

# pVITRO2-neo-mcs

A multigenic plasmid for high levels of expression

Catalog code: pvitro2-nmcs

<https://www.invivogen.com/pvitro2-mcs>

For research use only

Version 21J14-MM

## PRODUCT INFORMATION

### Contents

- 20 µg of pVITRO2-neo-mcs provided as lyophilized DNA

### Storage and stability

- Products are shipped at room temperature.  
- Lyophilized DNA should be resuspended upon receipt and stored at -20°C. Lyophilized DNA is stable for 3 months at -20°C. Resuspended DNA is stable for at least one 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

pVITRO is a family of plasmids developed mainly for *in vitro* studies. They allow the ubiquitous and constitutive co-expression of two genes of interest. pVITRO plasmids can be stably transfected in mammalian cells and the genes of interest are expressed at high levels. Each pVITRO plasmid is available with either two multiple cloning sites or two reporter genes. **pVITRO2-neo-mcs** plasmid is selectable in *E. coli* with kanamycin and in mammalian cells with G418. It contains two multiple cloning sites (MCS) for the convenient cloning of two cDNAs.

## PLASMID FEATURES

- **hFerH and hFerL composite promoters:** Ferritin is a 24 subunit protein composed of two subunit types, termed H (heavy) and L (light), which perform complementary functions in the protein. Ferritin is ubiquitously expressed. Its synthesis is highly regulated by the iron status of the cell. The iron regulation is achieved at the translational level through the interaction between the iron-responsive element (IRE), located in the 5' untranslated region (5'UTR) of the ferritin mRNAs, and the iron regulatory protein<sup>1</sup>. To eliminate the iron regulation of the ferritin promoters, the 5'UTR of FerH and FerL have been replaced by the 5'UTR of the mouse and chimpanzee elongation factor 1 (EF1) genes, respectively.
- **SV40 enhancer** which is comprised of a 72-base-pair repeat allows the enhancement of gene expression in a large host range. The enhancement varies from 2-fold in non-permissive cells to 20-fold in permissive cells<sup>2</sup>.
- **CMV enhancer:** The major immediate early enhancer of the human cytomegalovirus (HCMV), located between nucleotides -118 and -524, is composed of unique and repeated sequence motifs. The HCMV enhancer can substitute for the 72-bp repeats of SV40 and is severalfold more active than the SV40 enhancer<sup>3</sup>.
- **SV40 pAn:** the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA. The efficiency of this signal was first described by Carswell *et al.*<sup>4</sup>
- **pMB1 ori:** a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.
- **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 expression<sup>5</sup>.

- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- **Neo:** The *neo* gene from Tn5 confers resistance to Kanamycin in *E. coli* and G418 in mammalian cells. The *neo* gene is driven by the CMV enhancer/promoter in tandem with the bacterial EM7 promoter allowing selection in both mammalian cells and *E. coli*.
- **EF1 pAn** is a strong polyadenylation signal. InvivoGen uses a sequence starting after the stop codon of the EF1 cDNA and finishing after a bent structure rich in GT.
- **MCS1 and MCS2:** To facilitate cloning each multiple cloning site contains several restriction sites that are compatible with many other enzymes.  
**MCS1** includes *Agel*, *EcoRV*, *BamHI*, *SalI* and *AvrII*.
  - *Agel* is compatible with *BspEI* and *SgrAI*.
  - *EcoRV* (blunt-end restriction enzyme)
  - *BamHI* is compatible with *BglII*, *BstYI* and *BclI*.
  - *SalI* is compatible with *AvaI* and *XhoI*.
  - *AvrII* is compatible with *XbaI*, *SpeI* and *NheI*.**MCS2** includes *SgrAI*, *BglII*, *XhoI* and *NheI*.
  - *SgrAI* is compatible with *BspEI* and *Agel*.
  - *BglII* is compatible with *BamHI*, *BstYI* and *BclI*.
  - *XhoI* is compatible with *AvaI* and *SalI*.
  - *NheI* is compatible with *XbaI*, *SpeI* and *AvrII*.

1. Eisenstein R.S. & Munro H.N., 1990. Translational regulation of ferritin synthesis by iron. *Enzyme* 44(1-4):42-58. 2. Moreau P. *et al.*, 1981. The SV40 72 base repair repeat has a striking effect on gene expression both in SV40 and other chimeric recombinants. *Nucleic Acids Res.* 9(22):6047-68. 3. Boshart M. *et al.* 1985. A very strong enhancer is located upstream of an immediate early gene of human cytomegalovirus. *Cell* 141(2):521-30. 4. Carswell S. & Alwine J.C. 1989. Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. *Mol. Cell Biol.* 10: 4248-4258. 5. 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.

## METHODS

### Plasmid resuspension

Briefly 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 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 G418	Competent <i>E. coli</i> cells Selection antibiotic	gt116-11 ant-gn-1

## TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873

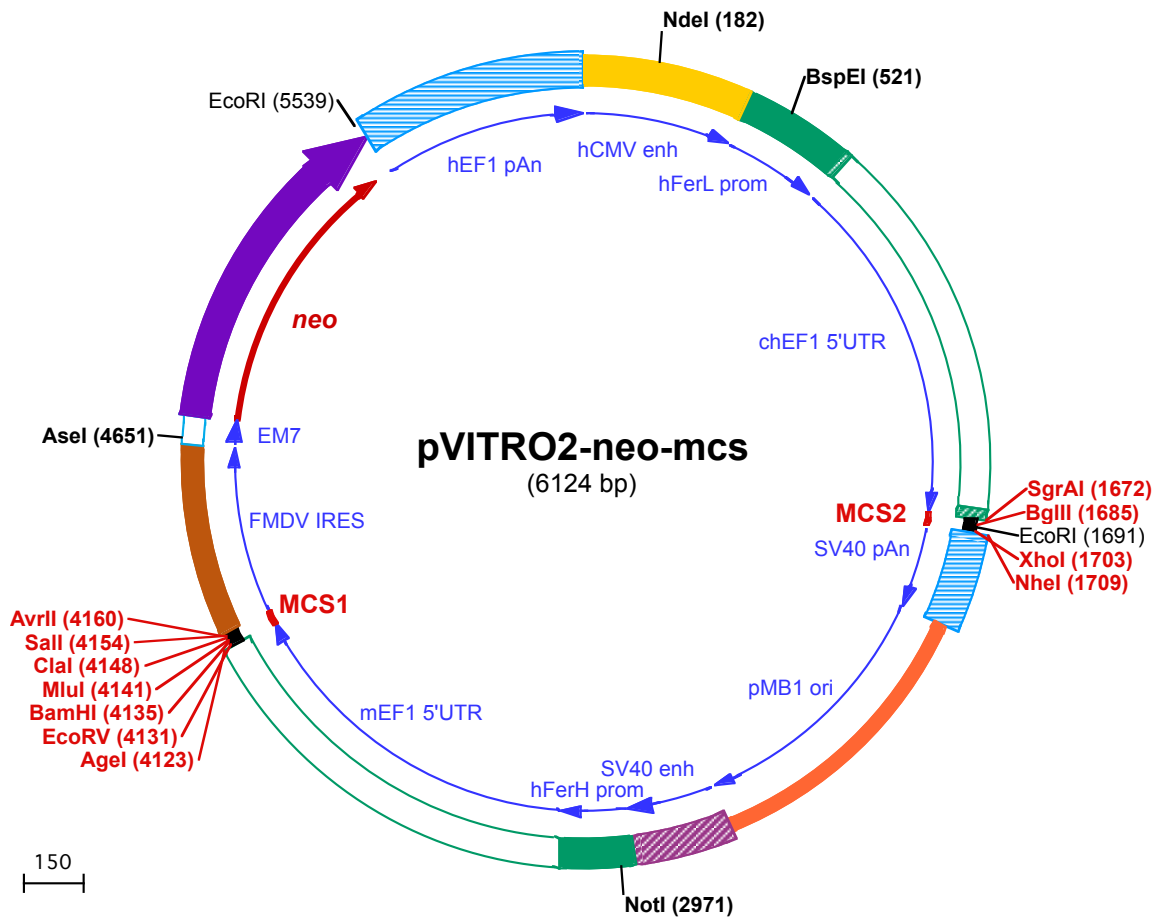
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1 CCTGCAGGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA

101 CGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAACTGCCACTTGGCAGTACATCAAGTGATCATATGCCAAGTACGCCCCC **NdeI (182)**

201 TATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATC

301 GCTATTACCATGATGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCGAAGTCTCCACCCCAATTGACGTCAATG

401 GGAGTTTGTTTTGACTAGTCAGGGGCCCAACCCCAAGCCCCATTTACAACACGCTGGCGCTACAGGCGGTGACTTCCCCTTGCTTTGGGGCGGG

501 GGGCTGAGACTCCTATGTGCTCCGGATTGGTCAGGCACGGCCTTCGGCCCCGCTCCTGCCACCGCAGATTGGCCGCTAGGCCTCCCGAGCGCCTGCC **BspEI (521)**

601 TCCGAGGGCCGGCGCACCATAAAAGAAGCCGCCCTAGCCACGTCCCTCGCAGTTCCGGGGTCCCGGGTCTGTCTCAAGCTTGCCGCCAGAACACAGG

701 taagtccgtgtgtggttcccgcgggctggcctctttacgggttatggccttgctgcttgaattacttccatgcccttggtgcagtacgtgattc

801 ttgatcccagcttccgggttgaagtgggtgggagagttcagggccttgcgcttaaggagccccttcgcctcgcttgagttgagggcctggcttgggcg

901 ctggggccgcccgtgctaactctggtggcaccttcgcgctgtctcgtgctttcgtactagcatttaaaatTTTTGATAACCAAGCTGCGACG

1001 cTTTTTTTctggcgagatagcttGtaaatgcgggccaaggatctgcacactggatatttgggttttggggccgcccggggcgagggggccctgctgctccc

1101 agcgcacatgttcggcgaggcggggcctgcgagcgcggccaccgagaatcggacggggtagtctcaactggccggcctgctctggtgcctggcctcgc

1201 gccgcccgtgatcgcggccctggcggaaggctggcccggctggcaccagttcgtgagcggaaagatggccgcttcccggccctgctgcagggagc

1301 tcaaaatggaggacgcggcgcgggagagcggcgggtagtcacccacacaaaggaaaaggccttctcctcatccgtcgttcatgtgactcca

1401 cggagtaccggcgccctcaggcacctcgattagttgtcagcttttggagtacgtcgtcttaggttggggggaggggttttatgcgatggagtttcc

1501 ccacactgagtgggtggagactgaagagttaggccagcttggcacttgatgtaattctccttggaaattgcccctttttagtggatcttgcctcattc

1601 tcaagcctcagacagtgttcaagttttttcttccatttcagGTGTCGTGAAACTACCCCTAAAAGCCACCGGCGTGCAGATCTGAATTCTTCG **SgrAI (1672)** **BglII (1685)** **EcoRI (1691)**

1701 AACTCGAGGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTG **NheI (1709)** **XhoI (1703)**

1801 TGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAAACAACAATTCATTTCATTTATGTTTCAGGTTACAGGGGAGGTGTGG

1901 GAGGTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGAAATGTTAATTAAGTACCATGACCAAAATCCCTAACGTGAGTTTTTCGTTCCACTG

2001 AGCGTCAGACCCGTAGAAAAGATCAAAGGATCTTCTTGATCCTTTTTTCTGCGGTAATCTGCTGCTTGAACAACAAAAACCACCGCTACCAGCG

2101 GTGGTTTGTGGCCGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTACGAGAGCGCAGATACCAATACTGTTCTTCTAGTGTAGCCGT

2201 AGTTAGGCCACCACTCAAGAACTCTGTAGCACCGCTACATACCTCGCTCTGTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCT

2301 TACCGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCCGGCTGAACGGGGTTCGTGCACACAGCCAGCTTGAGCGAACGACCTACA

2401 CCGAACTGAGATACCTACAGCGTGAAGTATGAGAAAGCGCCACGCTTCCGAAGGGAGAAAGCGGACAGGTATCCGGTAAGCGGCAGGGTCCGAACAGG

2501 AGAGCGCACGAGGGAGCTTCCAGGGGAAACGCCTGGTATCTTTATAGTCTGTCGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCG

2601 TCAGGGGGCGGAGCCTATGAAAAACGCCAGCAACCGGCCCTTTTACGGTTCCTGGCCTTTTGTGCTGACATGTTCTTAATTAACCTGC

2701 AGGGCCTGAAATAACCTCTGAAAGAGGAAGTGGTTAGGTACCTTCTGAGGCTGAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGAAAGTCC

2801 CCAGGCTCCCCAGCAGGCAGAAGTATGCAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGC

2901 AAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCACTAGTTCCGCCAGAGCGCGAGGGCCTCCAGCGGCCGCCCTCCCCACAGCAGGGGGCGGG **NotI (2971)**

3001 GTCCCGCCCCACCGAAGGAGCGGGCTCGGGGCGGGCGGCTGATTGGCCGGGGCGGGCTGACGCCGACCGGCTATAAGAGACCACAAGCGACCCG

3101 CAGGGCCAGACGTTCTTCGCCGAAGCTTGCCGTCAGAACGCAGGTGAGGGGCGGGTGTGGCTTCCGCGGGCCCGAGCTGGAGGTCTGCTCCGAGCGG  
3201 GCCGGGCCCCGCTGTCGTCGGCGGGGATTAGCTGCGAGCATTCCCGCTTCGAGTTGCGGGCGGCGGGGAGGCAGAGTGCAGAGCCTAGCGGCAACCCCG  
3301 TAGCCTCGCCTCGTGTCCGGCTTGAGGCTAGCGTGGTGTCCGCGCCGCCCGCGTGTACTCCGGCCGACTCTGGTCTTTTTTTTTTTTGTGTTGT  
3401 TGCCCTGCTGCCTTCGATTGCCGTTAGCAATAGGGGCTAACAAAGGGAGGTGCGGGGCTTGTCTGCCCGAGCCCGAGAGGTGATGTTGGGGAGGA  
3501 ATGGAGGGACAGGAGTGGCGGTGGGGCCCCCGCTTCGAGACACATGTCGACGCCACCTGGATGGGGCGAGGCTGGGGTTTTCCCGAAGCAACC  
3601 AGGCTGGGGTTAGCGTCCGAGGCCATGTGGCCCCAGCACCCGGCACGATCTGGCTTGGCGGCGCCGCTTGCCTGCCTCCCTAACTAGGGTGAGGCCA  
3701 TCCCGTCCGGCACCAGTTGCGTGCCTGAAAGATGGCCGCTCCCGGGCCCTGTTGCAAGGAGCTCAAATGGAGGACGCGGAGCCCGGTGGAGCGGGCG  
3801 GGTGAGTCAACCACAAAGGAAGAGGGCCTGGTCCCTACCGGCTGCTGCTTCTGTGACCCCGTGGTCTATCGGCCCAATAGTCACCTCGGGCTTT  
3901 TGAGCACGGCTAGTCGCGGGGGGGAGGGATGTAATGGCGTTGAGTTTGTTCACATTTGGTGGTGGAGACTAGTCAGGCCAGCCTGGCGCTGGAAG  
4001 TCATTTTTGGAATTTGCCCTTGTGTTTTGAGCGGAGCTAATTCTCGGGCTTCTTAGCGGTTCAAAGGTATCTTTAAACCCTTTTTAGGTGTTGTA

4101 AAACCACGCTAATTCAAAGCAACCGGTGATATCGGATCCACGCGTATCGATTGTCACCTAGGAGCAGGTTTCCCAATGACACAAAACGTGCAACT  
4201 GAAACTCCGCTGGTCTTCCAGGTCTAGAGGGTAACACTTTGACTGCGTTTGGCTCCACGCTCGATCCACTGGCGAGTGTAGTAACAGCACTGTTG  
4301 CTTCTAGCGGAGCATGACGGCCGTGGAACTCCTCCTTGGTAACAAGACCACGGGGCCAAAAGCCACGCCACAGGGCCCGTCATGTGTGCAACCC  
4401 CAGCACGGGACTTTACTGCGAAACCACTTTAAAGTGACATTGAACTGGTACCCACACTGGTACAGGCTAAGGATGCCCTTCAGGTACCCCGAGG  
4501 TAACACGCGACTCGGGATCTGAGAAGGGGACTGGGGCTTCTATAAAAGCGCTCGGTTTAAAAGCTTCTATGCCTGAATAGGTGACCGGAGGTGCGCA

4601 CCTTTCCTTGAATTACTGACCTATGAATACAACTGACTGTTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACTCACTATAGG  
4701 AGGGCCACCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCCGCTATGACTGGGCACAACAGACAATCGGCT  
4801 GCTCTGATGCCCGCTGTTCCGGCTGTCAGCGCAGGGGCGCCCGTCTTTTTGTCAAGACCACCTGTCGGTGCCTGAATGAACTGCAAGACGAGGC  
31▶ C S D A A V F R L S A Q G R P V L F V K T D L S G A L N E L Q D E A  
4901 AGCGCGCTATCGTGGCTGCCACGACGGGCTTCTTGGCAGCTGTGCTGACGTTGCTACTGAAGCGGAAGGGACTGGCTGCTATTGGCGAAGTV  
64▶ A R L S W L A T T G V P C A A V L D V V T E A G R D W L L L G E V  
5001 CCGGGCAGGATCTCCTGTCATCTCACCTTCTCCTGCCGAGAAAGTATCCATCATGGTGTGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCT  
98▶ P G Q D L L S S H L A P A E K V S I M A D A M R R L H T L D P A T  
5101 GCCATTGACCAAGCAACATCGCATCGAGGAGCAGTACTCGGATGGAAGCCGGTCTTGTGATCAGGATGATCTGGACGAAGAGCATCAGGG  
131▶ C P F D H Q A K H R I E R A R T R M E A G L V D Q D D L D E E H Q G  
5201 GCTCGGCCAGCCGAAGTTCGCCAGGCTCAAGGCGAGCATGCCGACGGCGAGGATCTCGTGTGACACATGGCGATGCCTGCTTCCGAATATCATG  
164▶ L A P A E L F A R L K A S M P D G E D L V V T H G D A C L P N I M  
5301 GTGAAAATGGCCGCTTTTCTGGATTATCGACTGTGGCCGGTGGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATTTGCTGAAG  
198▶ V E N G R F S G F I D C G R L G V A D R Y Q D I A L A T R D I A E  
5401 AGCTTGGCGGCAATGGGCTGACCGCTTCTCGTCTTACGGTATCGCCGCTCCGATTGCGAGCGCATCGCTTCTATCGCTTCTTACGAGTTCTT  
231▶ E L G G E W A D R F L V L Y G I A A P D S Q R I A F Y R L L D E F F

5501 CTGAGCGGACTCTGGGGTTCGAAATGACCGACCAAGCAATTGCTAGGATTATCCCTAATACCTGCCACCCACTTAAATCAGTGGTGAAGAACGG  
264▶ •  
5601 TCTCAGAACTGTTTGTTCATTGGCCATTTAAGTTTAGTAGTAAAAGACTGGTAAATGATAACAATGCATCGTAAAACCTTCAGAAGGAAAGGAGAATG  
5701 TTTTGTGACCACCTTTGTTTTCTTTTTGCGTGTGGCAGTTTTAAGTTATTAGTTTTTAAATCAGTACTTTTTAATGAAACAACCTTGACCAAAATT  
5801 TGTCACAGAATTTTGTAGACCATTAATAAAGTAAATGAGAAACCTGTGTGTTCTTTGGTCAACACCGAGACATTTAGGTGAAAGACATCTAATTCGG  
5901 TTTTACGAATCTGAAAACCTTGAATAATGTAATCTTGTAGTAAACACTTCTGGGTGGAGAATAGGGTGTTCCTCCCCACATAATTGGAAGGGGAAGG  
6001 AATATCATTTAAAGCTATGGGAGGTTGCTTTGATTACAACACTGGAGAGAAATGCAGCATGTTGCTGATTGCCTGTCACTAAAACAGGCCAAAACTGA  
6101 GTCCTTGGGTTGCATAGAAAGCTG