

pSELECT-CHis-zeo

Plasmid for the expression of polyhistidine (His)-C-terminal tagged proteins

Catalog # psetz-chis

For research use only

Version 20L01-MM

PRODUCT INFORMATION

Content:

- 20 µg of pSELECT-CHis-zeo plasmid provided as lyophilized DNA

- 3' to 5' orientation

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 plasmids are specifically designed for strong and constitutive expression of a gene of interest in a wide variety of cell lines. They allow the selection of stable transfectants and offer a variety of selectable markers. pSELECT plasmids contain two expression cassettes: the first drives the expression of the gene of interest and the second drives the expression of a large choice of dominant selectable markers for both *E. coli* and mammalian cells. They are both terminating with a strong polyadenylation signal (polyA) that separates the two expression cassettes 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 selectable marker.

pSELECT-Tag is a new family of expression plasmids designed to generate tagged proteins in order to facilitate their detection and/or purification. pSELECT-Tag features three well-known tags: the green fluorescent protein (GFP) gene, the human influenza hemagglutinin (HA) epitope and the polyhistidine (His) tag. pSELECT-Tag plasmids allow to add the tag either at the N or C terminus of the protein of interest.

C-terminal tag: the tag is cloned downstream of a multiple cloning site and followed by a Stop codon.

PLASMID FEATURES

First expression cassette

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

• **MCS:** The multiple cloning site contains the following restriction sites: 5' - Age I, Sal I, Eco47 III, Nco I, BamH I - 3'

Each restriction site is compatible with many other enzymes.

• **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*.

• **Sh ble gene** confers zeocin resistance. 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.

CLONING STRATEGY

For expression of a tagged protein, it is important to ensure to clone your gene-of-interest into the correct reading frame. In general it is recommended to use Age I/Bam HI restriction site combination for cloning into plasmids with the C-Tag. For the plasmids with C-Tag, check whether the start codon of your gene of interest is in the correct reading frame with the C-Tag.

Note: The Bam HI restriction site is compatible with Bgl II.



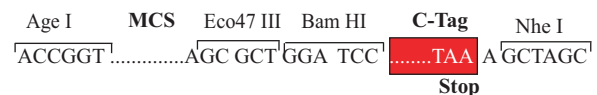
If it is not possible to use the Age I restriction site, it is possible to use another restriction site such as Sal I.



If it is not possible to use the Bam HI restriction site, it is possible to use another restriction site such as Nco I.



Alternatively, blunt end cloning can be achieved using the Eco 47 III site, which is upstream of the Nco I site. Blunt end PCR fragments can be obtained using T4 DNA polymerase or Klenow.



TECHNICAL SUPPORT

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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 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:

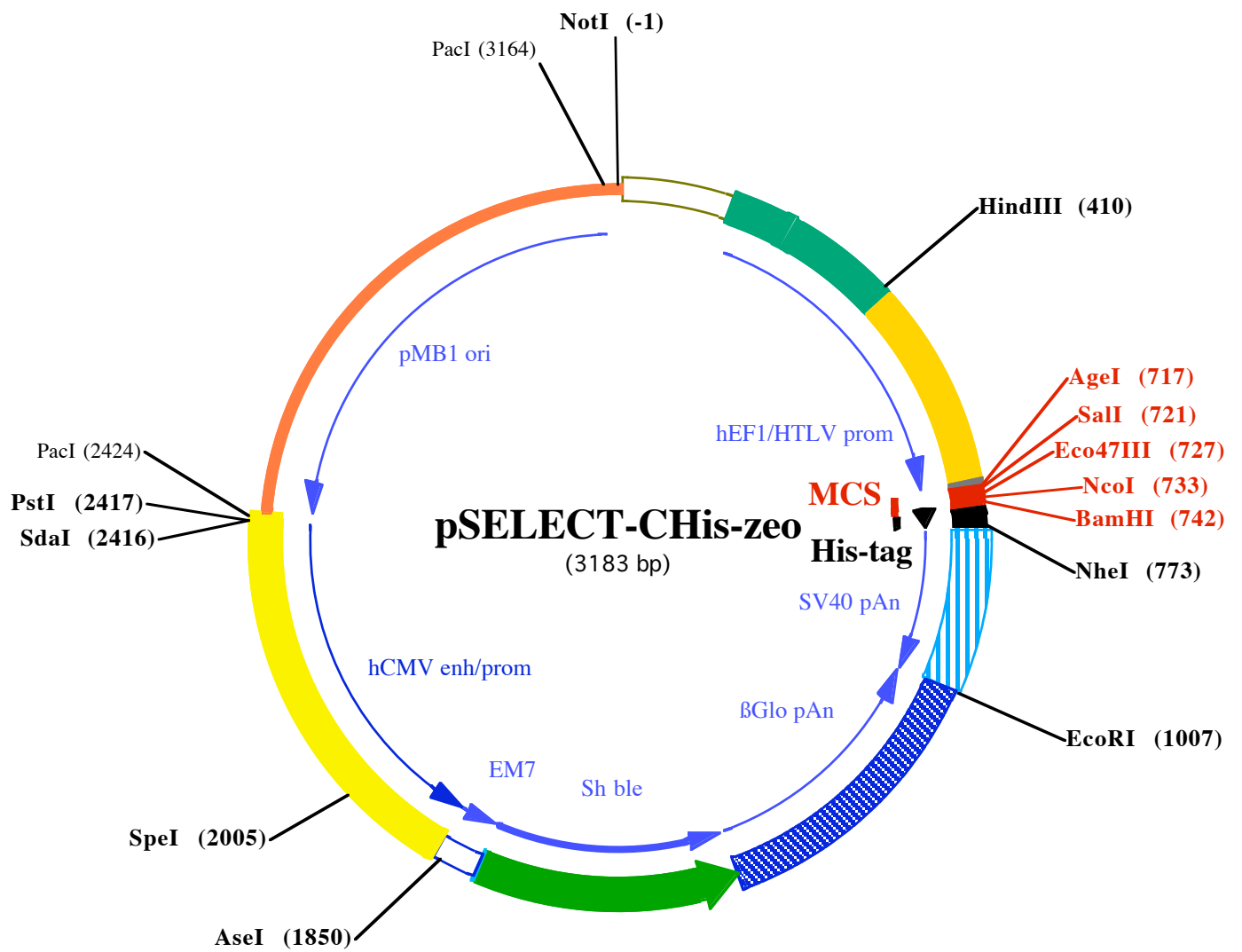
1. Kim, D.W. *et al.* (1990). *Gene* 2: 217-223.
2. Takebe, Y. *et al.* (1988). *Mol. Cell Biol.* 1: 466-472.
3. Carswell, S., and Alwine, J.C. (1989). *Mol. Cell Biol.* 10: 4248-4258.
4. Yu J & Russell JE. (2001). *Mol Cell Biol*, 21(17):5879-88.

RELATED PRODUCTS

Product	Catalog Code
pSELECT-NGFP-blasti	psetb-ngfp
pSELECT-CGFP-blasti	psetb-cgfp
pSELECT-NGFP-zeo	psetz-ngfp
pSELECT-CGFP-zeo	psetz-cgfp
pSELECT-NHA-blasti	psetb-nha
pSELECT-CHA-blasti	psetb-cha
pSELECT-NHA-zeo	psetz-nha
pSELECT-CHA-zeo	psetz-cha
pSELECT-NHis-blasti	psetb-nhis
pSELECT-NHis-zeo	psetz-nhis
pSELECT-CHis-blasti	psetb-chis

TECHNICAL SUPPORT

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NotI (-1)

1 GCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATAC
75 GCTCTCCATCAAAACAAAACGAAACAAAACAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAG
149 AACATTTCTCTATCGAAGGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCC

223 CCGAGAAGTTGGGGGAGGGGTCGGCAATTGAACGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGT

297 GATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAA

HindIII (410)

371 CGTTCTTTTTCGCAACGGGTTTGGCGCCAGAACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCCTTCACGCG

445 CCCGCCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCTGTGGTGCCTCC

519 TGAAGTGCCTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAG

593 CCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGTCAACTCTACGTCTTTGTTTCGTTTT

SalI (721) NcoI (733)

AgeI (717) Eco47III (727)

667 CTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCACCGGTCGACAGCGCTCCATGGC

BamHI (742)

NheI (773)

741 TGGGATCCGGCCATCATCATCACCATCACTAAAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGG

1↓ Gl ySer Gl yHi sHi sHi sHi sHi s •••

815 ACAAACCACAACACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAA

889 CCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTACAGGGGGAGGTG

EcoRI (1007)

963 TGGGAGTTTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAATTCTAAAATACAGCATAGCAAACTT

1037 TAACCTCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGC

1111 CAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTTCATGGAGTTTAAAGATATAGTGTATTTTCCCAAGTTTG

1185 AACTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCACATTCCCTTTTTAGTAAAATATTCAGAAA

1259 TAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCAT

1333 AATATCCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAAGCGA

1407 GCTTCTAGCTTATCCTCAGTCCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGTTCGCGCAGGGCGA

125↓ •••AspGl nGl uGl uAl aVal PheHi sVal CysAsnGl yAl aP roAspArgLeuAl aPh

1481 ACTCCC GCCCCACGGCTGCTCGCCGATCTCGGTCATGGCCGGCCCGAGGCGTCCCGAAGTTCGTGGACACG

105↓ eGl uArgGl yTrpProGl nGl uGl yI l eGl uThr MetAl aP roGl ySer Al aAspArgPheAsnThr Ser Val V

1555 ACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACACCCAGGCCAGGGTGTGTCCGGCACCAC

80↓ aI Gl uSer TrpGl uAl aTyrLeuGl uAspLeuGl yA rgVal T rpVal T rpAl aLeuThrAsnAspP roVal Val

1629 CTGGTCTGGACCGCTGATGAACAGGGTCACGTCGTCGCCGACCACACCGGCGAAGTCTCTCCACGAAGT

56↓ Gl nAspGl nValAl aSer I l ePheLeuThr Val AspAspArgVal Val Gl yAl aPheAspAspGl uVal PheAs

1703 CCCGGGAGAACCAGCCGGTCCGTCAGAACTCGACCGCTCCGGCGACGTCGCGCGCGGTGAGCACCCGGAACG

31↓ pArgSer PheGl yLeuArgAspThr T rpPheGl uValAl aGl yAl aVal AspArgAl aThr LeuVal P roValAl a

1777 GCACTGGTCAACTTGCCATGATGGCCCTCCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATG

6↓ l aSer Thr LeuLysAl aMet

AseI (1850)

1851 ATTAATTGTCAAACAGCGTGGATGGCGTCTCCAGC T TATCTGACGGTTCCTAAACGAGCTCTGCTTATATA

1924 GACCTCCCACCGTACACGCCTACCGCCCATTTGCGTCAATGGGGCGGAGTTGTTACGACATTTTGGAAAGTCCC

SpeI (2005)

1998 GTTGATTTA **CTAGT**CAAAAACAAACTCCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAA
2071 CCGCTATCCACGCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGT
2145 ACTGCCAAGTAGGAAAGTCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACG
2219 TCAATAGGGGGCGTACTTGGCATATGATACACTTGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCAC
2293 CCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGG

PacI (2424)

PstI (2417)

SdaI (2416)

2367 GGTTCGTTGGGCGGT**CAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCC TGCAG G TT AA TT**AAGAACAT
2439 GTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCC
2513 CCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAG
2587 GCGTTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGTTACCGGATACCTGTCCGCCTT
2661 TCTCCCTTCGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTTCGCT
2735 CCAAGCTGGGCTGTGTGCACGAACCCCCGTT**CAGCCC**GACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAG
2809 TCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGT
2883 AGGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCG
2957 CTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGC
3031 GGTGGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTC

PacI (3164)

3105 TACGGGGTCTGACGCTCAGTGGAAACGAAAAC**TACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTA**
3179 AATC A