

# pSELECT-NHA-zeo

Plasmid for the expression of human influenza hemagglutinin (HA)-N-terminal tagged proteins

Catalog # psetz-nha

For research use only

Version 20K30-MM

## PRODUCT INFORMATION

### Content:

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

### 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 for 3 months at -20°C. Resuspended DNA is stable for more than one year at -20°C.

### 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. N-terminal tag: the tag encompasses the Start codon and is followed by a multiple cloning site (MCS).

## PLASMID FEATURES

### First expression cassette

• **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter<sup>1</sup> 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<sup>2</sup>. 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<sup>3</sup>.

• **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 transcription<sup>4</sup>.

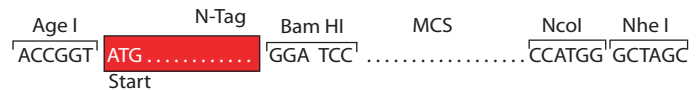
## CLONING STRATEGY

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 BamHI/NheI restriction site combination for cloning into plasmids with the N-Tag. For the plasmids with N-Tag, check whether your gene of interest and the start codon of the N-tag are in the correct reading frame.

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



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



Alternatively, blunt end cloning can be achieved using the Eco47III site, which is downstream of the BamHI 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 H<sub>2</sub>O. 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 DH5a.

### 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.

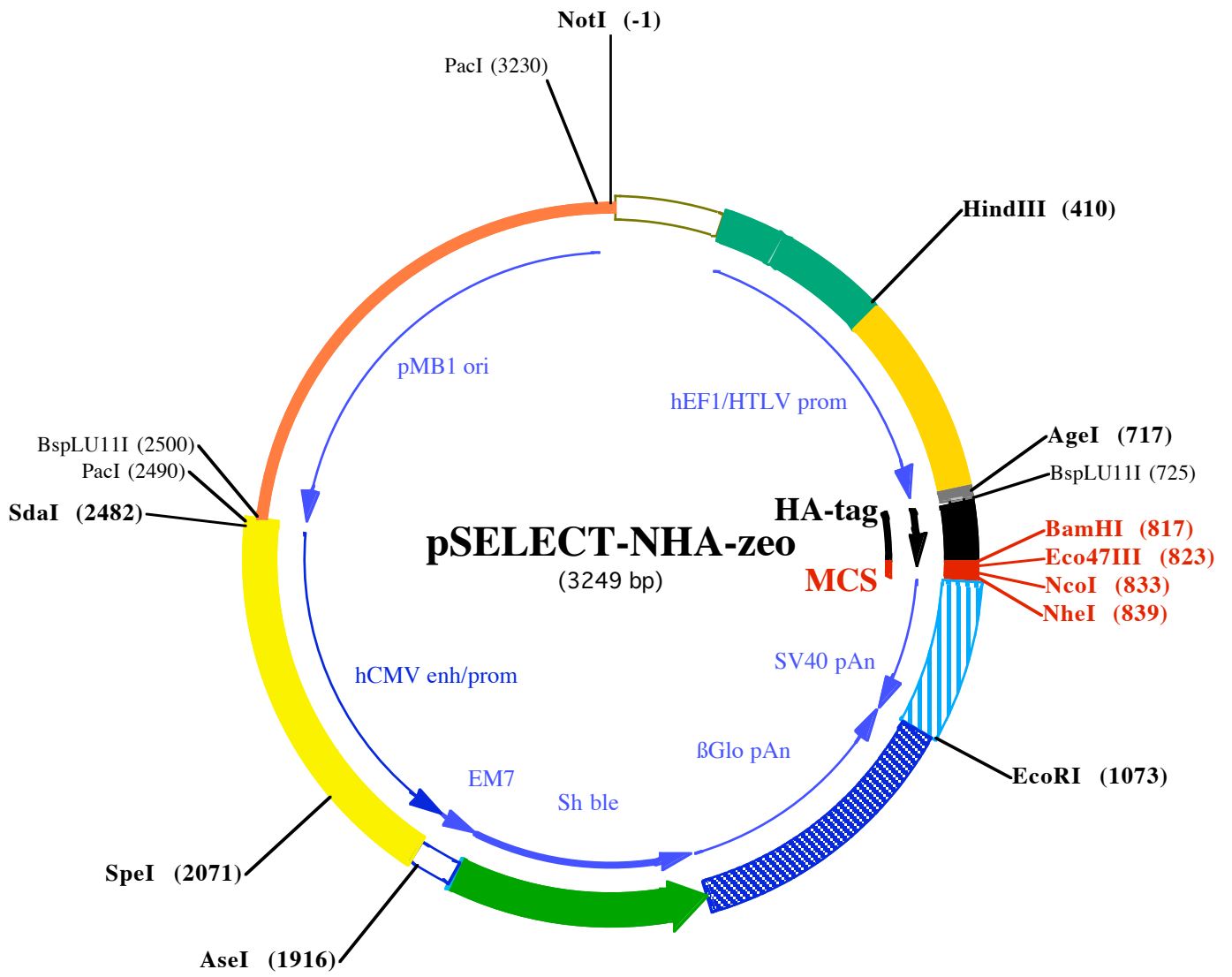
## RELATED PRODUCTS

Product	Catalog Code
pSELECT-NGFP-blasti	psetb-ngfp
pSELECT-CGFP-blasti	psetb-cgfp
pSELECT-NGFP-zeo	psetz-ngfp
pSELECT-CGFP-zeo	psetz-cgfp
pSELECT-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

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**NotI (-1)**

1 GCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATAC  
75 GCTCTCCATCAAAACAAAACGAAACAAAACAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAG  
149 AACATTTCTCTATCGAAGGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCC

223 CCGAGAAGTTGGGGGAGGGGTCGGCAATTGAACGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGT

297 GATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAA

**HindIII (410)**

371 CGTTCTTTTTCGCAACGGGTTTGC CGCCAGAACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCCTTACAGCG

445 CCCGCCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCC

519 TGAAGTGCCTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAG

593 CCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGTCTCAACTCTACGTCTTTGTTTCGTTTT

**BspLU11I (725)**

**AgeI (717)**

667 CTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCACCGGTCAACATGTATCCCTATG  
1 MetTyrPProTyrA

741 ATGTGCCAGACTATGCTGGCTATCCATATGATGTTCTGATTATGCTGGATACCCTTATGATGTGCCAGACTAT  
5 spValProAspTyrAlaGlyTyrProTyrAspValProAspTyrAlaGlyTyrProTyrAspValProAspTyr

**Eco47III (823) NheI (839)**

**BamHI (817) NcoI (833)**

815 GCCGGATCCAGCGCTGCAGCCATGGGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCA  
30 Al aGlySer

889 CAACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATA

963 AGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCCAGGGGAGGTGTGGGAGGT

**EcoRI (1073)**

1037 TTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAATTCTAAAATACAGCATAGCAAACTTTAACCTCC

1111 AAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGC

1185 ATTAGCTGTTTGCAGCCTCACCTTCTTTTCATGGAGTTTAAGATATAGTGTATTTTCCCAAGGTTTGAAGTAGCT

1259 CTTCAATTTCTTTATGTTTTAAATGCACTGACCTCCACATTCCTTTTTAGTAAAATATTCAGAAAATAATTTAA

1333 ATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCC

1407 CCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAG

1481 CTTATCCTCAGTCCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGGTGCGCGAGGGCGAACTCCCGC  
125 AspGlnGluAlaValPheHisValCysAsnGlyAlaProAspArgLeuAlaPheGluArgG

1555 CCCACGGCTGCTCGCCGATCTCGGTCTGCGCCGCGCCGGAGGCGTCCCGGAAGTTCTGTGGACACGACCTCCGA  
102 IlyTrpProGlnGluGlyIleGluThrMetAlaProGlySerAlaAspArgPheAsnThrSerValValGluSer

1629 CCACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACACCCAGGCCAGGGTGTGTCCGGCACCACTGGTCTCT  
78 TrpGluAlaTyrLeuGluAspLeuGlyArgValTrpValTrpAlaLeuThrAsnAspProValValGluAspGln

1703 GGACCGCGCTGATGAACAGGGTCACGTCGTCCCGGACCACCCGGCGAAGTCTCTCCACGAAGTCCCGGGAG  
53 nValAlaSerIlePheLeuThrValAspAspArgValValGlyAlaPheAspAspGluValPheAspArgSerP

1777 AACCCGAGCCGGTCCGTCCAGAAGTCCGCGCAGCTCGCGCGCGGTGAGCACCGGAACGGCACTGGT  
28 heGlyLeuArgAspThrTrpPheGluValAlaGlyAlaValAspArgAlaThrLeuValProValAlaSerThr

**AseI (1916)**

1851 CAAGTTGGCCATGATGGCCCTCCTATAGTGAGTCGATTATACTATGCCGATATACTATGCCGATGATTAATTG  
4 LeuLysAlaMet

1925 TCAAACAGCGTGGATGGCGTCTCCAGCTTATCTGACGGTTCACATAAACGAGCTCTGCTTATATAGACCTCCCA

1999 CCGTACACGCCTACCGCCATTTGCGTCAATGGGGCGGAGTTGTTACGACATTTTGGAAAGTCCCGTTGATTTA  
 2073 CTAGTCAAAACAAACTCCCATTGACGTCAATGGGTGGAGACTTGGAAATCCCCGTGAGTCAAACCGCTATCCA  
 2147 CGCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGT  
 2221 AGGAAAGTCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGG  
 2295 GCGTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGT  
 2369 CAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGGTCGTTGGG

PacI (2490)

**SdaI (2482)**

BspLU11I (2500)

2443 CGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGCAGGTTAATTAAGAACATGTGAGCAAAA  
 2515 GGCCAGCAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGA  
 2589 GCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCC  
 2663 CTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGTTACCGGATACCTGTCCGCCTTTCTCCCTTCG  
 2737 GGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTTCGCTCCAAGCTGGG  
 2811 CTGTGTGCACGAACCCCCGTTACGCCCACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGG  
 2885 TAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCT  
 2959 ACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAA  
 3033 GCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTT  
 3107 TTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCT  
 3181 GACGCTCAGTGGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATC A

PacI (3230)