

pMONO-zeo-mcs

Single expression cassette plasmid for the expression of one gene of interest

Catalog code: pmonoz-mcs

For research use only

Version 20K26-MM

PRODUCT INFORMATION

Content:

- 20 µg of pMONO-zeo-mcs 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. Lyophilized DNA is stable 12 months at -20°C. Resuspended DNA is stable more than one year at -20°C. Avoid repeated freeze-thaw cycles.
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

pMONO 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 choice of selectable markers. pMONO plasmids contain a unique transcription unit that drives the expression of the gene of interest and the selectable marker through an internal ribosome entry site (IRES). This dual gene expression system ensures that stable clones express the gene of interest. Transcription of the expression cassette is efficiently terminated by the late SV40 polyadenylation signal (polyA).

Note: The use of the late SV40 polyA allows you to silence your gene of interest by using the ready-made psiRNA-SV40pA (#psirna42-sv40pa), a plasmid expressing a short hairpin siRNA targeting the late SV40 polyA.

PLASMID FEATURES

- **SV40/FerH/mEF1α:** pMONO plasmids feature a composite ferritin promoter that confers strong and constitutive expression in a wide range of mammalian cells. The promoter is composed of the ferritin heavy chain (FerH) core promoter¹ fused at its 5' end to the SV40 enhancer, and at its 3' end to the intron-containing 5'UTR of the mouse elongation factor 1 alpha gene. This composite promoter yields similar levels of expression as the CMV promoter in all cell lines tested.
- **MCS:** The multiple cloning site contains the following restriction sites: 5' - Age I, EcoR V, BamH I, Mlu I, Cla I, Sal I, Avr II - 3'
Each restriction site is unique and compatible with many other enzymes, increasing the cloning options.
- **FMDV IRES:** The internal ribosome entry site of the Foot and Mouth Disease Virus enables the translation of two open reading frames from one mRNA with high levels of expression².

- **Zeo:** Resistance to Zeocin™ is conferred by the Sh ble gene from *Streptoalloteichus hindustanus*. In mammalian cells, the *Sh ble* gene is transcribed from the composite ferritin promoter as a polycistronic mRNA and translated through the FMDV IRES. In *E. coli*, *Sh ble* is transcribed from the bacterial EM7 promoter.
- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- **SV40 pAn:** the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA³.
- **Ori:** a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.

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 other commonly used laboratory *E. coli* strains, such as DH5α.

Zeocin™ usage

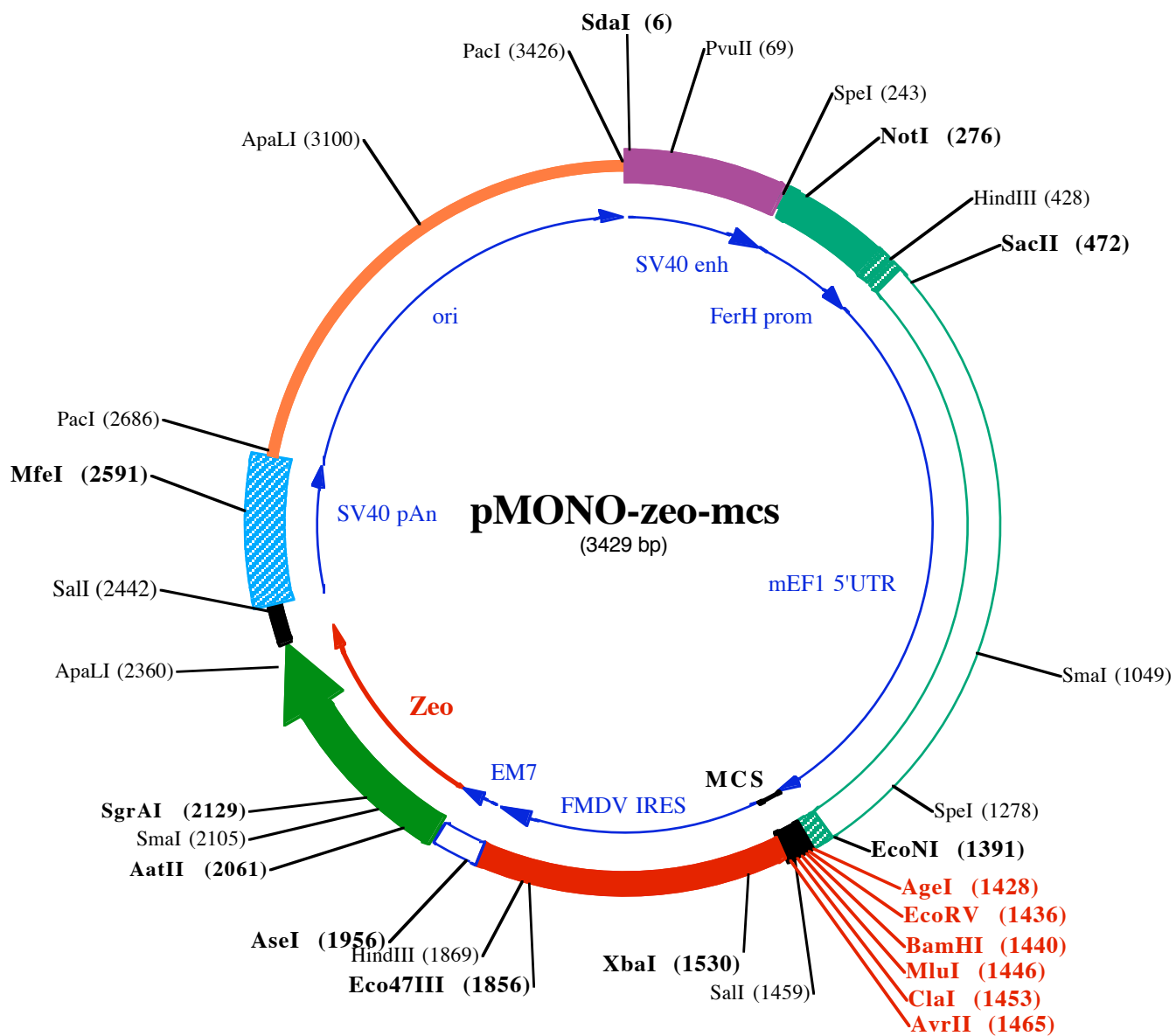
This antibiotic can be used for *E. coli* at 25 µg/ml in liquid or solid media and at 50-200 µg/ml to select Zeocin™-resistant mammalian cells.

References:

1. Eisenstein RS. and Munro HN. 1990. Translational regulation of ferritin synthesis by iron. *Enzyme* 44(1-4):42-58
2. Ramesh N *et al.* 1996. High-titer bicistronic retroviral vectors employing foot-and-mouth disease virus internal ribosome entry site. *Nucleic Acids Res.* 24(14):2697-700
3. Carswell S. & Alwine JC. 1989. Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. *Mol. Cell Biol.* 10: 4248-4258

TECHNICAL SUPPORT

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SdaI (6) **PvuII (69)**
1 CCTGCAGGGCTGAAATAACCTCTGAAAGAGGAACCTGGTTAGGTACCTTCTGAGGCTGAAAGAACCAGCTGTGGAATGTGTGTAGTTAGGGTGTGGAA
101 AGTCCCCAGGCTCCCCAGCAGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGAAAGTCCCCAGGCTCCCCAGCAGCAGAAG

SpeI (243) **NotI (276)**
201 TATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCACTAGTTCGCCAGAGCGCGGAGGGCTCCAGCGCCGCCCTCCCCACAGCAGGG
301 GCGGGTCCCGCGCCACCAGGAGCGGGCTCGGGCGGGCGCTGATTGGCCGGGCGGGCTGACGCCGACCGGCTATAAGAGACCACAAGCG

HindIII (428) **SacII (472)**
401 ACCCGCAGGGCCAGACGTTCTTCGCGAAGCTTCCGCTCAGAACGCAGGTGAGGGCGGGTGTGGCTTCGCGGGCCGCCAGCTGGAGGCTGCTCCG
501 AGCGGGCCGGGCCCGCTGCTGCTCGCGGGGATTAGTCTGCGAGCATTCCCGCTTCGAGTTGCGGGCGGGCGGGAGGACAGAGTGCAGGCTAGCGGCAA
601 CCCCAGTCCGCTCGCTGCTCGGGCTTGGAGCTAGCGTGGTGTCCGCGCCGCCCGCTGCTACTCCGGCCGACTCTGGCTTTTTTTTTTTTGT
701 GTTGTTCCTCTGCTCCTTCGATTGCCGTTACAGCAATAGGGCTAACAAAGGAGGGTGCAGGGCTTGTCTCGCCGAGACCCGAGAGGTCATGTTGGG
801 GAGGAATGGAGGGACAGGAGTGGCGGCTGGGGCCCGCCGCTTCGGAGCACATGTCCGACGCCACCTGGATGGGGCAGGCGTGGGTTTTTCCGGAAG
901 CAACCAGGCTGGGGTTAGCGTCCGAGGCCATGTGGCCCGCAGCCCGCAGCATCTGGCTTGGCGGCGCGCTTGCCTCCCTAACTAGGTTGA

SmaI (1049)
1001 GGCCATCCGCTCCGCGACCAGTTGCGTGGTGGAAAGATGGCCGCTCCCGGGCTTGTGCAAGGAGCTCAAATGGAGACGCGGACGCCGGTGGAGC
1101 GGGCGGTGAGTACCCACACAAAGGAGAGGGCTGGTCCCTCACCGGCTGCTGCTTCTGTGACCCCGTGGTCTATCGGCCCAATAGTCACCTCGG

SpeI (1278)
1201 GCTTTTGAGCACGGTAGTCGCGCGGGGGAGGGATGTAATGGCGTTGGAGTTTTCACATTTGGTGGTGGAGACTAGTCAGGCGCAGCCTGGCGCT

EcoNI (1391)
1301 GGAAGTCATTTTTGGAATTTGCCCTTGGTTTTGAGCGGAGCTAATTCTCGGGCTTCTTAGCGGTTCAAAGGTATCTTTAAACCTTTTTTAGGTGT

EcoRV (1436) MluI (1446) **Sall (1459)**
AgeI (1428) **BamHI (1440)** **ClaI (1453)** **AvrII (1465)**
1401 TGTGAAACCACCGCTAATTCAAAGCAACCGGTGATATCGATCCACGCGTATCGATTGTCGACCCCTAGGAGCAGGTTTCCCAATGACACAAACGTGC

XbaI (1530)
1501 AACTTGAACCTCCGCTGGTCTTCCAGGTCTAGAGGGTAACACTTTGACTGCGTTGGTCCACGCTCGATCCACTGGCGAGTGTAGTAACAGCAC
1601 TGTGTCTCTAGCGGAGCATGACGGCGTGGAACTCCTCCTTGGTAACAAGACCCACGGGGCCAAAAGCCACGCCACACGGGCCGTCATGTGTGC
1701 AACCCAGCAGCGGCACTTTACTGCGAAACCACTTAAAGTGACATTGAACTGGTACCCACACACTGGTGACAGGCTAAGGATGCCCTTCAGGTACCC

Eco47III (1856) **HindIII (1869)**
1801 CGAGGTAACACGCGACTCGGGATCTGAGAAGGGGACTGGGGCTTCTATAAAAGCGCTCGGTTTAAAAAGCTTCTATGCTGAATAGGTGACCGGAGGT

AseI (1956)
1901 CGGCACCTTCTTTGCAATTAAGTACCTGAATACAAGTACTGTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACTCACT

AatII (2061)
2001 ATAGGAGGGCCATCATGGCCAAGTTGACCAGTGCCGTTCCGGTGTCCGCGGACGTCGCGGAGCGGTCGAGTTCTGGACCGACGGCTCGGGT

SmaI (2105) **SgrAI (2129)**
2101 CTCCGGGACTTCGTGGAGGACGACTTCGCGGTTGGTCCGGGACGACGTGACCTGTTTCATCAGCGGTCAGGACAGGTTGGTCCGGACAACACC
2201 CTGGCTGGGTGGGTGGCGGCTGGACGAGCTGTACGCCGAGTGGTCCGAGGTCGTGTCCACGAACTCCGGGACGCTCCGGCCGGCCATGACCC
2301 AGATCGGCAGCAGCGTGGGGCGGGAGTTCCGCTTGCAGCGACCCGGCCGCAACTGCGTGCACCTTCTGTGGCCGAGGAGCAGGACTGACCGACCCGAC
2401 CAACACCGCGGTCGACGCGGCCGCGGGTCCGAGGGGGTCCGACGATCCAGACATGATAAGATACATTGATGAGTTGGACAACCACTAGAAAT

ApaLI (2360) **Sall (2442)**
2401 CAACACCGCGGTCGACGCGGCCGCGGGTCCGAGGGGGTCCGACGATCCAGACATGATAAGATACATTGATGAGTTGGACAACCACTAGAAAT

MfeI (2591)
2501 GCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACACCAACAAATGCAAT

PacI (2686)
2601 CATTTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAATGTTAATTAAGTCCATGAC
2701 CAAAATCCCTTAACGTGAGTTTTCTGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGGATCCTTTTTTCTGCGGTAATCTGC
2801 TGCTTCAAACAAAAAACCCGCTACCCAGCGGTGGTTTTGTTGCCGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTCCAGCAGAGCGC
2901 AGATACCAAACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAAGTCTGTAGCACCGCTACATACCTCGCTGCTAATCCTGTTACC

ApaLI (3100)
3001 AGTGGCTGCTGCCAGTGCGGATAAGTCTGTCTTACCGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGTTCCG
3101 TGACACAGCCAGCTTGGAGCGAACGACCTACACCAACTGAGATACCTACAGCGTGAAGTATGAGAAAGCGCCACGCTCCCGAAGGGAGAAAGCGG
3201 ACAGGTATCCGGTAAGCGGCAGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGAAACGCTGGTATCTTTATAGTCTGTGGGTTTCGCCA
3301 CCTCTGACTTGAGCGTCGATTTTTGTGATGCTCAGGGGGCGGAGCCTATGAAAAACCGCAGCAACCGCGCCTTTTTACGGTTCTGGCCTTTTGC

PacI (3426)
3401 TGGCCTTTTGTCTCATGTTCTTAATTA