invitrogen™

# pcDNA™ 4/*myc*-His A, B, and C

**Catalog no. V863-20**

**Rev. Date: 27 October 2010**

Manual part no. 25-0236

MAN0000078

User Manual

# Table of Contents

# Kit Contents and Storage

## Shipping and Storage

pcDNA™4/*myc*-His vectors are shipped on wet ice. Upon receipt, store vectors at –20°C.

## Kit Contents

All vectors are supplied as detailed below. **Store the vectors at –20°C.**

| Item | Composition | Amount |
|---|---|---|
| pcDNA™4/*myc*-His A, B, and C | 40 µL of 0.5 µg/µL vector in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 | 20 µg |
| pcDNA™4/*myc*-His/*lacZ* | 40 µL of 0.5 µg/µL vector in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 | 20 µg |

# Introduction

## Product Overview

### Description of the System

pcDNA™4/*myc*-His A, B, and C are 5.1 kb vectors designed for overproduction of recombinant proteins in mammalian cell lines. Features of the vectors allow purification and detection of expressed proteins (see pages 11-12 for more information). High-level stable and transient expression can be carried out in most mammalian cells. The vectors contain the following elements:

- Human cytomegalovirus immediate-early (CMV) promoter for high-level expression in a wide range of mammalian cells
- Three reading frames to facilitate in-frame cloning with a C-terminal peptide encoding the *myc* (*c-myc*) epitope and a polyhistidine (6xHis) metal-binding tag
- Zeocin™ resistance gene for selection of stable cell lines (Mulsant *et al.*, 1988) (see page 14 for more information).
- Episomal replication in cell lines that are latently infected with SV40 or that express the SV40 large T antigen (e.g., COS7).

The control plasmid, pcDNA™4/*myc*-His/*lac*Z 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™4/*myc*-His:

1. Consult the multiple cloning sites described on pages 3-4 to determine which vector (A, B, or C) to use for cloning 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 to 100 µg/mL ampicillin or 25 to 50 µg/mL Zeocin™ in Low Salt LB. For more information, see page 16.
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. For antibodies to the *myc* epitope or the C-terminal polyhistidine tag, see page 18.
7. To purify your recombinant protein, you may use metal-chelating resin such as ProBond™. ProBond™ resin is available separately (see page 17).

# Methods

## Cloning into pcDNA™4/*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´, DH5αF´, JM109, and INVαF´. 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 from Invitrogen as chemically competent or electrocompetent cells (see page 17).

**Transformation Method**

You may use any method of your choice for transformation. Chemical transformation is the most convenient for most researchers. Electroporation is the most efficient and the method of choice for large plasmids.

**Maintaining pcDNA™4/*myc*-His**

To propagate and maintain the pcDNA™4/*myc*-His vectors, use a small amount of the supplied 0.5 µg/µL stock solution in TE, pH 8.0 to transform a *rec*A, *end*A *E. coli* strain like TOP10F´, DH5α, JM109, or equivalent. Select transformants on LB plates containing 50 to 100 µg/mL ampicillin or 25 to 50 µg/mL Zeocin™ in Low Salt LB. Be sure to prepare a glycerol stock of each plasmid for long-term storage (see page 5).

**Cloning Considerations**

Your insert should contain a Kozak translation initiation sequence for proper initiation of translation (Kozak, 1987; Kozak, 1991; Kozak, 1990). An example of a Kozak consensus sequence is provided below. Note that other sequences are possible, but the A at position -3 and the G at position +4 are the most critical for function (shown in bold). The ATG initiation codon is shown underlined.

**A**NN<u>ATG</u>**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 3-4 to develop a cloning strategy.

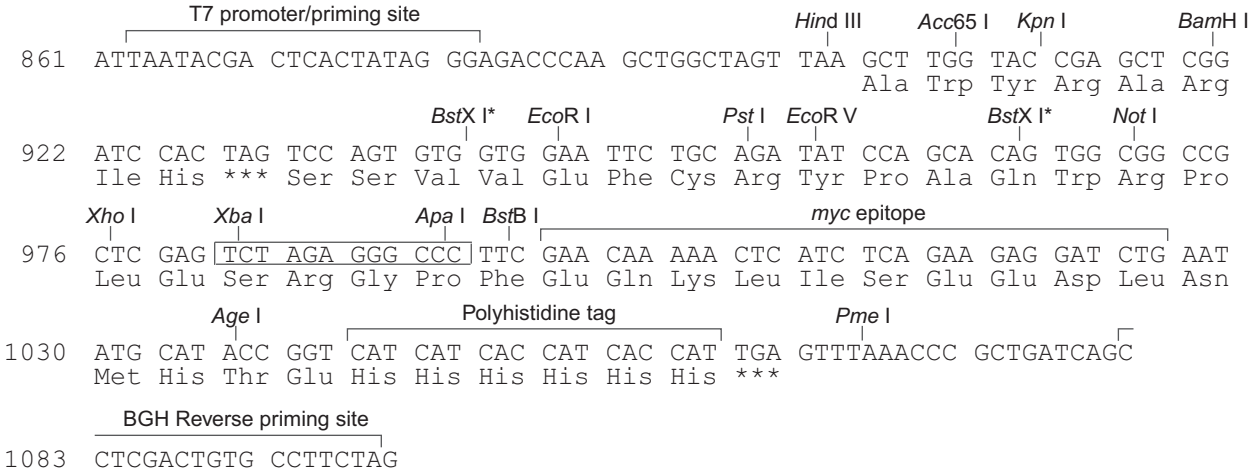
If you wish to express your protein WITHOUT the C-terminal peptide, be sure to include a stop codon.

*Continued on next page*

# Cloning into pcDNA™4/*myc*-His A, B, and C, Continued

## Multiple Cloning Site of Version A

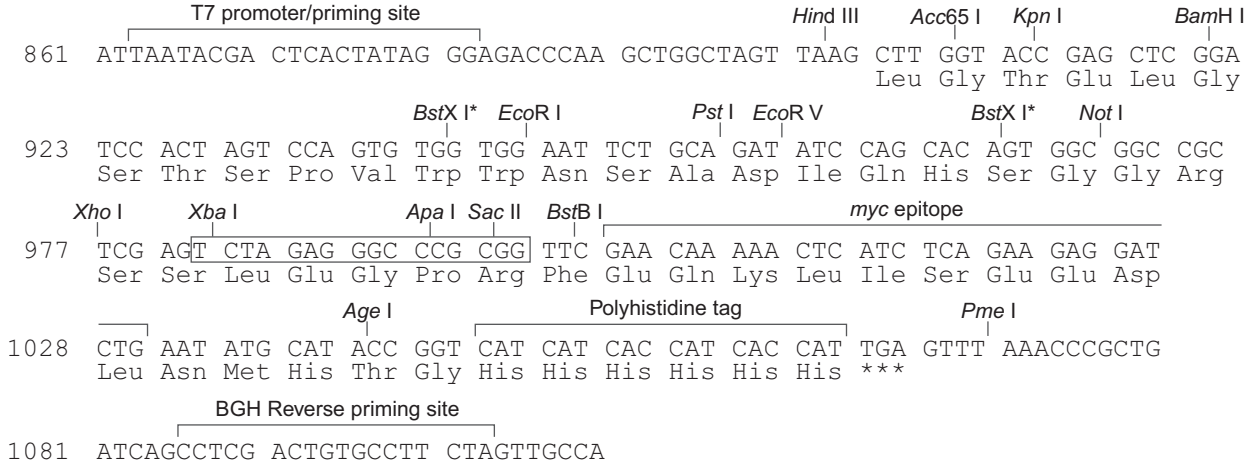
Below is the multiple cloning site for pcDNA™4/*myc*-His A. Restriction sites are labeled to indicate the cleavage site. The boxed nucleotides indicate the variable region. **Note that there is a stop codon between the *Bam*H I site and the *Bst*X I site.** The multiple cloning site has been confirmed by sequencing and functional testing. The vector sequence of pcDNA™4/*myc*-His A is available for downloading from our website (www.invitrogen.com) or from **Technical Support** (see page 19).




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

## Multiple Cloning Site of Version B

Below is the multiple cloning site for pcDNA™4/*myc*-His B. Restriction sites are labeled to indicate the cleavage site. The boxed nucleotides indicate the variable region. The multiple cloning site has been confirmed by sequencing and functional testing. The vector sequence of pcDNA™4/*myc*-His B is available for downloading from our website (www.invitrogen.com) or from **Technical Support** (see page 19).



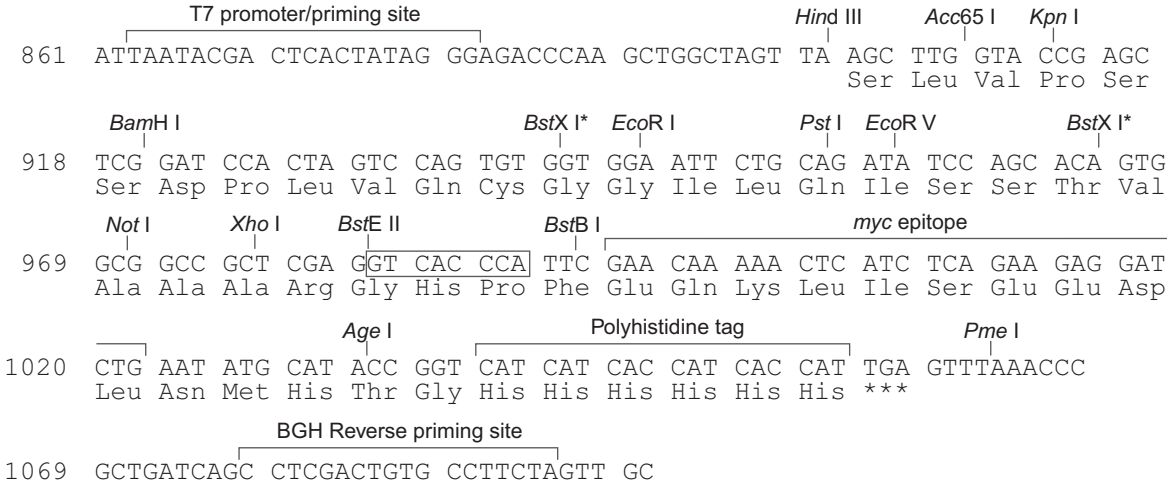

*Note that there are two *Bst*X I sites in the polylinker.

*Continued on next page*

# Cloning into pcDNA™4/*myc*-His A, B, and C, Continued

## Multiple Cloning Site of Version C

Below is the multiple cloning site for pcDNA™4/*myc*-His C. Restriction sites are labeled to indicate the cleavage site. The boxed nucleotides indicate the variable region. The multiple cloning site has been confirmed by sequencing and functional testing. The vector sequence of pcDNA™4/*myc*-His C is available for downloading from our website (www.invitrogen.com) or from **Technical Support** (see page 19).




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

## *E. coli* Transformation

Transform your ligation mixtures into a competent *rec*A, *end*A *E. coli* strain (e.g., TOP10F´, DH5α) and select on LB plates containing 50–100 µg/mL ampicillin or 25–50 µg/mL Zeocin™ in Low Salt LB (see page 16). Select 10–20 clones and analyze for the presence and orientation of your insert.

**Important**

Any *E. coli* strain that contains the complete Tn*5* transposable element (i.e., DH5αF´IQ, SURE, SURE2) encodes the *ble* (bleomycin resistance gene). These strains will confer resistance to Zeocin™. For the most efficient selection, we recommend that you choose an *E. coli* strain that does not contain the Tn*5* gene (i.e., TOP10, DH5α, DH10, etc.).

*Continued on next page*


We recommend that you sequence your construct with the T7 Forward and BGH Reverse primers to confirm that your gene is fused in frame with the *myc* epitope and the C-terminal polyhistidine tag. For ordering primers, see page 17.

### Preparing a Glycerol Stock

Once you have identified the correct clone, be sure to purify the colony and make a glycerol stock for long-term storage. It is also a good idea to keep a DNA stock of your plasmid at –20°C.

1. Streak the original colony out on an LB plate containing 50 µg/mL ampicillin or 25 µg/mL Zeocin™ in Low Salt LB. Incubate the plate at 37°C overnight.
2. Isolate a single colony and inoculate into 1–2 mL of LB with 50 µg/mL ampicillin.
3. Grow the culture to mid-log phase ($OD_{600}$ = 0.5–0.7).
4. Mix 0.85 mL of culture with 0.15 mL of sterile glycerol and transfer to a cryovial.
5. Store at –80°C.

# Transfection and Analysis

## Introduction

Once you have confirmed that your construct is in the correct orientation and fused in frame with the C-terminal peptide, you are ready to transfect your cell line of choice. We recommend that you include the positive control vector and a mock transfection to evaluate your results.

## 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 lipids, decreasing transfection efficiency. We recommend isolating plasmid DNA using the PureLink™ HiPure Miniprep Kit or the PureLink™ HiPure Midiprep Kit (see page 17 for ordering information).

## 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. We recommend 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* (see page 21).

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). Invitrogen offers the Lipofectamine™ 2000 Reagent for mammalian transfection For more details, call **Technical Support** (see page 19) or visit our website at www.invitrogen.com.

## Positive Control

pcDNA™4/*myc*-His/*lac*Z is provided as a positive control vector for mammalian cell transfection and expression (see page 13) and may be used to optimize transfection conditions for your cell line. The gene encoding β-galactosidase is expressed in mammalian cells under the control of the CMV promoter. A successful transfection will result in β-galactosidase expression that can be easily assayed (see below).

## 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. Invitrogen offers the β-Gal Assay Kit and the β-Gal Staining Kit for fast and easy detection of β-galactosidase expression (see page 17).

*Continued on next page*

### Detecting Fusion Proteins

Several antibodies are available from Invitrogen to detect expression of your fusion protein from pcDNA™4/*myc*-His (see page 18).

To detect fusion protein by western blot, you will need to prepare a cell lysate from transfected cells. We recommend that you perform a time course to optimize expression of the fusion protein (*e.g.* 24, 48, 72 hours, etc. after transfection). To lyse cells:

1. Wash cell monolayers (~$10^6$ cells) once with phosphate-buffered saline (PBS).
2. Scrape cells into 1 mL PBS and pellet the cells at 1,500 × *g* for 5 minutes.
3. Resuspend in 50 µL Cell Lysis Buffer (see page 16). Other lysis buffers may be suitable.
4. Incubate cell suspension at 37°C for 10 minutes to lyse the cells.
5. Centrifuge the cell lysate at 10,000 × g for 10 minutes to pellet nuclei and transfer the supernatant to a fresh tube. Assay the lysate for protein concentration.

   **Note:** Do not use protein assays utilizing Coomassie® Blue or other dyes. NP-40 interferes with the binding of the dye with the protein.
6. Add SDS-PAGE sample buffer to a final concentration of 1X and boil the sample for 5 minutes.
7. Load 20 µg of lysate onto an SDS-PAGE gel and electrophorese. Use the appropriate percentage of acrylamide to resolve your fusion protein.

**Note**

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

### Purification

You will need 5 × $10^6$ to 1 × $10^7$ **transfected** cells for purification of your protein on a 2 mL ProBond™ column (or other metal-chelating column). Refer to the manufacturer's instructions before attempting to purify your fusion protein. To prepare cells for lysis, refer to the protocol on page 10.

# Creating Stable Cell Lines

## Introduction

The pcDNA™4/*myc*-His vectors contain the Zeocin™ resistance gene for selection of stable cell lines using Zeocin™. We recommend that you test the sensitivity of your mammalian host cell to Zeocin™ as natural resistance varies among cell lines. General information and guidelines are provided below for your convenience. For more information about Zeocin™, refer to page 14.

## Effect of Zeocin™ on Sensitive and Resistant Cells

The method of killing with Zeocin™ is quite different from neomycin and hygromycin. Cells do not round up and detach from the plate. Sensitive cells will exhibit the following morphological changes upon exposure to Zeocin™:

- Vast increase in size
- Abnormal cell shape
- Presence of large empty vesicles in the cytoplasm (breakdown of the endoplasmic reticulum and golgi apparatus or scaffolding proteins)
- Breakdown of plasma and nuclear membrane (appearance of many holes in these membranes). Eventually, these "cells" will completely break down and only "strings" of protein will remain.

Zeocin™-resistant cells should continue to divide at regular intervals to form distinct colonies. There should not be any distinct morphological changes in Zeocin™-resistant cells when compared to cells not under selection with Zeocin™.

## Selection in Mammalian Cell Lines

To generate a stable cell line expressing your protein, you need to determine the minimum concentration of Zeocin™ required to kill your untransfected host cell line. Typically, concentrations between 50 and 1,000 µg/mL Zeocin™ are sufficient to kill the untransfected host cell line. Test a range of concentrations (see below) to ensure that you determine the minimum concentration necessary for your cell line.

1. Seed cells ($2 \times 10^5$ cells/60 mm plate) for each time point and allow cells to adhere overnight.
2. The next day, substitute culture medium with medium containing varying concentrations of Zeocin™ (e.g., 0, 50, 125, 250, 500, 750, and 1,000 µg/mL).
3. Replenish the selective medium every 3–4 days, and observe the percentage of surviving cells.
4. Count the number of viable cells at regular intervals to determine the appropriate concentration of Zeocin™ that prevents growth.

*Continued on next page*

### Possible Sites for Linearization

To obtain stable transfectants, you may choose to linearize your vector before transfection. While linearizing your vector may not improve the efficiency of transfection, it increases the chances that the vector does not integrate in a way that disrupts the gene of interest. The table below lists unique sites that may be used to linearize your construct prior to transformation. **Other restriction sites are possible. Note that the cleavage site is indicated for versions A, B, and C of pcDNA™4/*myc*-His.** Be sure that your insert does not contain the restriction enzyme site you wish to use to linearize your vector.

| Enzyme | Restriction Site (bp) (A,B,C) | Location | Supplier |
|---|---|---|---|
| Bgl II | 13 | Upstream of CMV promoter | Many |
| Mfe I | 161 | Upstream of CMV promoter | New England Biolabs |
| Nru I | 209 | Upstream of CMV promoter | Many |
| Mlu I | 229 | 5´ end of CMV promoter | Many |
| Bst1107 I | 2881 (A), 2885 (B), 2877 (C) | End of SV40 poly A | AGS*, Fermentas, Takara, Boehringer-Mannhiem |
| Eam1105 I | 4153 (A), 4157 (B), 4149 (C) | Ampicillin gene | AGS*, Fermentas, Takara |
| Fsp I | 4375 (A), 4379 (B), 4371 (C) | Ampicillin gene | Many |
| Pvu I | 4523 (A), 4527 (B), 4519 (C) | Ampicillin gene | Many |
| Sca I | 4633 (A), 4637 (B), 4629 (C) | Ampicillin gene | Many |
| Ssp I | 4957 (A), 4961 (B), 4953 (C) | Ampicillin gene | Many |

*Angewandte Gentechnologie Systeme

### Selecting Stable Integrants

Once the appropriate Zeocin™ concentration is determined, you can generate a stable cell line with your construct.

1. Transfect your cells using the appropriate protocol for your cell line. Include a sample of untransfected cells as a negative control.
2. After transfection, wash the cells once with 1X PBS and add fresh medium to the cells.
3. 48 hours after transfection, split the cells into fresh medium containing Zeocin™ at the appropriate concentration for your cell line. Split the cells such that they are no more than 25% confluent.
4. Replenish selective medium every 3–4 days until Zeocin™-resistant colonies are detected.
5. Pick and expand colonies.

*Continued on next page*

### Preparing Cells for Lysis

Use the procedure below to prepare cells for lysis prior to purification of your protein on ProBond™. You will need $5 \times 10^6$ to $1 \times 10^7$ cells for purification of your protein on a 2 mL ProBond™ column (see ProBond™ Purification System manual).

1. Seed cells in 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 to 5 minutes or by scraping the cells in PBS.
4. Inactivate the trypsin by diluting with fresh medium (if necessary) and transfer the cells to a sterile microcentrifuge tube.
5. Centrifuge the cells at 240 × *g* for 5 minutes. Resuspend the cell pellet in PBS.
6. Centrifuge the cells at 240 × *g* for 5 minutes. You may lyse the cells immediately or freeze in liquid nitrogen and store at –80°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.

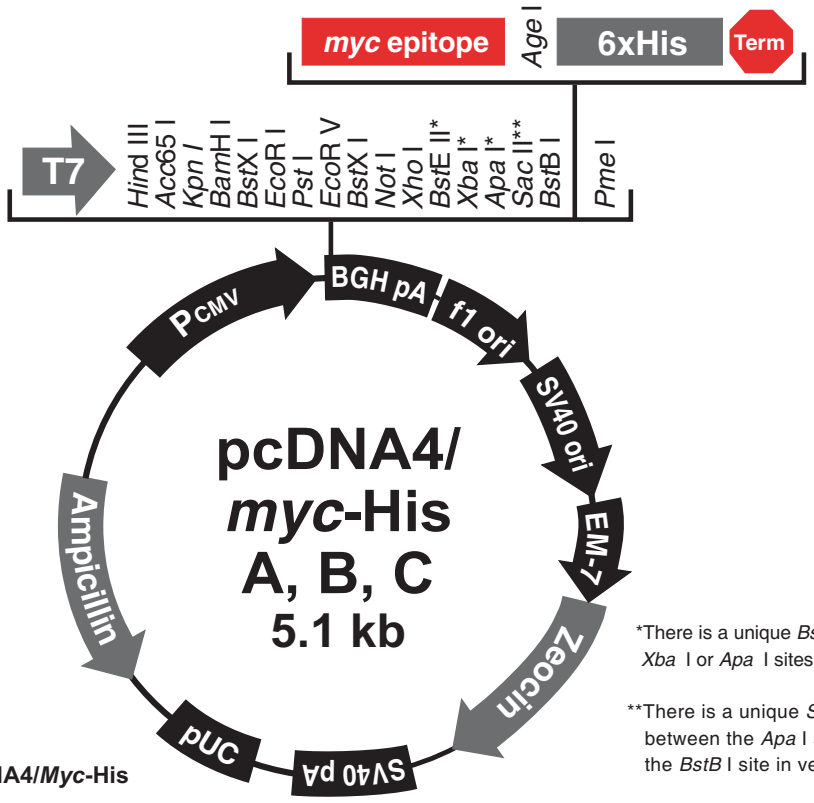
If you are using other metal-chelating resin, refer to the manufacturer's instruction for recommendations on sample preparation.

# Appendix

## pcDNA™4/*myc*-His Vector

### Map of pcDNA™4/*myc*-His

The figure below summarizes the features of the pcDNA™4/*myc*-His vectors. The vector sequences for pcDNA™4/*myc*-His A, B, and C are available for downloading from our website (www.invitrogen.com) or from **Technical Support** (see page 19).




*There is a unique *Bst*E II site, but no *Xba* I or *Apa* I sites in version C.

**There is a unique *Sac* II site between the *Apa* I site and the *BstB* I site in version B only.

**Comments for pcDNA4/*Myc*-His**
**5075 nucleotides**

CMV promoter: bases 209-863
T7 promoter/priming site: bases 863-882
Multiple cloning site: bases 902-999
*myc* epitope: bases 997-1026
Polyhistidine tag: bases 1042-1059
BGH reverse priming site: bases 1082-1099
BGH polyadenylation signal: bases 1085-1312
f1 origin: bases 1358-1786
SV40 promoter and origin: bases 1814-2122
EM-7 promoter: bases 2170-2225
Zeocin resistance gene: bases 2244-2618
SV40 polyadenylation signal: bases 2748-2878
pUC origin: bases 3261-3934
Ampicillin resistance gene: bases 4079-4939

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# pcDNA™4/*myc*-His Vector, Continued

## Features of pcDNA™4/*myc*-His

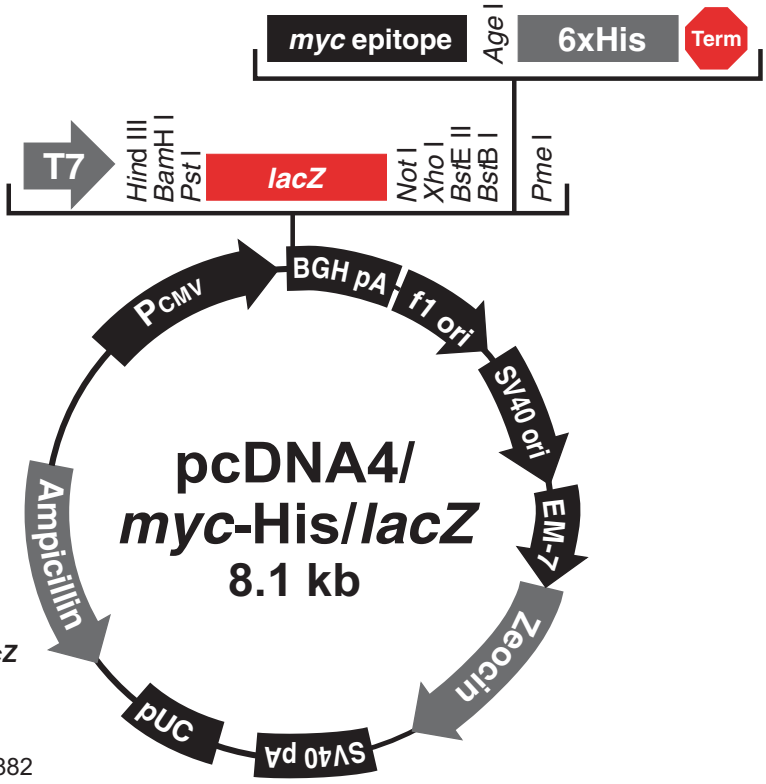
pcDNA™4/*myc*-His A (5075 bp), pcDNA™4/*myc*-His B (5079 bp), and pcDNA™4/*myc*-His C (5071 bp) contain the following elements. All features have been functionally tested.

| Feature | Benefit |
|---|---|
| Human cytomegalovirus (CMV) immediate-early promoter/enhancer | Permits 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 *myc* epitope and polyhistidine C-terminal tag. |
| *myc* epitope (Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu) | Allows detection of your recombinant protein with the Anti-*myc* Antibody the Anti-*myc*-HRP Antibody, or the Anti-*myc*-AP Antibody (Evans et al., 1985) (see page 18 for ordering). |
| C-terminal polyhistidine (6xHis) tag | Permits purification of your recombinant protein on metal-chelating resin such as ProBond™.<br>In addition, the C-terminal polyhistidine tag is the epitope for the Anti-His(C-term) Antibody, the Anti-His (C-term)-HRP Antibody and the Anti-His(C-term)-AP (Lindner et al., 1997) (see page 18). |
| BGH reverse priming site | Permits 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 Zeocin™ resistance gene and episomal replication in cells expressing the SV40 large T antigen. |
| EM-7 promoter | Synthetic promoter based on the bacteriophage T7 promoter for expression of the Zeocin™ resistance gene in *E. coli.* |
| Zeocin™ resistance gene | Selection of transformants in *E. coli* and stable transfectants in mammalian cells (Drocourt et al., 1990; Mulsant et al., 1988). |
| 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 transformants in *E. coli.* |

# pcDNA™4/*myc*-His/*lacZ*

## Map of Control Vector

pcDNA™4/*myc*-His/*lacZ* is a 8120 bp control vector containing the gene for β–galactosidase. This vector was constructed by ligating a 3,880 bp *Bam*H I-*Stu* I fragment containing the CMV promoter and the Zeocin™ resistance gene from pcDNA™4/*myc*-His B to a 4,240 bp *Bam*H I-*Stu* I fragment containing the *lacZ* gene, *myc* epitope, and polyhistidine tag from pcDNA™3.1/*myc*-His/*lacZ*. The figure below summarizes the features of the pcDNA™4/*myc*-His/*lacZ* vector. The vector sequence for pcDNA™4/*myc*-His/lacZ is available for downloading from our website (www.invitrogen.com) or by contacting **Technical Support** (see page 19).




**Comments for pcDNA4/*Myc*-His/*lacZ***
**8120 nucleotides**

CMV promoter: bases 209-863
T7 promoter/priming site: bases 863-882
LacZ ORF: bases 963-4019
*myc* epitope: bases 4044-4073
Polyhistidine tag: bases 4089-4106
BGH reverse priming site: bases 4129-4146
BGH polyadenylation signal: bases 4132-4359
f1 origin: bases 4405-4833
SV40 promoter and origin: bases 4861-5169
EM-7 promoter: bases 5217-5272
Zeocin resistance gene: bases 5291-5665
SV40 polyadenylation signal: bases 5795-5925
pUC origin: bases 6308-6981
Ampicillin resistance gene: bases 7126-7986

# Zeocin™

## Introduction

The pcDNA™4/*myc*-His vectors contain the Zeocin™ resistance gene for selection of stable cell lines using Zeocin™. We recommend that you test the sensitivity of your mammalian host cell to Zeocin™ as natural resistance varies among cell lines. General information and guidelines are provided in this section for your convenience.

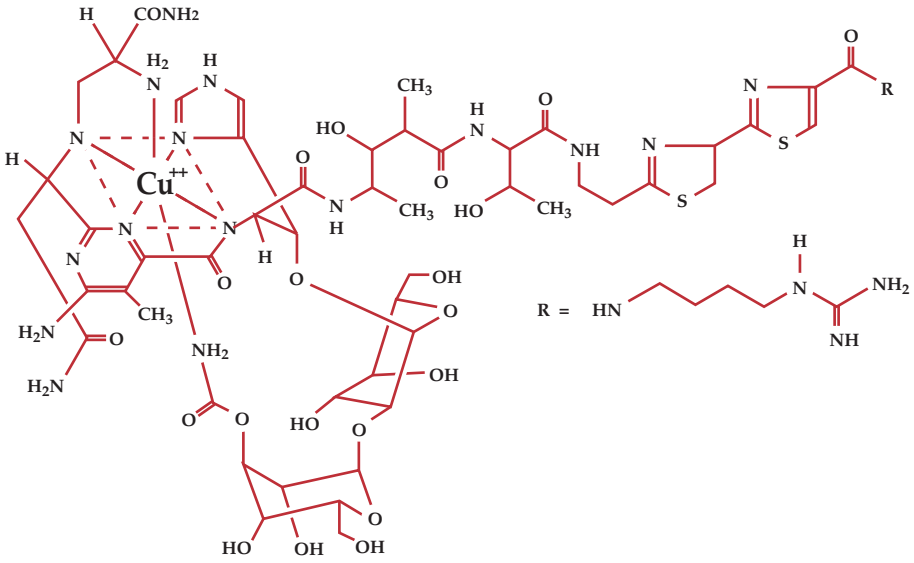
## Zeocin™

Zeocin™ is a member of the bleomycin/phleomycin family of antibiotics isolated from *Streptomyces*. Antibiotics in this family are broad spectrum antibiotics that act as strong anti-bacterial and anti-tumor drugs. They show strong toxicity against bacteria, fungi (including yeast), plants, and mammalian cells.

The Zeocin™ resistance protein has been isolated and characterized (Calmels *et al.*, 1991; Drocourt *et al.*, 1990). This protein, the product of the *Sh ble* gene (*Streptoalloteichus hindustanus* bleomycin gene), is a 13.7 kDa protein that binds Zeocin™ in a stoichiometric manner to inhibit its DNA strand cleavage activity. Expression of this protein in eukaryotic and prokaryotic hosts confers resistance to Zeocin™.

## Molecular Weight, Formula, and Structure

The formula for Zeocin™ is $C_{55}H_{86}O_{21}N_{20}S_2Cu$-HCl and the molecular weight is 1,527.5 daltons. Zeocin™ is an HCl salt. The diagram below shows the structure of Zeocin™.

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# Zeocin™, Continued

## Applications of Zeocin™

Zeocin™ is used for selection in mammalian cells (Mulsant *et al.*, 1988); plants (Perez *et al.*, 1989); yeast (Baron *et al.*, 1992); and prokaryotes (Drocourt *et al.*, 1990). Suggested concentrations of Zeocin™ for selection in mammalian cell lines and *E. coli* are listed below:

| Organism | Zeocin™ Concentration and Selective Medium |
|---|---|
| *E. coli* | 25–50 µg/mL in **low salt LB** medium* (see page 16 for recipe) |
| Mammalian Cells | 50–1,000 µg/mL (varies with cell line) |

*Efficient selection requires that the concentration of NaCl be no more than 5 g/liter (< 90 mM).

## Handling Zeocin™

- High salt and acidity or basicity inactivates Zeocin™. Therefore, we recommend that you reduce the salt in bacterial medium and adjust the pH to 7.5 to keep the drug active (see page 16).
- Store Zeocin™ at –20°C and thaw on ice before use.
- Zeocin™ is light sensitive. Store drug, plates, and medium containing drug in the dark.
- Wear gloves, a laboratory coat, and safety glasses or goggles when handling solutions containing Zeocin™.
- Zeocin™ is toxic. Do not ingest or inhale solutions containing the drug.

# Recipes

## Low Salt LB Medium with Zeocin™

For Zeocin™ to be active, the salt concentration of the medium must remain low (<90 mM) and the pH must be 7.5. For selection in *E. coli*, it is **imperative** that you prepare LB broth and plates using the following recipe. Note the lower salt content of this medium. Failure to use low salt LB medium will result in non-selection due to inactivation of the drug.

**Low Salt LB Medium:**

10 g Tryptone
**5 g NaCl**
5 g Yeast Extract

1. Combine the dry reagents above and add deionized, distilled water to 950 mL. Adjust pH to 7.5 with 5 M NaOH. Bring the volume up to 1 liter. For plates, add 15 g/L agar before autoclaving.
2. Autoclave on liquid cycle at 15 lbs/sq. in. and 121°C for 20 minutes.
3. Thaw Zeocin™ on ice and vortex before removing an aliquot.
4. Allow the medium to cool to at least 55°C before adding the Zeocin™ to 25 µg/mL final concentration.
5. Store plates at 4°C in the dark. Plates containing Zeocin™ are stable for 1-2 weeks.

## Cell Lysis Buffer

50 mM Tris, pH 7.8
150 mM NaCl
1% Nonidet P-40

1. This solution can be prepared from the following common stock solutions. For 100 mL, combine:

| | |
|---|---|
| 1 M Tris base | 5 mL |
| 5 M NaCl | 3 mL |
| Nonidet P-40 | 1 mL |

2. Bring the volume up to 90 mL with deionized water and adjust the pH to 7.8 with HCl.
3. Bring the volume up to 100 mL. Store at room temperature.

**Note:** Protease inhibitors may be added at the following concentrations:
1 mM PMSF
1 µg/mL pepstatin
1 µg/mL leupeptin

# Accessory Products

## Introduction

The following products may be used with the pcDNA™4/*myc*-His vectors. For details, visit www.invitrogen.com or contact **Technical Support** (page 19).

| Item | Amount | Catalog no. |
|---|---|---|
| ProBond™ Purification System | 6 × 2 mL precharged, prepacked ProBond™ resin columns and buffers for native and denaturing purification | K850-01 |
| ProBond™ Resin | 50 mL | R801-01 |
| | 150 mL | R801-15 |
| Electrocomp™ TOP10F´ | 5 × 80 µL | C665-55 |
| One Shot® TOP10F´ (chemically competent cells) | 21 × 50 µL | C3030-03 |
| EKMax™ Enterokinase | 250 units | E180-01 |
| PureLink™ HiPure Plasmid Miniprep Kit | 100 preps | K2100-03 |
| PureLink™ HiPure Plasmid Midiprep Kit | 25 preps | K2100-04 |
| β-Gal Assay Kit | 80 mL | K1455-01 |
| β-Gal Staining Kit | 1 kit | K1465-01 |
| Zeocin™ | 1 gram | R250-01 |
| | 5 grams | R250-05 |
| Lipofectamine™ 2000 Reagent | 0.75 mL | 11668-027 |

## Primers

For your convenience, Invitrogen offers a custom primer synthesis service. Visit www.invitrogen.com for more details.

*Continued on next page*

# Accessory Products, Continued

## Antibodies

If you do not have an antibody specific to your protein, Invitrogen offers the Anti-*myc*, or Anti-His(C-term) antibodies to detect your recombinant fusion protein. Horseradish peroxidase (HRP)- and alkaline phosphatase (AP)–conjugated antibodies are available for convenient one-step detection.

| Antibody | Epitope | Catalog no. |
|---|---|---|
| Anti-*myc* | Detects a 10 amino acid epitope derived from *c-myc* (Evan *et al.*, 1985): EQKLISEEDL | R950-25 |
| Anti-*myc*-HRP | | R951-25 |
| Anti-*myc*-AP | | R952-25 |
| Anti-His(C-term) | Detects the C-terminal polyhistidine tag (requires the free carboxyl group for detection) (Lindner et al., 1997): HHHHHH-COOH | R930-25 |
| Anti-His(C-term)-HRP | | R931-25 |
| Anti-His(C-term)-AP | | R932-25 |

# Technical Support

## Web Resources

Visit the Invitrogen website at **www.invitrogen.com** for:

- Technical resources, including manuals, vector maps and sequences, application notes, MSDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical support contact information
- Access to the Invitrogen Online Catalog
- Additional product information and special offers

## Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our website (www.invitrogen.com).

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## MSDS

Material Safety Data Sheets (MSDSs) are available on our website at www.invitrogen.com/msds.

## 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.invitrogen.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

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