invitrogen™

pcDNA™6/His A, B, and C

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User Manual

Table of Contents

Kit Contents and Storage

Shipping and Storage

pcDNA™6/His vectors are shipped on wet ice. Upon receipt, store vectors at –20ºC.

Contents

20 µg each of pcDNA™6/His A, B, and C are supplied at 0.5 µg/µL in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 in a total volume of 40 µL.

20 µg of pcDNA™6/His/*lacZ* is supplied at 0.5 µg/µL in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 in a total volume of 40 µL.

Introduction

Product Overview

Description of the System

pcDNA™6/His A, B, and C are 5.2 kb vectors designed for the overproduction of recombinant proteins in mammalian cell lines. Features of the vectors allow for the 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 the 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/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™6/His.

1. Consult the multiple cloning sites depicted 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.
2. 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.
3. Analyze your transformants for the presence of insert by restriction digestion.
4. Select a transformant with the correct restriction pattern and use sequencing to confirm that your gene is cloned in-frame with the N-terminal peptide.
5. Transfect your construct into a cell line of choice using your own method of transfection. Generate a stable cell line, if desired.
6. Test for expression of your recombinant gene by western blot analysis or functional assay. For an antibody to the Xpress™ epitope, see page 17.
7. To purify your recombinant protein, you may use a metal-chelating resin such as ProBond™. ProBond™ resin is available separately (see page 18 for ordering information).

Methods

Cloning into pcDNA™6/His A, B, and C

Before Starting

Diagrams are provided on pages 3–5 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, 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´, and INVαF´ (see page 17 for ordering information). We recommend that you propagate vectors containing inserts in *E. coli* strains that are recombination deficient (*rec*A) and endonuclease A deficient (*end*A).

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.

Maintaining pcDNA™6/His

To propagate and maintain the pcDNA™6/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´ or INVαF´. Select transformants on LB plates containing 50 to 100 µg/mL ampicillin or 50 µg/mL blasticidin. Prepare a glycerol stock of each plasmid for long-term storage (see page 6).

Note

The pcDNA™6/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 pages 3–5 to develop a cloning strategy.

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

1. Digest pcDNA™6/His A, B, or C with *Kpn* I.
2. Create blunt ends with T4 DNA polymerase and dNTPs. See (Ausubel *et al.*, 1994) for a detailed protocol.
3. 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.

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

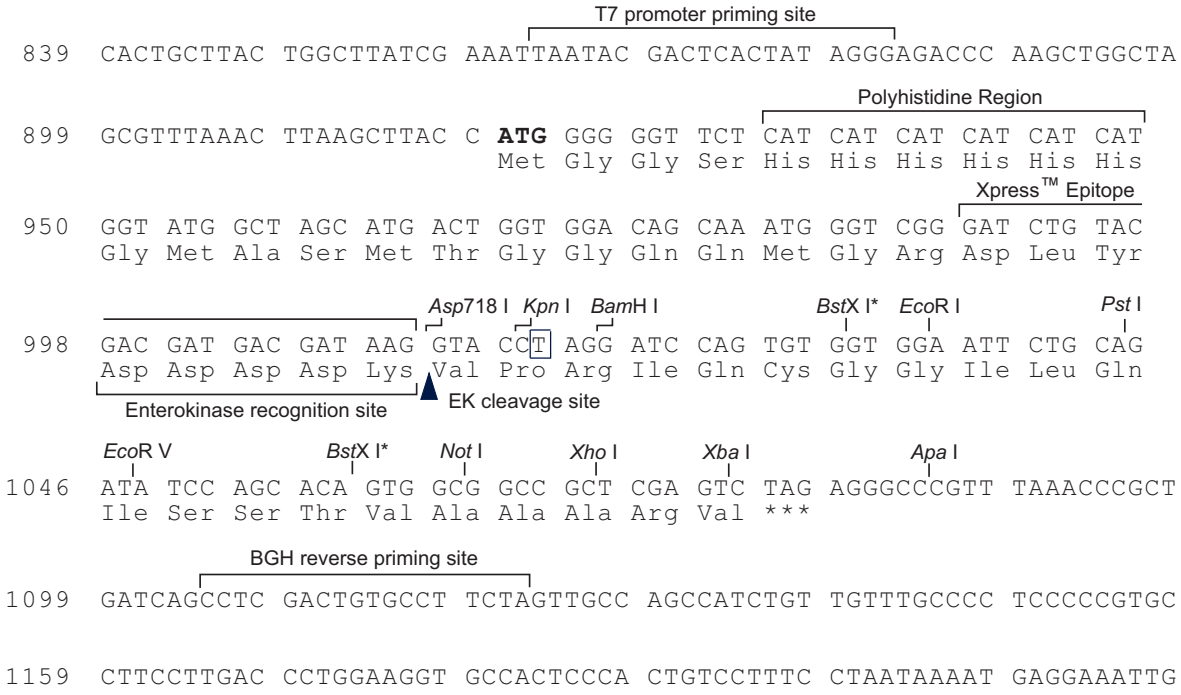
Kozak Sequence for Mammalian Expression

If you will be recombining your entry clone with a destination vector for mammalian expression, your insert should contain a Kozak consensus sequence with an ATG initiation codon for proper initiation of translation (Kozak, 1987; Kozak, 1990; Kozak, 1991). 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)NN<u>ATG</u>**G**

Multiple Cloning Site of Version A

Below is the multiple cloning site for pcDNA™6/His A. Restriction sites are labeled to indicate the cleavage site. The boxed nucleotide indicates the variable region. **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 sequence of pcDNA™6/His A is available at www.invitrogen.com or by request from Technical Support (s**ee page 19).



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

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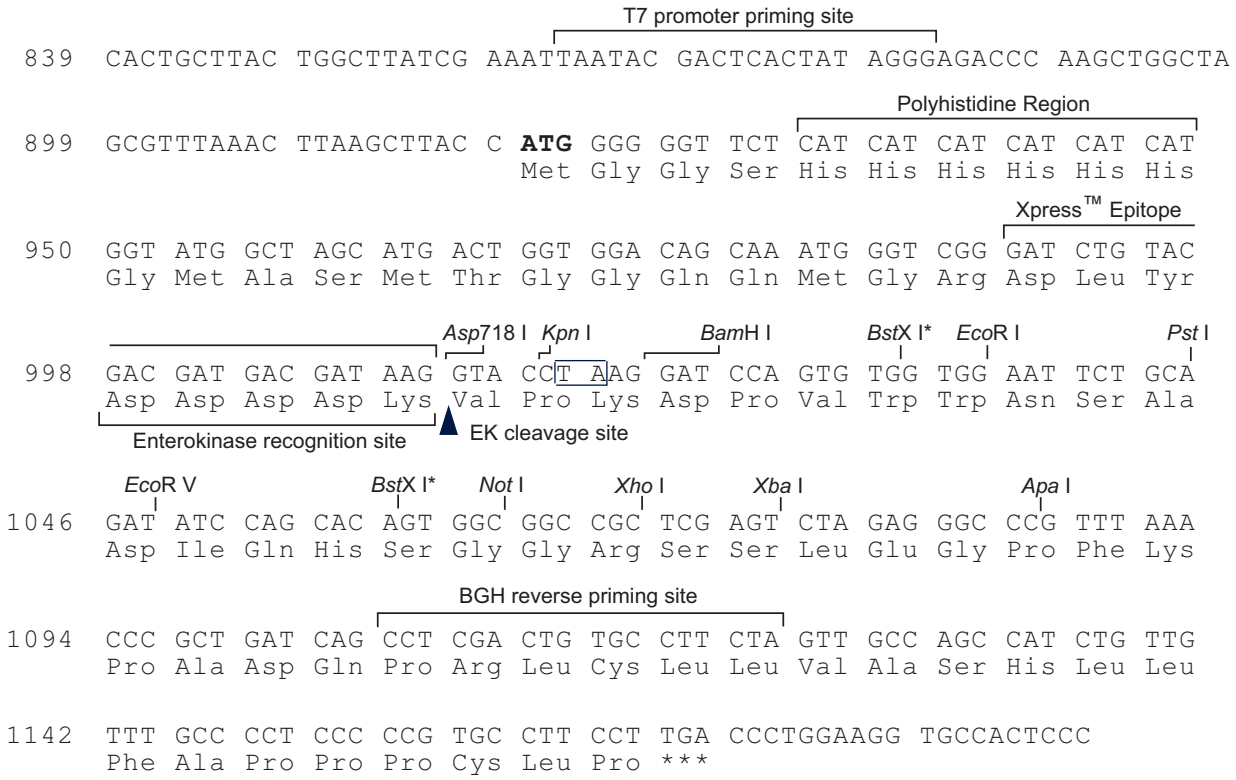
Cloning into pcDNA™6/His A, B, and C, Continued

Multiple Cloning Site of Version B

Below is the multiple cloning site for pcDNA™6/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/His B is available at www.invitrogen.com or by request from Technical Support (**see page 19).



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

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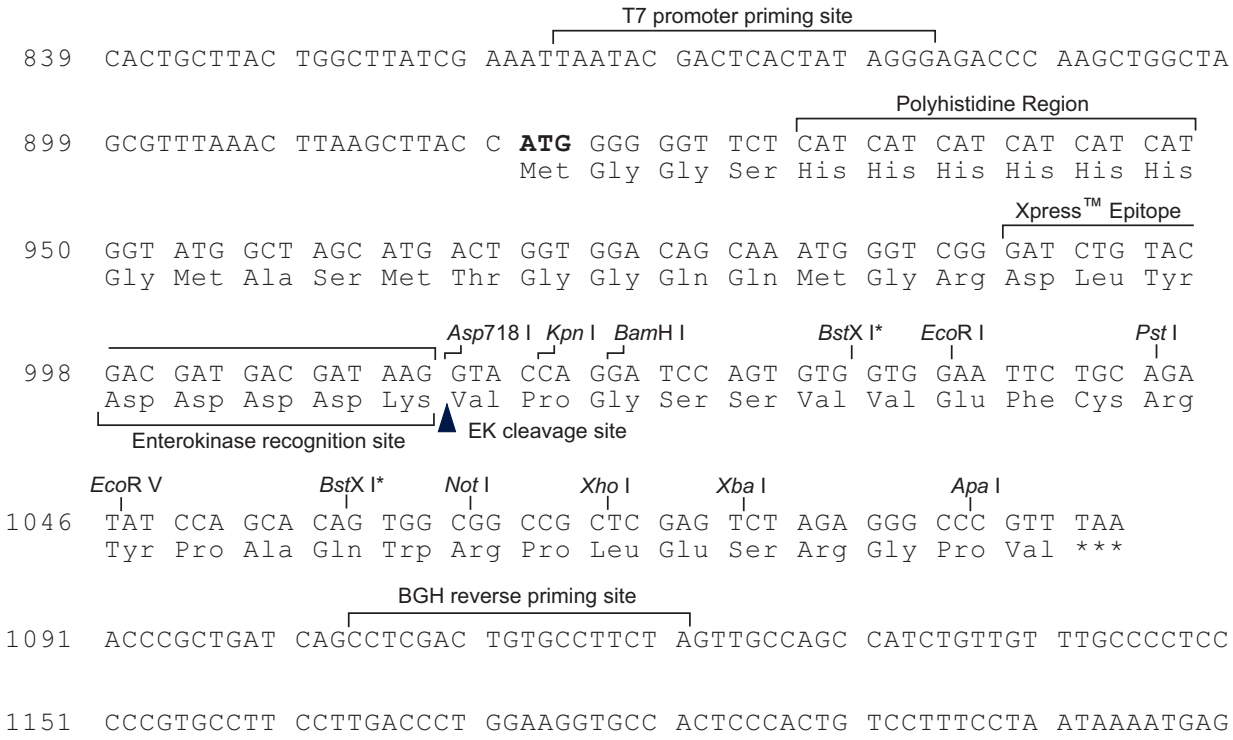
Cloning into pcDNA™6/His A, B, and C, Continued

Multiple Cloning Site of Version C

Below is the multiple cloning site for pcDNA™6/His C. Restriction sites are labeled to indicate the cleavage site. The multiple cloning site has been confirmed by sequencing and functional testing. **The sequence of pcDNA™6/His C is available at www.invitrogen.com or by request from Technical Support (**see page 19).



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

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

E. coli Transformation

Transform the ligation mixtures into a competent *rec*A, *end*A *E. coli* strain (e.g. TOP10F´, INVαF') 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.

Sequence your construct with the T7 Forward and BGH Reverse primers (see page 17 for ordering information) to confirm that your gene is fused in-frame with the Xpress™ epitope and the N-terminal polyhistidine tag. Refer to the diagrams on pages 3–5 for the sequences and locations of the priming sites. For your convenience, Invitrogen offers a custom primer service. For more information, visit www.invitrogen.com or call Technical Support (see page 19).

Primer	Sequence
T7 Forward	5′-TAATACGACTCACTATAGGG-3′
BGH Reverse	5′-TAGAAGGCACAGTCGAGG-3′

Preparing a Glycerol Stock

After identifying 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.

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

Applying Selective Pressure

Take some (if not all) of the following precautions to prevent your clone from being "overrun" by background contaminants:

- **Use carbenicillin instead of ampicillin.** Carbenicillin is more stable than ampicillin, and allows for a longer period of selective pressure.
- **Increase the antibiotic concentration.** More antibiotic means that your clones will not be overwhelmed by β-lactamase buildup.
- **Periodically refresh plate media.** If you suspect that tubes/plates may be beginning to fail, spin them down, remove the old media, and replenish the wells with fresh LB media plus glycerol and antibiotic.

Streak clones on selective (preferably carbenicillin) LB agar plates. After about 12 hours, isolate colonies for downstream usage. This will isolate your desired clones from potential background contaminants.

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 lipid complexing, decreasing transfection efficiency. We recommend isolating DNA using the PureLink™ HiPure Miniprep Kit, the PureLink™ HiPure Midiprep Kit (see page 17 for ordering information), or CsCl gradient centrifugation.

Methods of Transfection

For established cell lines (e.g., HeLa), consult original references or the supplier of your cell line for the optimal method of transfection. Precisely follow 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 22).

For high efficiency transfection in a broad range of mammalian cells, use Lipofectamine™ 2000 Reagent available from Invitrogen (see page 17). For more information on Lipofectamine™ 2000 and other transfection reagents, visit our web site at www.invitrogen.com or contact Technical Support (see page 19).

Positive Control

pcDNA™6/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.

Assay for β-galactosidase Activity

You may assay for β-galactosidase expression by activity assay using cell-free lysates (Miller, 1972) or by staining the cells for activity. Invitrogen offers the β-Gal Assay Kit and the β-Gal Staining Kit for fast and easy detection of β-galactosidase expression (see page 17 for ordering information).

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Detecting Fusion Proteins

The Anti-Xpress™ Antibody is available from Invitrogen (see page 17) and can be used to detect expression of your fusion protein from pcDNA™6/His. To detect the fusion protein by western blot, 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 below) or other suitable lysis buffer.
4. Incubate cell suspension at 37°C for 10 minutes to lyse the cells.
5. Centrifuge at 10,000 × *g* for 10 minutes to pellet nuclei and transfer the post-nuclear lysate to a new 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. Prepare the solution from the following common stock solutions.

   For 100 mL, combine:

Stock Solution	Volume
1 M Tris base	5 mL
5 M NaCl	3 mL
Nonidet P-40	1 mL

2. Bring the volume to 90 mL with deionized water and adjust the pH to 7.8 with HCl.
3. Bring the volume 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 your protein.

Purification

You will need 5×10^6 to 1×10^7 of **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, see the protocol on page 13.

Creating Stable Cell Lines

Introduction

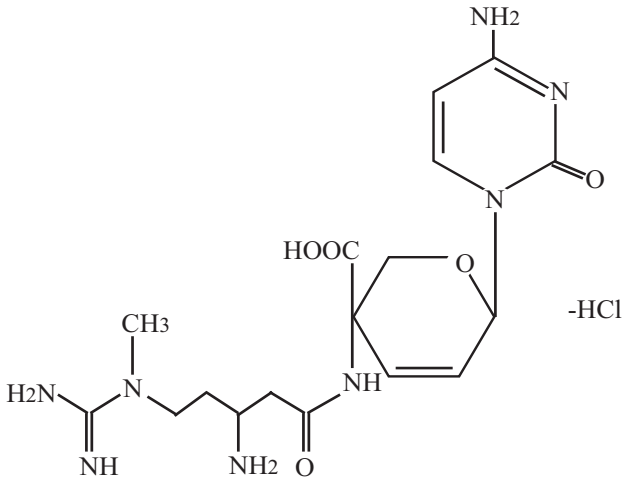
The pcDNA™6/His vectors contain the blasticidin resistance gene for selection of stable cell lines using blasticidin. Test the sensitivity of your mammalian host cell to blasticidin as natural resistance varies among cell lines. General information and guidelines are provided below for your convenience.

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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Preparing and Storing Stock Solutions

Blasticidin may be obtained from Invitrogen in 50 mg aliquots (see page 17 for ordering information). Prepare stock solutions of 5 to 10 mg/mL of blasticidin in water as described below.

- 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 likelihood that the vector will 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.** Be sure that your insert does not contain the restriction enzyme site you wish to use to linearize your vector.

Enzyme	Location	Supplier
Bgl II	Upstream of CMV promoter	Invitrogen*
Mfe I	Upstream of CMV promoter	New England Biolabs
*Bst*1107 I	End of SV40 poly A	Roche
*Eam*1105 I	Ampicillin gene	Roche
Fsp I	Ampicillin gene	New England Biolabs
Sca I	Ampicillin gene	Invitrogen*
Ssp I	Backbone	Invitrogen*

* see page 17 for ordering information

Continued on next page

Selection in Mammalian Cell Lines

To generate a stable cell line expressing your protein, first 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 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 blasticidin (e.g., 0, 1, 3, 5, 7.5, and 10 µg/mL).
3. Replenish the selective medium every 3–4 days. Cells sensitive to blasticidin will detach from the plate. Dead cells will accumulate in the medium.
4. Count the number of viable cells at regular intervals to determine the appropriate concentration of blasticidin that prevents growth.

Selecting Stable Integrants

Once the appropriate blasticidin concentration is determined, 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 blasticidin 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 blasticidin-resistant colonies are detected.
5. Pick and expand colonies.

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Preparing Cells for Lysis

Use the procedure below to prepare cells for lysis prior to purifying your protein on ProBond™ (see page 18). You will need 5×10^6 to 1×10^7 cells for purification of your protein on a 2 mL ProBond™ column (see the ProBond™ Protein Purification manual).

1. Seed cells in five T-75 flasks or 2 to 3 T-175 flasks.
2. Grow the cells in selective medium until they are 80–90% confluent.
3. Harvest the cells by treating with trypsin-EDTA for 2 to 5 minutes or by scraping the cells in PBS.
4. Inactivate the trypsin by diluting with fresh medium (if necessary) and transfer the cells to a sterile microcentrifuge tube.
5. Centrifuge the cells at 250 × *g* for 5 minutes. Resuspend the cell pellet in PBS.
6. Centrifuge the cells at 250 × *g* for 5 minutes. You may lyse the cells immediately or freeze in liquid nitrogen and store at −80°C until needed.

Lysing Cells

If you are using ProBond™ resin, refer to the ProBond™ Protein Purification manual for details about sample preparation for chromatography.

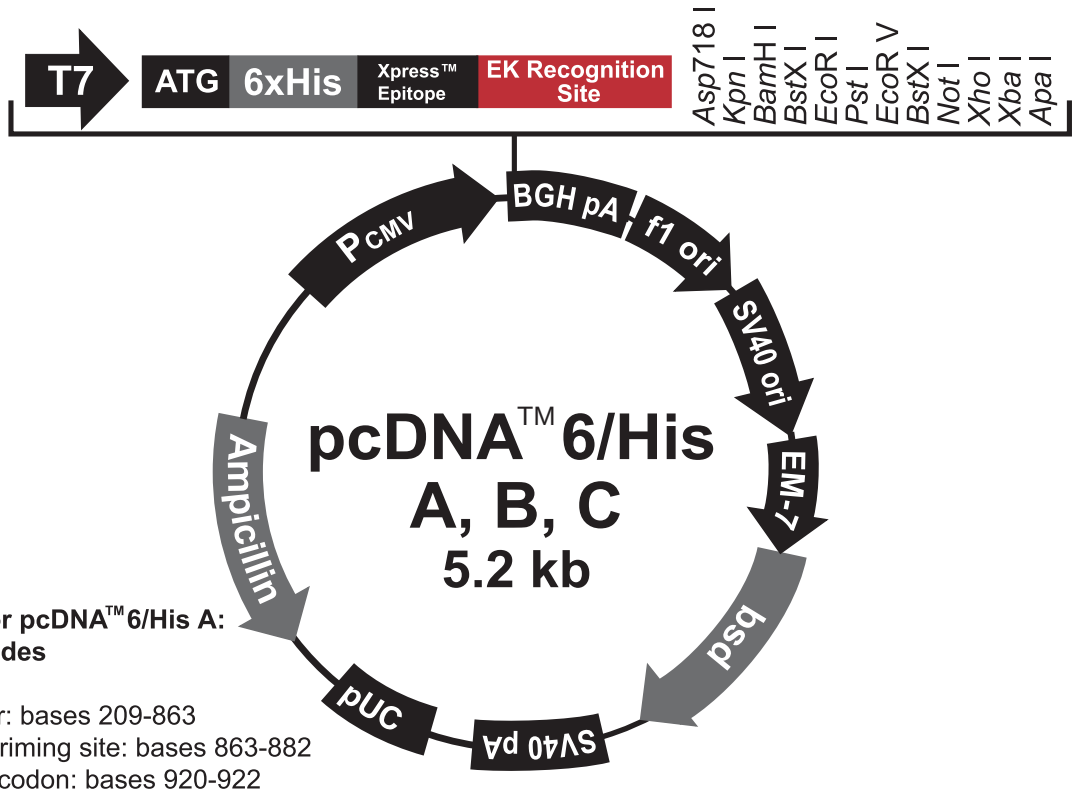
If you are using another metal-chelating resin, refer to the manufacturer's instructions for recommendations on sample preparation.

Appendix

pcDNA™6/His Vector

Map of pcDNA™6/His

The figure below summarizes the features of the pcDNA™6/His vectors. **The sequences for pcDNA™6/His A, B, and C are available at www.invitrogen.com or by request from Technical Support (see page 19).**



Comments for pcDNA™6/His A: 5149 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
BGH reverse priming site: bases 1105-1122
BGH polyadenylation signal: bases 1108-1335
f1 origin: bases 1389-1809
SV40 promoter and origin: bases 1837-2144
EM-7 promoter: bases 2192-2247
Blasticidin resistance gene: bases 2266-2664
SV40 polyadenylation signal: bases 2822-2952
pUC origin: bases 3335-4008
bla promoter bases: 5014-5112 (C)
Ampicillin (*bla*) resistance gene: bases 4153-5013 (C)
(C) = Complementary strand

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pcDNA™6/His Vector, Continued

Features of pcDNA™6/His

pcDNA™6/His A (5,150 bp), pcDNA™6/His B (5,151 bp), and pcDNA™6/His C (5,149 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 recombinant protein on metal-chelating resin such as ProBond™.
Xpress™ epitope tag	Allows for the detection of an 8 amino acid epitope (Asp-Leu-Tyr-Asp-Asp-Asp-Asp-Lys) on the recombinant protein with the Anti-Xpress™ Antibody.
Enterokinase cleavage site	Allows for the removal of the N-terminal polyhistidine tag from the recombinant protein using an enterokinase such as EKMax™ Enterokinase (see page 17).
Multiple cloning site in three reading frames	Allows for the insertion of your gene and facilitates cloning in-frame with the Xpress™ epitope and N-terminal polyhistidine tag.
BGH reverse priming site	Permits sequencing through the insert.
Bovine growth hormone (BGH) polyadenylation signal	Efficient transcription termination and polyadenylation of mRNA (Goodwin & Rottman, 1992).
f1 origin	Allows rescue of single-stranded DNA.
SV40 early promoter and origin	Allows for 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.*

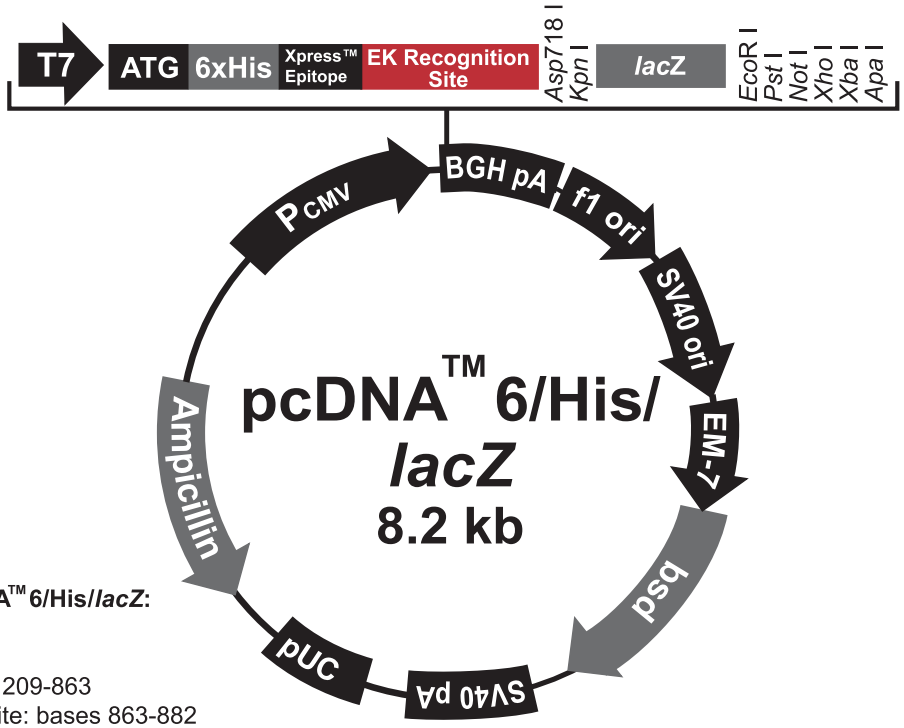
pcDNA™6/His/*lacZ*

Description

pcDNA™6/His/*lacZ* is a 8,213 bp control vector containing the gene for β-galactosidase. This vector was constructed by ligating a 3,082 bp *Kpn* I-*Eco*R I fragment containing the *lacZ* gene from pcDNA4™/His/*lacZ* to a 5,131 bp *Kpn* I-*Eco*R I fragment containing the CMV promoter, Xpress™ epitope, and polyhistidine tag from pcDNA™6/His B.

Map of Control Vector

The figure below summarizes the features of the pcDNA™6/His/*lacZ* vector. **The nucleotide sequence for pcDNA™6/His/*lacZ* is available at www.invitrogen.com or by request from Technical Support (see page 19).**



Comments for pcDNA™6/His/*lacZ*: 8212 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
BGH reverse priming site: bases 4168-4185
BGH polyadenylation signal: bases 4171-4398
f1 origin: bases 4444-4872
SV40 promoter and origin: bases 4900-5207
EM-7 promoter: bases 5255-5310
Blasticidin resistance gene: bases 5329-5727
SV40 polyadenylation signal: bases 5885-6015
pUC origin: bases 6398-7071
bla promoter bases: 8077-8175 (C)
Ampicillin (*bla*) resistance gene: bases 7216-8076 (C)
(C)= Complementary strand

Accessory Products

Additional Products

The following additional products may be used with the pcDNA™6/His vectors. For more information, visit www.invitrogen.com or contact Technical Support (see page 19).

Item	Quantity	Cat. no.
Electrocomp™ Kit (TOP10F´)	2 × 20 reactions	C665-11
	6 × 20 reactions	C665-24
One Shot® TOP10F´ Chemically Competent *E. coli*	20 × 50 µL	C3030-03
One Shot® INVαF' Chemically Competent *E. coli*	20 × 50 µL	C2020-03
	40 × 50 µL	C2020-06
Ampicillin	200 mg	11593-027
Blasticidin	50 mg	R210-01
Carbenicillin	5 g	10177-012
T7 promoter primer	2 µg	N560-02
BGH Reverse primer	2 µg	N575-02
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
β–Gal Assay Kit	1 kit	K1455-01
β–Gal Staining Kit	1 kit	K1465-01
Anti-Xpress™ Antibody	50 µL	R910-25
EKMax™ Enterokinase	250 units	E180-01

Restriction Enzymes

For your convenience, Invitrogen offers an extensive selection of restriction enzymes, including the following:

- *Bgl II*
- *Ssp* I
- *Sca* I

Visit www.invitrogen.com for more details.

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Accessory Products, Continued

ProBond™ Resin

Ordering information for ProBond™ resin is provided below.

Item	Quantity	Cat. no.
ProBond™ Purification System	12 mL precharged ProBond™ resin, 6 columns, and buffers for native and denaturing purification	K850-01
ProBond™ Resin	50 mL	R801-01
	150 mL	R801-15

Other Mammalian Expression Vectors

Invitrogen offers 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 polyhistidine epitopes for detection and either the neomycin, blasticidin, or Zeocin™ resistance genes. All vectors utilize the polyhistidine tag for purification. For more information on the mammalian expression vectors available, visit www.invitrogen.com or contact Technical Support (see page 19).

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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References

Andersson, S., Davis, D. L., Dahlbäck, H., Jörnvall, H., and Russell, D. W. (1989) Cloning, Structure, and Expression of the Mitochondrial Cytochrome P-450 Sterol 26-Hydroxylase, a Bile Acid Biosynthetic Enzyme. J. Biol. Chem. *264*, 8222-8229

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1994) *Current Protocols in Molecular Biology*, Greene Publishing Associates and Wiley-Interscience, New York

Boshart, M., Weber, F., Jahn, G., Dorsch-Häsler, K., Fleckenstein, B., and Schaffner, W. (1985) A Very Strong Enhancer is Located Upstream of an Immediate Early Gene of Human Cytomegalovirus. Cell *41*, 521-530

Goodwin, E. C., and Rottman, F. M. (1992) The 3´-Flanking Sequence of the Bovine Growth Hormone Gene Contains Novel Elements Required for Efficient and Accurate Polyadenylation. J. Biol. Chem. *267*, 16330-16334

Izumi, M., Miyazawa, H., Kamakura, T., Yamaguchi, I., Endo, T., and Hanaoka, F. (1991) Blasticidin S-Resistance Gene (*bsr*): A Novel Selectable Marker for Mammalian Cells. Exp. Cell Res. *197*, 229-233

Kimura, M., Takatsuki, A., and Yamaguchi, I. (1994) Blasticidin S Deaminase Gene from *Aspergillus terreus* (*BSD*): A New Drug Resistance Gene for Transfection of Mammalian Cells. Biochim. Biophys. ACTA *1219*, 653-659

Kozak, M. (1987) An Analysis of 5´-Noncoding Sequences from 699 Vertebrate Messenger RNAs. Nucleic Acids Res. *15*, 8125-8148

Kozak, M. (1990) Downstream Secondary Structure Facilitates Recognition of Initiator Codons by Eukaryotic Ribosomes. Proc. Natl. Acad. Sci. USA *87*, 8301-8305

Kozak, M. (1991) An Analysis of Vertebrate mRNA Sequences: Intimations of Translational Control. J. Cell Biology *115*, 887-903

Miller, J. H. (1972) *Experiments in Molecular Genetics*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Nelson, J. A., Reynolds-Kohler, C., and Smith, B. A. (1987) Negative and Positive Regulation by a Short Segment in the 5´-Flanking Region of the Human Cytomegalovirus Major Immediate-Early Gene. Molec. Cell. Biol. *7*, 4125-4129

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, Second Ed., Cold Spring Harbor Laboratory Press, Plainview, New York

Takeuchi, S., Hirayama, K., Ueda, K., Sakai, H., and Yonehara, H. (1958) Blasticidin S, A New Antibiotic. The Journal of Antibiotics, Series A *11*, 1-5

Yamaguchi, H., Yamamoto, C., and Tanaka, N. (1965) Inhibition of Protein Synthesis by Blasticidin S. I. Studies with Cell-free Systems from Bacterial and Mammalian Cells. J. Biochem (Tokyo) *57*, 667-677

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