

pMONO-neo-GFP

A GFP-expression plasmid selectable with Kanamycin/G418

Catalog code: pmonon-gfp

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

For research use only

Version 19L13-MM

PRODUCT INFORMATION

Contents

- 20 µg of pMONO-neo-gfp 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 transfecants 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.

pMONO-GFP plasmids feature a new allele of the GFP gene called LGFP. They can be used as control vectors or for cloning of an open reading frame, as the LGFP gene is flanked by two unique restriction sites: Bsp HI at the 5' end that encompasses the start codon, and Avr II at the 3' end.

PLASMID FEATURES

- **SV40/FerH/mEF1 α :** 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.
- **LGFP:** This red-shifted variant of the jellyfish GFP gene encodes a green fluorescent protein that absorbs blue light (major peak at 480 nm) and emits green light (major peak at 505 nm).
- **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 G418	Competent <i>E. coli</i> cells Selection antibiotic	gt116-11 ant-gn-1

TECHNICAL SUPPORT

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

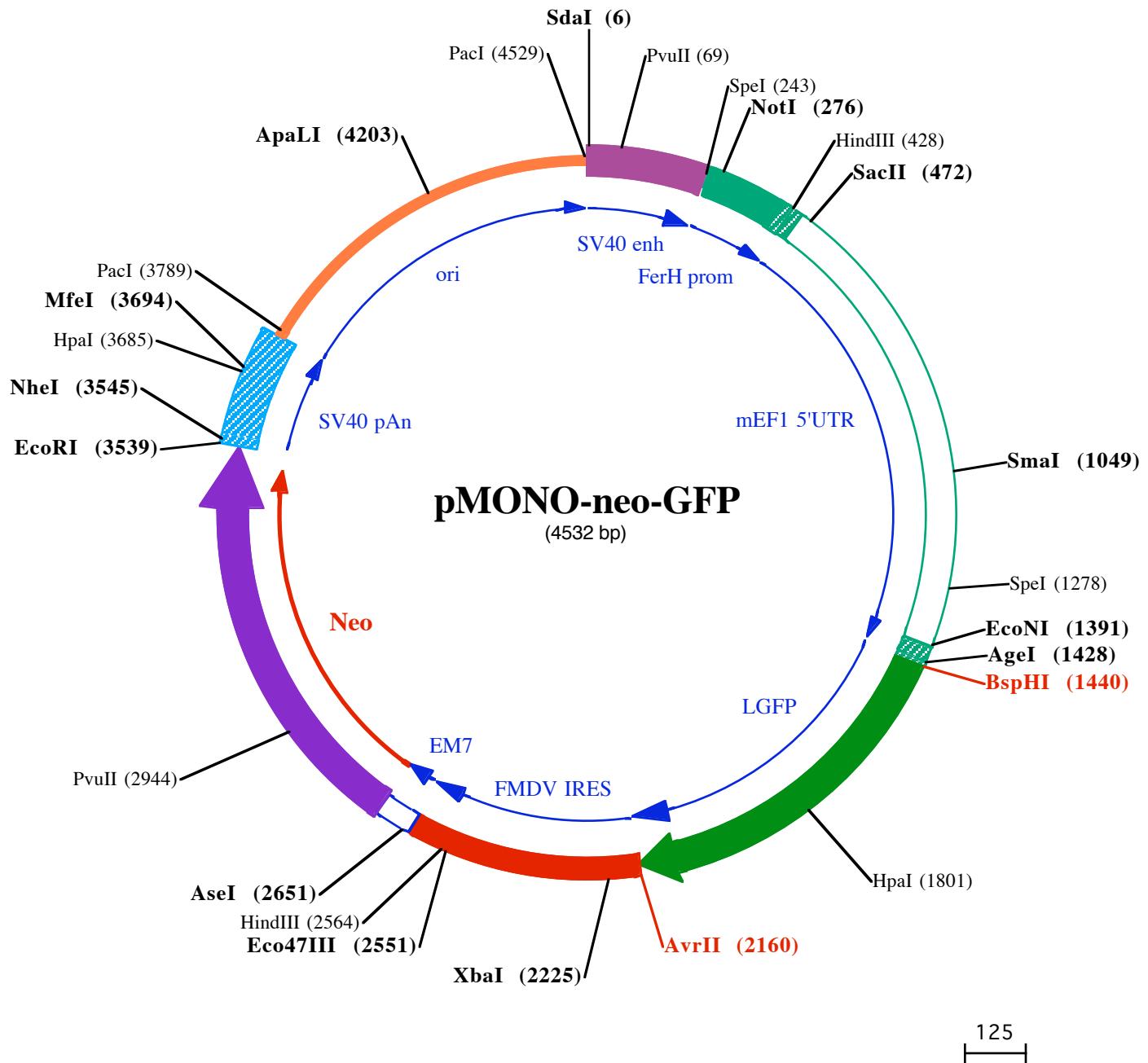
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SdaI (6)

1 CCTCGAGGCCCTGAAATAACCTCTGAAAGAGGAACCTGGTAGGTACCTCTGAGGCTGAAAGAACAGCTGTTGAAATGTGTCAGTTAGGGTGTGAA

101 AGTCCCAGGCTCCAGCAGGAGAATGCAAGCATGCATCTCAATTAGTCAGCAACCAGCTGTTGAAAGTCCCCAGCAGGAGAAG

201 TATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGCTGTTGAAAGTCCCCAGCAGGAGAAG

SpeI (243) → NotI (276)

301 GCGGGGTCGCCAGCCACCGAAGGAGCAGGGCTGGGGCGGCCTGATTGGCGGGCGGCCTGACGCCAGCGCTATAAGAGACCAAGCG

401 ACCCGCAGGGCCAGACGTTCTCGCCAGCTGCGTCAAGAACGCAGGTGAGGGCGGGTGTGGCTCCGGCCGAGCTGGAGGTCTGCTCG

HindIII (428) → SacII (472)

501 AGCGGGCCGGCCCGCTGTCGCGGGATTAGCTGCGAGCATTCCGCTTGAGTTGCGGGCGCGGGAGGAGTGCAGGCTAGCGCAA

601 CCCCGTAGCCTCGCTCGTCCGCTTGAGGCCAGCGTGGTGTCCGCCCGCGTCACTCCGCCACTCTGGCTTTTTTTTGT

701 GTTGTGCCCCCTGCTGCTCGATTGCGTTCAAGAACAGGGAGGGTGCAGGGCTTGCTCGCCGGAGCCGGAGAGGTATGGTGGG

801 GAGGAATGGAGGGACAGGAGTGGCGGCTGGGGCCCTCGAGCACATGTCGACGCCACCTGGATGGCGAGGCTGGGGTTTCCGAAG

901 CAACCGAGCTGGGTTAGCGTCCGAGGCCATGTGGCCAGCACCGCAGATCTGGCTTGGCGCCGCGTGCCTCCACTAGGGTGA

1001 GGCCATCCCGTCGGCACCGAGTTGCGTGCAGGAAAGATGGCCGCTCCGGCCCTGTTGCAAGGAGCTAAATGGAGGACGCCAGCCGGTGGAGC

1101 GGGCGGGTAGTCACCCACAAAGGAAGAGGGCTGGTCCCTCACCGCTGCTGCTTCTGTGACCCGTGGTCTATCGCCGAATAGTCACCTCG

1201 GCTTTGAGCACGGCTAGTCGCGGGGGAGGGATGTAATGGCGTTGAGTTGTCACATTGGTGGGAGACTAGTCAGGCCAGCTGGCGT

SmaI (1049)

1301 GGAAGTCATTTGAAATTGTCCTTGAGTTGAGCGGAGCTAATTCTGGCTTCTAGCGGTTCAAAGGTATCTTAAACCCCTTTAGGTGT

AgeI (1428) BspHI (1440)

1401 TGTGAAAACCACCGCTAATTCAAAGCAACCGTCGACGTGAGCAAGGGAGAAGAACTCTTACTGGTGTGTCCTGAGCTGGATGG

→ 1► Met Ser Lys Gl y Gl u Gl u Leu Phe Thr Gl y Val Val Pro l e Leu Val Gl u Leu Asp Gl

1501 TGATGTGAATGGCCACAATTCTGTGCTGGTGAAGGTGAAGGGAGATGCAACTTATGGAAAGCTGACTCTGAAGTTCATTGTACAACAGGAAGCTG

2► y Asp Val Asn Gl y Hi s Lys Phe Ser Val Ser Gl y Gl u Gl y Asp Al a Thr Tyr Gl y Lys Leu Thr Leu Phe Lys Th r Gl y Lys Leu

1601 CCAGTGCCTTGGCCAACCTGGTACCTGGTACCCCTGACTTATGGTCAATGTTCACTGGTACCGAGTACCTGACCACATGAAGCAGCATGACTTAAATCTG

54► Pro Val Pro Trp Pro Thr Leu Val Thr Leu Thr Tyr Gl y Val Gl n Cys Phe Ser Arg Tyr Pro Asp Hi s Met Lys Gl n Hi s Asp Phe Phe Lys Ser A

HpaI (1801)

1701 CAATGCCAGAAGGTTATTCAGGAGAGGACAATTCTTAAAGGTGATGGAAATTATAAGACAAGGGCAGAAGTGAAGTTGAAGGTGATACAGTGG

87► Ia Met Pro Gl y Tyr Val Gl n Gl u Arg Th r l e Phe Lys Asp Asp Gl y Asn Tyr Lys Th r Arg Al a Gl u Val Lys Phe Gl u Gl y Asp Th r Leu Va

1801 TAACAGAAATTGAGCTGAAAGGCATTGTTAAAGGAAGATGGAAACATTCTGGGTCACAAGCTGGAGTACAACATAATTCTCACATGTTACATTG

120► I Asn Arg l e Gl u Leu Lys Gl y l e Asp Phe Lys Gl y Asn l e Leu Gl y Hi s Lys Leu Gl y Tyr Asn Ser Hi s Asn Val Ty r l e Met

1901 GCAGATAAGCAGAAGAATGGAAATTAGTTAACAGATTAGACACAAACATTGAGGATGGACTCTGCAACTGGCAGACCAATTACAGCAGAACACCC

154► Al a Asp Lys Gl n Lys Asn Gl y l e Lys Val Asn Phe Lys l e Arg Hi s Asn l e Gl u Asp Gl y Ser Val Gl n Leu Al a Asp Hi s Tyr Gl n Gl n Asn Th r P

2001 CTATTGGTATGGCCAGTTCTCCTCCAGATAATCACTATCTGGCAGTCACTGGCTCTGTCAAAGACCCTAATGAGAAAAGAGACCACATGGCCT

187► rolle Gl y Asp Gl y Pro Val Leu Leu Pro Asp Asn Hi s Tyr Leu Arg Th r Gl n Ser Al a Leu Ser Lys Asp Pro Asn Gl u Lys Arg Asp Hi s Met Val Le

AvrII (2160)

2101 CCTGGAGTTGTGACAGCAGCGAGGAATTACTCTGGAAATGGATGGATGAGCTGTACAAGTAAACCTAGGAGCAGGTTCCCAATGACACAAAACGTGCAACTT

220► u Leu Gl u Phe Val Th r Al a Al a Gl y l e Th r Leu Gl y Met Asp Gl u Leu Tyr Lys ***

XbaI (2225)

2201 GAAACTCCGCCTGGTCTTCCAGGTCTAGAGGGTAACACTTGACTGCGTTGGCTCCACGCTCGATCCACTGGCAGTGTAGTAACAGCACTGTT

2301 CTTCGTAGCGGAGCATGACGCCGTGGAACTCCTCTTGGTAACAAGGACCCACGGGGCAAAGCCACGCCACACGGCCGTCATGTGCAACCC

2401 CAGCACGGCAGTTACTGCAAACCACTTAAAGTGACATTGAAACTGGTACCCACACACTGGTACAGGCTAAGGATGCCCTCAGTACCCGAGG

Eco47III (2551) HindIII (2564)

2501 TAACACCGACACTCGGGATCTGAGAAGGGACTGGGCTCTATAAAAGCGCTGGTTAAAAGCTTCTATGCCATAGTAAAGTGACCGGAGGTGGCA

AseI (2651)

2601 CCTTTCTTCAATTACTGACCCATGAATACAACGTACTGTTGACAATTATCATGGCATAGTATATGCCATAGTATAATACGACTCACTATAGG

→ 2► Met l e Gl u Gl n Asp Gl y Leu Hi s Al a Gl y Ser Pro Al a Al a Trp Val Gl u Arg Leu Phe Gl y Tyr Asp Trp Al a Gl n Gl n Th r l e Gl y C

2701 AGGGCCACCATGATTGAAACAAGATGGATTGCAAGCAGGGTCTCCGGCCGCTTGGTGGAGAGGCTATTGGCTATGACTGGCACAACAGACAATGGCT

31► y Asp Al a Val Phe Arg Leu Ser Al a Gl n Gl y Ar g P ro Val Leu Phe Val Lys Th r Asp Leu Ser Gl y Al a Leu Asn Gl u Leu Gl n Asp Gl u Al

PvuII (2944)

2901 AGCGCGCTATCGGGCACGAGGGCTTCTGGCAGCTGTGCTGAGCTGTTGCACTGAAGGGAGGACTGGCTATTGGCAAGT

64► a Al a Arg Leu Ser Trp Leu Al a Th r Th r Gl y Val Pro Cys Al a Al a Val Leu Asp Val Val Th r Gl u Al a Gl y Ar g Asp Trp Leu Leu Gl y Gl u Val

3001 CGGGGGAGGATCTCTGTCATCTACCTGCTCTGCCAGAAAGTATCCATGGCTATGCCAGTGAATGCCGCGCTGCATACGCTTGTGATCCGGCTACCT

98► Pro Gl y Gl n Asp Leu Leu Ser Ser Hi s Leu Al a Pro Al a Gl u Lys Val Ser l l e Met Al a Asp Al a Met t Arg Arg Leu Hi s Th r Leu Asp Pro Al a Th r C

3101 GCCCATTGACCACCAAGCAGAACATCGCATGAGCAGCACGTACTGGATGGAAGCCGGTCTGTCAGGATGATCTGGACGAAGAGCATCAGGG

131► ys Pro Phe Asp Hi s Gl n Al a Lys Hi s Arg l e Gl u Arg Al a Arg Th r Arg Met Gl u Al a Gl y Leu Val Asp Gl u Asp Asp Leu Asp Gl u Gl u Hi s Gl n Gl

3201 GCTCGGCCAGCCAACTGTCGCCAGGCTCAAGGCAGCATGCCGACGGCAGGATCTCGTGTACACATGGGATGCCCTGCTGCCAATATCATG
 164▶ yLeuAl aProAl aGl uLeuPheAl aArgLeuLysAl aSer Met ProAspAl yGl uAspLeuVal Val Thr His Gl yAspAl aCysLeuProAsnI IeMet
 3301 GTGGAAAATGGCCGCTTTCTGGATTCTCGACTGTGGCCGGCTGGGTGCGGACCGCTATCAGGACATAGCGTTGGCTACCGTGATATTGCTGAAG
 198▶ Val Gl uAsnGl yArgPheSer Gl yPhel IeAspCysGl yArgLeuGl yVal Al aAspArgTyrGl nAspI IeAl aLeuAl aThr ArgAspI IeAl aGl uG
 3401 AGCTTGGCCGGCAATGGCTGACCGCTTCTCGTCTTACGGTATCGCCGCTCCGATTGCGCAGGCATCGCCTCTATGCCTTGTACGGAGTTCTT
 231▶ IuLeuGl yGl yGl uTrpAl aAspArgPheLeuVal LeuTyrGl y I IeAl aAl aProAspSer Gl nArgI IeAl aPheTyrArgLeuLeuAspGl uPhePh

NheI (3545)

EcoRI (3539)

3501 CTGAGCGGGACTCTGGGTTCGAAATGACCGACCAAGC GAATT CGTAGCTGGCAGACATGATAAGATAACATTGATGAGTTGGACAACCACAACTAG
 264▶ e***

HpaI (3685) MfeI (3694)

3601 AATCGAGTAAAAAAATGCTTATTGTGAAATTGTGATGCTATTGCTTATTGTAACCATTATAAGCTGCAATAAACAAAGTTAACAAACAATTG

PacI (3789)

3701 ATTCACTTATGTTCAAGGTTCAAGGGGAGGTGTGGAGGTTTTAAAGCAAGTAAACCTCTACAAATGTGGATGGAAATGTTAACCTAGCCAT →

3801 GACCAAAATCCCTAACGTGAGTTCTGGTCACTGAGCGTCAGACCCCCGTAGAAAAGATCAAAGGATCTTCTGAGATCCTTTCTGCCGTAATC

3901 TGCTGCTTCAAACAAAAAACCCGCTACCAGCGTGGTTGTTGCCGATCAAGAGCTACCAACTCTTTCCGAAGGTAACGGCTCAGCAGAG

4001 CGCAGATACCAAATACTGTTCTCTAGTGTAGCCGTAGTTAGGCCACCACTCAAGAACTCTGAGCACCGCTACATACCTCGCTCTGTAATCCTGTT

4101 ACCAGTGGCTGCTGCCAGTGGGATAAGTCGTCTTACGGGTTGGACTCAAGACGATAGTTACCGATAAGGCGCAGCGGTCGGCTGAACGGGGGT

ApaLI (4203)

4201 TCGTCACACAGCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGGCTATGAGAAAGGCCACGCTCCGAAGGGAGAAAGG

4301 CGGACAGGTATCCGTAAGCGCAGGGTCGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGAAACGCCCTGGTATCTTATAGTCTGCGGTTTCG

4401 CCACCTCTGACTTGAGCGTCGATTTGTGATGCTCGTCAAGGGGGCGGAGCCTATGGAAAACGCCAGCAACCGGCCCTTTACGGTCTGCCCTT

PacI (4529)

4501 TGCTGGCCTTTGCTCACATGTTCTTAATTAA →