invitrogen™

pcDNA™6/*myc*-His A, B, and C

Catalog no. V221-20

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User Manual

Table of Contents

Kit Contents and Storage

Shipping and Storage

pcDNA™6/*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.**

Vector	Composition	Amount
pcDNA™6/*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™6/*myc*-His/*lacZ*	40 µl of 0.5 µg/µl vector in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0	20 µg

Accessory Products

Introduction

The products listed below are designed to help you detect and purify your recombinant fusion proteins expressed from pcDNA™6/*myc*-His vectors. In addition, Invitrogen has a wide variety of mammalian expression vectors, many of which can be utilized with pcDNA™6/*myc*-His to express multiple proteins in the same cell (see next page). For more information, www.invitrogen.com or contact **Technical Support** (see page 17).

Product	Amount	Catalog no.
ProBond™ Purification System	6 purifications	K850-01
ProBond™ Resin	50 ml	R801-01
	150 ml	R801-15
PureLink™ HiPure Plasmid Miniprep Kit	100 preps	K2100-03
PureLink™ HiPure Plasmid Midiprep Kit	25 preps	K2100-04
Electrocomp™ TOP10F′	2 × 20 rxns 6 × 20 rxns	C665-11 C665-24
One Shot™ TOP10F′ (chemically competent cells)	20 × 50 µl	C3030-03
β-Gal Assay Kit	1 kit	K1455-01
β-Gal Staining Kit	1 kit	K1465-01
Blasticidin	50 mg	R210-01

Antibodies for Detection

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

Antibody	Epitope	Catalog no.
Anti-*myc*	Detects 10 amino acid epitope derived from *c-myc* (Evans *et al.*, 1985): EQKLISEEDL	R950-25
Anti-*myc*-HRP		R951-25
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

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 our website (www.invitrogen.com) or call **Technical Support** (page 17).

Introduction

Overview

Description of the System

pcDNA™6/*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 14–15 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.
- Blasticidin resistance gene (*bsd*) for selection of stable cell lines (Kimura *et al.*, 1994).
- 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™6/*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™6/*myc*-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 C-terminal *myc* epitope and the polyhistidine tag. Ligate your insert into the appropriate vector and transform into *E. coli*. Select transformants on 50–100 µg/ml ampicillin or 50 µg/ml blasticidin.
- Analyze your transformants for the presence of the 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 C-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 antibodies to the *myc* epitope or the C-terminal polyhistidine tag, see the next page.
- To purify your recombinant protein, you may use metal-chelating resin such as ProBond™. ProBond™ resin is available separately (see page v).

Methods

Cloning into pcDNA™6/*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 published references (Ausubel *et al.*, 1994; Sambrook *et al.*, 1989).

E. coli Strain

Many *E. coli* strains are suitable for the propagation of pcDNA™6/*myc*-His vectors, including TOP10F´, DH5-αF′™, JM109 and INVαF′. We recommend that you propagate vectors containing inserts in *E. coli* strains that are recombinant deficient (*rec*A) and endonuclease A-deficient (*end*A).

For your convenience, TOP10F′ is available as chemically competent or electrocompetent cells from Invitrogen (see page v).

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™6/*myc*-His

To propagate and maintain the pcDNA™6/*myc*-His vectors, use 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 50–100 µg/ml ampicillin or 50 µg/ml blasticidin. Be sure to prepare a glycerol stock of each plasmid for long-term storage (see page 6 for recipe).

Cloning Considerations

Your insert should contain a Kozak consensus sequence with an ATG initiation codon for proper initiation of translation (Kozak, 1987; Kozak 1990). An example of a Kozak consensus sequence is provided below. Other sequences are possible, but the G or A at position –3 and the G at position +4 (shown in bold) illustrates the most commonly occurring sequence with strong consensus. Replacing one of the two bases at these positions provides moderate consensus, while having neither results in weak consensus. The ATG initiation codon is shown underlined.

(G/A)NNATGG

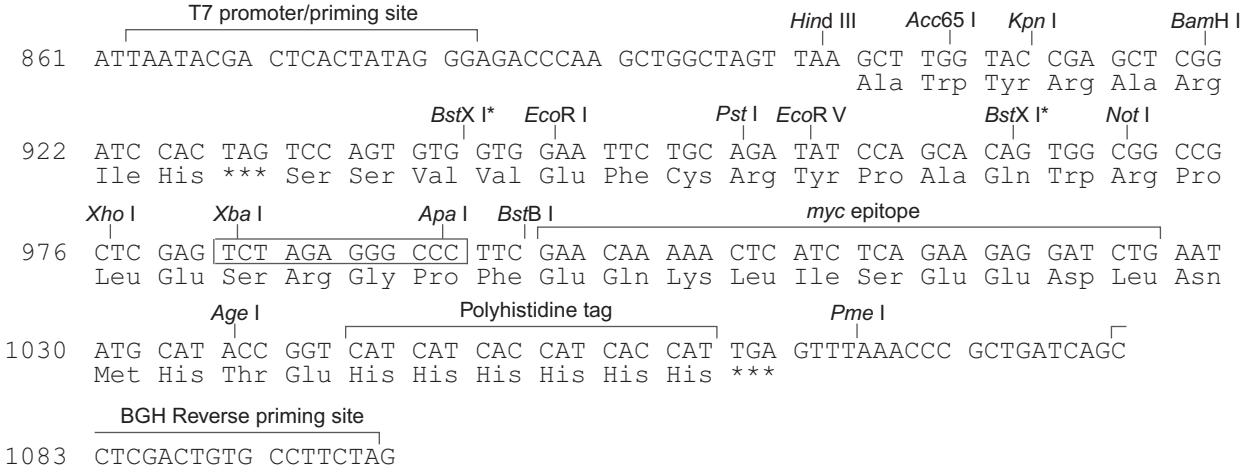
If you wish to express your protein WITHOUT the C-terminal peptide, be sure to include a stop codon.

Continued on next page

Cloning into pcDNA™6/*myc*-His A, B, and C, Continued

Multiple Cloning Site of Version A

Below is the multiple cloning site for pcDNA™6/*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 sequence of pcDNA™6/*myc* -His A is available for downloading from our website at www.invitrogen.com or from **Technical Support** (page 17).



*Note that there are two *BstXI* sites in the polylinker.

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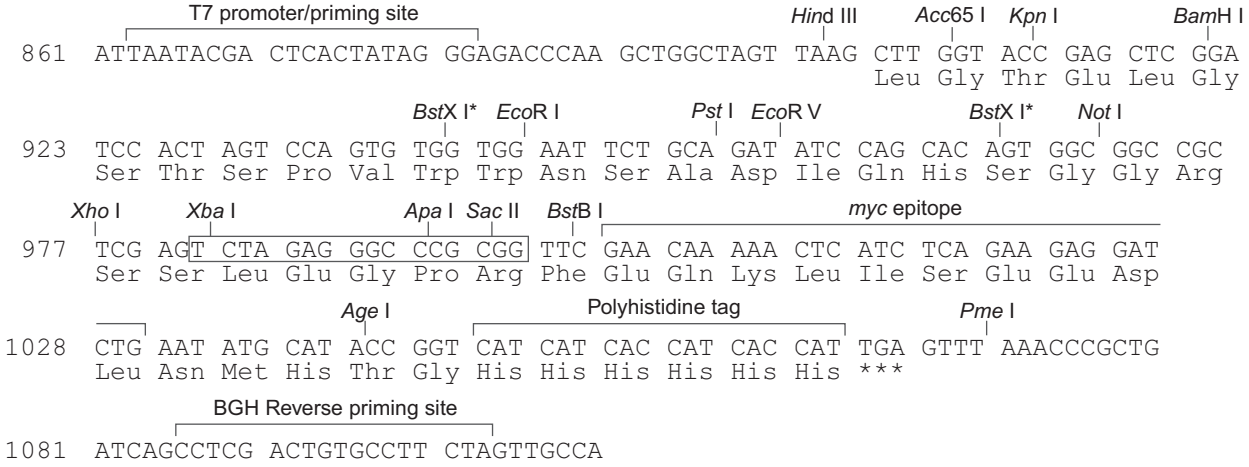
Cloning into pcDNA™6/*myc*-His A, B, and C, Continued

Multiple Cloning Site of Version B

Below is the multiple cloning site for pcDNA™6/*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 sequence of pcDNA™6/*myc* -His B is available for downloading from our website at www.invitrogen.com or from **Technical Support** (page 17).



*Note that there are two *BstXI* sites in the polylinker.

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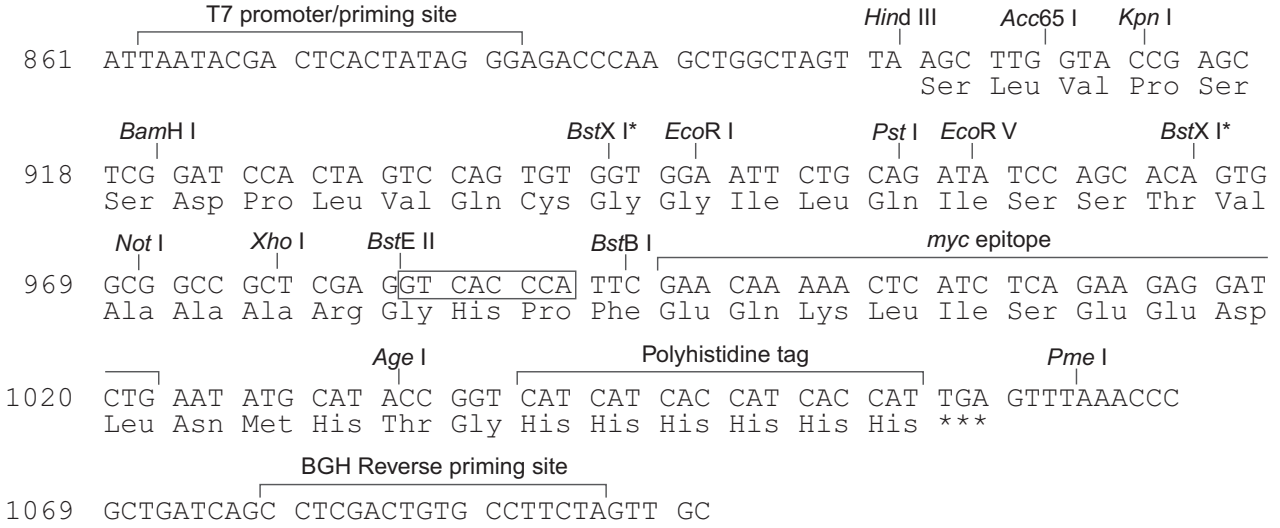
Cloning into pcDNA™6/*myc*-His A, B, and C, Continued

Multiple Cloning Site of Version C

Below is the multiple cloning site for pcDNA™6/*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 sequence of pcDNA™6/*myc* -His C is available for downloading from our website at www.invitrogen.com or from **Technical Support** (page 17).



*Note that there are two *BstXI* 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 50 µg/ml blasticidin. Select 10–20 clones and analyze for the presence and orientation of your insert.

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.

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.

- Streak the original colony out on an LB plate containing 50 µg/ml ampicillin or 50 µg/ml blasticidin. Incubate the plate at 37°C overnight.
- Isolate a single colony and inoculate into 1–2 ml of LB containing 50 µg/ml ampicillin.
- 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 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 lipid complexing, decreasing transfection efficiency. We recommend isolating plasmid DNA using the PureLink™ HiPure Miniprep Kit or the PureLink™ HiPure Midiprep Kit (see page v).

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 of transfection include calcium phosphate (Chen & Okayama, 1987; Wigler *et al*., 1977), lipid-mediated (Felgner *et al*., 1989; Felgner & Ringold, 1989) and electroporation (Chu *et al*., 1987; Shigekawa & Dower, 1988). For high efficiency transfection in a broad range of mammalian cells, we recommend using Lipofectamine™ 2000 Reagent available from Invitrogen. For more information on Lipofectamine™ 2000 and other transfection reagents available, visit our website at www.invitrogen.com or contact **Technical Support** (page 17).

Positive Control

pcDNA™6/*myc*-His/*lac*Z is provided as a positive control vector for mammalian cell transfection and expression (see page 16) 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 v).

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Detection of Fusion Proteins

Several antibodies are available from Invitrogen to detect expression of your fusion protein from pcDNA™6/*myc*-His (see page v).

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 recipe below). 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.

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

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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 13.

Creating Stable Cell Lines

Introduction

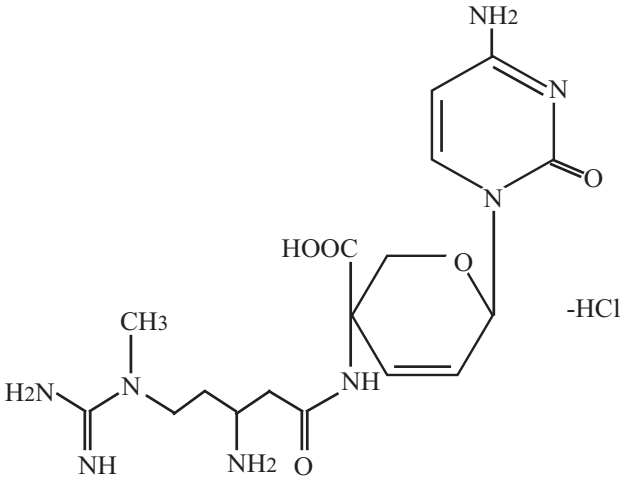
The pcDNA™6/*myc*-His vectors contain the blasticidin resistance gene for selection of stable cell lines using blasticidin. We recommend that you test the sensitivity of your mammalian host cell to blasticidin, as natural resistance varies among cell lines. General information and guidelines are provided below for your convenience.

Blasticidin

Blasticidin S HCl is a nucleoside antibiotic isolated from *Streptomyces griseochromogenes* that inhibits protein synthesis in both prokaryotic and eukaryotic cells (Takeuchi *et al.*, 1958; Yamaguchi *et al.*, 1965). Resistance is conferred by expression of either one of two blasticidin S deaminase genes: *bsd* from *Aspergillus terreus* (Kimura *et al.*, 1994) or *bsr* from *Bacillus cereus* (Izumi *et al.*, 1991). These deaminases convert blasticidin S to a non-toxic deaminohydroxy derivative (Izumi *et al.*, 1991).

Molecular Weight, Formula and Structure

The formula for blasticidin is $C_{17}H_{26}N_8O_5$-HCl and the molecular weight is 458.9. The diagram below shows the structure of blasticidin.



Handling Blasticidin

Always wear gloves, mask, goggles, and protective clothing (*e.g.*, a laboratory coat) when handling blasticidin. Weigh out blasticidin and prepare solutions in a hood.

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Preparing and Storing Stock Solutions

Blasticidin may be obtained from Invitrogen in 50-mg aliquots (see page v). Blasticidin is soluble in water. Water is generally used to prepare stock solutions of 5–10 mg/ml.

- Dissolve blasticidin in sterile water and filter-sterilize the solution.
- Aliquot in small volumes suitable for one-time use (see last point below) and freeze at –20°C for long-term storage or store at +4°C for short term storage.
- Aqueous stock solutions are stable for 1–2 weeks at +4°C and 6–8 weeks at –20°C.
- The pH of the aqueous solution should not exceed 7 to prevent inactivation of blasticidin.
- Do not subject stock solutions to freeze/thaw cycles (**do not store in a frost-free freezer**).
- Upon thawing, use what you need and discard the unused portion.

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™6/*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	162	Upstream of CMV promoter	New England Biolabs
Mlu I	229	5´ end of CMV promoter	Many
*Bst*1107 I	2932 (A), 2936 (B), 2928 (C)	End of SV40 poly A	AGS*, Fermentas, Takara, Boehringer-Mannhiem
*Eam*1105 I	4204 (A), 4208 (B), 4200 (C)	Ampicillin gene	AGS*, Fermentas, Takara
Fsp I	4426 (A), 4430 (B), 4422 (C)	Ampicillin gene	Many
Sca I	4684 (A), 4688 (B), 4680 (C)	Ampicillin gene	Many
Ssp I	5008 (A), 5012 (B), 5004 (C)	Ampicillin gene	Many

* Angewandte Gentechnologie Systeme

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Creating Stable Cell Lines, Continued

Selection in Mammalian Cell Lines

To generate a stable cell line expressing your protein, you need to determine the minimum concentration of blasticidin required to kill your untransfected host cell line. Typically, concentrations between 2 and 10 μg/ml blasticidin 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.

- Seed cells (2×10^5 cells/60 mm plate) for each time point and allow the cells to adhere overnight.
- The next day, substitute culture medium with medium containing varying concentrations of blasticidin (*e.g.*, 0, 1, 3, 5, 7.5, and 10 μg/ml).
- Replenish the selective medium every 3–4 days. Cells sensitive to blasticidin will round up and detach from the plate. Dead cells will accumulate in the medium.
- Count the number of viable cells at regular intervals to determine the appropriate concentration of blasticidin that prevents growth.

Selection of Stable Integrants

Once the appropriate blasticidin concentration is determined, you can generate a stable cell line with your construct. Colonies can generally be identified in 7–10 days with complete selection and expansion in 2 weeks.

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

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Preparing Cells for Lysis

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

1. Seed cells in either five T-75 flasks or two-to-three 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 1,500 rpm for 5 minutes. Resuspend the cell pellet in PBS.
6. Centrifuge the cells at 1,500 rpm 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 manual for details about sample preparation for chromatography.

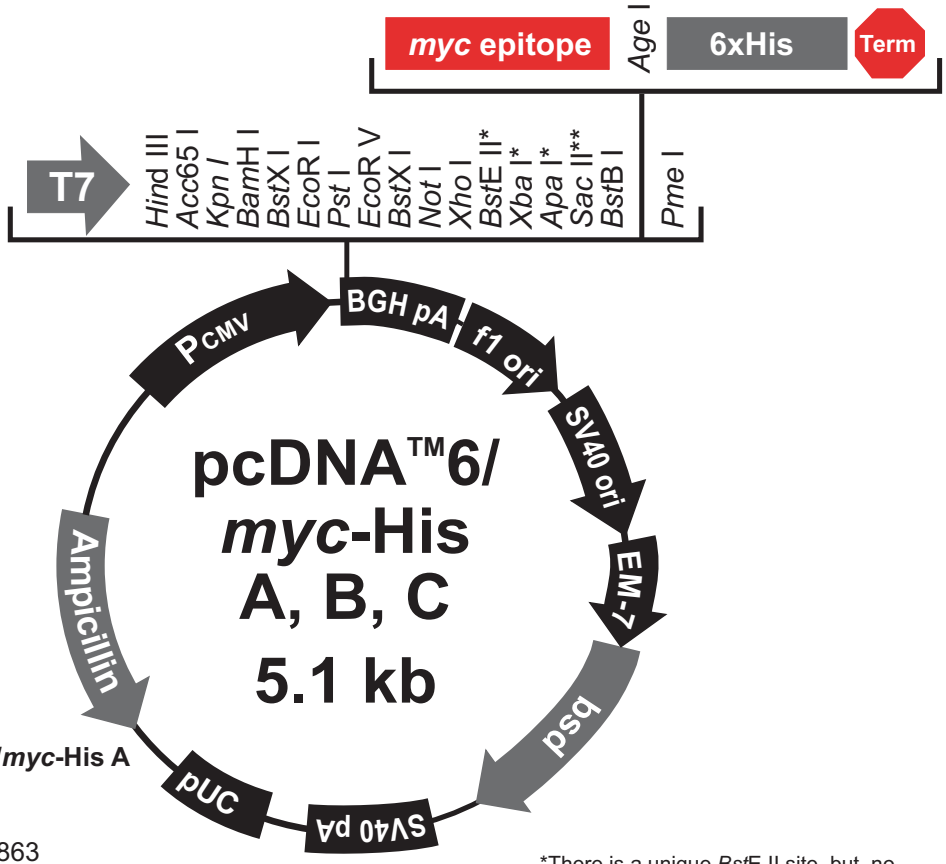
If you are using other metal-chelating resin, refer to the manufacturer's instruction for recommendations on sample preparation.

Appendix

pcDNA™6/*myc*-His Vector

Map of pcDNA™6/*myc*-His

The figure below summarizes the features of the pcDNA™6/*myc*-His vectors. The sequences for pcDNA™6/*myc*-His A, B, and C are available for downloading from our website (www.invitrogen.com) or from **Technical Support** (see page 17).



*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 *Bst*B I site in **version B only.

Comments for pcDNA™6/*myc*-His A
5126 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 1081-1295
f1 origin: bases 1358-1771
SV40 promoter and origin: bases 1813-2121
EM-7 promoter: bases 2169-2224
Blasticidin resistance gene: bases 2249-2641
SV40 polyadenylation signal: bases 2799-2929
pUC origin: bases 3312-3985
Ampicillin resistance gene: bases 4130-4991

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pcDNA™6/*myc*-His Vector, Continued

Features of pcDNA™6/*myc*-His

pcDNA™6/*myc*-His A (5,126 bp), pcDNA™6/*myc*-His B (5,130 bp), and pcDNA™6/*myc*-His C (5,122 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 or Anti-*myc*-HRP Antibody (Evans *et al.*, 1985).
C-terminal polyhistidine tag	Permits 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 (Lindner *et al.*, 1997) and the Anti-His (C-term)-HRP Antibody.
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 blasticidin 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 blasticidin resistance gene in *E. coli.*
Blasticidin resistance gene (*bsd*)	Selection of stable transfectants in mammalian cells (Kimura *et al.*, 1994).
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.*

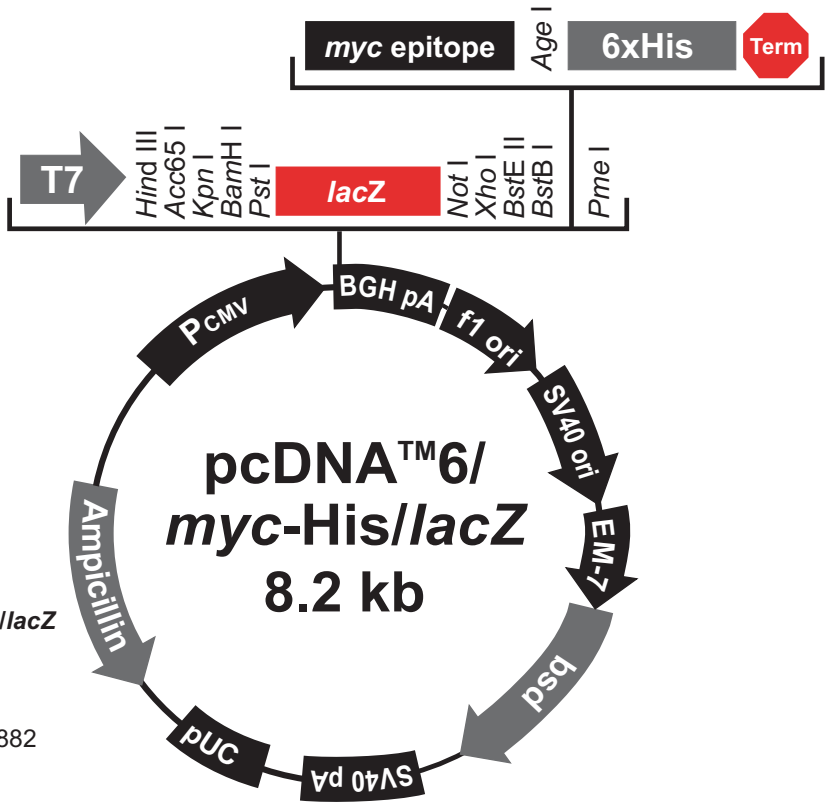
pcDNA™6/*myc*-His/*lacZ*

Map of pcDNA™6/*myc*-His/*lacZ*

pcDNA™6/*myc*-His/*lacZ* is a 8173-bp control vector containing the gene for β-galactosidase. This vector was constructed by ligating a 3,932-bp *Bam*H I-*Stu* I fragment containing the blasticidin resistance gene from pcDNA™6/*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™6/*myc*-His/*lacZ* vector.

The nucleotide sequence for pcDNA™6/*myc*-His/*lacZ* is available for downloading from our website (www.invitrogen.com) or by contacting **Technical Support** (see page 17).



Comments for pcDNA™6/*myc*-His/*lacZ*
8173 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 4444-4857
SV40 promoter and origin: bases 4922-5246
EM-7 promoter: bases 5216-5271
Blasticidin resistance gene: bases 5296-5688
SV40 polyadenylation signal: bases 5846-5976
pUC origin: bases 6359-7032
Ampicillin resistance gene: bases 7177-8038

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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