# pSELECT-zeo-HSV1tk

## A plasmid encoding a CpG-free HSV1tk

Catalog code: psetz-hsv1tk

### For research use only

Version 20L01-MM-02

## PRODUCT INFORMATION

### Content:

- 20 µg of **pSELECT-zeo-HSV1tk** 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 resuspended upon receipt and stored at -20˚C.
Resuspended DNA is stable more than one year at -20˚C.
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

pSELECT-zeo plasmids contain genes that have been chemically synthetised. 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 HSV1tk 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.

**To express the synthetic gene in mammalian cells:** 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 cassette is driven by the eukaryote CMV enhancer/promoter in tandem with the bacterial EM7 promoter.

## PLASMID FEATURES

### First expression cassette

• **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (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.
• **HSV1tk CpG-free:** The Herpes Simplex Virus 1 (HSV1) thymidine kinase gene, engineered to contain no CpG motif.
• **SV40 pAn:** the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA[3].
• **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.*
• **ßGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription[4].

## 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.

### References:

1. Kim, D.W. *et al.* (1990). Gene 2: 217-223.
2. Takebe, Y. *et al.* (1988). Mol. Cell Biol. 1: 466-472.
3. Carswell, S., and Alwine, J.C. (1989). Mol. Cell Biol. 10: 4248-4258.
4. Yu J & Russell JE. (2001). Mol Cell Biol, 21(17):5879-88.

**InvivoGen**
www.invivogen.com

pSelect-zeo- HSV1tk
(4274 bp)

SgfI (6)
PvuI (7)
MfeI (82)
Psp1406I (203)
PvuII (239)
HindIII (245)
Bsu36I (291)
KasI (535)
AgeI (552)
BstEII (555)
NcoI (560)
SphI (585)
BbsI (656)
StuI (682)
BstAPI (690)
BsrGI (716)

EagI (4110)
NotI (4109)
SwaI (4099)
PacI (4090)
AlwNI (3771)
ApaLI (3674)

BspLU11I (3360)
PacI (3350)
PstI (3343)
SdaI (3342)
NdeI (3164)
SnaBI (3059)
SpeI (2931)
SacI (2833)
AseI (2776)
SfiI (2716)
BssHII (2680)
BsrBI (2664)
XmaI (2628)
SgrAI (2601)

EagI (2385)
ApaLI (2371)
DraIII (2365)
EcoO109I (2247)
SwaI (2186)
SspI (2172)

AlwNI (2023)
EcoRI (1933)
MfeI (1848)
HpaI (1837)
BstXI (1667)
NheI (1699)
Ppu10I (1525)
NsiI (1525)
BsrGI (1472)
BbsI (1189)
Acc65I (1088)

hEF1-HTLV prom
HSV1tk (CpG-free)
pMB1 ori
hCMV enh/prom
EM7
Sh ble
ßglo pAn
SV40 pAn

100

InvivoGen
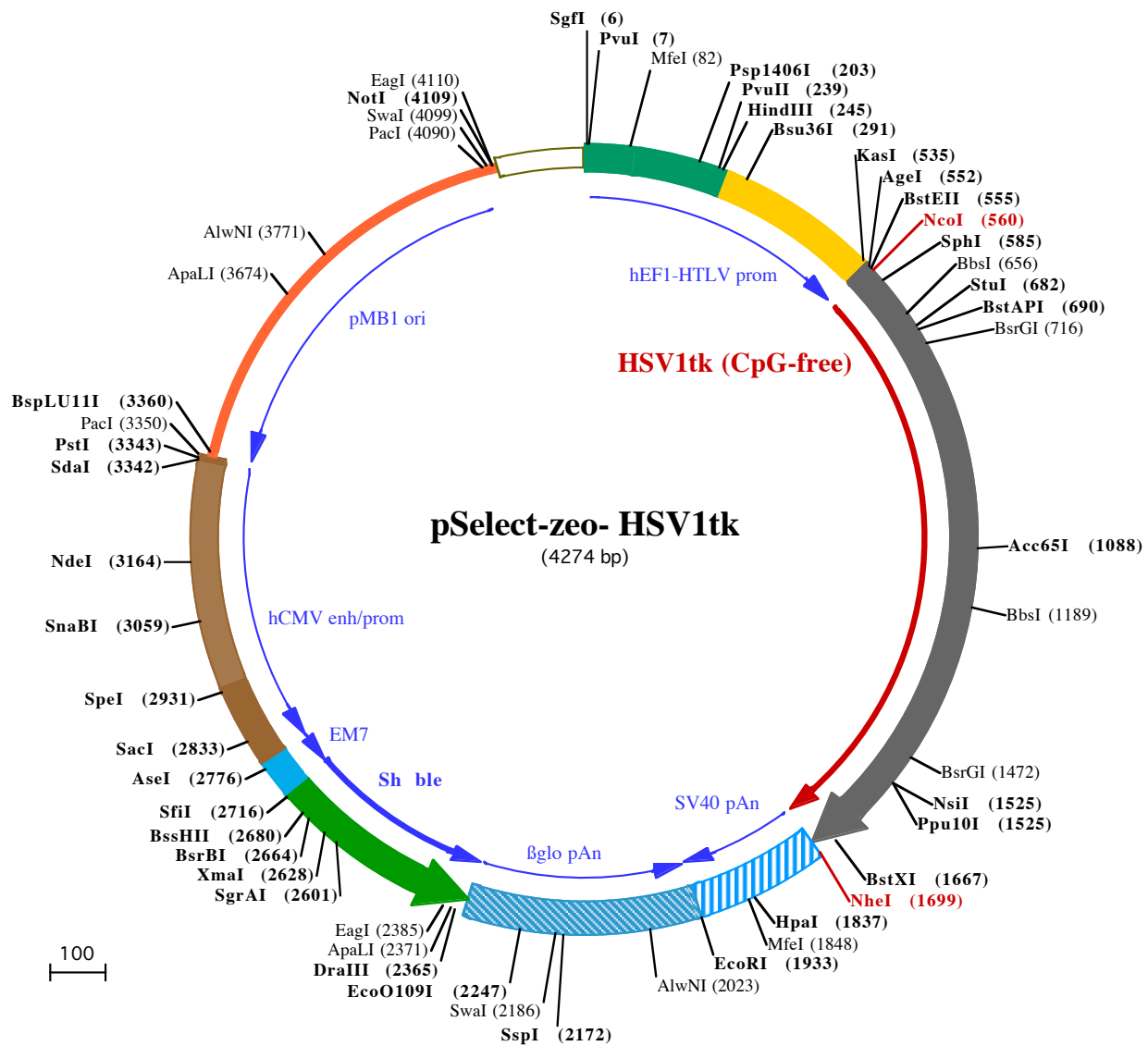
PvuI (7)
SgfI (6)
MfeI (82)

```
  1  GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACGGGTGCCTA

101  GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCC
```

HindIII (245)
Psp1406I (203)                    PvuII (239)                                              Bsu36I (291)

```
201  GTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCC

301  GCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC

401  GGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTT
```

NcoI (560)
BstEII (555)
KasI (535)        AgeI (552)                                    SphI (585)

```
501  TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCAccggtcacCATGGCTTCTTACCCTGGACACCAGCATGCTTCTGCCTT
                                                              1 ▶ M  A  S  Y  P  G  H  Q  H  A  S  A  F
```
                                                           BbsI (656)                    StuI (682)BstAPI (690)
```
601  TGACCAGGCTGCCAGATCCAGGGGCCACTCCAACAGGAGAACTGCCCTAAGACCCAGAAGACAGCAGGAAGCCACTGAGGTGAGGCCTGAGCAGAAGATG
 13 ▶ D  Q  A  A  R  S  R  G  H  S  N  R  R  T  A  L  R  P  R  R  Q  Q  E  A  T  E  V  R  P  E  Q  K  M
```
                BsrGI (716)
```
701  CCAACCCTGCTGAGGGTGTACATTGATGGACCTCATGGCATGGGCAAGACCACCACCACTCAACTGCTGGTGGCACTGGGCTCCAGGGATGACATTGTGT
 47 ▶ P  T  L  L  R  V  Y  I  D  G  P  H  G  M  G  K  T  T  T  T  Q  L  L  V  A  L  G  S  R  D  D  I  V

801  ATGTGCCTGAGCCAATGACCTACTGGAGAGTGCTAGGAGCCTCTGAGACCATTGCCAACATCTACACCACCCAGCACAGGCTGGACCAGGGAGAAATCTC
 80 ▶ Y  V  P  E  P  M  T  Y  W  R  V  L  G  A  S  E  T  I  A  N  I  Y  T  T  Q  H  R  L  D  Q  G  E  I  S

901  TGCTGGAGATGCTGCTGTGGTGATGACCTCTGCCCAGATCACAATGGGAATGCCCTATGCTGTGACTGATGCTGTTCTGGCTCCTCACATTGGAGGAGAG
113 ▶ A  G  D  A  A  V  V  M  T  S  A  Q  I  T  M  G  M  P  Y  A  V  T  D  A  V  L  A  P  H  I  G  G  E
```
                                                                     Acc65I (1088)
```
1001 GCTGGCTCTTCTCATGCCCCTCCACCTGCCCTGACCCTGATCTTTGACAGACACCCCATTGCAGCCCTGCTGTGCTACCCAGCAGCAAGGTACCTCATGG
147 ▶ A  G  S  S  H  A  P  P  P  A  L  T  L  I  F  D  R  H  P  I  A  A  L  L  C  Y  P  A  A  R  Y  L  M
```
                                                                                  BbsI (1189)
```
1101 GCTCCATGACCCCACAGGCTGTGCTGGCTTTTGTGGCCCTGATCCCTCCAACCCTCCCTGGCACCAACATTGTTCTGGGAGCACTGCCTGAAGACAGACA
180 ▶ G  S  M  T  P  Q  A  V  L  A  F  V  A  L  I  P  P  T  L  P  G  T  N  I  V  L  G  A  L  P  E  D  R  H

1201 CATTGACAGGCTGGCAAAGAGGCAGAGACCTGGAGAGAGACTGGACCTGGCCATGCTGGCTGCAATCAGAAGGGTGTATGGACTGCTGGCAAACACTGTG
213 ▶ I  D  R  L  A  K  R  Q  R  P  G  E  R  L  D  L  A  M  L  A  A  I  R  R  V  Y  G  L  L  A  N  T  V

1301 AGATACCTCCAGTGTGGAGGCTCTTGGAGAGAGGACTGGGGACAGCTCTCTGGAACAGCAGTGCCCCCTCAAGGAGCTGAGCCCCAGTCCAATGCTGGTC
247 ▶ R  Y  L  Q  C  G  G  S  W  R  E  D  W  G  Q  L  S  G  T  A  V  P  P  Q  G  A  E  P  Q  S  N  A  G
```
                                                                     BsrGI (1472)
```
1401 CAAGACCCCACATTGGGGACACCCTGTTCACCCTGTTCAGAGCCCCTGAGCTGCTGGCTCCCAATGGAGACCTGTACAATGTGTTTGCCTGGGCTCTGGA
280 ▶ P  R  P  H  I  G  D  T  L  F  T  L  F  R  A  P  E  L  L  A  P  N  G  D  L  Y  N  V  F  A  W  A  L  D
```
Ppu10I (1525)
NsiI (1525)
```
1501 TGTTCTAGCCAAGAGGCTGAGGTCCATGCATGTGTTCATCCTGGACTATGACCAGTCCCCTGCTGGATGCAGAGATGCTCTGCTGCAACTAACCTCTGGC
313 ▶ V  L  A  K  R  L  R  S  M  H  V  F  I  L  D  Y  D  Q  S  P  A  G  C  R  D  A  L  L  Q  L  T  S  G
```
                                                          BstXI (1667)                          NheI
```
1601 ATGGTGCAGACCCATGTGACCACCCCTGGCAGCATCCCCACCATCTGTGACCTAGCCAGAACCTTTGCCAGGGAGATGGGAGAGGCCAACTAAACCTGAG
347 ▶ M  V  Q  T  H  V  T  T  P  G  S  I  P  T  I  C  D  L  A  R  T  F  A  R  E  M  G  E  A  N  •

1701 CTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTAT
```
                 HpaI (1837)    MfeI (1848)
```
1801 TGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGGAGGTGTGGGAGGTTTTT
```
                 EcoRI (1933)
```
1901 TAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATTCTAAAATACAGCATAGCAAAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTTCTG
```
                 AlwNI (2023)
```
2001 AGGGATGAATAAGGCATAGGCATCAGGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTTAAGATATAGTGTATTTTC
```
                                                          SspI (2172)        SwaI (2186)
```
2101 CCAAGGTTTGAACTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCCACATTCCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCA
```
                 EcoO109I (2247)
```
2201 TTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAA
```
                                                          ApaLI (2371)
                                               DraIII (2365)              EagI (2385)
```
2301 CCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGGTCGCGCA
                                            125 ◀ •  D  Q  E  E  A  V  F  H  V  C  N  G  A  P  D  R  L

2401 GGGCGAACTCCCGCCCCCACGGCTGCTCGCCGATCTCGGTCATGGCCGGCCCGGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTA
107 ◀ A  F  E  R  G  W  P  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

2501 CAGCTCGTCCAGGCCGCGCACCCACACCCAGGCAGGGTGTTGTCCGGCCACCTGGTCCTGGACCGCGCTGATGAACAGGGTCACGTCGTCCCGGACC
 74 ◀ L  E  D  L  G  R  V  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
```

SgrAI  (2601)          XmaI  (2628)                                        BsrBI  (2664)     BssHII  (2680)

2601  ACACCGGCGAAGTCGTCCTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCGGTCCAGAACTCGACCGCTCCGGCGACGTCGCGCGCGGTGAGCACCGGAA
  40◄ V  G  A  F  D  D  E  V  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

                    SfiI  (2716)                                                                     AseI  (2776)

2701  CGGCACTGGTCAACTTGGCCATGATGGCCCTCCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAAAACAGCGTGGAT
   7◄ A  S  T  L  K  A  M     ←                                                  ←

                                      SacI  (2833)

2801  GGCGTCTCCAGCTTATCTGACGGTTCACTAAACGAGCTCTGCTTATATAGACCTCCCACCGTACACGCCTACCGCCCATTTGCGTCAATGGGGCGGAGTT

                                        SpeI  (2931)

2901  GTTACGACATTTTGGAAAGTCCCGTTGATTTACTAGTCAAAACAAACTCCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAACCGC

                                                SnaBI  (3059)

3001  TATCCACGCCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAGGTCA

                                              NdeI  (3164)

3101  TGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGT

3201  TTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGGTCGTTG

                                                  PacI (3350)
                                  PstI  (3343)
                            SdaI  (3342)        BspLU11I  (3360)

3301  GGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAA

3401  AAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTA

3501  TAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAA

                                                    ApaLI (3674)

3601  GCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGA

                                                    AlwNI (3771)

3701  CCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCG

3801  AGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTA

3901  CCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAA

                                                      PacI (4090)   SwaI

4001  AGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACA

                    EagI  (4110)
                    NotI  (4109)

4101  TTTAAATCAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAA

4201  AACGAAACAAAACAAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA