pSELECT-zeo-HSV1-tk::Sh

An expression plasmid coding for a CpG-free cytotoxic/resistance fusion gene

Catalog code: psetz-hsv1tksh

Version 20L01-MM

PRODUCT INFORMATION

Contents:
• 20 *μ*g of pSELECT-zeo-HSV1-tk::Sh 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 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 full-length ORF sequencing.
• Plasmid DNA was purified by ion exchange chromatography.

GENERAL PRODUCT USE

pSelect-zeo plasmids contain genes that have been chemically synthesized. The DNA sequence of these genes was modified by optimizing the codon usage, reducing or eliminating the CpG motifs and avoiding secondary DNA structures without changing the amino acid sequence of the wild type proteins.

pSELECT-zeo plasmids may be used:

To subclone the synthetic gene into another vector. To facilitate subcloning, the HSV1-tk::Sh gene is flanked by two unique restriction sites: Nco I at the 5' end that encompasses the Start codon, and Nhe I at the 3'end.

As a gene reporter plasmid. pSELECT-zeo is a mammalian expression plasmid selectable in *E. coli* and mammalian cells with Zeocin™, as the *Sh ble* gene in the second expression casssette is driven by the eukaryote CMV enhancer/promoter in tandem with the bacterial EM7 promoter.

PLASMID FEATURES

• ori: a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.

First expression cassette

• hEF1-HTLV prom is a composite promoter comprising the Elongation Factor-1alpha (EF-1α) core promoter[1] 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[2]. 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.

• HSV1-tk::Sh CpG-free: InvivoGen has engineered a fusion gene between the Herpes Simplex Virus 1 (HSV1) thymidine kinase gene and the *Sh ble* gene conferring Zeocin™ resistance. Both genes have been modified and contain no CpG motifs.

• SV40 pAn: the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA[3].

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*.

• ßGlo pAn: The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription[4].

1. **Kim D.** *et al.*, **1990.** Use of the human elongation factor 1α promoter as a versatile and efficient expression system Gene 91(2):217-23. **2. Takebe, Y.** *et al.*, **1988.** R 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. 1:466-72. **3. Carswell S. & Alwine J., 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 J. 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.

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 H_2O. 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.

InvivoGen
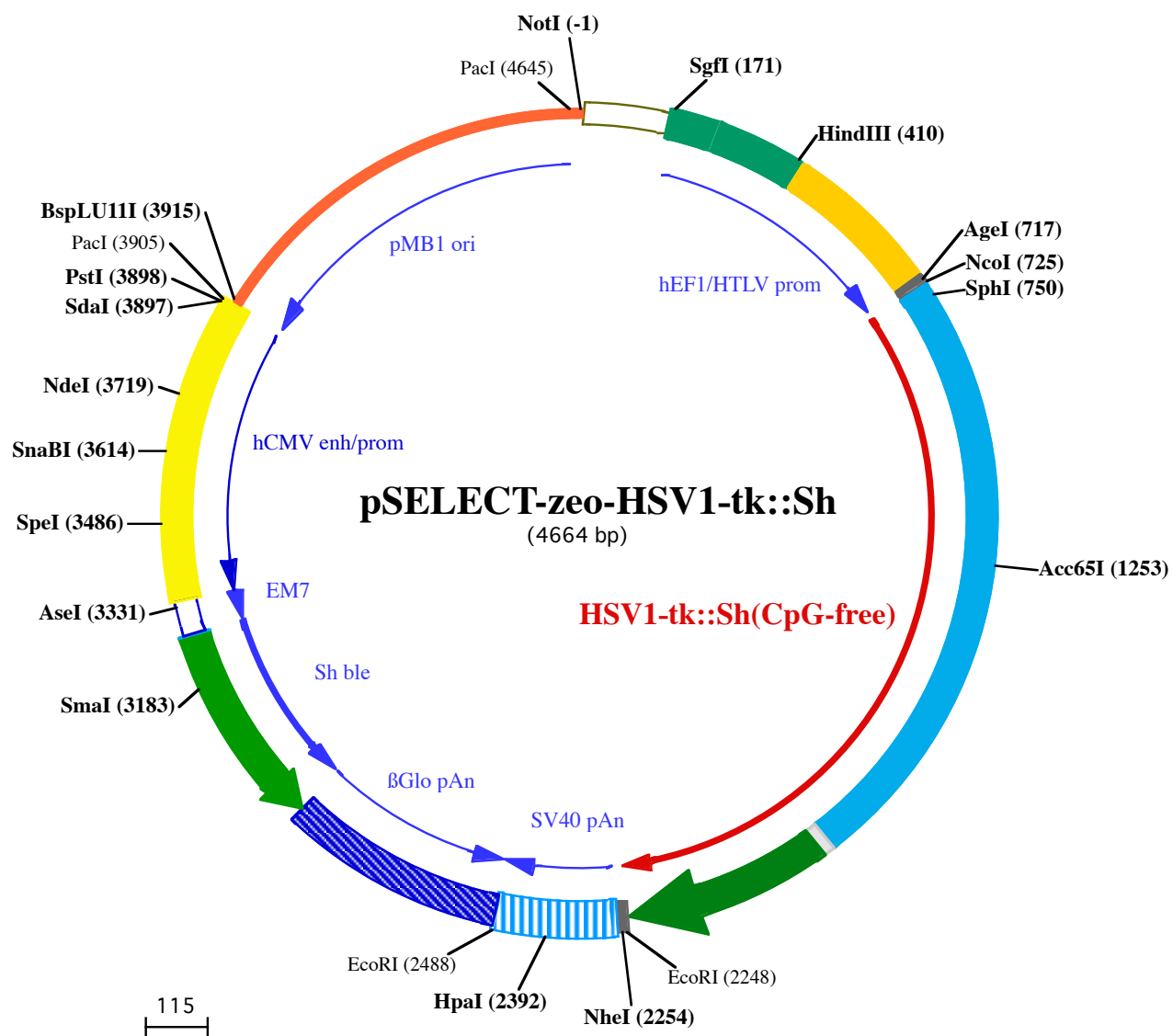www.invivogen.com

pSELECT-zeo-HSV1-tk::Sh
(4664 bp)

HSV1-tk::Sh(CpG-free)

NotI (-1)
PacI (4645)
SgfI (171)
HindIII (410)
BspLU11I (3915)
PacI (3905)
PstI (3898)
SdaI (3897)
AgeI (717)
NcoI (725)
SphI (750)
NdeI (3719)
SnaBI (3614)
SpeI (3486)
AseI (3331)
SmaI (3183)
Acc65I (1253)
EcoRI (2488)
HpaI (2392)
NheI (2254)
EcoRI (2248)

pMB1 ori
hEF1/HTLV prom
hCMV enh/prom
EM7
Sh ble
ßGlo pAn
SV40 pAn

115

NotI (-1)

1 GCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACA

101 AAACAAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCA

SgfI (171)

201 GAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACGGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATG

301 TCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAG

HindIII (410)

401 AACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCT

501 CCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTA

601 GACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTTTCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACC

NcoI (725)
AgeI (717)   **SphI (750)**

701 GGCGCCTACCTGAGATCACCGGTCACCATGGCTTCTTACCCTGGACACCAGCATGCTTCTGCCTTTGACCAGGCTGCCAGATCCAGGGGCCACTCCAACA
        1 ▸ M A S Y P G H Q H A S A F D Q A A R S R G H S N

801 GGAGAACTGCCCTAAGACCCAGAAGACAGCAGGAAGCCACTGAGGTGAGGCCTGAGCAGAAGATGCCAACCCTGCTGAGGGTGTACATTGATGGACCTCA
25 ▸ R R T A L R P R R Q Q E A T E V R P E Q K M P T L L R V Y I D G P H

901 TGGCATGGGCAAGACCACCACCACTCAACTGCTGGTGGCACTGGGCTCCAGGGATGACATTGTGTATGTGCCTGAGCCAATGACCTACTGGAGAGTGCTA
58 ▸ G M G K T T T T Q L L V A L G S R D D I V Y V P E P M T Y W R V L

1001 GGAGCCTCTGAGACCATTGCCAACATCTACACCACCCAGCACAGGCTGGACCAGGGAGAAATCTCTGCTGGAGATGCTGCTGTGGTGATGACCTCTGCCC
92 ▸ G A S E T I A N I Y T T Q H R L D Q G E I S A G D A A V V M T S A

1101 AGATCACAATGGGAATGCCCTATGCTGTGACTGATGCTGTTCTGGCTCCTCACATTGGAGGAGAGGCTGGCTCTTCTCATGCCCCTCCACCTGCCCTGAC
125 ▸ Q I T M G M P Y A V T D A V L A P H I G G E A G S S H A P P P A L T

Acc65I (1253)

1201 CCTGATCTTTGACAGACACCCCATTGCAGCCCTGCTGTGCTACCCAGCAGCAAGGTACCTCATGGGCTCCATGACCCCACAGGCTGTGCTGGCTTTTGTG
158 ▸ L I F D R H P I A A L L C Y P A A R Y L M G S M T P Q A V L A F V

1301 GCCCTGATCCCTCCAACCCTCCCTGGCACCAACATTGTTCTGGGAGCACTGCCTGAAGACAGACACATTGACAGGCTGGCAAAGAGGCAGAGACCTGGAG
192 ▸ A L I P P T L P G T N I V L G A L P E D R H I D R L A K R Q R P G

1401 AGAGACTGGACCTGGCCATGCTGGCTGCAATCAGAAGGGTGTATGGACTGCTGGCAAACACTGTGAGATACCTCCAGTGTGGAGGCTCTTGGAGAGAGGA
225 ▸ E R L D L A M L A A I R R V Y G L L A N T V R Y L Q C G G S W R E D

1501 CTGGGGACAGCTCTCTGGAACAGCAGTGCCCCCTCAAGGAGCTGAGCCCCAGTCCAATGCTGGTCCAAGACCCCACATTGGGGACACCCTGTTCACCCTG
258 ▸ W G Q L S G T A V P P Q G A E P Q S N A G P R P H I G D T L F T L

1601 TTCAGAGCCCCTGAGCTGCTGGCTCCCAATGGAGACCTGTACAATGTGTTTGCCTGGGCTCTGGATGTTCTAGCCAAGAGGCTGAGGTCCATGCATGTGT
292 ▸ F R A P E L L A P N G D L Y N V F A W A L D V L A K R L R S M H V

1701 TCATCCTGGACTATGACCAGTCCCCTGCTGGATGCAGAGATGCTCTGCTGCAACTAACCTCTGGCATGGTGCAGACCCATGTGACCACCCCTGGCAGCAT
325 ▸ F I L D Y D Q S P A G C R D A L L Q L T S G M V Q T H V T T P G S I

1801 CCCCACCATCTGTGACCTAGCCAGAACCTTTGCCAGGGAGATGGGAATCTCTGGAGCCAATGGAGTCATGGCCAAGTTGACCAGTGCTGTCCCAGTGCTC
358 ▸ P T I C D L A R T F A R E M G I S G A N G V M A K L T S A V P V L

1901 ACAGCCAGGGATGTGGCTGGAGCTGTTGAGTTCTGGACTGACAGGTTGGGGTTCTCCAGAGATTTTGTGGAGGATGACTTTGCAGGTGTGGTCAGAGATG
392 ▸ T A R D V A G A V E F W T D R L G F S R D F V E D D F A G V V R D

2001 ATGTGCACCCTGTTCATCTCAGCAGTCCAGGACCAGGTGGTGCCTGACAACACCCTGGCTTGGGTGTGGGTGAGAGGACTGGATGAGCTGTATGCTGAGTG
425 ▸ D V T L F I S A V Q D Q V V P D N T L A W V W V R G L D E L Y A E W

2101 GAGTGAGGTGGTCTCCACCAACTTCAGGGATGCCAGTGGCCCTGCCATGACAGAGATTGGAGAGCAGCCCTGGGGGAGAGAGTTTGCCCTGAGAGACCCA
458 ▸ S E V V S T N F R D A S G P A M T E I G E Q P W G R E F A L R D P

NheI (2254)
EcoRI (2248)

2201 GCAGGCAACTGTGTGCACTTTGTGGCAGAGGAGCAGGACTGAGGGATAAGAATTCGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAA
492 ▸ A G N C V H F V A E E Q D •

HpaI (2392)

2301 CCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAA

EcoRI (2488)

2401 CAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATTCTAAAAT

2501 ACAGCATAGCAAAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCA

2601 TTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTTAAGATATAGTGTATTTTCCCAAGGTTTGAACTAGCTCTTCATTTCTTTATGTTTTAAATGCAC

2701 TGACCTCCCACATTCCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCT

2801 CAAGGCCCTTCATAATATCCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTAT

2901 CCTCAGTCCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGGTCGCGCAGGGCGAACTCCCGCCCCCACGGCTGCTCGCCGATCTCGGTCATGG
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 Q E G I E T M A

3001 CCGGCCCGGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACACCCAGGCCAGGGTGTTGTC
92 ◂ 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 W V W A L T N D

SmaI (3183)

3101 CGGCACCACCTGGTCCTGGACCGCGCTGATGAACAGGGTCACGTCGTCCCGGACCCACACCGGCGAAGTCGTCCTCCACGAAGTCCCGGGAGAACCCGAGC
59 ◂ 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 F D R S F G L

3201 CGGTCGGTCCAGAACTCGAACCGCTCCGGCGACGTCGCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTTGGCCATGATGGCCCTCCTATAGTGAGTCG
25 ◂ 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 (3331)

3301 TATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAAAACAGCGTGGATGGCGTCTCCAGCTTATCTGACGGTTCACTAAACGAGCTCTGCTTA

SpeI (3486)

3401 TATAGACCTCCCACCGTACACGCCTACCGCCCATTTGCGTCAATGGGGCGGAGTTGTTACGACATTTTGGAAAGTCCCGTTGATTTACTAGTCAAAACAA

3501 ACTCCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAACCGCTATCCACGCCCATTGATGTACTGCCAAAACCGCATCATCATGGTA

SnaBI (3614)

3601 ATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGT

NdeI (3719)

3701 CAATAGGGGGCGTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTAT

PstI (3898)
SdaI (3897)

3801 TGGCGTTACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCT

PacI (3905)   BspLU11I (3915)

3901 GCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGA

4001 CGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCT

4101 CCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGG

4201 TGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGT

4301 AAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAAC

4401 TACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCA

4501 CCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGC

PacI (4645)

4601 TCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCA