

pSELECT-zeo-Fcy::fur

A plasmid encoding a CpG-free cytotoxic fusion gene

Catalog code: psetz-fcyfur

For research use only

Version 20K30-MM

PRODUCT INFORMATION

Content:

- 20 µg of pSELECT-zeo-Fcy::fur 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. Lyophilized DNA is stable 3 months 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 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-Fcy::fur encodes a synthetic CpG-free suicide gene. Suicide genes code for enzymes that convert non-toxic compounds (prodrugs) into toxic products.

pSELECT-zeo-Fcy::fur can also be used to subclone this synthetic suicide gene into another vector. To facilitate subcloning, the Fcy::fur 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.

PLASMID FEATURES

First expression cassette

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

• **Fcy::fur**: Synthetic CpG-free *S. cerevisiae* cytosine deaminase-uracil phosphoribosyl transferase fusion gene. InvivoGen has fused the gene encoding uracil phosphoribosyl transferase (UPRT) to the cytosine deaminase (CD) gene. CD converts cytosine and its analogue, 5-fluorocytosine (5-FC), to uracil and 5-fluorouracil (5-FU) respectively. 5-FU is a toxic compound commonly used as anticancer drug. UPRT converts 5-FU to 5-FUMP, an irreversible inhibitor of thymidylate synthase. CD::UPRT fusions are 100 times more efficient than the CD genes alone and provide greater bystander effect.

• **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.

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*.

• **βGlo pAn**: The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription⁴.

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. & Alwine, J.C. (1989). *Mol. Cell Biol.* 10: 4248-4258.

4. Yu J & Russell JE. (2001). *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 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

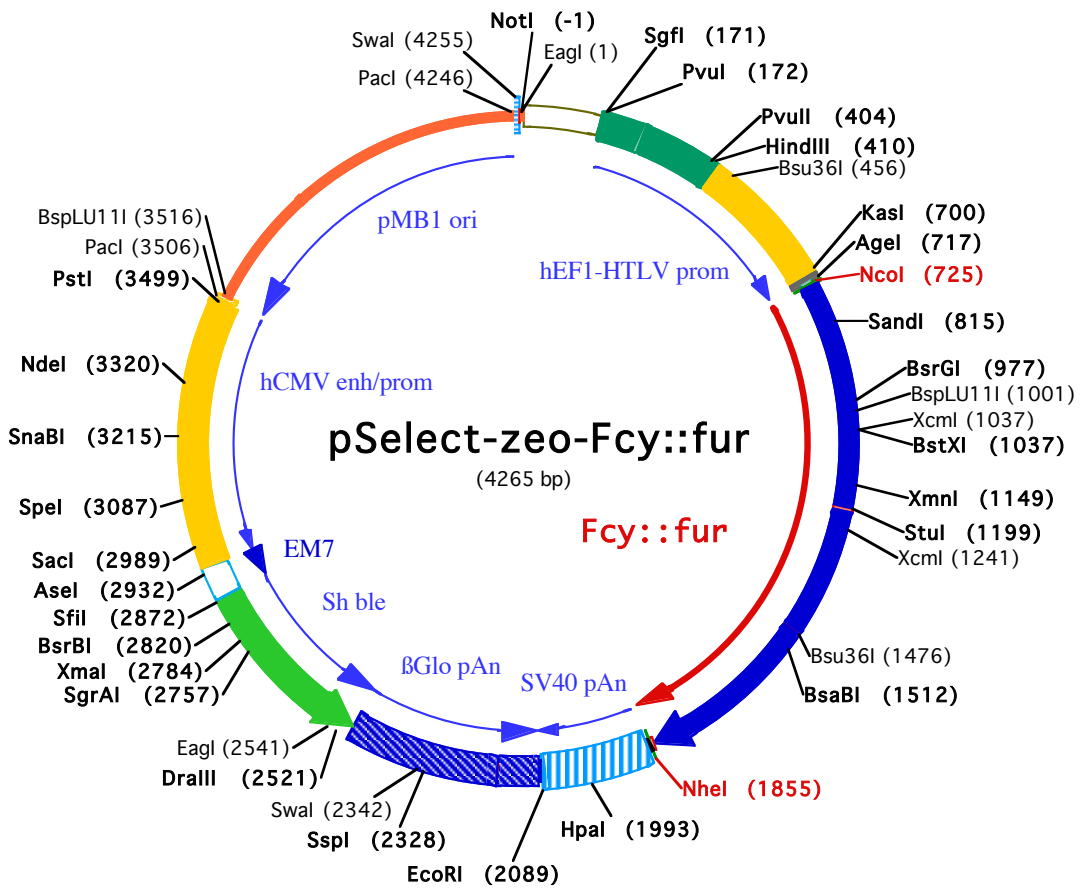
InvivoGen USA (Toll-Free): 888-457-5873

InvivoGen USA (International): +1 (858) 457-5873

InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Hong Kong: +852 3622-3480

E-mail: info@invivogen.com



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EagI (1)
NotI (-1)
1 GCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACA
PvuI (172)
SgfI (171)
101 AAACAAACTAGCAAAATAGGCTGTCCCCAGTGAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGATCTCGGATCGCTCCGGTGCCCGTCAGTGGGCA
201 GAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGTCCGCAATTGAACGGTGCCTAGAGAAGGTGGCGGGGTAACCTGGGAAAGTGAT
300 GTCGTGACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCCCGTGAACGTTCTTTTTTCGCAACGGGTTTCCGCCCA
HindIII (410)
PvuII (404)
Bsu36I (456)
400 GAACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCCTTACGCGCCCGCCCTACCTGAGGCGCCATCCACGCCGTTGAGTCGCGTTCTGCCGCC
500 TCCCGCTGTGGTGCCTCTGAACTGCGTCCGCGCTAGGTAAGTTAAAGCTCAGGTCGAGACCGGCCCTTTGTCGGCGCTCCCTTGGAGCTACCT
600 AGACTCAGCCGGCTCTCCACGCTTTGCCTGACCTGCTTGTCTCAACTCTACGCTTTGTTTCGTTTTCTGTTCTGCGCCGTTACAGATCCAAGCTGTGAC
KasI (700) Agel (717) NcoI (725)
700 CGGCGCTACCTGAGATCACCGgtcacCATGGTCACAGGAGGCATGGCTTCAAAGTGGGACCAGAAGGGCATGGACATTGCCTATGAGGAGGCTGCTCTG
1 M V T G G M A S K W D Q K G M D I A Y E E A A L
800 GGCTACAAGGAGGGGAGGGTCCCAATTGGTGGCTGCCTCATCAACAAGAGTGGCAGTGTCTGGGACGGGCCACAACATGAGTTCCAGAAGGGCA
25 G Y K E G G V P I G G C L I N N K D G S V L G R G H N M R F Q K G
900 GTGCCACCCTGCATGGGAGATCAGCACCTGGAGAAGTGTGGCAGGCTGGAGGGCAAGGTCTACAAGGACACCCTGTACACCACCCTCAGCCCTTG
58 S A T L H G E I S T L E N C G R L E G K V Y K D T T L Y T T L S P C
BsrGI (977)
1000 TGACATGTGCACAGGGCCATCATCATGTATGGCATTCCAGGTGTGTGGTGGGAGAGAATGTCAACTTCAAGTCAAAGGAGAGAAGTACCTCCAGACC
91 D M C T G A I I M Y G I P R C V V G E N V N F K S K G E K Y L Q T
XcmI (1037)
1100 AGGGGCCATGAGTGGTGTGGTGGATGATGAGAGGTGCAAGAAGATTATGAAGCAGTTCATTGATGAGAGACCCAGGACTGGTTTGGAGCATTGGGG
125 R G H E V V V V D D E R C K K I M K Q F I D E R P Q D W F E D I G
XmnI (1149)
1200 AGGCCTTGAGCCCTTCAAGAATGTGTACCTCCTCCCCAGACCAACCACTCTGGACTCTACACCATCATCAGGAACAAGAACCACCAGGCCAGA
158 E A S E P F K N V Y L L P Q T N Q L L G L Y T I I R N K N T T R P D
1300 CTTATCTTCTACAGTACAGGATCATCAGGCTCCTGGTGGAGGAGGGCCCAACCACCTCCCTGTGCAAGGAGGATTTGGAGAGTACACCAATGAG
191 F I F Y S D R I I R L L V E E G L N H L P V Q K Q I V E T D T N E
Bsu36I (1476)
1400 AACTTTGAGGGAGTGTCTTTCATGGGCAAGATTTGTGGGTGTCCATTGTGAGGGCTGGGAGAGCATGGAGCAGGCCTGAGGGACTGTTGACAGGAGTG
225 N F E G V S F M G K I C G V S I V R A G E S M E Q G L R D C C R S
BsaBI (1512)
1500 TGAGGATTGGCAAGATCCTGATCCAGAGGATGAGGAGACTGCCTGCCAAGCTGTTCTATGAGAAGCTCCCTGAAGACATCTCTGAGAGGTATGTCTT
258 V R I G K I L I Q R D E E T A L P K L F Y E K L P E D I S E R Y V F
1600 CCTCCTGGACCCATGCTGGCAACTGGAGGCTCTGCAATCATGGCACTGAGGTGCTCATCAAGAGGGGAGTCAAGCCTGAGAGGATCTACTTCTCAAC
291 L L D P M L A T G G S A I M A T E V L I K R G V K P E R I Y F L N
1700 CTCATCTGCTCAAAGGAGGGCATTGAGAAGTACCATGCTGCCTCCCTGAAGTGGAGTGTCACTGGGCTCTGGACAGGGGCTGGATGAGAACAAGT
325 L I C S K E G I E K Y H A A F P E V R I V T G A L D R G L D E N K
NheI (1855)
1800 ACCTGGTCCCTGGCCTGGGAGACTTTGGGACAGATACTACTGTGTCAAACCTGAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACA
358 Y L V P G L G D F G D R Y Y C V •
HpaI (1993)
1900 AACCAACTAGAATGCAGTGAATAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAAC
EcoRI (2089)
2000 AACAACAATTGCATTATTTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAATTCTAAA
2100 ATACAGCATAGCAAACTTTAACTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGCTGTTGCCAATGTG
2200 CATTAGCTGTTTGCAGCCTCACCTTCTTCATGGAGTTTAAAGATATAGTGTATTTTCCCAAGGTTTGAACTAGCTCTTCATTTCTTTATGTTTTAAATGC
SspI (2328) SwaI (2342)
2300 ACTGACCTCCACATTCCTTTTTAGTAAAATATTCAGAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATAGGCAGAAATCCAGATG
2400 CCAAGGCCCTTCATAATATCCCCAGTTTGTAGTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCAGGCTTCTAGCTT
1274 •
DrallI (2521) EagI (2541)
2500 ATCTCAGTCTGCTCCTCTGCCACAAAGTGCACGCAAGTTCGCGGGCGGGTCCGCGAGGGGAACTCCCGCCCCACGGCTGCTCGCCGATCTCGGTCA
126 G • 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
2600 GGCCGGCCCGGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGCGTACAGCTCGTCCAGGCCGCGACCCACCCAGGCCAGGGTGTG
93 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 W V W A L T N
SgrAI (2757) XmaI (2784)
2700 TCCGGACCACTGGTCTGGACCGCGTGTGATGAACAGGTTCACGCTGCTCCGGACCAACCGGCGAAGTCTCTCCACGAAGTCCCGGAGAACCCGA
59 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 F D R S F G L
BsrBI (2820) SfiI (2872)
2800 GCCGGTCCGTCAGAACTCGACCGCTCCGGCGACGTCGCGCGGGTGGACACCGGAACGGCACTGGTCAACTTGGCCATGATGGCCCTCCTATAGTGA
26 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 (2932) SacI (2989)
2900 CGTATTACTATGCCGATATACTATGCCGATGATTAATTTGTCAAACAGCGTGGATGGCGTCTCCAGCTTATCTGACGGTTCCTAAACGAGCTCTGCT

SpeI (3087)

3000 TATATAGACCTCCACCGTACACGCCTACCGCCATTTCGTCATATGGGGCGGAGTTGTTACGACATTTTGGAAAGTCCCGTTGATTACTAGTCAAAC

3100 AAACCTCCATTGACGTCAATGGGGTGGAGACTTGAAATCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTACTGCCAAAACCGCATCATCATGG

SnaBI (3215)

3200 TAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTACCCTGTCATTGAC

NdeI (3320)

3300 GTCAATAGGGGGCTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCT

3400 ATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTACCCTAAGTTATGTAACGC

Pacl (3506)

PstI (3499)

BspLU11I (3516)

3500 CTGCAGGTTAATTAAAGAACATGTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAAAGGCCGCTTCTGCGGTTTTTCCATAGGCTCCGCCCCCT

3600 GACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCCT

3700 CTCCTGTTCCGACCCCTGCCGTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTC

3800 GGTGTAGGTCGTTGCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTCAGCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCG

3900 GTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTA

4000 ACTACGGTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAAC

4100 CACCGCTGGTAGCGGTGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTGATCTTTTCTACGGGGTCTGAC

Pacl (4246)Swal (4255)

4200 GCTCAGTGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCA