D K M R

StuI (1256)

1201 GAAGCTCTCAGCTTTGTTCCATCCTGGAACCTGACCTGCAAGGACTCCCTCCTCAGGCCTTGAAGGAAATACATCCCTACTTGGTGATGGACCTGGAG
158 K L F S F V P S W N L T C K D S L L Q A L K E I H P Y L V M D L E

BamHI (1306)

1301 CAGAGCGGATCCGGTGGAGGTGCCATGGTTTCTAAGGGAGAAGAACTCTTACTGGTGTGTCCCAATTCTGGTTGAGCTGGATGTTGAAATGGCC
192 Q S G S G G G A M V S K G E E L F T G V V P I L V E L D G D V N G
1401 ACAAACTCTGTGTCTGGTGAAGGTGAAGGAGATGCAACTTATGAAAGCTGACTCTGAAGTTCATTTGTACAACAGGAAAGCTGCCAGTGCCTTGGCC
225 H K F S V S G E G E G D A T Y G K L T L K F I C T T G K L P V P W P

Acc65I (1544)

1501 AACTCTGGTGACCACCCTGACTTATGGTGTCAATGTTTCAGCAGGTACCCTGACCACATGAAGCAGCATGACTTCTTTAAATCTGCAATGCCAGAAGGT
258 T L V T T L T Y G V Q C F S R Y P D H M K Q H D F F K S A M P E G
1601 TATGTTCAAGGAGGACAATCTTCTTTAAGGATGATGGAAATTATAAGACAAGGGCAGAAAGTGAAGTTTGAAGGTGATACACTGGTTAACAGAATTGAGC
292 Y V Q E R T I F F K D D G N Y K T R A E V K F E G D T L V N R I E
1701 TGAAGGCATTGATTTTAAAGGAAGTGAAGAACATTCTGGGTACAAGTGGAGTACAATAATTCTCACAATGTTTACATTATGGCAGATAAGCAGAG
325 L K G I D F K E D G N I L G H K L E Y N Y N S H N V Y I M A D K Q R
1801 GAATGGAATTAAGGCTAATTTCAAGATTAGACACAACATTGAGGATGGATCTGTCCAAGTGGCAGACCATTACCAGCAGAACACCCTATTGGTGATGGC
358 N G I K A N F K I R H N I E D G S V Q L A D H Y Q Q N T P I G D G
1901 CCAGTTCCTCCAGATAATCACTATCTCAGCACTCAATCTGCTCTGTCCAAAGACCCTAATGAGAAAAGAGACCACATGGTCTCCTGGAGTTTGTGA
392 P V L L P D N H Y L S T Q S A L S K D P N E K R D H M V L L E F V

NheI (2045)

2001 CAGCAGCAGGAATTAAGTCTGGGAATGGATGAGCTGTACAAGTAAAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGACAAACCACAAC
425 T A A G I T L G M D E L Y K
2101 GAATGCAGTGAATAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTG

EcoRI (2279)

2201 CATTCAATTTATGTTTCAGGTTCAAGGGGAGGTGTGGGAGTTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAATTTCTAAAATACAGCATAG
2301 CAAAACCTTAACCTCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGATTAGCTGTT
2401 TGCAGCCTCACCTTCTTTCATGGAGTTAAGATATAGTGTATTTTCCAAAGTTTGAAGTGTCTTCAATTTCTTTATGTTTTAAATGCACTGACCTCCC

SspI (2518)

2501 ACATTCCCTTTTATAGTAAAATATTCAGAAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCT
2601 TCATAATATCCCCAGTTTGTAGTGGACTTAGGGAACAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCC
2701 TGCTCCTCTGCCCAAAGTGCACGCAGTTGCCGGCCGGTTCGCGCAGGGCGAACTCCCGCCCCACGGCTGCTCGCCGATCTCGGTATGGCCGGCCCCG
122 Q E E A V 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
2801 AGCGTCCCGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACACCCAGGCCAGGGTGTGTCCGGCACCAC
89 A D R F N 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

XmaI (2974)

2901 CTGGTCTGGACCGCTGATGAACAGGGTCACGTCGTCGCCGACACACCGCGAAGTCGTCTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCCGGTC
56 Q D Q V A 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
3001 CAGAACTCGACCGCTCCGGCGACGTCGCGCGGGTGAACACCGGAAACGGCACTGGTCAACTGGCCATGATGGCCCTCTATAGTGAGTCGTATTATACT
22 W F E V A G A V D R A T L V P V A S T L K A M

AseI (3122)

3101 ATGCCGATATACTATGCCGATGATTAATTGTCAAACACAGCGTGGATGGCGTCTCCAGCTTATCTGACGGTTCCTAAACGAGCTCTGCTTATATAGACCT

SpeI (3277)

3201 CCCACCGTACACGCCTACCGCCATTGCGTCAATGGGGCGGAGTTGTTACGACATTTGGAAAGTCCCGTTGATTTACTAGTCAAAAACAACTCCCATT
3301 GACGTCATGGGGTGGAGACTTGAAATCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTAAGTCCAAAACCGCATCATCATGGTAATAGCGATG

SnaBI (3405)

3401 ACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTACCGTCATTGACGTCATAGGGG

NdeI (3510)

3501 GCGTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGAGTTACCGTAAATACTCCACCCATTGACGTCATGGAAAGTCCCTATTGGCGTTAC

PaeI (3696)

SdaI (3688)

3601 TATGGGAACATACGTCATTATTGACGTCATGGGCGGGGTCTGGGCGGTGAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGCAGGTTAA

BspLU11I (3706)

3701 TTAAGAACATGTGAGCAAAAAGCCAGCAAAAAGCCAGGAACCGTAAAAAGCCGCGTTGCTGGCGTTTTCCATAGGCTCCGCCCCCTGACGAGCATCA

3801 CAAAAATCGACGCTCAAGTCAGAGTGGCGAAACCCGACAGGACTATAAGATACCAGGCGTTCCCCCTGGAAGCTCCCTCGTGGCTCTCCTGTTCCG

3901 ACCCTGCCGTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCG

4001 TTCGCTCAAGCTGGGCTGTGTGCACGAACCCCGTTACGCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCAACCCGGTAAGACACGA

4101 CTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCTAC

4201 ACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACAAACCACCGCTGGTA

4301 GCGGTGGTTTTTTTGTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGGTCTGACGCTCAGTGGAA

PaeI (4436)

4401 CGAAAACCTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCA