

pSELECT-GFP-LC3

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

Catalog # psetz-gfp-lc3

For research use only

Version # 10K02-MM

PRODUCT INFORMATION

Content:

- 20 µg of pSELECT-GFP-LC3 plasmid provided as lyophilized DNA
- 4 pouches of *E. coli* Fast-Media® Zeo (2 TB and 2 Agar)

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 3 months at -20°C. Resuspended DNA is stable more than one year at -20°C.
Store *E. coli* Fast-Media® Zeo at room temperature. Fast-Media® pouches are stable 18 months when stored properly.

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-LC3 is a mammalian expression vector containing the human LC3B gene fused at its 5' end to the green fluorescent protein (GFP) gene. This plasmid is selectable in bacteria and mammalian cells using Zeocin™. Expression of the GFP-LC3 fusion gene allows to visualize autophagosome formation in real time in live cells. During autophagosome formation, GFP-LC3 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-LC3 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::LC3B** fusion gene was generated by fusing a GFP variant to the 5' end of the human 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 *Streptococcus 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 transcription¹.

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.

Selection of bacteria with *E. coli* Fast-Media®

Fast-Media® is a **fast and convenient** way to prepare liquid and solid media for bacterial culture by using only a microwave. Fast-Media® is a TB (liquid) or LB (solid) based medium that already contains the antibiotic. Fast-Media® Zeo can be ordered separately (cat. code: fas-zn-l (liquid), cat. code: fas-zn-s (solid)).

Method:

- 1- Pour the contents of a Fast-Media® pouch into a clean borosilicate glass bottle or flask.
- 2- Add 200 ml of distilled water to the flask
- 3- Heat in a microwave on MEDIUM power setting (about 400Watts), until bubbles start appearing (approximately 3 minutes). **Do not heat a closed container. Do not autoclave Fast-Media®.**
- 4- Swirl gently to mix the preparation. **Be careful, the bottle and media are hot, use heatproof pads or gloves and care when handling.**
- 5- Reheat the media for 30 seconds and gently swirl again. Repeat as necessary to completely dissolve the powder into solution. But be careful to avoid overboiling and volume loss.
- 6- Let agar medium cool to 45°C before pouring plates. Let liquid media cool to 37°C before seeding bacteria.

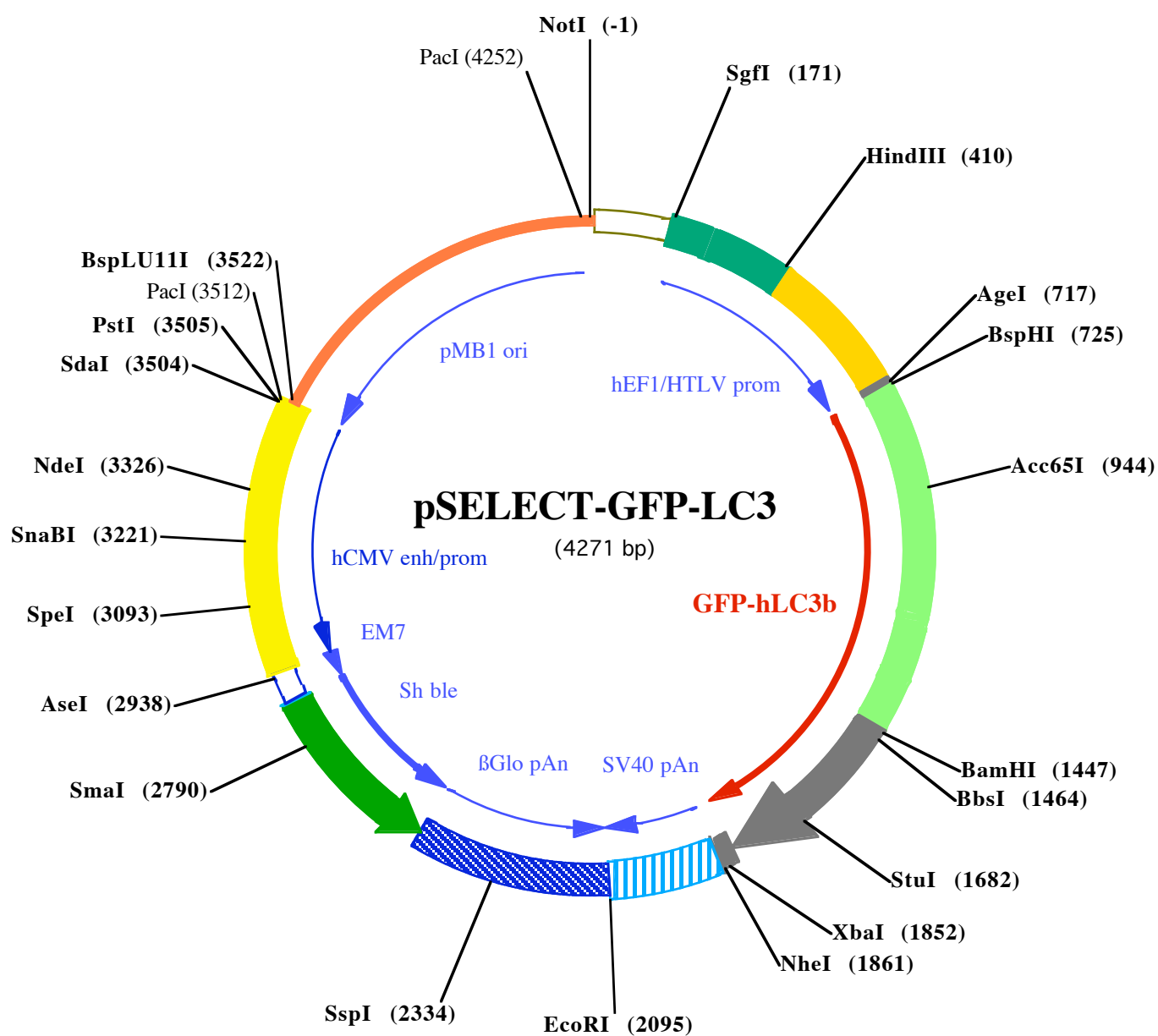
Note: Do not reheat solidified Fast-Media® as the antibiotic will be permanently destroyed by the procedure.

TECHNICAL SUPPORT

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NotI (-1)

1 GCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATAC
75 GCTCTCCATCAAAACAAAACGAAACAAAACAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAG

SgfI (171)

149 AACATTTCTCTATCGAAGGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCC

223 CCGAGAAGTTGGGGGAGGGGTGCGCAATTGAACGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGT

297 GATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAA

HindIII (410)

371 CGTTCTTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCCTTCACGCG

445 CCGCGCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCTGTGGTGCCTCC

519 TGAAGTGCCTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGAG

593 CCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGTCTCAACTCTACGTCTTTGTTTCGTTTT

BspHI (725)**AgeI (717)**

667 CTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCACCGGTCATCATGAGCAAGGGAG

1 MetSer LysGlyG

741 AAGAAGCTTTTACTGGTGTGTCCCAATTCTGGTTGAGCTGGATGGTGAATGGCCACAAATCTCTGTG

5 IuGluLeuPheThr GlyVal ValProIleLeuValGluLeuAspGlyAspValAsnGlyHisLysPheSerVal

815 TCTGGTGAAGGTGAAGGAGATGCAACTTATGAAAGCTGACTCTGAAGTTCATTTGTACAACAGGAAAGCTGCC

30 SerGlyGluGlyGluGlyAspAlaThrTyrGlyLysLeuThrLeuLysPheIleCysThrThrGlyLysLeuP

Acc65I (944)

889 AGTGCCTTGGCCAACTCTGGTGACCACCCTGACTTATGGTGTTCATGTTTCAGCAGGTACCCTGACCACATGA

54 oValProTrpProThrLeuValThrThrLeuThrTyrGlyValGlnCysPheSerArgTyrProAspHisMetL

963 AGCAGCATGACTTCTTTAAATCTGCAATGCCAGAAGGTTATGTTCAGGAGAGGACAATCTTCTTTAAGGATGAT

79 ysGlnHisAspPhePheLysSerAlaMetProGluGlyTyrValGlnGluArgThrIlePhePheLysAspAsp

1037 GGAAATTATAAGACAAGGGCAGAAGTGAAGTTGAAGGTGATACACTGGTTAACAGAATTGAGCTGAAAGGCAT

104 GlyAsnTyrLysThrArgAlaGluValLysPheGluGlyAspThrLeuValAsnArgIleGluLeuLysGlyIle

1111 TGATTTTAAGGAAGATGGAACATTCTGGGTACAAAGCTGGAGTACAACATAATTCTCACAATGTTTACATTA

128 eAspPheLysGluAspGlyAsnIleLeuGlyHisLysLeuGluTyrAsnTyrAsnSerHisAsnValTyrIleM

1185 TGGCAGATAAGCAGAGGAATGGAATTAAGGCTAATTTCAAGATTAGACACAACATTGAGGATGGATCTGTCCAA

153 etAlaAspLysGlnArgAsnGlyIleLysAlaAsnPheLysIleArgHisAsnIleGluAspGlySerValGln

1259 CTGGCAGACCATTACCAGCAGAACACCCCTATTGGTGTGAGTGGCCAGTTCTCCTCCAGATAATCACTATCTCAG

178 LeuAlaAspHisTyrGlnGlnAsnThrProIleGlyAspGlyProValLeuLeuProAspAsnHisTyrLeuSe

1333 CACTCAATCTGCTCTGTCCAAAGACCCTAATGAGAAAAGAGACCACATGGTCCTCCTGGAGTTTGTGACAGCAG

202 rThrGlnSerAlaLeuSerLysAspProAsnGluLysArgAspHisMetValLeuLeuGluPheValThrAlaA

BamHI (1447)**BbsI (1464)**

1407 CAGGAATTACTCTGGGAATGGATGAGCTGTACAAGGGAGGTGGATCCATGCCGTGCGAGAAGACCTTCAAGCAG

227 laGlyIleThrLeuGlyMetAspGluLeuTyrLysGlyGlyGlySerMetProSerGluLysThrPheLysGln

1481 CGCCGCACCTTCGAACAAAGAGTAGAAGATGTCCGACTTATTCGAGAGCAGCATCCAACCAAAATCCCGGTGAT

252 ArgArgThrPheGluGlnArgValGluAspValArgLeuIleArgGluGlnHisProThrLysIleProValIle

1555 AATAGAACGATACAAGGGTGAGAAGCAGCTTCCTGTTCTGGATAAAACAAAGTTCCTTGACCTGACCATGTCA

276 elIleGluArgTyrLysGlyGluLysGlnLeuProValLeuAspLysThrLysPheLeuValProAspHisValA

StuI (1682)

1629 ACATGAGTGAGCTCATCAAGATAATTAGAAGGCGCTTACAGCTCAATGCTAATCAGGCCTTCTCCTGTTGGTG

301 snMetSerGluLeuIleLysIleIleArgArgArgLeuGlnLeuAsnAlaAsnGlnAlaPhePheLeuLeuVal

1703 AACGGACACAGCATGGTCAGCGTCTCCACACCAATCTCAGAGGTGTATGAGAGTGAGAAAGATGAAGATGGATT

326 AsnGlyHisSerMetValSerValSerThrProIleSerGluValTyrGluSerGluLysAspGluAspGlyPh

1777 CCTGTACATGGTCTATGCCTCCAGGAGACGTTCCGGATGAAATTGTCAAGTGTAAAACAGAAAAAATGCAGCT

350 eLeuTyrMetValTyrAlaSerGlnGluThrPheGlyMetLysLeuSerVal ●●●

NheI (1861)**XbaI (1852)**

1851 CTTCTAGAATTGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAG

1925 TGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACA

1999 AGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAAGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGT

EcoRI (2095)

2073 AAAACCTCTACAAATGTGGTATGGAATTCTAAAATACAGCATAGCAAACTTTAACCTCCAAATCAAGCCTCTA

2147 CTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCA

2221 GCCTCACCTTCTTTTCATGGAGTTTAAGATATAGTGTATTTTCCCAAGGTTTGAAGTCTCTTCATTTCTTTAT

SspI (2334)

2295 GTTTTAAATGCACTGACCTCCCACATTCCCTTTTTAGTAAATATTAGAAATAATTTAAATACATCATTGCAA

2369 TGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCCAGTTTAGTAGTT

2443 GGAAGTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCC

1254 ●●●AspG

2517 TGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGGTGCGCAGGGCGAACTCCCGCCCCACGGCTGCTC

1224 I nG I uG I uA I aVa I PheH i sVa I CysAsnG I yA I aP roAspArgLeuA I aPheG I uArgG I yTrpP roG I nG I u

2591 GCCGATCTCGGTCATGGCCGGCCCGAGGCGTCCCGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACA

984 G I y I l eG I uThr MetA I aP roG I ySer A I aAspArgPheAsnThr Ser Va I Va I G I uSer TrpG I uA I aTyrLe

2665 GCTCGTCCAGGCCGCGCACCCACACCCAGGCCAGGGTGTGTCCGGCACCACTGGTCTTGACCGCGCTGATG

734 uG I uAspLeuG I yArgVa I TrpVa I TrpA I aLeuThrAsnAspP roVa I Va I G I nAspG I nVa I A I aSer I l eP

SmaI (2790)

2739 AACAGGGTCACGTCGTCCCGGACCACACCGGCGAAGTCTGCTCCACGAAGTCCCGGGAGAACCCGAGCCGGTC

484 heLeuThr Va I AspAspArgVa I Va I G I yA I aPheAspAspG I uVa I PheAspArgSer PheG I yLeuArgAsp

2813 GGTCCAGAAGTTCGACCGCTCCGGCGACGTCGCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTTGGCCATGA

244 Thr TrpPheG I uVa I A I aG I yA I aVa I AspArgA I aThr LeuVa I P roVa I A I aSer Thr LeuLysA I aMet

AseI (2938)

2887 TGGCCCTCCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAAACAGCGTGG

2961 ATGGCGTCTCCAGCTTATCTGACGGTTCCTAAACGAGCTCTGCTTATATAGACCTCCACCGTACACGCCTA

SpeI (3093)

3034 CCGCCCATTTGCGTCAATGGGGCGGAGTTGTTACGACATTTTGGAAAGTCCCGTTGATTTACTAGTCAAAACA

3107 AACTCCCATTTGACGTCATGGGGTGGAGACTTGGAAATCCCGTGAGTCAAACCGCTATCCACGCCCATTTGATG

SnaBI (3221)

3181 TACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCA

NdeI (3321)

3255 TAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCATAGGGGGCGTACTTGCCA

3329 TATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCATGGAAAGTC

3403 CCTATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCATGGGCGGGGTCGTTGGGCGGTCAGCCAGG

PacI (3512)

PstI (3505)

SdaI (3504)

BspLU11I (3522)

3477 CGGGCCATTTACCGTAAGTTATGTAACGCCTGAGGTTAA TT AAGAACATGTGAGCAAAAGGCCAGCAAAA

3548 GGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTTCATAGGCTCCGCCCCCTGACGAGCATCACAAA

3622 ATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCC

3696 CTCGTGCGCTCTCCTGTTCCGACCCTGCCGTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGC

3770 GCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTGCTCCAAGCTGGGCTGTGTGCACG

3844 AACCCCCCGTTTACGCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGAC

3918 TTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTT

3992 GAAGTGGTGGCCTAACTACGGCTACACTAGAGAAGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCT
4066 TCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTCGAAG
4140 CAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTG
PacI (4252)
4214 GAACGAAAACACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATC A