

# pSELECT-GFP-mLC3

A mammalian expression plasmid containing the murine LC3B gene fused at 5' end to the GFP gene

Catalog code: psetz-gfplc3

For research use only

Version 20L01-MM

## PRODUCT INFORMATION

### Content:

- 20 µg of pSELECT-GFP-mLC3 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 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-GFP-mLC3 is a mammalian expression vector containing the murine LC3B gene fused at its 5' end to the green fluorescent protein (GFP) gene. This plasmid is selectable in bacteria and mammalian cells with Zeocin™. Expression of the GFP-mLC3 fusion gene allows to visualize autophagosome formation in real time in live cells. During autophagosome formation, GFP-mLC3 is processed and recruited to the autophagosome membrane, where it can be imaged as cytoplasmic puncta by high resolution fluorescence microscopy. The percentage of GFP-mLC3 positive cells can be determined and is indicative of autophagosome formation.

The same plasmid is available with the GFP gene alone as a control. This control plasmid is called pSELECT-NGFP-zeo (cat. code: psetz-ngfp). This plasmid is selectable in bacteria and mammalian cells with Zeocin™.

## PLASMID FEATURES

### First expression cassette

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

- **GFP::mLC3B** fusion gene was generated by fusing a GFP variant 5' of the murine LC3B gene. A synthetic intron was added between both moieties to increase the activity of GFP. This hybrid protein absorbs blue light (major peak at 480 nm) and emits green light (major peak at 505 nm).

- **SV40 pAn:** the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA.

- **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 transcription<sup>1</sup>.

## 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 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

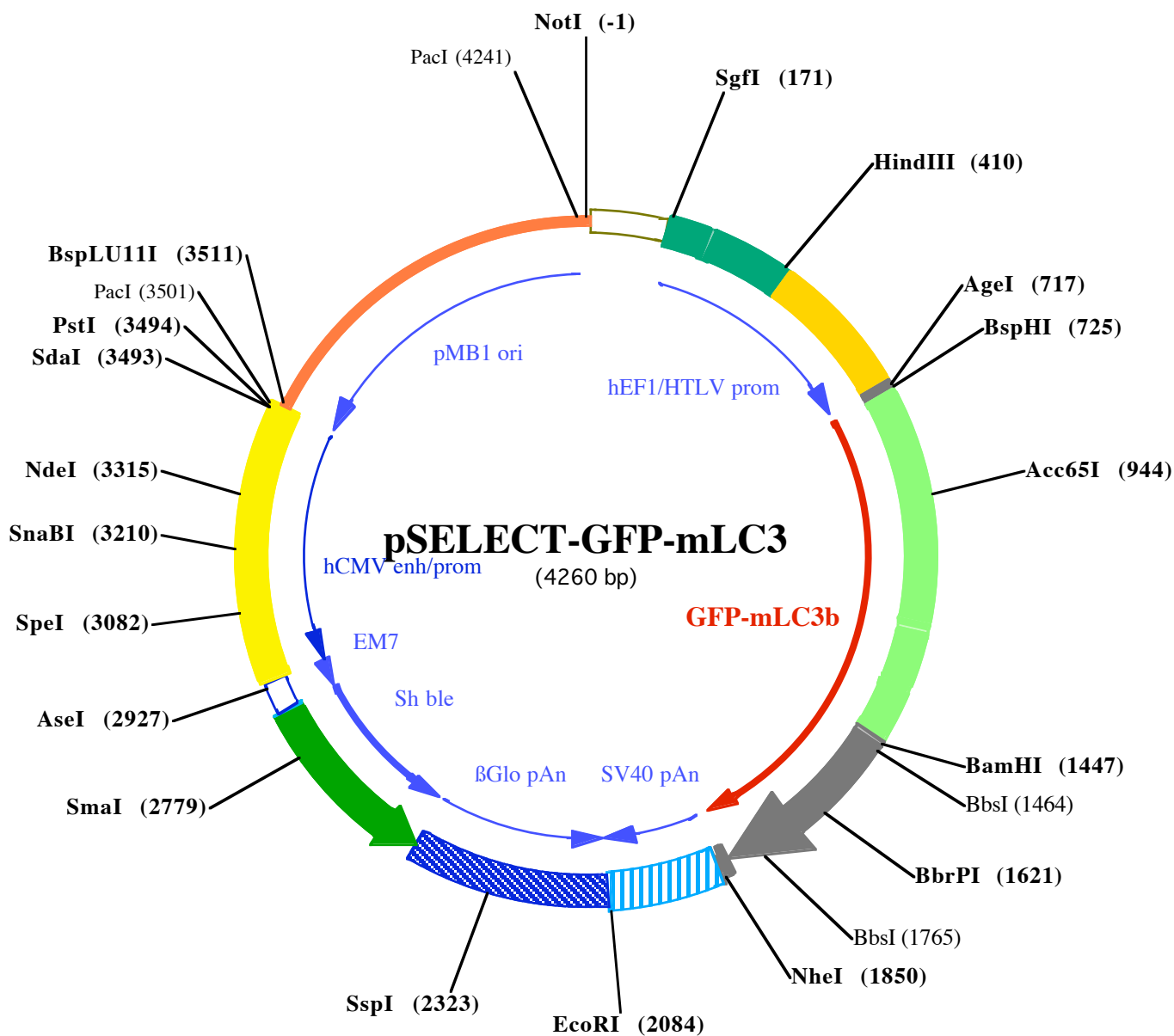
InvivoGen USA (International): +1 (858) 457-5873

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

InvivoGen Hong Kong: +852 3622-3480

E-mail: [info@invivogen.com](mailto:info@invivogen.com)





**NotI (-1)**

1 GCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTGTGTGAATCGTAACTAACATAC  
75 GCTCTCCATCAAAACAAAACGAAACAAAACAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAG

**SgfI (171)**

149 AACATTTCTCTATCGAAGGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCC

223 CCGAGAAGTTGGGGGAGGGGTGCGCAATTGAACGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGT

297 GATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAA

**HindIII (410)**

371 CGTTCCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCCTTCACGCG

445 CCCGCCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCC

519 TGAAGTGCCTCCGCCGTCTAGGTAAGTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGAG

593 CCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGTCAACTCTACGTCTTTGTTTTGTTTT

**BspHI (725)**

**AgeI (717)**

667 CTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCACCGGTCATCATGAGCAAGGGAG

1 MetSer LysGlyG

741 AAGAACTCTTTACTGGTGTGTCCAATTCTGGTTGAGCTGGATGGTGTGAATGGCCACAAATCTCTGTG

5 IuGIuLeuPheThrGlyValValProIleLeuValGluLeuAspGlyAspValAsnGlyHisLysPheSerVal

815 TCTGGTGAAGGTGAAGGAGATGCAACTTATGAAAGCTGACTCTGAAGTTCATTTGTACAACAGGAAAGCTGCC

30 SerGlyGluGlyGluGlyAspAlaThrTyrGlyLysLeuThrLeuLysPheIleCysThrThrGlyLysLeuP

**Acc65I (944)**

889 AGTGCCTTGCCAACTCTGGTGACCACCCTGACTTATGGTGTCAATGTTTCAGCAGGTACCCTGACCACATGA

54 oValProTrpProThrLeuValThrThrLeuThrTyrGlyValGlnCysPheSerArgTyrProAspHisMetL

963 AGCAGCATGACTTCTTTAAATCTGCAATGCCAGAAGGTTATGTTTCAGGAGAGGACAATCTTCTTTAAGGATGAT

79 ysGlnHisAspPhePheLysSerAlaMetProGluGlyTyrValGlnGluArgThrIlePhePheLysAspAsp

1037 GGAAATTATAAGACAAGGGCAGAAGTGAAGTTTGAAGGTGATACTGGTTAACAGAATTGAGCTGAAAGGCAT

104 GlyAsnTyrLysThrArgAlaGluValLysPheGluGlyAspThrLeuValAsnArgIleGluLeuLysGlyIle

1111 TGATTTTAAGGAAGATGGAACATTCTGGGTCAACAAGCTGGAGTACAACATAATTCTCACAATGTTTACATTA

128 eAspPheLysGluAspGlyAsnIleLeuGlyHisLysLeuGluTyrAsnTyrAsnSerHisAsnValTyrIleM

1185 TGGCAGATAAGCAGAGGAATGGAATTAAGGCTAATTTCAAGATTAGACACAACATTGAGGATGGATCTGTCCAA

153 eTalaAspLysGlnArgAsnGlyIleLysAlaAsnPheLysIleArgHisAsnIleGluAspGlySerValGln

1259 CTGGCAGACCATTACCAGCAGAACCCTATTGGTGTGAGTGGCCAGTTCCTCCTCCAGATAATCACTATCTCAG

178 LeuAlaAspHisTyrGlnGlnAsnThrProIleGlyAspGlyProValLeuLeuProAspAsnHisTyrLeuSe

1333 CACTCAATCTGCTCTGTCCAAAGACCCTAATGAGAAAAGAGACCACATGGTCTCCTGGAGTTTGTGACAGCAG

202 rThrGlnSerAlaLeuSerLysAspProAsnGluLysArgAspHisMetValLeuLeuGluPheValThrAlaA

**BamHI (1447)**

**BbsI (1464)**

1407 CAGGAATTACTCTGGGAATGGATGAGCTGTACAAGGGAGGTGGATCCATGCCGTCCGAGAAGACCTTCAAGCAG

227 laGlyIleThrLeuGlyMetAspGluLeuTyrLysGlyGlyGlySerMetProSerGluLysThrPheLysGln

1481 CGCCGGAGCTTTGAACAAAGAGTGAAGATGTCCGGCTCATCCGGGAGCAGCACCCACCAAGATCCCAGTGAT

252 ArgArgSerPheGluGlnArgValGluAspValArgLeuIleArgGluGlnHisProThrLysIleProValIle

**BbrPI (1621)**

1555 TATAGAGCGATACAAGGGGAGAAGCAGCTGCCCGTCTGGACAAGACCAAGTTCCTGGTGCCTGACCACGTGA

276 eIleGluArgTyrLysGlyGluLysGlnLeuProValLeuAspLysThrLysPheLeuValProAspHisValAla

1629 ACATGAGCGAGCTCATCAAGATAATCAGACGGCGCTTGAGCTCAATGCTAACCAAGCCTTCTCCTCCTGGTG

301 snMetSerGluLeuIleLysIleIleArgArgArgLeuGlnLeuAsnAlaAsnGlnAlaPhePheLeuLeuVal

**BbsI (1765)**

1703 AATGGGCACAGCATGGTGTGTGTCCTCCATCTCCGAAGTGTACGAGAGTGAGAGAGATGAAGACGGCTT

326 AsnGlyHisSerMetValSerValSerThrProIleSerGluValTyrGluSerGluArgAspGluAspGlyPh

1777 CCTGTACATGGTTTATGCCTCGCAGGAGACATTCGGGACAGCAATGGCTGTGTAAGACTCCAACAAAGCCAATG

350 eLeuTyrMetValTyrAlaSerGlnGluThrPheGlyThrAlaMetAlaVal ●●●

**NheI (1850)**

1851 GCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGACAAACCACAACCTAGAATGCAGTGAACAAAATG

1925 CTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAAGTAAACAACA

1999 ACAATTGCATTCAATTTATGTTTCAGGTTCAAGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTAC

**EcoRI (2084)**

2073 AAATGTGGTATGGAATTCTAAAATACAGCATAGCAAAACTTTAACCTCCAATCAAGCCTCTACTTGAATCCTT

2147 TTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTC

2221 TTTCATGGAGTTAAGATATAGTGTATTTTCCAAGGTTTGAAGTACTAGCTCTTCATTTCTTTATGTTTTAAATGC

**SspI (2323)**

2295 ACTGACCTCCCACATTCCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAAT

2369 GTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGA

2443 ACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCCTGCTCCTCTGC

2517 CACAAAGTGCACGCAGTTGCCGGCCGGGTCGCGCAGGGCGAACTCCCGCCCCACGGCTGCTCGCCGATCTCGG

2591 TCATGGCCGGCCGGAGGCGTCCCGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGG

2665 CCGCGCACCCACACCAGGCCAGGGTGTGTCCGGCACCACTGGTCTGGACCGCGCTGATGAACAGGGTCAC

**SmaI (2779)**

2739 GTCGTCCCGGACCACACCGGCGAAGTCGTCCTCCACGAAGTCCCGGAGAACCCGAGCCGGTCCGTCCAGAACT

2813 CGACCGTCCGGCGACGTCGCGCGCGGTGAGCACCGAACGGCACTGGTCAACTTGCCATGATGGCCCTCTA

**AseI (2927)**

2887 TAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAAACAGCGTGGATGGCGTCTCC

2961 AGCTTATCTGACGGTTCATAAACGAGCTCTGCTTATATAGACCTCCACCGTACACGCCTACCGCCATTTG

**SpeI (3082)**

3034 CGTCAATGGGGCGGAGTTGTTACGACATTTTGGAAAGTCCCGTTGATTTACTAGTCAAAAACAACTCCCATTG

3107 ACGTCAATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTACTGCCAAAA

**SnaBI (3210)**

3181 CCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTAAGTCCCAAGTAGGAAAAGTCCCATAAAGGTCATGT

**NdeI (3315)**

3255 ACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGCGTACTTGGCATATGATACT

3329 TGATGTACTGCCAAGTGGGCAGTTTACCCTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGT

3403 TACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGTCGTTGGGCGGTACGCCAGGCGGGCCATTTA

**PacI (3501)**

**PstI (3494)**

**SdaI (3493)**

**BspLU11I (3511)**

3477 CCGTAAGTTATGTAACGCCTGCAGGTTAA TTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACC

3548 GTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCA

3622 AGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTTCCCCCTGGAAGCTCCCTCGTGCGCTC

3696 TCCTGTTCCGACCCTGCCGTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATA

3770 GCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTT

3844 CAGCCCCACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACT

3918 GGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGC

3992 CTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGA

4066 GTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTAC

4140 GCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGAACGAAAAC

PacI (4241)

4214 CACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATC A