

pMONO-neo-mcs

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

Catalog code: pmonon-mcs

<https://www.invivogen.com/pmono-neo>

For research use only

Version 19L13-MM

PRODUCT INFORMATION

Contents

- 20 µg of pMONO-neo-mcs plasmid provided as lyophilized DNA

Storage and stability

- Product is shipped at room temperature.
- Lyophilized DNA should be stored at -20°C.
- Resuspended DNA should be stored at -20°C and is stable for at least 1 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

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 (#psirna3gz21-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.

- **Neo:** The *neo* gene from Tn5 confers resistance to Kanamycin in *E. coli* and G418 in mammalian cells. In mammalian cells, the *neo* gene is transcribed from the composite ferritin promoter as a polycistronic mRNA and translated through the FMDV IRES. In *E. coli*, *neo* 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.

1. Eisenstein RS. & 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

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

Bacterial antibiotic selection

Kanamycin (not provided) is normally used for *E. coli* at a final concentration of 50 µg/ml in liquid or solid media.

Mammalian antibiotic selection

G418 is normally used at a concentration of 400 µg/ml. However, the optimal concentration needs to be determined for your cells.

RELATED PRODUCTS

Product	Description	Cat. Code
ChemiComp GT116 cells	Competent <i>E. coli</i> cells	gt116-11
G418	Selection antibiotic	ant-gn-1

TECHNICAL SUPPORT

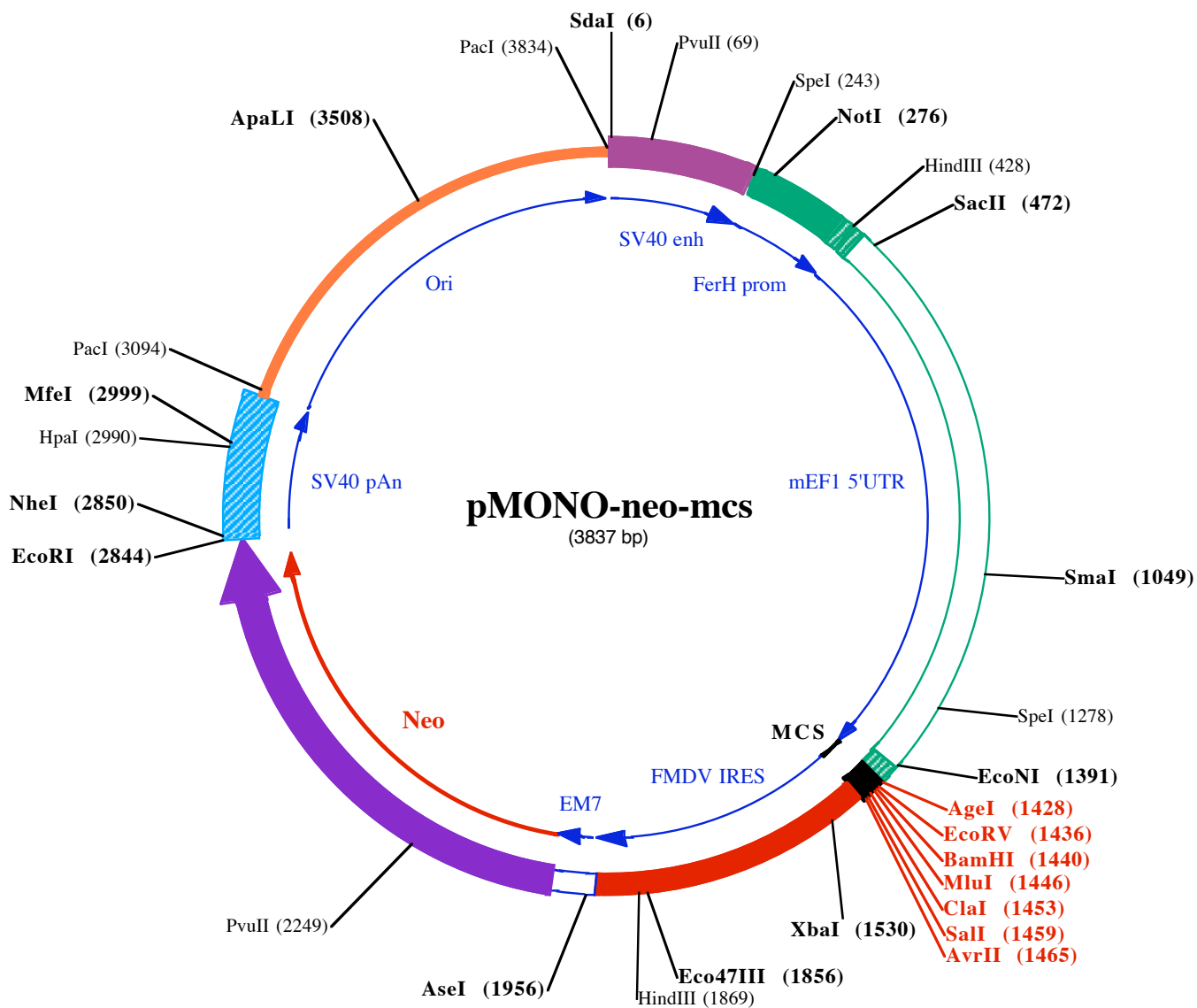
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InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Hong Kong: +852 3622-3480

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100

SdaI (6) **PvuII (69)**

1 CCTGCAGGGCCTGAAATAACCTCTGAAAGAGGAACCTGGTTAGGTACCTTCTGAGGCTGAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGGAA

101 AGTCCCAGGCTCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGAAAGTCCCAGGCTCCCAGCAGGCAGAAG

201 TATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCC **SpeI (243)** **NotI (276)**

301 GCGGGTCCCAGCCACCGAAGGAGCGGGCTCGGGCGGGCGGGCTGATTGGCCGGGCGGGCTGACGCCGACGCGGCTATAAGAGACCACAAGCG

401 ACCCGCAGGGCCAGACGTTCTTCGCCGAAGCTTCCCGTCAGAACGCAGGTGAGGGGCGGGTGTGGCTTCCGCGGGCCCGAGCTGGAGGTCTGCTCCG

501 AGCGGGCCGGCCCCGCTGTCGTGCGGGGATTAGCTGCGAGCATTCCCGTTCGAGTTGCGGGCGGCGGGAGGCAGAGTGCAGGGCTAGCGGCAA

601 CCCCAGTCCGCTCGTGTCCGGCTTGGGCTAGCGTGGTGTCCGCGCCGCCCGCTGCTACTCCGGCCGACTCTGGTCTTTTTTTTTTTTGT

701 GTTGTGCCCTGCTGCCTTCGATTGCCGTTAGCAATAGGGGTAACAAAGGAGGGTGCGGGGCTTCTCGCCGAGCCGGAGAGGTATGTTGGG

801 GAGGAATGGAGGACAGGAGTGGCGGCTGGGGCCGCCGCTTCGGAGCAGTGTCCGACGCCACTGGATGGGGCAGGCTGGGGTTTTTCCGAAG

901 CAACCAGGCTGGGGTTAGCGTCCGAGGCCATGTGGCCCCAGCACCCGGCACGATCTGGCTTGGCGGCGCGCTTGCCTGCCTCCTAACTAGGGTGA

1001 GGCCATCCCGTCCGGCACCAGTTGCGTGCCTGAAAGATGGCGCTCCGGGCGCTTGTCAAGGAGCTCAAATGGAGGACGCGGAGCCGGTGGAGC

1101 GGGCGGTGAGTCACCCACAAAGGAGAGGGCTGGTCCCTACCGGCTGCTCTCTGTGACCCGTTGCTCTATCGCCGCAATAGTCACCTCGG

1201 GCTTTTGGACACGGCTAGTCGCGGGGGGGAGGGATGTAATGGCGTTGGAGTTTGTTCACATTTGGTGGGTGGAGACTAGTCAGGCCAGCTGGCGCT

1301 GGAAGTCATTTTTGGAATTTGCTCCCTTGTAGTTTTGAGCGGAGCTAATTCTCGGGCTTCTTAGCGGTTCAAAGGTATCTTTTAAACCTTTTTTAGGTG

1401 TGTGAAACCACCGTAATTCAAAGCAACCGGTGATATCGGATCCACGCGTATCGATTGTCGACCTAGGAGCAGGTTTCCCAATGACACAAAACGTGC

1501 AACTTGAACTCCGCTGGTCTTCCAGGTCTAGAGGGGTAACACTTTGACTGCGTTTGGCTCCACGCTCGATCCACTGGCGAGTGTTAGTAACAGCAC

1601 TGTGCTTCTGAGCGGAGCATGACGGCGTGGAACTCCTCCTTGGTAACAAGGACCCACGGGGCCAAAAGCCACGCCACACGGGCCGTCATGTGTGC

1701 AACCCAGCAGCGGACTTTACTGCGAAACCCTTTAAAGTGACATTGAACTGGTACCACACACTGGTGACAGGCTAAGGATGCCCTTACGGTACCC

1801 CGAGGTAACACGCGACTCGGGATCTGAGAAGGGGACTGGGGCTTCTATAAAGCGCTCGGTTTAAAGCTTCTATGCCTGAATAGGTGACCGGAGGT

1901 CGGCACCTTCTTTGCAATTAAGTACCTGACTGTTTGGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACTCACT

2001 ATAGGAGGGCCACCATGATTGAACAAGATGGATTGCACGAGTTCTCCGGCGCTTGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAA

2101 CGGCTGCTCTGATGCCGCGTGTCCGGCTGTCAGCGCAGGGGCGCCGCTTCTTTTGTCAAGACCGACTGTCGGTGCCTGAATGAAGTCAAGAC

2201 GAGGCAGCGCGCTATCGTGGCTGGCCACGCGGGCTTCTTGGCAGCTGTGCTCGACGTTGCTACTGAAGCGGAAGGGACTGGCTGCTATTGGGGC

63> Gl uAl aAl aArgLeuSer TrpLeuAl aThr Thr Gl yVal P roCysAl aAl aVal l LeuAspVal Val Thr Gl uAl aGl yArgAspTrpLeuLeuGl yG

2301 AAGTCCGGGGCAGGATCTCTGTATCTACCTTGTCTCTCCGAGAAAGTATCCATCATGGCTGATGCAATGGCGCGGCTGCATACGCTTGTCCGGC

96> l uVal P roGl yGl nAspLeuLeuSer Ser Hi sLeuAl aP roAl aGl uLysVal l Ser l l eMe tAl aAspAl aMe tArgArgLeuHi s Thr LeuAspP roAl

2401 TACCTGCCATTGACCAAGCAACATCGCATCGAGCGAGCAGTACTCGGATGGAAGCCGGCTTGTGCTGATCAGGATGATCTGGACGAAGGCAT

129> a Thr CysP roPheAspHi sGl nAl aLysHi sArg l l eGl uArgAl aArgThr ArgMe tGl uAl aGl yLeuVal l AspGl nAspAspLeuAspGl uGl uHi s

2501 CAGGGCTCGCGCCAGCCGAAGTGTTCGCCAGGCTCAAGGCGAGCATGCCGACGGCGAGGATCTGCTGTCGACATGGCGATGCTGCTGCCGAATA

163> Gl nGl yLeuAl aP roAl aGl uLeuPheAl aArgLeuLysAl aSer Me tP roAspGl yGl uAspLeuVal Val Thr Hi sGl yAspAl aCysLeuP roAsn l

2601 TCATGGTGGAAAATGGCCGCTTTCTGGATTTCGACTGTGGCGGCTGGGTGTGGCGGACCGTATCAGGACATAGCGTTGGCTACCCGTGATATTGC

196> l eMe tVal l Gl uAsnGl yArgPheSer Gl yPhe l l eAspCysGl yArgLeuGl yVal l Al aAspArgTyrGl nAsp l l eAl aLeuAl aThr ArgAsp l l eAl

2701 TGAAGAGCTTGGCGGCAATGGGCTGACCGCTTCTCTGCTTTACGGTATCGCCGCTCCCGATTGCGAGCGCATCGCTTCTATCGCTTCTTGGACGAG

229> aGl uGl uLeuGl yGl uTrpAl aAspArgPheLeuVal l LeuTyrGl y l l eAl aAl aP roAspSer Gl nArg l l eAl aPheTyrArgLeuLeuAspGl u

2801 TTCTTCTGAGCGGACTCTGGGTTGAAATGACCGACCAAGCGAATTCGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACA

263> PhePhe•••

2901 ACTAGAATGCAGTAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACATTATAAGTGAATAAACAAAGTTAACAAACA

3001 ATTGCAATCATTTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGTTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATGTTAATTAAC

HpaI (2990) **MfeI (2999)**

PacI (3094)

3101 GCCATGACAAAATCCCTTAACGTGAGTTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTCTGCGCG
3201 TAATCTGCTGCTTGCAAACAAAAAACACCGCTACCAGCGGTGGTTTTGTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTCCAG
3301 CAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATC
3401 CTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGCTTACCAGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGG
ApaLI (3508)
3501 GGGGTTCTGTCACACAGCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAAGCGCCACGCTTCCGAAGGGAG
3601 AAAGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGAAACGCCTGGTATCTTTATAGTCTGTGCGGG
3701 TTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGG
Pacl (3834)
3801 CCTTTTGCTGGCCTTTTGCTCACATGTTCTTAATTAA