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-blasti** plasmid provided as lyophilized DNA
- 1 ml of ZeocinÊ (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 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 ß-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 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.
• **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 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.*
• ***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*.
• **ßGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription[4].

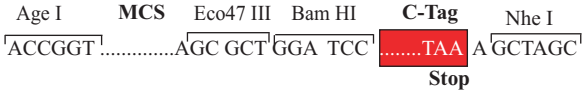
CLONING STRATEGY

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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InvivoGen Europe: +33 (0) 5-62-71-69-39
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E-mail: info@invivogen.com

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.

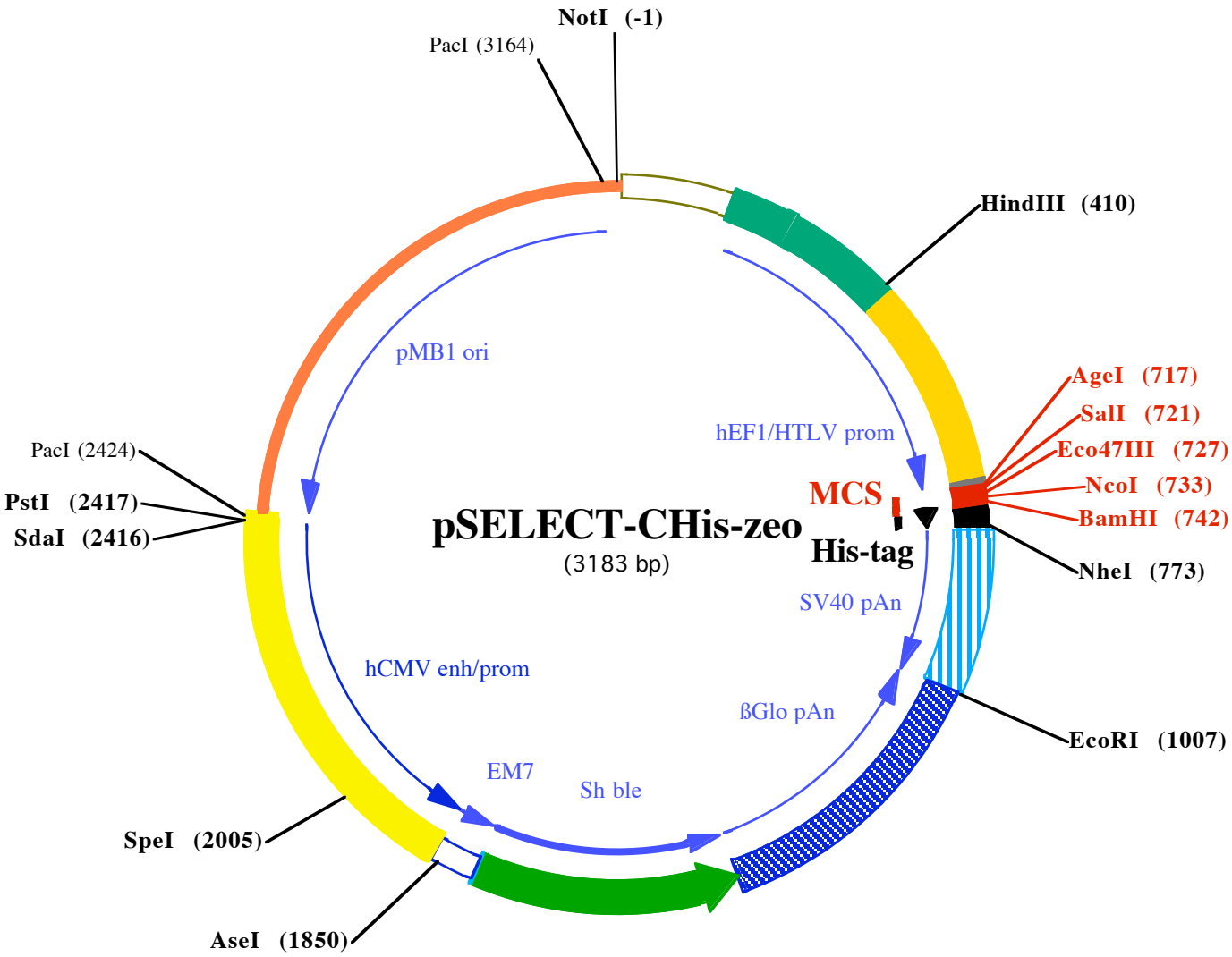
Zeocin™ usage
This antibiotic can be used for *E. coli* at 25 µg/ml in liquid or solid media and at 50-200 µg/ml to select Zeocin™-resistant mammalian cells.

References:

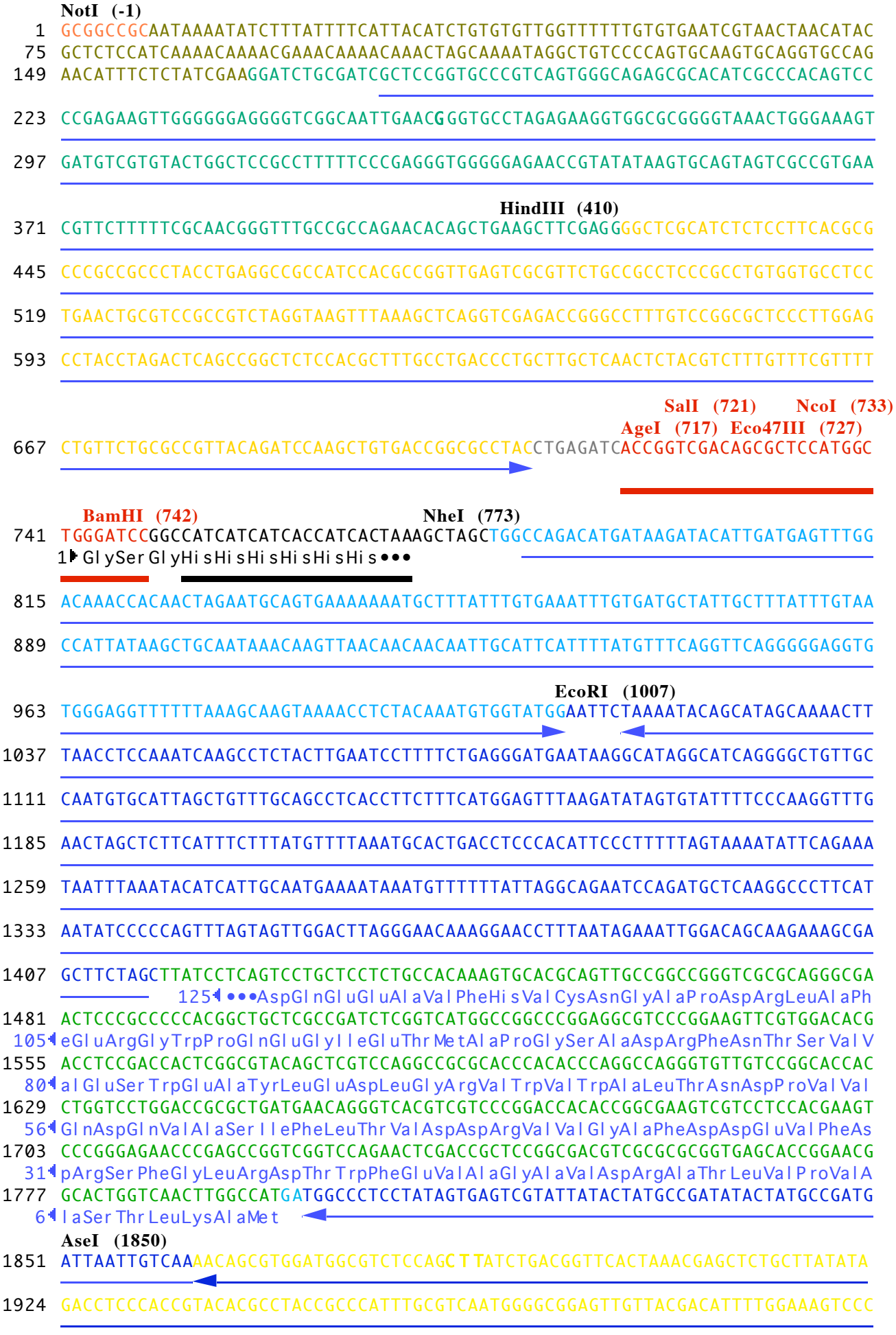
1. Kim, D.W. *et al.* (1990). Gene 2: 217-223.
2. Takebe, Y. *et al.* (1988). Mol. Cell Biol. 1: 466-472.
3. Carswell, S., and Alwine, J.C. (1989). Mol. Cell Biol. 10: 4248-4258.
4. Yu J & Russell JE. (2001). Mol Cell Biol, 21(17):5879-88.

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


NotI (-1)
PacI (3164)
HindIII (410)
pMB1 ori
AgeI (717)
SalI (721)
Eco47III (727)
NcoI (733)
BamHI (742)
NheI (773)
hEF1/HTLV prom
MCS
His-tag
PacI (2424)
PstI (2417)
SdaI (2416)
pSELECT-CHis-zeo
(3183 bp)
SV40 pAn
hCMV enh/prom
ßGlo pAn
EcoRI (1007)
EM7
Sh ble
SpeI (2005)
AseI (1850)

InvivoGen


NotI (-1)
HindIII (410)
SalI (721)
NcoI (733)
AgeI (717)
Eco47III (727)
BamHI (742)
NheI (773)
EcoRI (1007)
AseI (1850)


SpeI (2005)
1998 GTTGATTTACTAGTCAAAACAAACTCCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAA
2071 CCGCTATCCACGCCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGT
2145 ACTGCCAAGTAGGAAAGTCCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACG
2219 TCAATAGGGGGCGTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCAC
2293 CCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGG
PacI (2424)
PstI (2417)
SdaI (2416)
2367 GGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGCAGGTTAATTAAGAACAT
2439 GTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCC
2513 CCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAG
2587 GCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTT
2661 TCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCT
2735 CCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAG
2809 TCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGT
2883 AGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCG
2957 CTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGC
3031 GGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTC
PacI (3164)
3105 TACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTA
3179 AATCA