USER GUIDE

invitrogen™
by life technologies™

pcDNA™4/His A, B, and C

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Kit Contents and Storage

Contents

40 µL of 0.5 µg/µL each pcDNA™4/His A, B, and C in TE, pH 8.0.
40 µL of 0.5 µg/µL pcDNA™4/His/*lacZ* in TE, pH 8.0.

Shipping and Storage

Plasmids are shipped at room temperature. Store plasmids at −30°C to −10°C.

Introduction

Overview

Introduction

pcDNA™4/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 page 15–16 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 an N-terminal peptide encoding the Xpress™ epitope and a polyhistidine metal-binding peptide.
- Zeocin™ resistance gene for selection of stable cell lines (Mulsant *et al.*, 1988) (see page 11 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/His/*lacZ*, 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/His.

- 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 N-terminal Xpress™ epitope and the polyhistidine tag.
- Ligate your insert into the appropriate vector and transform into *E. coli*. Select transformants on 50 to 100 µg/mL ampicillin or 25–50 µg/mL Zeocin™ Selection Reagent.
- Analyze your transformants for the presence of insert by restriction digestion.
- Select a transformant with the correct restriction pattern and use sequencing to confirm that your gene is cloned in-frame with the N-terminal peptide.
- Transfect your construct into the cell line of choice using your own method of transfection.
- Test for expression of your recombinant gene by western blot analysis or functional assay. For antibody to the Xpress™ epitope, see page 18.
- To purify your recombinant protein, you may use metal-chelating resin such as ProBond™. ProBond™ resin is available separately. See page 18 for ordering information.

Methods

Clone into pcDNA™4/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 as chemically competent or electrocompetent cells (see page 18 for ordering information).

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.

Maintenance of pcDNA™4/His

Transform a *rec*A, *end*A *E. coli* strain like TOP10F´, DH5α, JM109, or equivalent. Select transformants on LB plates containing 50–100 μg/mL ampicillin or 25–50 μg/mL Zeocin™ Selection Reagent in Low Salt LB. Be sure to prepare a glycerol stock of each plasmid for long-term storage (see page 7 for recipe).

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Clone into pcDNA™4/His A, B, and C, Continued

Note

The pcDNA™4/His vectors are fusion vectors. To ensure proper expression of your recombinant protein, you must clone your gene in frame with the ATG at base pairs 920–922. This will create a fusion with the N-terminal polyhistidine tag, Xpress™ epitope, and the enterokinase cleavage site. The vector is supplied in three reading frames to facilitate cloning. See below and pages 4–5 to develop a cloning strategy.

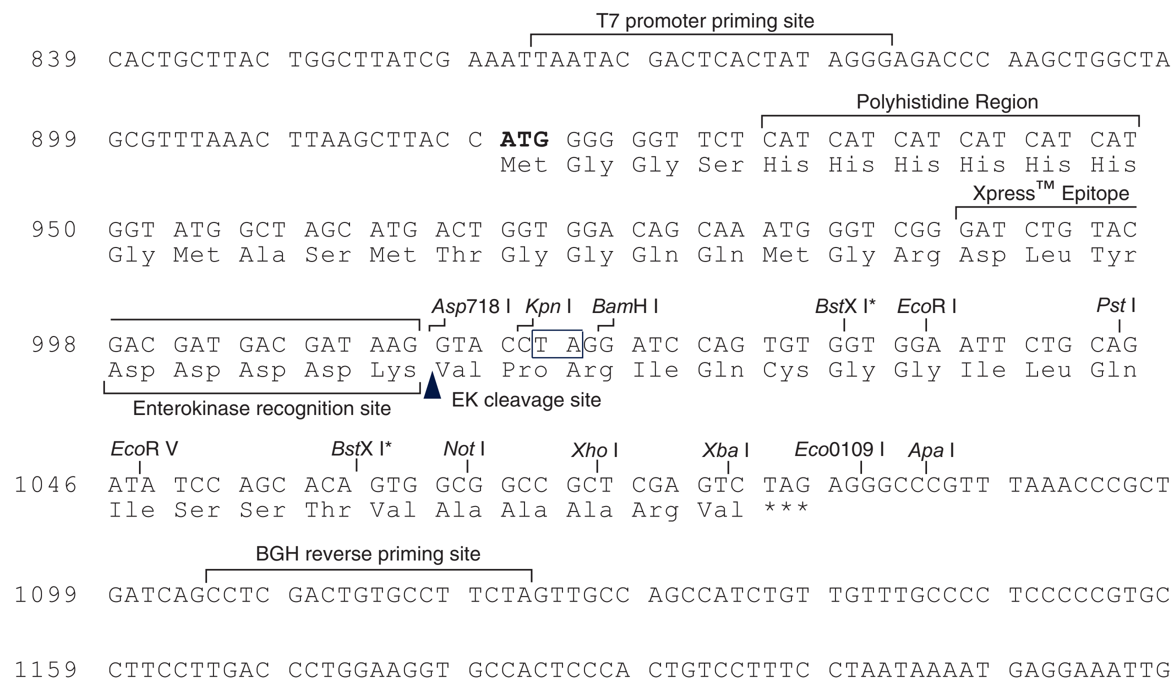
If you intend to clone as close as possible to the enterokinase cleavage site, follow the guidelines below:

- Digest pcDNA™4/His A, B, or C with *Kpn* I.
- Create blunt ends with T4 DNA polymerase and dNTPs.
- Clone your blunt-ended insert in frame with the lysine codon (AAG) of the enterokinase recognition site.

Following enterokinase cleavage, no vector-encoded amino acid residues will be present in your protein.

Multiple Cloning Site of Version A

The following graphic shows the multiple cloning site for pcDNA™4/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 *Xba* I site and the *Eco*0109 I site.** The multiple cloning site has been confirmed by sequencing and functional testing. **The sequence of pcDNA™4/His A is available from www.lifetechnologies.com or from Technical Support.** See page 19 for more information.



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

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Multiple Cloning Site of Version B

The following graphic shows the multiple cloning site for pcDNA™4/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 sequence of pcDNA™4/His B is available from www.lifetechnologies.com or from Technical Support.** See page 19 for more information.

```
                                        T7 promoter priming site
 839  CACTGCTTAC TGGCTTATCG AAATTAATAC GACTCACTAT AGGGAGACCC AAGCTGGCTA

                                                         Polyhistidine Region
 899  GCGTTTAAAC TTAAGCTTAC C ATG GGG GGT TCT CAT CAT CAT CAT CAT CAT
                              Met Gly Gly Ser His His His His His His

                                                                Xpress™ Epitope
 950  GGT ATG GCT AGC ATG ACT GGT GGA CAG CAA ATG GGT CGG GAT CTG TAC
      Gly Met Ala Ser Met Thr Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr

                              Asp718 I  Kpn I    BamH I    BstX I*  EcoR I    Pst I
 998  GAC GAT GAC GAT AAG GTA CCT AAG GAT CCA GTG TGG TGG AAT TCT GCA
      Asp Asp Asp Asp Lys Val Pro Lys Asp Pro Val Trp Trp Asn Ser Ala
      Enterokinase recognition site  ▲ EK cleavage site

        EcoR V          BstX I*  Not I   Xho I   Xba I   Eco0109 I  Apa I
1046  GAT ATC CAG CAC AGT GGC GGC CGC TCG AGT CTA GAG GGC CCG TTT AAA
      Asp Ile Gln His Ser Gly Gly Arg Ser Ser Leu Glu Gly Pro Phe Lys

                              BGH reverse priming site
1094  CCC GCT GAT CAG CCT CGA CTG TGC CTT CTA GTT GCC AGC CAT CTG TTG
      Pro Ala Asp Gln Pro Arg Leu Cys Leu Leu Val Ala Ser His Leu Leu

1142  TTT GCC CCT CCC CCG TGC CTT CCT TGA CCCTGGAAGG TGCCACTCCC
      Phe Ala Pro Pro Pro Cys Leu Pro ***
```

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

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Multiple Cloning Site of Version C

The following graphic shows the multiple cloning site for pcDNA™4/His C. Restriction sites are labeled to indicate the cleavage site. The boxed nucleotide indicates the variable region. The multiple cloning site has been confirmed by sequencing and functional testing. **The sequence of pcDNA™4/His C is available from www.lifetechnologies.com or from Technical Support.** See page 19 for more information.

```
                                       T7 promoter priming site
 839  CACTGCTTAC TGGCTTATCG AAATTAATAC GACTCACTAT AGGGAGACCC AAGCTGGCTA

                                                     Polyhistidine Region
 899  GCGTTTAAAC TTAAGCTTAC C ATG GGG GGT TCT CAT CAT CAT CAT CAT CAT
                              Met Gly Gly Ser His His His His His His

                                                          Xpress(TM) Epitope
 950  GGT ATG GCT AGC ATG ACT GGT GGA CAG CAA ATG GGT CGG GAT CTG TAC
      Gly Met Ala Ser Met Thr Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr

                          Asp718 I Kpn I BamH I    BstX I*  EcoR I      Pst I
 998  GAC GAT GAC GAT AAG GTA CC[A] GGA TCC AGT GTG GTG GAA TTC TGC AGA
      Asp Asp Asp Asp Lys Val Pro Gly Ser Ser Val Val Glu Phe Cys Arg
      Enterokinase recognition site  EK cleavage site

      EcoR V       BstX I*  Not I    Xho I    Xba I  Eco0109 I  Apa I
1046  TAT CCA GCA CAG TGG CGG CCG CTC GAG TCT AGA GGG CCC GTT TAA
      Tyr Pro Ala Gln Trp Arg Pro Leu Glu Ser Arg Gly Pro Val ***

                      BGH reverse priming site
1091  ACCCGCTGAT CAGCCTCGAC TGTGCCTTCT AGTTGCCAGC CATCTGTTGT TTGCCCCTCC

1151  CCCGTGCCTT CCTTGACCCT GGAAGGTGCC ACTCCCACTG TCCTTTCCTA ATAAAATGAG
```

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

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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™ Selection Reagent in Low Salt LB medium (see the following section). Select 10–20 clones and analyze for the presence and orientation of your insert.

Low Salt LB Medium with Zeocin™ Selection Reagent

For Zeocin™ Selection Reagent 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 the 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™ Selection Reagent on ice and vortex before removing an aliquot.
4. Allow the medium to cool to at least 55°C before adding the Zeocin™ Selection Reagent to 25 µg/mL final concentration.
5. Store plates at 4°C in the dark. Plates containing Zeocin™ Selection Reagent are stable for 1–2 weeks.

IMPORTANT!

Any *E. coli* strain that contains the complete Tn*5* transposable element (i.e. DH5αF´IQ, SURE, SURE2) encodes the *ble* gene (bleomycin resistance gene). These strains will confer resistance to Zeocin™ Selection Reagent. 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.).

We recommend that you sequence your construct with the T7 Forward and BGH Reverse sequencing primers to confirm that your gene is fused in frame with the N-terminal polyhistidine tag and the Xpress™ epitope. See the diagrams on pages 3–5 for location and sequences of primer binding sites.

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Clone into pcDNA™4/His A, B, and C, Continued

Prepare a Glycerol Stock

After identifying 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 in case the glycerol stock dies.

- Streak the original colony out on an LB plate containing 50 μg/mL ampicillin or 25 μg/mL Zeocin™ Selection Reagent in Low Salt LB. Incubate the plate at 37°C overnight.
- Isolate a single colony and inoculate into 1–2 mL of LB containing 50 μg/mL ampicillin or 25 μg/mL Zeocin™ Selection Reagent.
- Grow the culture to mid-log phase (OD_{600} = 0.5–0.7).
- Mix 0.85 mL of culture with 0.15 mL of sterile glycerol and transfer to a cryovial.
- 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 N-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 18 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* (Ausubel *et al.*, 1994).

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). For high efficiency transfection in a broad range of mammalian cells, we recommend using Lipofectamine™ 2000 Reagent. For more information on Lipofectamine™ 2000 and other transfection reagents, visit **www.lifetechnologies.com** or contact Technical Support (see page 19).

Positive Control

pcDNA™4/His/*lacZ* is provided as a positive control vector for mammalian cell transfection and expression (see page 17) 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 the following section).

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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. Life Technologies offers the β-Gal Assay Kit and the β-Gal Staining Kit for fast and easy detection of β-galactosidase expression (see page 18 for ordering information).

Detect Fusion Proteins

The Anti-Xpress™ Antibody is available from Life Technologies to detect expression of your fusion protein from pcDNA™4/His (see page 2).

To detect the 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 (50 mM Tris-HCl, pH 7.8, 150 mM NaCl, 1% Nonidet P-40, see the recipe in the following section). Other cell lysis buffers are suitable.
4. Incubate cell suspension at 37°C for 10 minutes to lyse the cells. **Note:** You may prefer to lyse the cells at room temperature or on ice if degradation of your protein is a potential problem.
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.

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Cell Lysis Buffer

50 mM Tris-HCl, pH 7.8

150 mM NaCl

1% Nonidet P-40

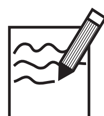
1. This solution can be prepared from the following common stock solutions. For 100 mL, combine:

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



Note

The N-terminal peptide containing the Xpress™ epitope and the polyhistidine tag will add approximately 3.4 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 14.

Create Stable Cell Lines

Introduction

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

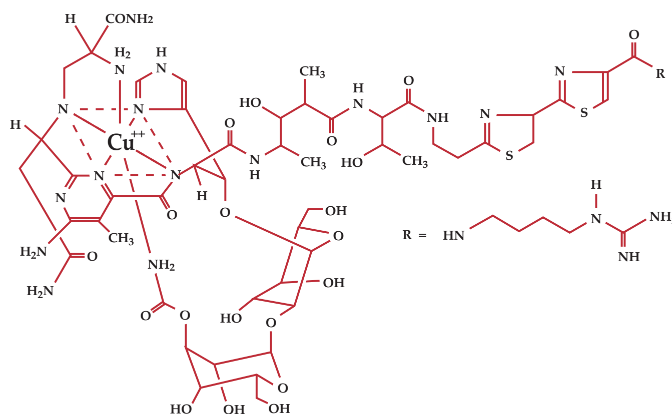
Zeocin™ Selection Reagent

Zeocin™ Selection Reagent 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™ Selection Reagent in a stoichiometric manner to inhibit its DNA strand cleavage activity. Expression of this protein in eukaryotic and prokaryotic hosts confers resistance to Zeocin™ Selection Reagent.

Molecular Weight, Formula, and Structure

The formula for Zeocin™ Selection Reagent is $C_{55}H_{86}O_{21}N_{20}S_2Cu$-HCl. The molecular weight is 1527.5. The structure of Zeocin™ Selection Reagent is:



Applications of Zeocin™ Selection Reagent

Zeocin™ Selection Reagent 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™ Selection Reagent for selection in mammalian cell lines and *E. coli* are listed in the following table:

Organism	Zeocin™ Concentration and Selective Medium
E. coli	25–50 µg/mL in **low salt LB** medium* (see page 7 for recipe)
Mammalian Cells	50–1000 µg/mL (varies with cell line)

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

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Handling Zeocin™ Selection Reagent

- High salt and acidity or basicity inactivate Zeocin™ Selection Reagent. 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 7).
- Store Zeocin™ Selection Reagent at −20°C and thaw on ice before use.
- Zeocin™ Selection Reagent 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™ Selection Reagent.
- Zeocin™ Selection Reagent is toxic. Do not ingest or inhale solutions containing the drug.

Ordering Information

You may purchase Zeocin™ Selection Reagent from Life Technologies. For your convenience, the drug is prepared in autoclaved, deionized water and available in 1.25 mL aliquots at a concentration of 100 mg/mL. The stability of Zeocin™ Selection Reagent is guaranteed for six months, if stored at −20°C. See page 18 for ordering information.

Effect of Zeocin™ Selection Reagent on Sensitive and Resistant Cells

Zeocin™ Selection Reagent's method of killing 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™ Selection Reagent:

- Vast increase in size (similar to the effects of cytomegalovirus infecting permissive cells)
- Abnormal cell shape
- Presence of large empty vesicles in the cytoplasm (breakdown of the endoplasmic reticulum and golgi apparatus, or other 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 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 Reagent.

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Selection in Mammalian Cell Lines

To generate a stable cell line expressing your protein, you need to determine the minimum concentration of Zeocin™ Selection Reagent required to kill your untransfected host cell line. Typically, concentrations between 50 and 1000 μg/mL Zeocin™ Selection Reagent are sufficient to kill the untransfected host cell line. Test a range of concentrations to ensure that you determine the minimum concentration necessary for your cell line.

1. Seed cells (2×10^5 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™ Selection Reagent (e.g. 0, 50, 125, 250, 500, 750, and 1000 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™ Selection Reagent that prevents growth.

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 following table lists unique sites that may be used to linearize your construct prior to transformation. **Other restriction sites are possible. Note that for the enzymes listed below, the cleavage site is indicated for versions A, B, and C of pcDNA™4/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
*Bst*1107 I	2904 (A), 2905 (B), 2903 (C)	End of SV40 poly A	AGS*, Fermentas, Takara, Boehringer-Mannhiem
Fsp I	4398 (A), 4399 (B), 4397 (C)	Ampicillin gene	Many
Pvu I	4546 (A), 4547 (B), 4545 (C)	Ampicillin gene	Many
Sca I	4656 (A), 4657 (B), 4655 (C)	Ampicillin gene	Many
Ssp I	4980 (A), 4981 (B), 4979 (C)	Ampicillin gene	Many

*Angewandte Gentechnologie Systeme.

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Create Stable Cell Lines, Continued

Select 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™ Selection Reagent 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.

Prepare Cells for Lysis

Use the following procedure to prepare cells for lysis prior to purification of your protein on ProBond™. You will need 5×10^6 to 1×10^7 cells for purification of your protein on a 2 mL ProBond™ column (see ProBond™ Protein Purification 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 with fresh medium (if necessary) and transfer the cells to a sterile microcentrifuge tube.
5. Centrifuge the cells at 1500 rpm for 5 minutes. Resuspend the cell pellet in PBS.
6. 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 ™ Protein Purification manual for details about sample preparation for chromatography.

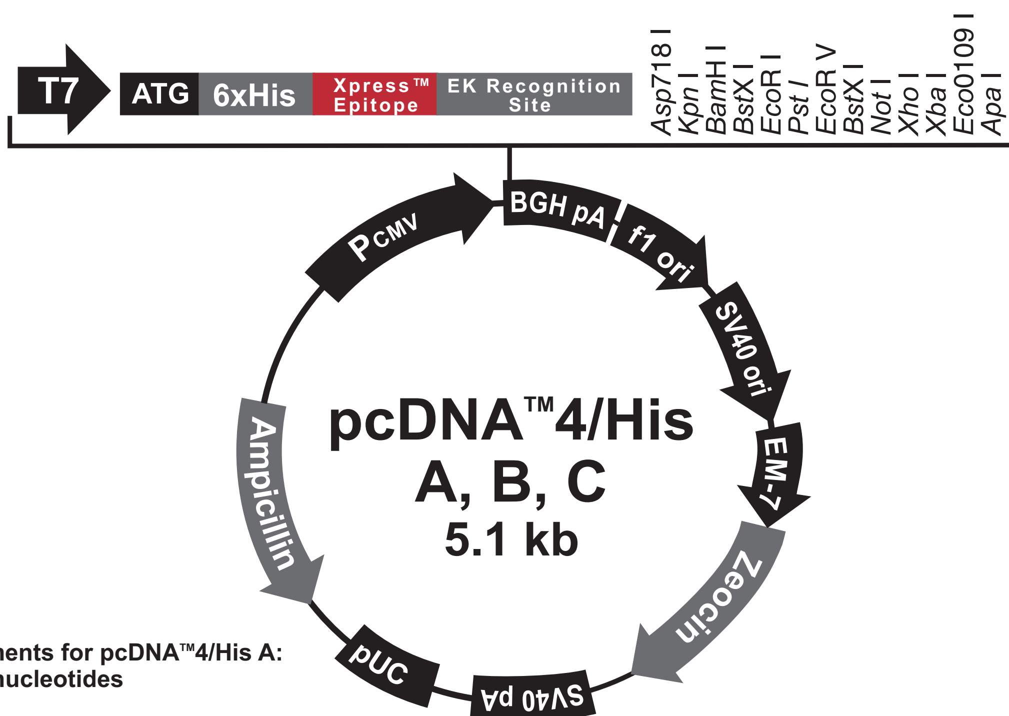
If you are using other metal-chelating resin, refer to the manufacturer's instruction for recommendations on sample preparation.

Appendix

pcDNA™4/His Vector

Map of pcDNA™4/His

The following figure summarizes the features of the pcDNA™4/His vectors. The sequences for pcDNA™4/His A, B, and C are available from **www.lifetechnologies.com** or from Technical Support (see page 19).



Comments for pcDNA™4/His A:
5097 nucleotides

CMV promoter: bases 209-863
T7 promoter/priming site: bases 863-882
ATG initiation codon: bases 920-922
Polyhistidine tag: bases 932-949
Xpress™ epitope: bases 989-1012
Enterokinase recognition site:bases 998-1012
Multiple cloning site: bases 1012-1085
BGH reverse priming site: 1105-1122
BGH polyadenylation sequence: bases 1108-1335
f1 origin: bases 1381-1809
SV40 promoter and origin: bases 1858-2144
EM-7 promoter: bases 2192-2247
Zeocin resistance gene: bases 2266-2640
SV40 polyadenylation sequence: bases 2770-2900
pUC origin: bases 3283-3956
Ampicillin resistance gene: bases 4101-4961

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Features of pcDNA™4/His

pcDNA™4/His A (5097 bp), pcDNA™4/His B (5098 bp), and pcDNA™4/His C (5096 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
N-terminal polyhistidine tag	Allows purification of your recombinant protein on metal-chelating resin such as ProBond™
Xpress™ epitope tag	Allows detection of your recombinant protein with the Anti-Xpress™ Antibody (Catalog no. R910-25)
Enterokinase cleavage site	Allows removal of the N-terminal polyhistidine tag from your recombinant protein using an enterokinase such as EnterokinaseMax™ (Catalog no. E180-01)
Multiple cloning site in three reading frames	Allows insertion of your gene and facilitates cloning in frame with the Xpress™ epitope and polyhistidine N-terminal tag
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 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 (ß-lactamase)	Selection of transformants in *E. coli*

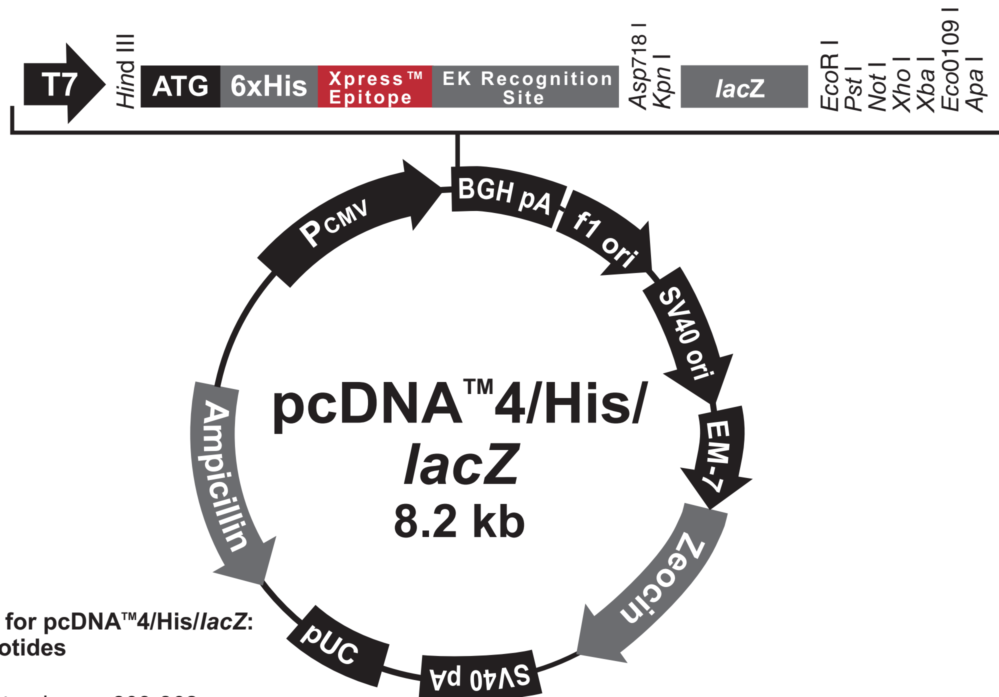
pcDNA™4/His/*lacZ*

Description

pcDNA™4/His/*lacZ* is a 8161 bp control vector containing the gene for β-galactosidase. This vector was constructed by ligating a 2518 bp *Sca* I-*Stu* I fragment containing the Zeocin™ resistance gene to a 5643 bp *Sca* I-*Stu* I fragment containing the CMV promoter, *lacZ* gene, Xpress™ epitope, and polyhistidine tag from pcDNA™3.1/His/*lacZ*.

Map of Control Vector

The following figure summarizes the features of the pcDNA™4/His/*lacZ* vector. The sequence for pcDNA™4/His/*lacZ* is available from **www.lifetechnologies.com** or by contacting Technical Support (see page 19).



Comments for pcDNA™4/His/*lacZ*:
8161 nucleotides

CMV promoter: bases 209-863
T7 promoter/priming site: bases 863-882
ATG initiation codon: bases 920-922
Polyhistidine tag: bases 932-949
Xpress™ epitope: bases 989-1012
Enterokinase recognition site:bases 998-1012
lacZ ORF: bases 1035-4087
BGH reverse priming site: 4168-4185
BGH polyadenylation sequence: bases 4171-4398
f1 origin: bases 4444-4872
SV40 promoter and origin: bases 4921-5208
EM-7 promoter: bases 5256-5311
Zeocin resistance gene: bases 5330-5704
SV40 polyadenylation sequence: bases 5834-5964
pUC origin: bases 6347-7020
Ampicillin resistance gene: bases 7165-8025

Accessory Products

Additional products

The products listed in the following table are designed to help you detect and purify your recombinant fusion protein expressed from pcDNA™4/His. In addition, Life Technologies has a wide variety of mammalian expression vectors, many of which can be utilized with pcDNA™4/His to express multiple proteins in the same cell.

Product	Quantity	Catalog no.
Electrocomp™ Kits	2 x 20 reactions	C665-11
One Shot® TOP10F´ (chemically competent cells)	20 x 50 µL	C3030-03
T7 Promoter Primer	2 µg	N560-02
PureLink® HiPure Plasmid Miniprep Kit	100 preps	K2100-03
PureLink® HiPure Plasmid Midiprep Kit	25 preps	K2100-04
Lipofectamine™ 2000 Reagent	1.5 mL	11668-019
ß-Gal Assay Kit	80 mL	K1455-01
ß-Gal Staining Kit	1 kit	K1465-01
Zeocin™ Selection Reagent	8 x 1.25 mL	R250-01
	50 mL	R250-05

Antibody for Detection

If you do not have an antibody to your protein, Life Technologies offers the Anti-Xpress™ Antibody (Catalog no. R910-25) to detect your recombinant fusion protein. This antibody detects an 8 amino acid epitope (Asp-Leu-Tyr-Asp-Asp-Asp-Asp-Lys).

ProBond™ Resin

Ordering information for ProBond™ resin is provided in the following table.

Product	Amount	Catalog no.
ProBond™ Purification System	6 purifications	K850-01
ProBond™ Resin	50 mL	R801-01
	150 mL	R801-15

Other Mammalian Expression Vectors

We have a wide variety of mammalian expression vectors utilizing the CMV or EF-1α promoters. Vectors are available with the Xpress™ (N-terminal), *c-myc* (C-terminal), V5 (C-terminal), or C-terminal polyhistidine epitopes for detection and either the neomycin, blasticidin, or Zeocin™ resistance genes. All vectors utilize the polyhistidine tag for purification using ProBond™ resin. For more information on the mammalian expression vectors available, see www.lifetechnologies.com or call Technical Support (page 19).

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 product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

Purchaser Notification

Limited Use Label License No. 22: Vectors and Clones Containing Sequences Coding for Histidine Hexamer

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