pcDNA6/His© A, B, and C

Catalog no. V222-20

Version B

001102

25-0237

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General Information

Contents

20 µg each pcDNA6/His A, B, and C, lyophilized
20 µg pcDNA6/His/*lacZ*, lyophilized

Shipping/Storage

Lyophilized plasmids are shipped at room temperature and stored at -20°C.

Product Qualification

Invitrogen qualifies the vectors by restriction digest. Restriction digestion must demonstrate the correct banding patterns when electrophoresed on an agarose gel.

Restriction Enzyme	pcDNA6/His A	pcDNA6/His B	pcDNA6/His C	pcDNA6/His/*lacZ*
Avr II	4028 bp, 1122 bp	5151 bp	5149 bp	8213 bp
*Bsu*36 I	No cut	5151 bp	No cut	7976 bp, 237 bp
Hind III	5150 bp	5151 bp	5149 bp	8213 bp
Mlu I	5150 bp	5151 bp	5149 bp	4904bp, 2104 bp, 780 bp, 425 bp
Nsi I	5078 bp, 72 bp	5079 bp, 72 bp	5077 bp, 72 bp	8141 bp, 72 bp
Pvu II	3349 bp, 1117 bp, 684 bp	3350 bp, 1117 bp, 684 bp	3348 bp, 1117 bp, 684 bp	3114 bp, 2557 bp, 1117 bp, 684 bp, 378 bp, 363 bp

Purchaser Notification

Blasticidin and the Blasticidin Selection Marker

Blasticidin and the blasticidin resistance gene are sold under patent license and may be used **for research purposes only.** Inquiries for commercial use should be directed to:

Kaken Pharmaceutical Company, Ltd. S

Bunkyo Green Court, Center Office Building, 19-20 Fl.

28-8 Honkomagome 2-chome

Bunkyo-ku, Tokyo 113-8650

Phone: 81 3-5977-5008

Fax: 81 3-5977-5008

BGH Polyadenylation Signal

The BGH polyadenylation sequence is licensed under U.S. Patent No. 5,122,458 **for research purposes only**. Inquiries for commercial use should be directed to:

Bennett Cohen, Ph.D.

Research Corporation Technologies

101 North Wilmot Road, Suite 600

Tucson, AZ 85711-3335

Phone: 520-748-4400

Fax: 520-748-0025

CMV Promoter

Use of the CMV promoter is covered under U.S. Patent Nos. 5,168,062 and 5,385,839 owned and licensed by the University of Iowa Research Foundation and may be used **for research purposes only**. Commercial users must obtain a license to these patents directly from the University of Iowa Research Foundation. Inquiries for commercial use should be directed to:

Brenda Akins

University of Iowa Research Foundation (UIRF)

214 Technology Innovation Center

Iowa City , IA 52242

Tel: 319-335-4549

Methods

Overview

Introduction

pcDNA6/His A, B, and C are 5.2 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 an N-terminal peptide encoding the Xpress™ 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, pcDNA6/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 pcDNA6/His.

- Consult the multiple cloning sites described on pages 4-6 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 50 µg/ml blasticidin.
- 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. Generate a stable cell line, if desired.
- Test for expression of your recombinant gene by western blot analysis or functional assay. For antibody to the Xpress™ epitope, please see the next page.
- To purify your recombinant protein, you may use metal-chelating resin such as ProBond™. ProBond™ resin is available separately (see next page for ordering information).

Accessory Products

Introduction

The products listed below are designed to help you detect and purify your recombinant fusion protein expressed from pcDNA6/His. In addition, Invitrogen has a wide variety of mammalian expression vectors, many of which can be utilized with pcDNA6/His to express multiple proteins in the same cell (please see below).

Antibody for Detection

If you do not have an antibody to your protein, Invitrogen 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 below.

Item	Amount	Catalog no.
ProBond™ Protein Purification System	6 x 2 ml precharged, prepacked ProBond™ resin columns and buffers for native and denaturing purification	K850-01
ProBond™ Protein Purification Kit with Anti-Xpress™ Antibody	1 Kit	K851-01
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, please see our web site (www.invitrogen.com) or call Technical Service (page 17).

Cloning into pcDNA6/His© A, B, and C

Introduction

Diagrams are provided on pages 4-6 to help you ligate your gene of interest in frame with the N-terminal peptide. General considerations for cloning and transformation are listed below.

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, please refer to *Molecular Cloning: A Laboratory Manual* (Sambrook *et al.*, 1989) or *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994) (See **References**, page 19).

E. coli Strain

Many *E. coli* strains are suitable for the growth of this vector including TOP10F´ (Catalog no. C615-00), DH5αF´, JM109 (Catalog no. C666-00), and INVαF´ (Catalog no. C658-00). 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 from Invitrogen.

Item	Quantity	Catalog no.
Electrocomp™ TOP10F´	5 x 80 µl	C665-55
Ultracomp™ TOP10F´ (chemically competent cells)	5 x 300 µl	C665-03
One Shot® TOP10F´ (chemically competent cells)	21 x 50 µl	C3030-03

Transformation Method

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

Maintenance of pcDNA6/His

To propagate and maintain the pcDNA6/His vectors, we recommend resuspending each vector in 20 µl sterile water to prepare a 1 µg/µl stock solution. Store the stock solution at -20°C.

Use this stock solution to transform a *rec*A, *end*A *E. coli* strain like TOP10F´, DH5αF´, JM109, or equivalent. Select transformants on LB plates containing 50 to 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 7).

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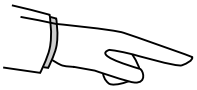
Cloning into pcDNA6/His© A, B, and C, continued



NOTE

The pcDNA6/His vectors are fusion vectors. To ensure proper expression of your recombinant fusion 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 5-6 to develop a cloning strategy.

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

- Digest pcDNA6/His A, B, or C with *Kpn* I.
- Create blunt ends with T4 DNA polymerase and dNTPs. See (Ausubel *et al.*, 1994) for a detailed protocol.
- 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

Below is the multiple cloning site for pcDNA6/His A. Restriction sites are labeled to indicate the cleavage site. The boxed nucleotide indicates the variable region. **Please note that there is a stop codon between the *Xba* I site and the *Apa* I site.** The multiple cloning site has been confirmed by sequencing and functional testing. **The complete sequence of pcDNA6/His A is available for downloading from our web site (http://www.invitrogen.com) or from Technical Service.** See page 17 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
```

*Asp*718 I *Kpn* I *Bam*H I *Bst*X I* *Eco*R I *Pst* I

```
 998  GAC GAT GAC GAT AAG GTA CCT AGG ATC CAG TGT GGT GGA ATT CTG CAG
      Asp Asp Asp Asp Lys Val Pro Arg Ile Gln Cys Gly Gly Ile Leu Gln
```

Enterokinase recognition site EK cleavage site

*Eco*R V *Bst*X I* *Not* I *Xho* I *Xba* I *Apa* I

```
1046  ATA TCC AGC ACA GTG GCG GCC GCT CGA GTC TAG AGGGCCCGTT TAAACCCGCT
      Ile Ser Ser Thr Val Ala Ala Ala Arg Val ***
```

pcDNA3.1/BGH reverse priming site

```
1099  GATCAGCCTC GACTGTGCCT TCTAGTTGCC AGCCATCTGT TGTTTGCCCC TCCCCCGTGC

1159  CTTCCTTGAC CCTGGAAGGT GCCACTCCCA CTGTCCTTTC CTAATAAAAT GAGGAAATTG
```

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

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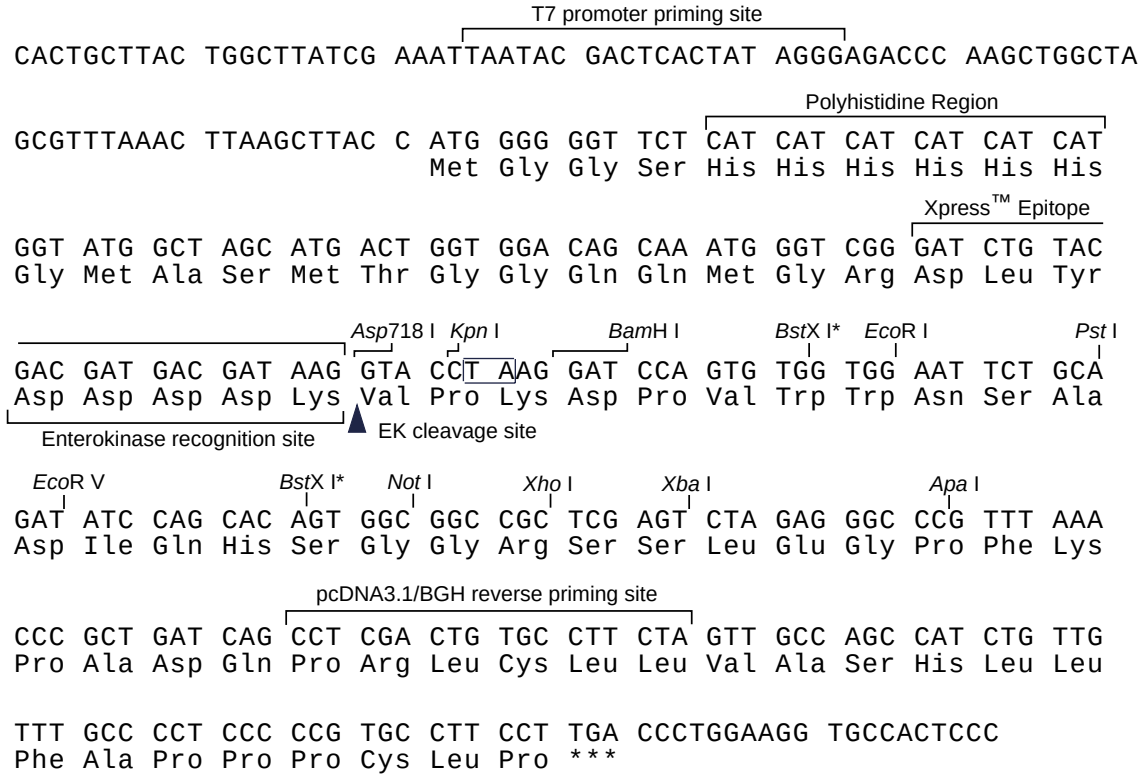
Cloning into pcDNA6/His© A, B, and C, continued

Multiple Cloning Site of Version B

Below is the multiple cloning site for pcDNA6/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 complete sequence of pcDNA6/His B is available for downloading from our web site (http://www.invitrogen.com) or from Technical Service.** See page 17 for more information.



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

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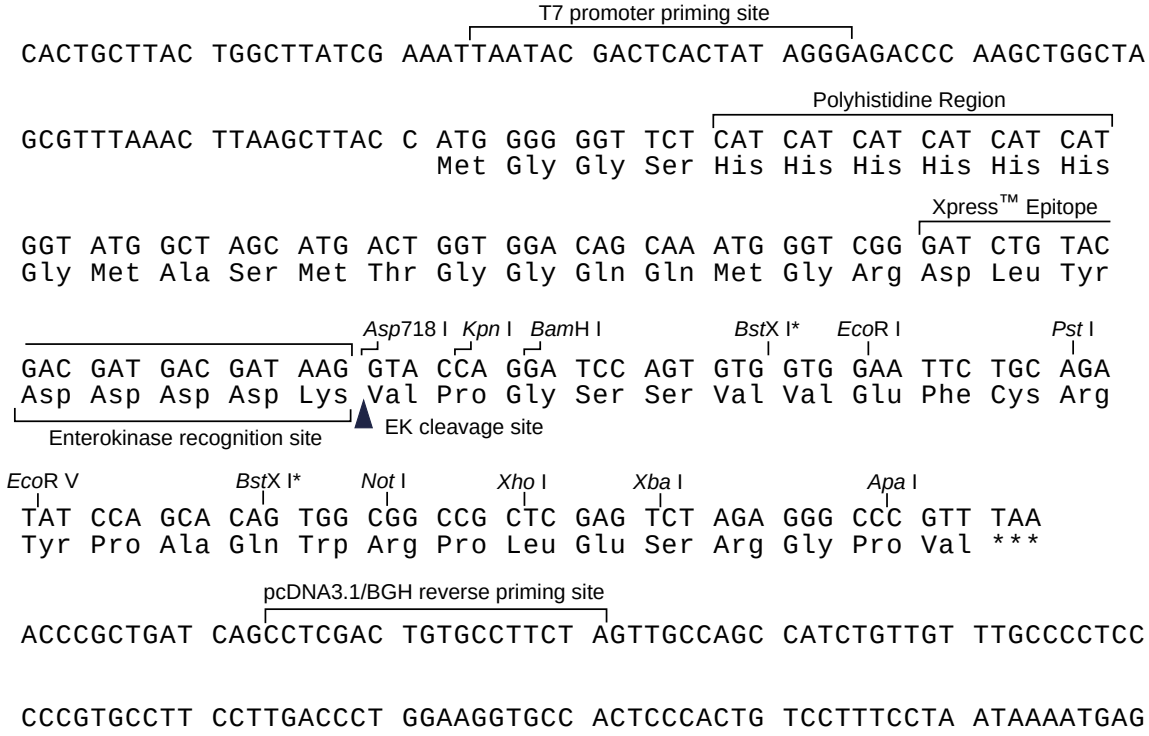
Cloning into pcDNA6/His© A, B, and C, continued

Multiple Cloning Site of Version C

Below is the multiple cloning site for pcDNA6/His C. Restriction sites are labeled to indicate the cleavage site**.** The multiple cloning site has been confirmed by sequencing and functional testing. **The complete sequence of pcDNA6/His C is available for downloading from our web site (http://www.invitrogen.com) or from Technical Service.** See page 17 for more information.



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

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Cloning into pcDNA6/His© A, B, and C, continued

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 pcDNA3.1/ BGH Reverse primers (Catalog nos. N560-02 and N575-02, respectively) to confirm that your gene is fused in frame with the N-terminal polyhistidine tag and the Xpress™ epitope.

Preparing a Glycerol Stock

Once you have identified the correct clone, 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 or 50 µg/ml blasticidin.
- 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 S.N.A.P.™ MiniPrep Kit (10-15 µg DNA, Catalog no. K1900-01), the S.N.A.P.™ MidiPrep Kit (10-200 µg, Catalog no. K1910-01), or CsCl gradient centrifugation.

Methods of Transfection

For established cell lines (e.g. HeLa), please 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* (**Reference** section, page 19).

Invitrogen offers a wide variety of transfection reagents including the Calcium Phosphate Transfection Kit for mammalian cell transfection. For more information, call Technical Service (see page 17) or visit our web site at www.invitrogen.com.

Positive Control

pcDNA6/His/*lacZ* 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 next page).

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Transfection and Analysis, continued

Assay for β-galactosidase Activity

You may assay for β-galactosidase expression by activity assay using cell-free lysates (Miller, 1972) or by staining the cells for activity. Invitrogen offers the β-Gal Assay Kit (Catalog no. K1455-01) and the β-Gal Staining Kit (Catalog no. K1465-01) for fast and easy detection of β-galactosidase expression.

Detection of Fusion Proteins

The Anti-Xpress™ Antibody is available from Invitrogen to detect expression of your fusion protein from pcDNA6/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 1500 x g for 5 minutes.
3. Resuspend in 50 µl Cell Lysis Buffer (see recipe below). Other cell lysis buffers are suitable.
4. Incubate cell suspension at 37°C for 10 minutes to lyse the cells.
5. Centrifuge the cell lysate at 10,000 x g for 10 minutes to pellet nuclei and transfer the supernatant to a fresh tube. Assay the supernatant 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-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

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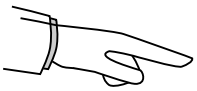


NOTE

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 x 10^6 to 1 x 10^7 **transfected** cells for purification of your protein on a 2 ml ProBond™ column (or other metal-chelating column). Please refer to the manufacturer's instructions before attempting to purify your fusion protein. To prepare cells for lysis, please refer to the protocol on page 13.

Creation of Stable Cell Lines

Introduction

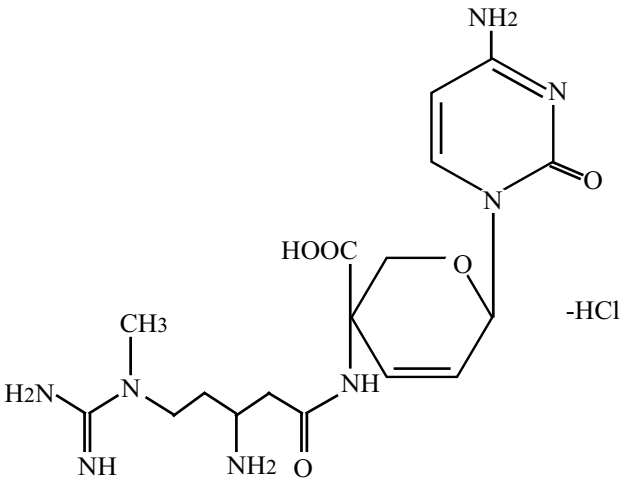
The pcDNA6/His vectors contain the blasticidin resistance gene for selection of stable cell lines using blasticidin. We recommend that you test the sensitivity of your mammalian host cell to blasticidin as natural resistance varies among cell lines. General information and guidelines are provided below for your convenience.

Blasticidin

Blasticidin S HCl is a nucleoside antibiotic isolated from *Streptomyces griseochromogenes* which 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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Creation of Stable Cell Lines, continued

Preparing and Storing Stock Solutions

Blasticidin may be obtained from Invitrogen (Catalog no. R210-01) in 50 mg aliquots. Blasticidin is soluble in water. Water is generally used to prepare stock solutions of 5 to 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.
- 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. Please note that for the enzymes listed below, the cleavage site is indicated for versions A, B, and C of pcDNA6/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
*Bst*1107 I	2956 (A), 2957 (B), 2955 (C)	End of SV40 poly A	AGS*, Fermentas, Takara, Boehringer-Mannhiem
*Eam*1105 I	4228 (A), 4229 (B), 4227 (C)	Ampicillin gene	AGS*, Fermentas, Takara
Fsp I	4450 (A), 4451 (B), 4449 (C)	Ampicillin gene	Many
Sca I	4708 (A), 4709 (B), 4707 (C)	Ampicillin gene	Many
Ssp I	5032 (A), 5033 (B), 5031 (C)	Backbone	Many

*Angewandte Gentechnologie Systeme

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Creation of 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 x 10^5 cells/60 mm plate) (approximately 25% confluency) for each time point and allow 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.

- 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.

Preparation of Cells for Lysis

Use the procedure below to prepare cells for lysis prior to purification of your protein on ProBond™. You will need 5 x 10^6 to 1 x 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 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 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, please refer to the ProBond™ Protein Purification manual for details about sample preparation for chromatography.

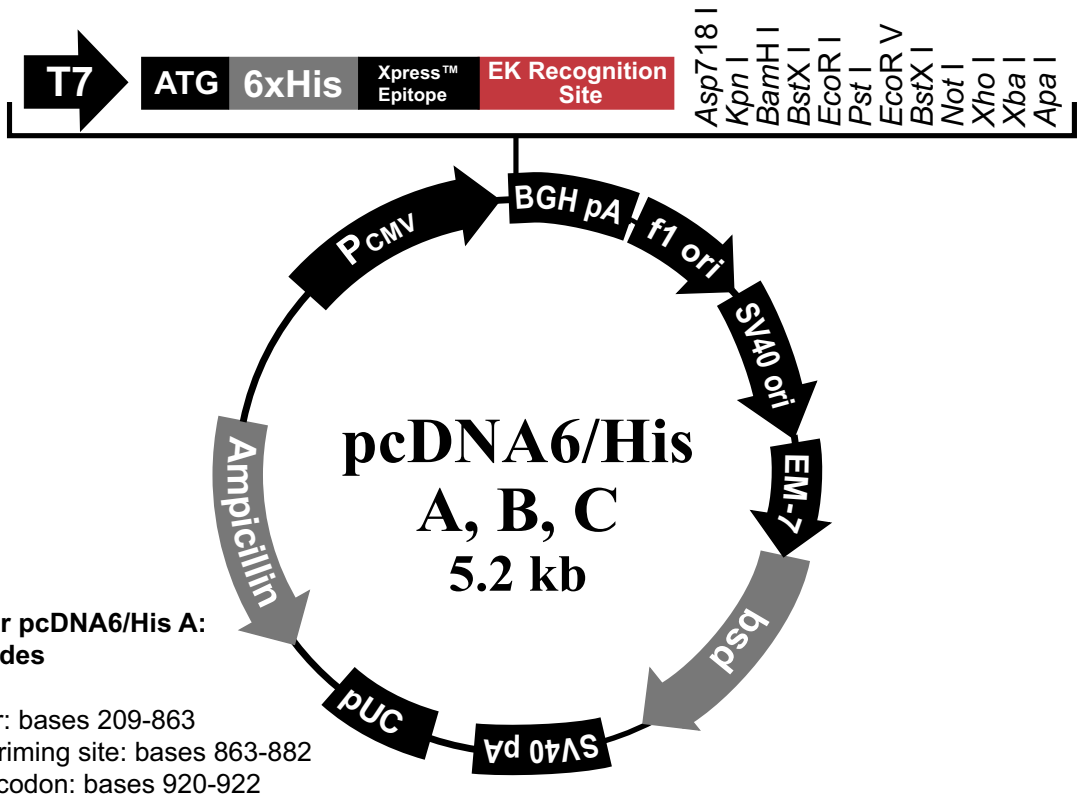
If you are using other metal-chelating resin, please refer to the manufacturer's instruction for recommendations on sample preparation.

Appendix

pcDNA6/His© Vector

Map of pcDNA6/His

The figure below summarizes the features of the pcDNA6/His vectors. **The sequences for pcDNA6/His A, B, and C are available for downloading from our World Wide Web site (http://www.invitrogen.com) or from Technical Service (see page 17).**



Comments for pcDNA6/His A: 5150 nucleotides

CMV promoter: bases 209-863
T7 promoter/priming site: bases 863-882
ATG initiation codon: bases 920-922
Polyhistidine region: bases 932-949
Xpress™ epitope: bases 989-1012
Enterokinase recognition site: bases 998-1012
Multiple cloning site: bases 1012-1085
pcDNA3.1/BGH reverse priming site: bases 1105-1122
BGH polyadenylation signal: bases 1108-1335
f1 origin: bases 1381-1809
SV40 promoter and origin: bases 1837-2145
EM-7 promoter: bases 2193-2248
Blasticidin resistance gene: bases 2267-2665
SV40 polyadenylation signal: bases 2823-2953
pUC origin: bases 3336-4009
Ampicillin resistance gene: bases 4154-5014

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pcDNA6/His© Vector, continued

Features of pcDNA6/His

pcDNA6/His A (5150 bp), pcDNA6/His B (5151 bp), and pcDNA6/His C (5149 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
N-terminal polyhistidine tag	Permits purification of your recombinant protein on metal-chelating resin such as ProBond™
Xpress™ epitope tag	Allow 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 N-terminal polyhistidine tag
pcDNA3.1/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 transformants in *E. coli* and 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*

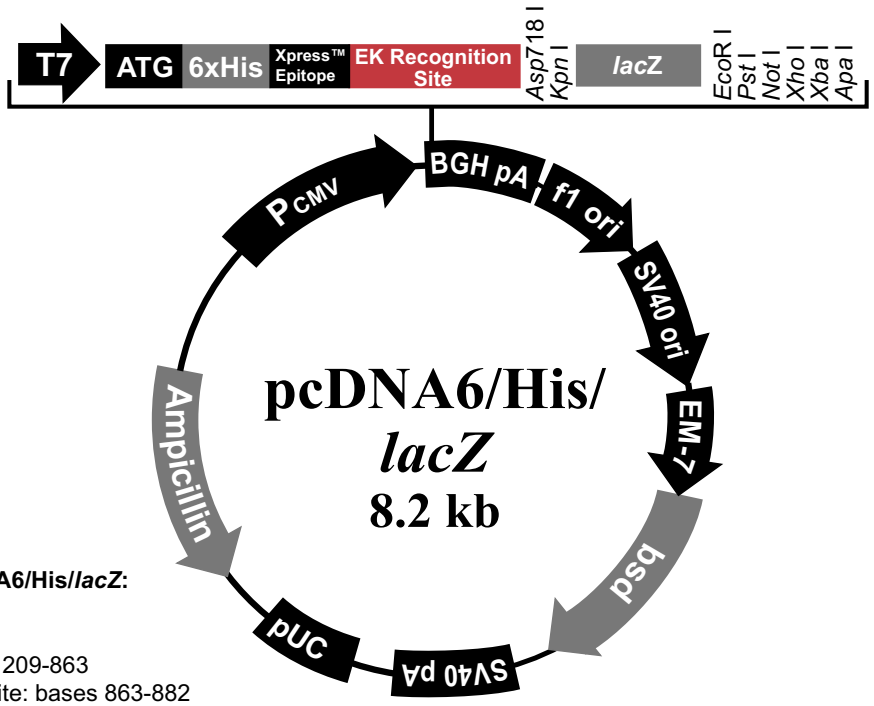
pcDNA6/His©/lacZ

Description

pcDNA6/His/*lacZ* is a 8213 bp control vector containing the gene for β-galactosidase. This vector was constructed by ligating a 3082 bp *Kpn* I-*Eco*R I fragment containing the *lacZ* gene from pcDNA4/His/*lacZ* to a 5131 bp *Kpn* I-*Eco*R I fragment containing the CMV promoter, Xpress™ epitope, and polyhistidine tag from pcDNA6/His B.

Map of Control Vector

The figure below summarizes the features of the pcDNA6/His/*lacZ* vector. **The complete nucleotide sequence for pcDNA6/His/*lacZ* is available for downloading from our World Wide Web site (http://www.invitrogen.com) or by contacting Technical Services (see the next page).**



Comments for pcDNA6/His/*lacZ*:
8213 nucleotides

CMV promoter: bases 209-863
T7 promoter/priming site: bases 863-882
ATG initiation codon: bases 920-922
Polyhistidine region: bases 932-949
Xpress™ epitope: bases 989-1012
Enterokinase recognition site: bases 998-1012
LacZ ORF: bases 1037-4087
pcDNA3.1/BGH reverse priming site: bases 4168-4185
BGH polyadenylation signal: bases 4171-4398
f1 origin: bases 4444-4872
SV40 promoter and origin: bases 4900-5208
EM-7 promoter: bases 5256-5311
Blasticidin resistance gene: bases 5330-5728
SV40 polyadenylation signal: bases 5886-6016
pUC origin: bases 6399-7072
Ampicillin resistance gene: bases 7217-8077

Technical Service

World Wide Web

Visit the Invitrogen Web Resource using your World Wide Web browser. At the site, you can:

- Get the scoop on our hot new products and special product offers
- View and download vector maps and sequences
- Download manuals in Adobe™ Acrobat™ (PDF) format
- Explore our catalog with full color graphics
- Obtain citations for Invitrogen products
- Post a question at one of our many user forums
- Request catalog and product literature

Once connected to the Internet, launch your web browser (Netscape 3.0 or newer), then enter the following location (or URL):

http://www.invitrogen.com

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

Technical Service

If you need technical information or help, please e-mail, call, or fax us:

Location	Email	Telephone/Fax
United States Canada	tech_service@invitrogen.com	Voice: 1-800-955-6288 Fax: 1-760-602-6500
Mexico South America	tech_service@invitrogen.com	Voice: 01-760-603-7200 Fax: 01-760-602-6500
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The Netherlands

continued on next page

Technical Service, continued

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