pSELECT-GFP-mLC3

A mammalian expression plasmid containing the murine LC3B gene fused at 5’end to the GFP gene
Catalog code: psetz-gfpmclc3

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 ß-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**: 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 Streptococcius 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.

- **ßGlo pAn**: The human beta-globin 3’UTR and polyadenylation sequence allows efficient arrest of the transgene transcription.

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.
pSELECT-GFP-mLC3

SV40

pAn

Sh ble

pMB1 ori

hCMV enh/prom

hEF1/HTLV prom

EM7

GFP-mLC3b

NotI (-1)

SgfI (171)

HindIII (410)

BspHI (725)

AgeI (717)

Acc65I (944)

BbsI (1464)

BbrPI (1621)

BbsI (1765)

NheI (1850)

BamHI (1447)

PstI (3494)

SdaI (3493)

PacI (3501)

BspLU11I (3511)

NdeI (3315)

SnaBI (3210)

SpeI (3082)

AseI (2927)

SmaI (2779)

SspI (2323)

EcoRI (2084)

PstI (3494)

Acc65I (944)

BbrPI (1621)

BbsI (1765)

NheI (1850)

BamHI (1447)

BbsI (1464)

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SspI (2323)

EcoRI (2084)

pSELECT-GFP-mLC3

(4260 bp)
CTA ACT ACG GCT A CACTAGA AGA AGAACGTATTTTG TATCT GCCTT GCTGTA A GCC A GTT A C CTT TCG GA AAA AAGA


PacI (4241)

CAC GT TA A G G A T T T T G T C AT G G C T A G T T A A T T A C A T T A A T C A