USER GUIDE

invitrogen™
by life technologies™

pcDNA™3.1/*myc*-His(-) A, B, and C

Catalog number V855-20

Revision date 2 March 2012
Publication Part number 28-0128

MAN0000634

For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

life technologies™

Contents

Kit Contents and Storage

Shipping and Storage

pcDNA™3.1/*myc*-His vectors are shipped at room temperature. Upon receipt, store vectors at -20°C.

Kit Contents

20 µg each of pcDNA™3.1/*myc*-His(-) A, B, and C are supplied at 0.5 µg/µL in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 in a total volume of 40 µL.

20 µg of pcDNA™3.1/*myc*-His(-)/*lacZ* is supplied at 0.5 µg/µL in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 in a total volume of 40 µL.

For research use only. Not intended for any human or animal therapeutic or diagnostic use.

Introduction

Product Overview

Description of the System

pcDNA™3.1/*myc*-His(-) A, B, and C are 5.5 kb vectors designed for overproduction of recombinant proteins in mammalian cell lines. The vectors are supplied in three reading frames to facilitate in frame cloning, with a C-terminal peptide containing a polyhistidine metal-binding tag and the *myc* (*c-myc*) epitope. The human cytomegalovirus immediate-early (CMV) promoter provides high-level expression in a wide range of mammalian cells. In addition, the vector will replicate episomally in cell lines that are latently infected with SV40 or that express the SV40 large T antigen (e.g. COS7). High-level stable and non-replicative transient expression can be carried out in most mammalian cells. The control plasmid, pcDNA™3.1/*myc*-His(-)/*lac*Z, is the pcDNA™3.1/*myc*-His(-) A vector with a 3.2 kb fragment containing the β-galactosidase gene cloned in frame with the C-terminal peptide (see page 9). It is included for use as a positive control for transfection, expression, and detection in the cell line of choice.

Experimental Outline

Use the following outline to clone and express your gene of interest in pcDNA™3.1/*myc*-His(-).

1. Consult the multiple cloning sites described on pages 3–5 to determine which vector (A, B, or C) should be used to clone your gene in frame with the C-terminal *myc* epitope and the polyhistidine tag.
2. Ligate your insert into the appropriate vector and transform into *E. coli*. Select transformants on 50–100 µg/mL ampicillin.
3. Analyze your transformants for the presence of insert by restriction digestion.
4. Select a transformant with the correct restriction pattern and use sequencing to confirm that your gene is cloned in frame with the C-terminal peptide.
5. Transfect your construct into the cell line of choice using your own method of transfection. Generate a stable cell line, if desired.
6. Test for expression of your recombinant gene by western blot analysis or functional assay. If you do not have an antibody to your protein, you may use the Anti-*myc* Antibody or the Anti-His (C-term) Antibody to detect your recombinant protein (see page 11 for ordering).
7. To purify your recombinant protein, you may use metal-chelating resin such as ProBond™. ProBond™ resin is available separately (see page 11 for ordering).

Methods

Cloning into pcDNA™3.1/*myc*-His(-) A, B, and C

General Molecular Biology Techniques

For help with DNA ligations, *E. coli* transformations, restriction enzyme analysis, purification of single-stranded DNA, DNA sequencing, and DNA biochemistry, refer to *Molecular Cloning: A Laboratory Manual* (Sambrook *et al.*, 1989) or *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994).

E. coli Strain

Many *E. coli* strains are suitable for the growth of this vector including TOP10F´. We recommend that you propagate vectors containing inserts in *E. coli* strains that are recombination deficient (*rec*A) and endonuclease A-deficient (*end*A).

For your convenience, TOP10F´ is available as chemically competent or electrocompetent cells for purchase (see page 11 for ordering).

Maintenance of pcDNA™3.1/*myc*-His(-)

To propagate and maintain the pcDNA™3.1/*myc*-His(-) vectors, use the supplied stock solution in TE, pH 8.0 to transform a *rec*A, *end*A *E. coli* strain like TOP10, TOP10F´, DH5α™, JM109, or equivalent. Select transformants on LB plates containing 50–100 µg/mL ampicillin. Be sure to prepare a glycerol stock of your plasmid.

Cloning Considerations

Your insert should contain a Kozak translation initiation sequence for proper initiation of translation (Kozak, 1987; Kozak, 1990). An example of a Kozak consensus sequence is provided below. Note that other sequences are possible, but the G or A at position –3 and the G at position +4 are the most critical nucleotides for function (shown in bold). The ATG initiation codon is shown underlined.

(G/A)NNATG**G**

To express your gene as a recombinant fusion protein, you must clone your gene in frame with the C-terminal peptide. The vector is supplied in three reading frames to facilitate cloning. See pages 3–5 to develop a cloning strategy.

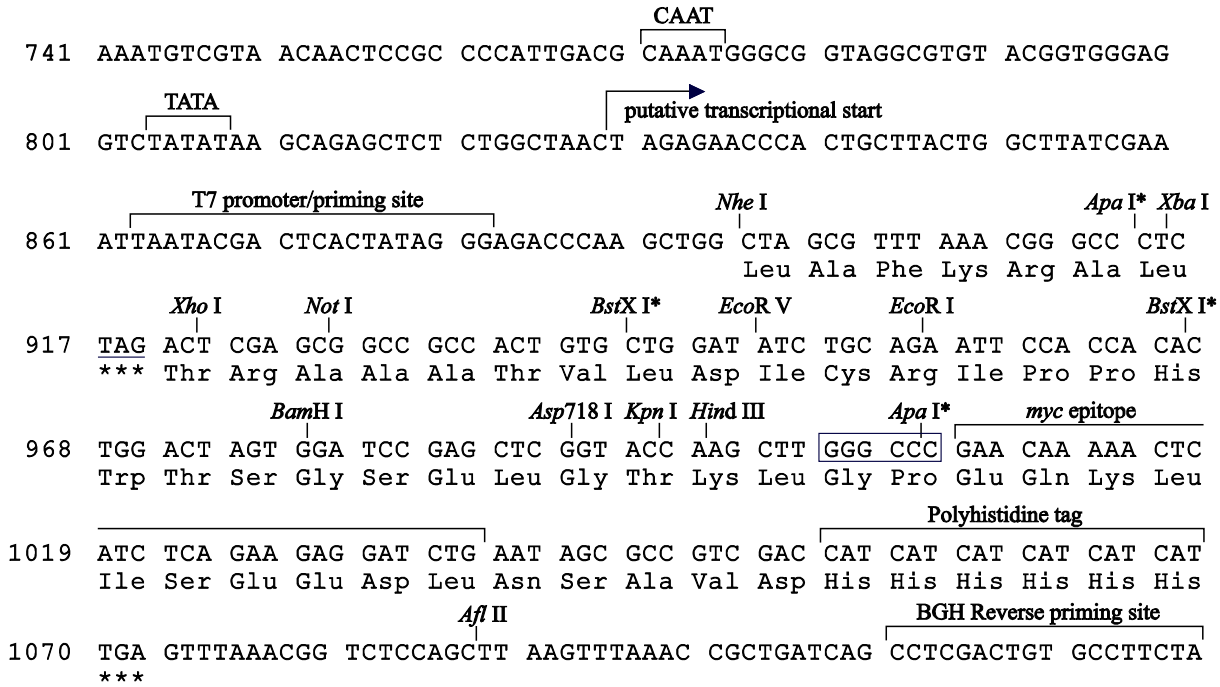
If you wish to express your protein **without** the C-terminal peptide, be sure to include a stop codon.

Continued on next page

Cloning into pcDNA™3.1/*myc*-His(-) A, B, and C, Continued

Multiple Cloning Site of Version A

Below is the multiple cloning site for pcDNA™3.1/*myc*-His(-) A. Restriction sites are labeled to indicate the cleavage site. The boxed nucleotides indicate the variable region. **Note that there is a stop codon after the *Xba* I site.** The multiple cloning site has been confirmed by sequencing and functional testing. The vector sequence of pcDNA™3.1/*myc*-His(–) A is available for downloading from www.lifetechnologies.com/support or from Technical Support (see page 12).



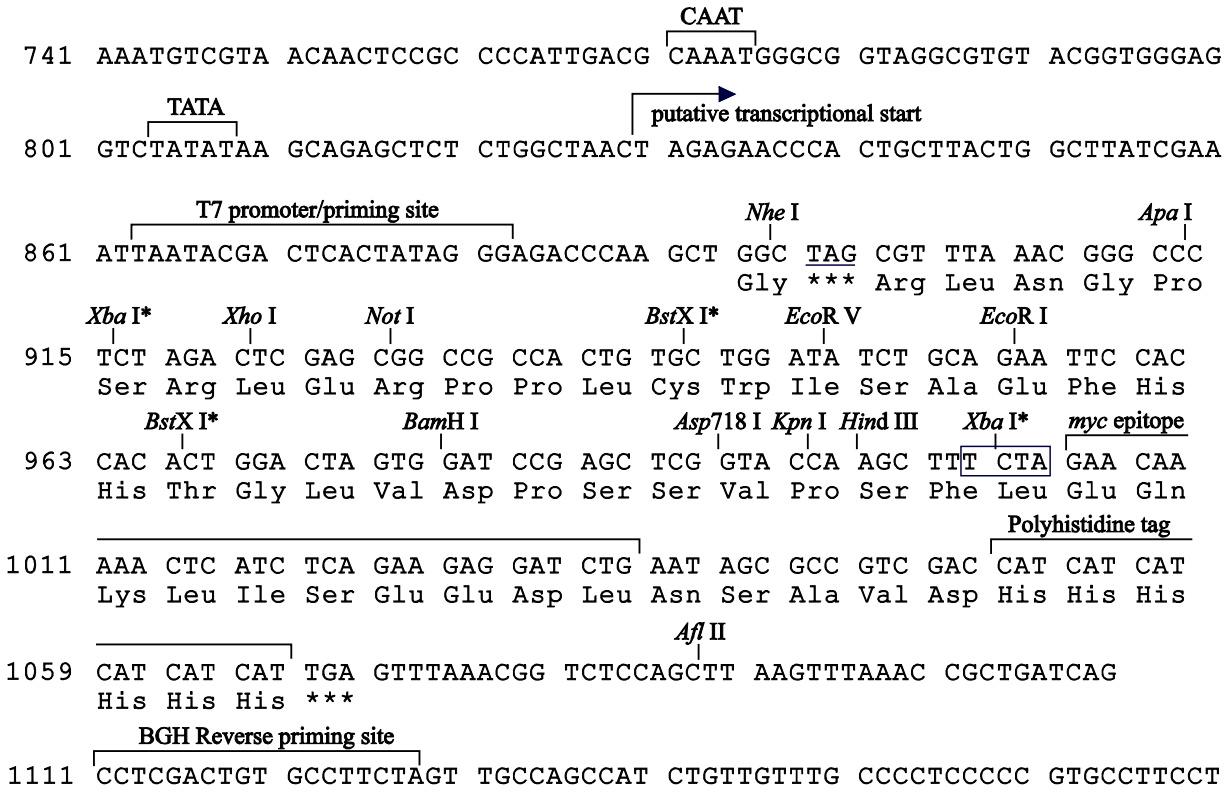
*Note that there are two *Bst*X I sites and two *Apa* I sites in the polylinker.

Continued on next page

Cloning into pcDNA™3.1/*myc*-His(-) A, B, and C, Continued

Multiple Cloning Site of Version B

Below is the multiple cloning site for pcDNA™3.1/*myc*-His(-) B. Restriction sites are labeled to indicate the cleavage site. The boxed nucleotides indicate the variable region. **Note that there is a stop codon after the *Nhe* I site.** The multiple cloning site has been confirmed by sequencing and functional testing. The vector sequence of pcDNA™3.1/*myc*-His(-) B is available for downloading from www.lifetechnologies.com/support or from Technical Support (see page 12).



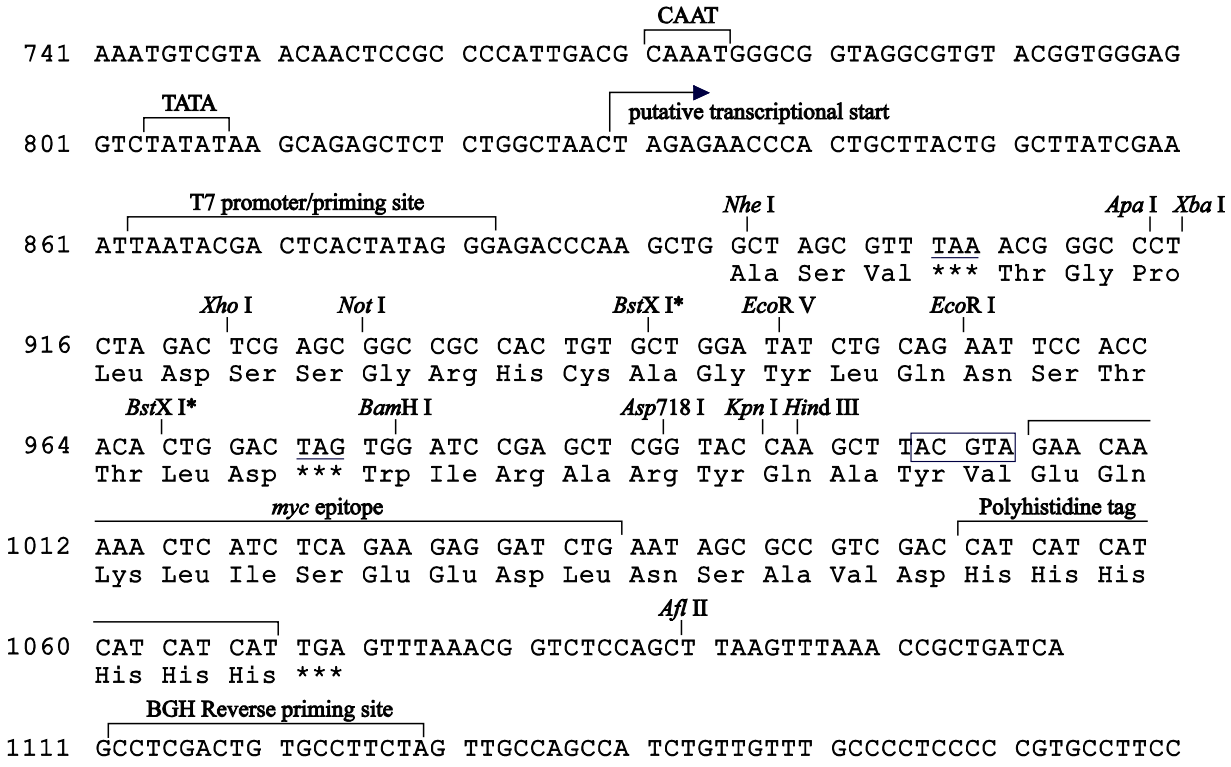
*Note that there are two *Bst*X I sites and two *Xba* I sites in the polylinker.

Continued on next page

Cloning into pcDNA™3.1/*myc*-His(-) A, B, and C, Continued

Multiple Cloning Site of Version C

Below is the multiple cloning site for pcDNA™3.1/*myc*-His(-) C. Restriction sites are labeled to indicate the cleavage site. The boxed nucleotides indicate the variable region. **Note that there are two stop codons. One is located between the *Nhe* I site and the *Apa* I site, and the other is located before the *Bam*H I site.** This means the 3´ cloning site must be either *Bam*H I, *Kpn* I (*Asp*718 I), or *Hin*d III if you wish to clone your gene into pcDNA™3.1/*myc*-His(-) C so that it is expressed without interruption. The multiple cloning site has been confirmed by sequencing and functional testing. The vector sequence of pcDNA™3.1/*myc*-His(-) C is available for downloading from www.lifetechnologies.com/support or from Technical Support (see page 12).



*Note that there are two *BstX* I sites in the polylinker.

General Guidelines and Special Information

E. coli Transformation

Transform your ligation mixtures into a competent *rec*A, *end*A *E. coli* strain (e.g. TOP10, TOP10F´, DH5α™) and select on LB plates containing 50–100 µg/mL ampicillin. Select 10–20 clones and analyze for the presence and orientation of your insert.

We recommend that you sequence your construct to confirm that your gene is fused in frame with the *myc* epitope and the C-terminal polyhistidine tag. We suggest using the T7 Promoter and BGH Reverse primer sequences. Refer to the diagrams on pages 3–5 for the sequence and location of the primer binding sites.

For your convenience, we offer a custom primer synthesis service. For more information, refer to www.lifetechnologies.com or contact Technical Support (see page 12).

Plasmid Preparation

Plasmid DNA for transfection into eukaryotic cells must be very clean and free from phenol and sodium chloride. Contaminants will kill the cells and salt will interfere with lipid complexing decreasing transfection efficiency. We recommend isolating DNA using the PureLink® HiPure Miniprep Kit or the PureLink® HiPure Midiprep Kit (see page 11 for ordering information), or CsCl gradient centrifugation.

Methods of Transfection

For established cell lines (e.g. HeLa), consult original references or the supplier of your cell line for the optimal method of transfection. It is recommended that you follow exactly the protocol for your cell line. Pay particular attention to medium requirements, when to pass the cells, and at what dilution to split the cells. Further information is provided in *Current Protocols in Molecular Biology*.

Methods for transfection include calcium phosphate (Chen and Okayama, 1987; Wigler *et al.*, 1977), lipid-mediated (Felgner *et al.*, 1989; Felgner and Ringold, 1989) and electroporation (Chu *et al.*, 1987; Shigekawa and Dower, 1988). The Calcium Phosphate Transfection Kit and a large selection of reagents for transfection are available for purchase. For more information on the reagents available, visit www.lifetechnologies.com/support or call Technical Support (see page 12).

Positive Control

pcDNA™3.1/*myc*-His(-)/*lac*Z is provided as a positive control vector for mammalian transfection and expression (see page 9) and may be used to optimize transfection conditions for your cell line. The gene encoding β-galactosidase is expressed in mammalian cells under the CMV promoter. A successful transfection will result in β-galactosidase expression that can be easily assayed (see next page).

Continued on next page

Assay for β-galactosidase Activity

You may assay for β-galactosidase expression by activity assay using cell-free lysates (Miller, 1972) or by staining the cells for activity. The β-Gal Assay Kit and the β-Gal Staining Kit are available for purchase for fast, easy detection of β-galactosidase expression (see page 11 for ordering).

Geneticin® Selective Antibiotic

Geneticin® blocks protein synthesis in mammalian cells by interfering with ribosomal function. It is an aminoglycoside, similar in structure to neomycin, gentamycin, and kanamycin. Expression of the bacterial aminoglycoside phosphotransferase gene (APH), derived from Tn*5*, in mammalian cells results in detoxification of Geneticin® (Southern and Berg, 1982).

Geneticin® Selection Guidelines

Geneticin® is available for purchase (see page 11 for ordering). Use as follows:

- Prepare Geneticin® in a buffered solution (e.g. 100 mM HEPES, pH 7.3).
- Use 100–1000 µg/mL of Geneticin® in complete medium.
- Calculate concentration based on the amount of active drug (check the lot label).
- Test varying concentrations of Geneticin® on your cell line to determine the concentration that kills your cells (kill curve). Cells differ in their susceptibility to Geneticin®.

Cells will divide once or twice in the presence of lethal doses of Geneticin®, so the effects of the drug take several days to become apparent. Complete selection can take from 3–6 weeks of growth in selective medium.

Preparation of Cells for Purification

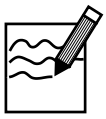
Use the procedure below to purify recombinant protein from a stable cell line. You will need 5×10^6 to 1×10^7 cells for purification on a 2 mL ProBond™ column (see ProBond™ Purification System manual).

1. Seed cells in either five T-75 flasks or 2 to 3 T-175 flasks.
2. Grow the cells in selective medium until they are 80–90% confluent.
3. Harvest the cells by treating with trypsin-EDTA for 2–5 minutes or by scraping the cells in PBS.
4. Inactivate the trypsin by diluting in fresh medium (if necessary), and transfer the cells to a sterile microcentrifuge tube.
5. Centrifuge the cells at 1500 rpm for 5 minutes. You may lyse the cells immediately or freeze in liquid nitrogen and store at –70°C until needed.

Lysis of Cells

If you are using ProBond™ resin, refer to the ProBond™ Purification System manual for details about sample preparation for chromatography.

If you are using other resin, refer to the manufacturer's instruction for recommendations on sample preparation.



Note

The C-terminal peptide containing the *myc* epitope and the polyhistidine tag will add approximately 3 kDa to the size of your protein.

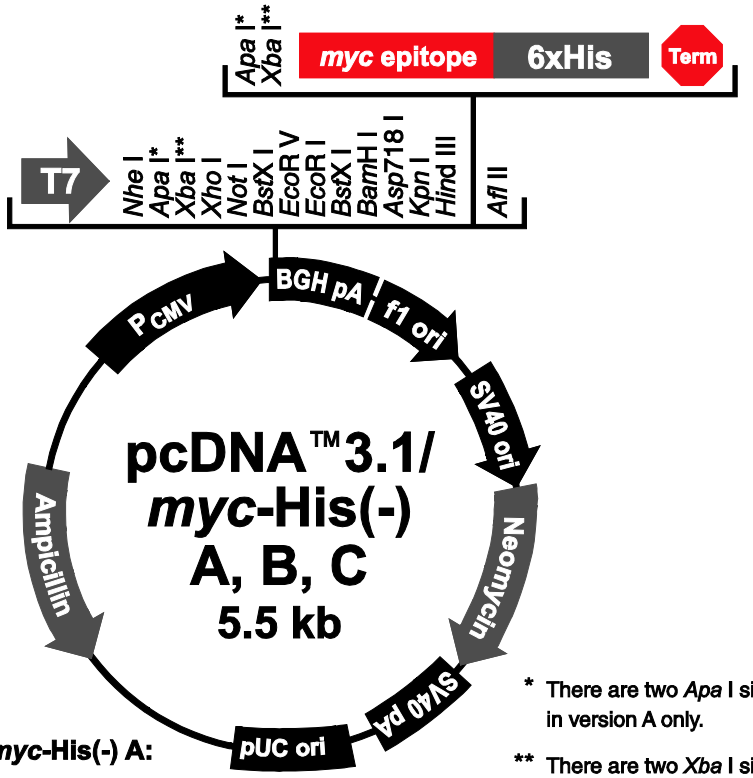
The size of the LacZ/myc-His fusion protein is approximately 121 kDa.

Appendix

Map of pcDNA™3.1/*myc*-His(-) A, B, and C Vectors

Map of pcDNA™3.1/*myc*-His(-)

The figure below summarizes the features of the pcDNA™3.1/*myc*-His(-) vectors. The sequences for pcDNA™3.1/*myc*-His(-) A, B, and C are available for downloading from www.lifetechnologies.com or from Technical Support (see page 12). Details of the multiple cloning sites for pcDNA™3.1/*myc*-His(-) A, B, and C are shown on pages 3–5.



* There are two *Apa* I sites in version A only.

** There are two *Xba* I sites in version B only.

Comments for pcDNA™3.1/*myc*-His(-) A: 5522 nucleotides

CMV promoter: bases 209-863
T7 promoter/priming site: bases 863-882
Multiple cloning site: bases 895-1006
myc epitope: bases 1007-1036
Polyhistidine tag: bases 1052-1069
BGH reverse priming site: bases 1113-1130
BGH polyadenylation signal: bases 1116-1343
f1 origin: bases 1389-1817
SV40 promoter and origin: bases 1844-2152
Neomycin resistance gene: bases 2227-3021
SV40 polyadenylation signal: bases 3195-3325
pUC origin: bases 3708-4381
Ampicillin resistance gene: bases 4526-5386 (complementary strand)

Features of pcDNA™3.1/*myc*-His(-) A, B, and C Vectors

Features of pcDNA™3.1/*myc*-His(-)

pcDNA™3.1/*myc*-His(-) A (5522 bp), pcDNA™3.1/*myc*-His(-) B (5520 bp), and pcDNA™3.1/*myc*-His(-) C (5521 bp) contain the following elements. All features have been functionally tested.

Feature	Benefit
Human cytomegalovirus (CMV) immediate-early promoter/enhancer	Allows efficient, high-level expression of your recombinant protein (Andersson *et al.*, 1989; Boshart *et al.*, 1985; Nelson *et al.*, 1987)
T7 promoter/priming site	Allows for *in vitro* transcription in the sense orientation and sequencing through the insert
Multiple cloning site in three reading frames	Allows insertion of your gene and facilitates cloning in frame with the C-terminal polyhistidine tag
myc epitope (*c-myc*)	Allows detection of your recombinant protein with the Anti-*myc* Antibody or Anti-*myc*-HRP Antibody (see page 11 for ordering) (Evan *et al.*, 1985)
C-terminal polyhistidine tag	Allows purification of your recombinant protein on metal-chelating resin such as ProBond™ In addition, the C-terminal polyhistidine tag is the epitope for the Anti-His (C-term) Antibody and the Anti-His (C-term)-HRP Antibody (see page 11 for ordering).
BGH reverse priming site	Allows sequencing through the insert
Bovine growth hormone (BGH) polyadenylation signal	Efficient transcription termination and polyadenylation of mRNA (Goodwin and Rottman, 1992)
f1 origin	Allows rescue of single-stranded DNA
SV40 early promoter and origin	Allows efficient, high-level expression of the neomycin resistance gene and episomal replication in cells expressing SV40 large T antigen
Neomycin (Geneticin®) resistance gene	Selection of stable transfectants in mammalian cells (Southern and Berg, 1982)
SV40 polyadenylation signal	Efficient transcription termination and polyadenylation of mRNA
pUC origin	High-copy number replication and growth in *E. coli*
Ampicillin resistance gene (β-lactamase)	Selection of vector in *E. coli*

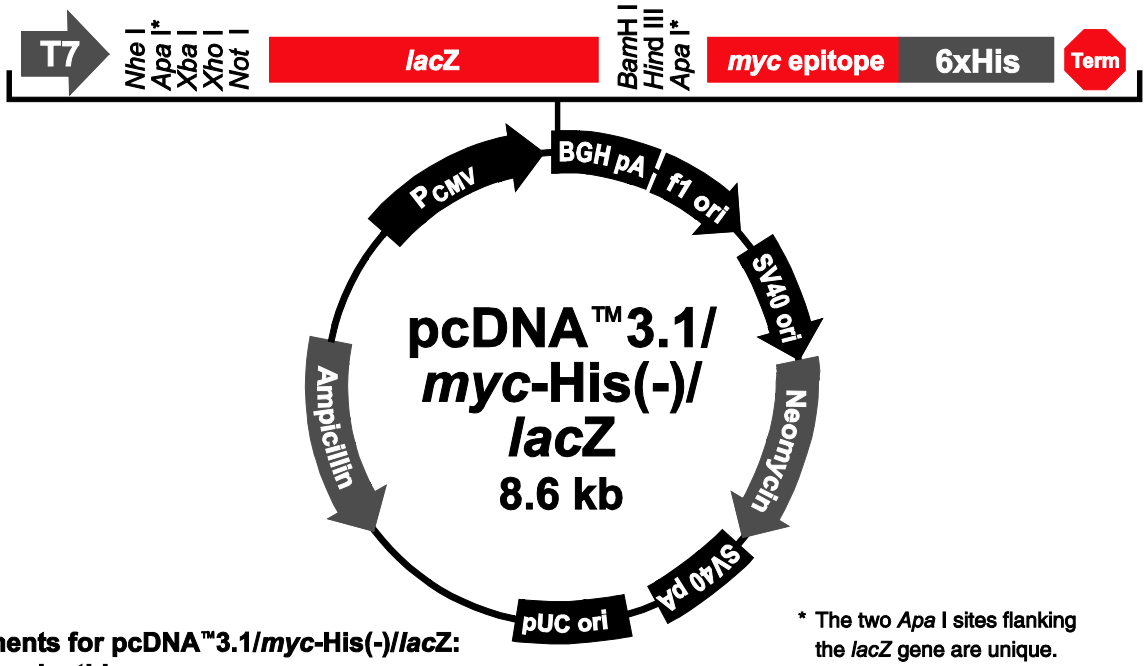
Map of pcDNA™3.1/*myc*-His(-)/*lacZ*

Description

pcDNA™3.1/*myc*-His(-)/*lacZ* is a 8592 bp control vector containing the gene for β-galactosidase. pcDNA™3.1/*myc*-His(-) A was digested with *Eco*R V. A 3.2 kb blunt *Sfu* I-*Not* I fragment containing the β-galactosidase gene was then ligated into pcDNA™3.1/*myc*-His(-) A in frame with the C-terminal peptide.

Map of Control Vector

The figure below summarizes the features of the pcDNA™3.1/*myc*-His(-)/*lacZ* vector. The nucleotide sequence for pcDNA™3.1/*myc*-His(-)/*lacZ* is available for downloading from www.lifetechnologies.com or from Technical Support (see page 12).



* The two *Apa* I sites flanking the *lacZ* gene are unique.

Comments for pcDNA™3.1/*myc*-His(-)/*lacZ*: 8592 nucleotides

CMV promoter: bases 209-863
T7 promoter/priming site: bases 863-882
LacZ ORF: bases 954-4010
myc epitope: bases 4077-4106
Polyhistidine tag: bases 4122-4139
BGH reverse priming site: bases 4183-4200
BGH polyadenylation signal: bases 4186-4413
f1 origin: bases 4459-4887
SV40 promoter and origin: bases 4914-5222
Neomycin resistance gene: bases 5297-6091
SV40 polyadenylation signal: bases 6265-6395
pUC origin: bases 6778-7451
Ampicillin resistance gene: bases 75967-8456 (complementary strand)

Accessory Products

Additional Products

Many of the reagents suitable for use with pcDNA™3.1/*myc*-His vectors are available for purchase. Ordering information for these reagents is provided below.

Item	Quantity	Cat. no.
Electrocomp™ TOP10F´	5 × 80 µL	C665-55
One Shot® TOP10F´ Chemically Competent *E. coli*	21 × 50 µL	C3030-03
β–Gal Assay Kit	1 kit	K1455-01
β–Gal Staining Kit	1 kit	K1465-01
Geneticin®	1 g 5 g 20 mL (50 mg/mL) 100 mL (50 mg/mL)	11811-023 11811-031 10131-035 10131-027

Detection of Recombinant Fusion Proteins

You can detect expression of your recombinant fusion protein from pcDNA™3.1/*myc*-His using the Anti-*Myc* and Anti-His antibodies available for purchase.

Epitope	Antibody	Cat. no.
c-myc	Anti-*Myc*	R950-25
	Anti-*Myc*-HRP	R951-25
C-terminal polyhistidine tag	Anti-His(C-term)	R930-25
	Anti-His(C-term)-HRP	R931-25

Purification of Recombinant Protein

The presence of the polyhistidine tag in pcDNA™3.1/*myc*-His allows purification of your recombinant fusion protein using a nickel-charged agarose resin such as ProBond™. Ordering information is provided below.

Item	Quantity	Cat. no.
ProBond™ Purification System	6 purifications	K850-01
ProBond™ Purification System with Anti-*myc*-HRP Antibody	1 Kit	K852-01
ProBond™	50 mL	R801-01
	150 mL	R801-15

Technical Support

Obtaining support

For the latest services and support information for all locations, go to www.lifetechnologies.com/support.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (techsupport@lifetech.com)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at www.lifetechnologies.com/support.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to www.lifetechnologies.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

Limited warranty

Life Technologies Corporation is committed to providing our customers with high-quality goods and services. Our goal is to ensure that every customer is 100% satisfied with our products and our service. If you should have any questions or concerns about a Life Technologies product or service, contact our Technical Support Representatives.

All Life Technologies products are warranted to perform according to specifications stated on the certificate of analysis. The Company will replace, free of charge, any product that does not meet those specifications. This warranty limits the Company's liability to only the price of the product. No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. The Company reserves the right to select the method(s) used to analyze a product unless the Company agrees to a specified method in writing prior to acceptance of the order.

Life Technologies makes every effort to ensure the accuracy of its publications, but realizes that the occasional typographical or other error is inevitable. Therefore the Company makes no warranty of any kind regarding the contents of any publications or documentation. If you discover an error in any of our publications, report it to our Technical Support Representatives.

Life Technologies Corporation shall have no responsibility or liability for any special, incidental, indirect or consequential loss or damage whatsoever. The above limited warranty is sole and exclusive. No other warranty is made, whether expressed or implied, including any warranty of merchantability or fitness for a particular purpose.

Purchaser Notification

Limited Use Label License No: Research Use Only

The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact outlicensing@lifetech.com or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

Limited Use Label License 6x His Tag

This product is licensed from Hoffmann-La Roche, Inc., Nutley, NJ and/or Hoffmann-LaRoche Ltd., Basel, Switzerland and is provided only for use in research. Information about licenses for commercial use is available from QIAGEN GmbH, Max-Volmer-Str. 4, D-40724 Hilden, Germany.

References

Andersson, S., Davis, D. L., Dahlbäck, H., Jörnvall, H., and Russell, D. W. (1989). Cloning, Structure, and Expression of the Mitochondrial Cytochrome P-450 Sterol 26-Hydroxylase, a Bile Acid Biosynthetic Enzyme. J. Biol. Chem. *264*, 8222-8229.

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1994). Current Protocols in Molecular Biology (New York: Greene Publishing Associates and Wiley-Interscience).

Boshart, M., Weber, F., Jahn, G., Dorsch-Häsler, K., Fleckenstein, B., and Schaffner, W. (1985). A Very Strong Enhancer is Located Upstream of an Immediate Early Gene of Human Cytomegalovirus. Cell *41*, 521-530.

Chen, C., and Okayama, H. (1987). High-Efficiency Transformation of Mammalian Cells by Plasmid DNA. Mol. Cell. Biol. *7*, 2745-2752.

Chu, G., Hayakawa, H., and Berg, P. (1987). Electroporation for the Efficient Transfection of Mammalian Cells with DNA. Nucleic Acids Res. *15*, 1311-1326.

Evan, G. I., Lewis, G. K., Ramsay, G., and Bishop, V. M. (1985). Isolation of Monoclonal Antibodies Specific for *c-myc* Proto-oncogene Product. Mol. Cell. Biol. *5*, 3610-3616.

Felgner, P. L., Holm, M., and Chan, H. (1989). Cationic Liposome Mediated Transfection. Proc. West. Pharmacol. Soc. *32*, 115-121.

Felgner, P. L., and Ringold, G. M. (1989). Cationic Liposome-Mediated Transfection. Nature *337*, 387-388.

Goodwin, E. C., and Rottman, F. M. (1992). The 3´-Flanking Sequence of the Bovine Growth Hormone Gene Contains Novel Elements Required for Efficient and Accurate Polyadenylation. J. Biol. Chem. *267*, 16330-16334.

Kozak, M. (1987). An Analysis of 5´-Noncoding Sequences from 699 Vertebrate Messenger RNAs. Nucleic Acids Res. *15*, 8125-8148.

Kozak, M. (1990). Downstream Secondary Structure Facilitates Recognition of Initiator Codons by Eukaryotic Ribosomes. Proc. Natl. Acad. Sci. USA *87*, 8301-8305.

Miller, J. H. (1972). Experiments in Molecular Genetics (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory).

Nelson, J. A., Reynolds-Kohler, C., and Smith, B. A. (1987). Negative and Positive Regulation by a Short Segment in the 5´-Flanking Region of the Human Cytomegalovirus Major Immediate-Early Gene. Mol. Cell. Biol. *7*, 4125-4129.

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, Second Edition (Plainview, New York: Cold Spring Harbor Laboratory Press).

Shigekawa, K., and Dower, W. J. (1988). Electroporation of Eukaryotes and Prokaryotes: A General Approach to the Introduction of Macromolecules into Cells. BioTechniques *6*, 742-751.

Southern, P. J., and Berg, P. (1982). Transformation of Mammalian Cells to Antibiotic Resistance with a Bacterial Gene Under Control of the SV40 Early Region Promoter. J. Molec. Appl. Gen. *1*, 327-339.

Wigler, M., Silverstein, S., Lee, L.-S., Pellicer, A., Cheng, Y.-C., and Axel, R. (1977). Transfer of Purified Herpes Virus Thymidine Kinase Gene to Cultured Mouse Cells. Cell *11*, 223-232.

Headquarters
5791 Van Allen Way | Carlsbad, CA 92008 USA | Phone +1 760 603 7200 | Toll Free in USA 800 955 6288
For support visit www.invitrogen.com/support or email techsupport@invitrogen.com

www.lifetechnologies.com

life technologies™