pcDNA™3.1/myc-His(-) A, B, and C

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# Contents

Kit Contents and Storage.................................................................................................................................................. iv

**Introduction** ............................................................................................................................................................... 1
  
  Product Overview ........................................................................................................................................................ 1

**Methods** ...................................................................................................................................................................... 2
  
  Cloning into pcDNA™3.1/ myc-His(-) A, B, and C ................................................................................................. 2
  
  General Guidelines and Special Information........................................................................................................... 6

**Appendix** .................................................................................................................................................................. 8
  
  Map of pcDNA™3.1/ myc-His(-) A, B, and C Vectors ............................................................................................... 8
  
  Features of pcDNA™3.1/ myc-His(-) A, B, and C Vectors......................................................................................... 9
  
  Map of pcDNA™3.1/ myc-His(-)/ lacZ ....................................................................................................................... 10
  
  Accessory Products ...................................................................................................................................................... 11
  
  Technical Support......................................................................................................................................................... 12
  
  Purchaser Notification ............................................................................................................................................. 13
  
  References................................................................................................................................................................. 14
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.

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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(-)/lacZ, 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 (recA) and endonuclease A-deficient (endA). 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 recA, endA 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)NNATGG

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
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 BstX I sites and two Apa I sites in the polylinker.*
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](http://www.lifetechnologies.com/support) or from Technical Support (see page 12).

*Note that there are two BstX I sites and two Xba I sites in the polylinker.*
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 NheI site and the ApaI site, and the other is located before the BamHI site. This means the 3' cloning site must be either BamHI, KpnI (Asp718 I), or HindIII 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 recA, endA 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(-)/lacZ 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*
### General Guidelines and Special Information, Continued

#### 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 Tn5, 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 × 10⁶ to 1 × 10⁷ 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.

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

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.

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

- CMV promoter: bases 209-863
- T7 promoter/priming sites: bases 869-882
- Multiple cloning site: bases 895-1006
- myc epitope: bases 1007-1038
- Polysine tag: bases 1052-1069
- BGH reverse priming site: bases 1113-1136
- BGH polyadenylation signal: bases 1116-1343
- t1 origin: bases 1369-1817
- SV40 promoter and origin: bases 1844-2152
- Neomycin resistance gene: bases 2227-3021
- SV40 polyadenylation signal: bases 3185-3325
- pUC origin: bases 3708-4381
- Ampicillin resistance gene: bases 4528-5386 (complementary strand)
Features of pcDNA™3.1/myc-His(-) A, B, and C Vectors

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.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human cytomegalovirus (CMV) immediate-early promoter/enhancer</td>
<td>Allows efficient, high-level expression of your recombinant protein (Andersson et al., 1989; Boshart et al., 1985; Nelson et al., 1987)</td>
</tr>
<tr>
<td>T7 promoter/priming site</td>
<td>Allows for in vitro transcription in the sense orientation and sequencing through the insert</td>
</tr>
<tr>
<td>Multiple cloning site in three reading frames</td>
<td>Allows insertion of your gene and facilitates cloning in frame with the C-terminal polyhistidine tag</td>
</tr>
<tr>
<td>myc epitope (c-myc)</td>
<td>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)</td>
</tr>
<tr>
<td>C-terminal polyhistidine tag</td>
<td>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).</td>
</tr>
<tr>
<td>BGH reverse priming site</td>
<td>Allows sequencing through the insert</td>
</tr>
<tr>
<td>Bovine growth hormone (BGH) polyadenylation signal</td>
<td>Efficient transcription termination and polyadenylation of mRNA (Goodwin and Rottman, 1992)</td>
</tr>
<tr>
<td>f1 origin</td>
<td>Allows rescue of single-stranded DNA</td>
</tr>
<tr>
<td>SV40 early promoter and origin</td>
<td>Allows efficient, high-level expression of the neomycin resistance gene and episomal replication in cells expressing SV40 large T antigen</td>
</tr>
<tr>
<td>Neomycin (Geneticin®) resistance gene</td>
<td>Selection of stable transfectants in mammalian cells (Southern and Berg, 1982)</td>
</tr>
<tr>
<td>SV40 polyadenylation signal</td>
<td>Efficient transcription termination and polyadenylation of mRNA</td>
</tr>
<tr>
<td>pUC origin</td>
<td>High-copy number replication and growth in E. coli</td>
</tr>
<tr>
<td>Ampicillin resistance gene (β-lactamase)</td>
<td>Selection of vector in E. coli</td>
</tr>
</tbody>
</table>
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 EcoR 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.

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).

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

CMV promoter: bases 209-883
T7 promoter/priming site: bases 883-882
LacZ ORF: bases 864-4010
myc epitope: bases 4077-4106
Polyhistidine tag: bases 4122-4139
BGH reverse priming site: bases 4153-4200
BGH polyadenylation signal: bases 4189-4413
fl origin: bases 4459-4487
SV40 promoter and origin: bases 4914-5222
Neomycin resistance gene: bases 5287-6091
SV40 polyadenylation signal: bases 6235-6386
pUC origin: bases 6779-7451
Ampicillin resistance gene: bases 75967-8458 (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.

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

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.

<table>
<thead>
<tr>
<th>Epitope</th>
<th>Antibody</th>
<th>Cat. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-myc</td>
<td>Anti-Myc</td>
<td>R950-25</td>
</tr>
<tr>
<td></td>
<td>Anti-Myc-HRP</td>
<td>R951-25</td>
</tr>
<tr>
<td>C-terminal polyhistidine tag</td>
<td>Anti-His(C-term)</td>
<td>R930-25</td>
</tr>
<tr>
<td></td>
<td>Anti-His(C-term)-HRP</td>
<td>R931-25</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Cat. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ProBond™ Purification System</td>
<td>6 purifications</td>
<td>K850-01</td>
</tr>
<tr>
<td>ProBond™ Purification System with Anti-myca-HRP Antibody</td>
<td>1 Kit</td>
<td>K852-01</td>
</tr>
<tr>
<td>ProBond™</td>
<td>50 mL</td>
<td>R801-01</td>
</tr>
<tr>
<td></td>
<td>150 mL</td>
<td>R801-15</td>
</tr>
</tbody>
</table>
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.

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References


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