invitrogen™

pcDNA™3.1/V5-His A, B, and C

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User Manual

Contents

Contents and Storage

Contents

20 µg each of pcDNA™3.1/V5-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™3.1/V5-His/*lac*Z 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.

Shipping and Storage

pcDNA™3.1/V5-His vectors are shipped on wet ice. Upon receipt, store vectors at –20ºC.

Methods

Cloning into pcDNA™3.1/V5-His A, B, and C

Introduction

pcDNA™3.1/V5-His A, B, and C are 5.5 kb vectors derived from pcDNA™3.1(+) and designed for high-level expression, purification, and detection of recombinant proteins in mammalian hosts. High-level stable and non-replicative transient expression can be carried out in most mammalian cells. The vectors contain the following elements:

- Three reading frames to facilitate in-frame cloning with a C-terminal tag encoding the V5 epitope and a polyhistidine metal-binding peptide
- Human cytomegalovirus (CMV) immediate-early promoter for high-level expression in a wide range of mammalian cells
- 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™3.1/V5-His/*lac*Z, contains a 3.2 kb fragment containing the β-galactosidase gene cloned in frame with the C-terminal peptide (see page 11). pcDNA™3.1/V5-His/*lac*Z is included for use as a positive control for transfection, expression, purification, and detection in the cell line of choice.

Experimental Outline

Use the following outline to clone and express your gene of interest in pcDNA™3.1/V5-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 V5 epitope and polyhistidine tag.
- Ligate your insert into the appropriate vector and transform into *E. coli*. Select transformants with 50 to 100 µg/ml ampicillin.
- 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 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. If you do not have an antibody to your protein, Invitrogen offers Anti-V5 antibodies or Anti-His(C-term) antibodies to detect your recombinant protein. See page 13 for ordering information.
- To purify your recombinant protein, you may use metal-chelating resin such as ProBond™. ProBond™ resin is available separately (see page 13 for ordering information).

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Cloning into pcDNA™3.1/V5-His A, B, and C, Continued

Before Starting

Diagrams are provided on pages 3–5 to help you ligate your gene of interest in frame with the C-terminal peptide. General considerations are listed below for additional information. For information on transformation and transfection, see page 6.

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).

Maintaining pcDNA™3.1/V5-His

Many *E. coli* strains are suitable for the growth of this vector. To propagate and maintain pcDNA™3.1/V5-His A,B, and C, use 10 ng of the vector to transform a *rec*A (recombination deficient), *end*A (endonuclease A deficient) *E. coli* strain like TOP10, TOP10F′, DH5α™-T1^R, DH10B™, or equivalent (see page 12 for ordering information). Select the transformants on LB plates containing 50 to 100 µg/ml ampicillin.

For long-term storage, prepare a glycerol stock of your plasmid containing *E. coli* strain.

Kozak Sequence for Mammalian Expression

If you are 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, 1991; 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.

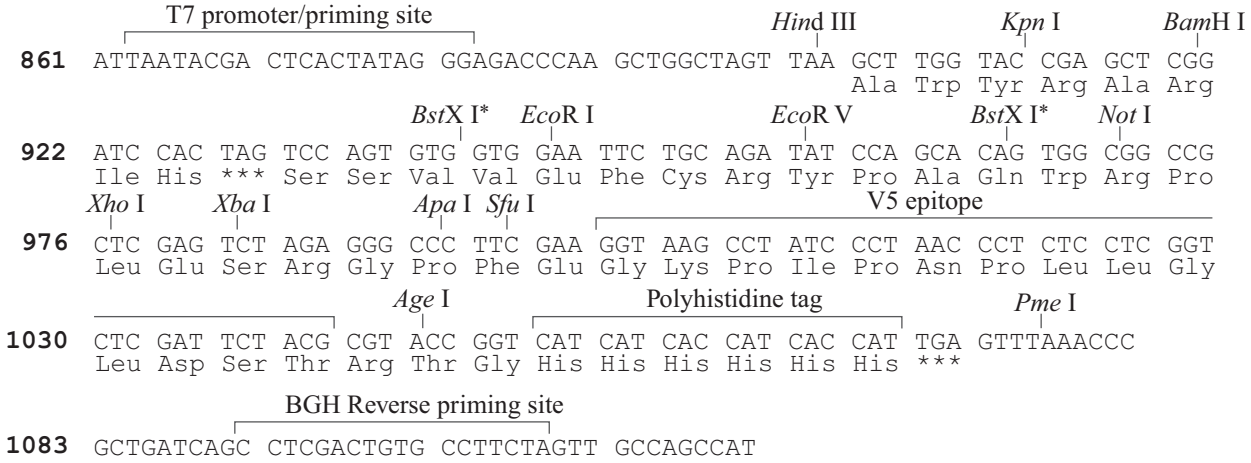
(G/A)NN<u>ATG</u>**G**

Continued on next page

Cloning into pcDNA™3.1/V5-His A, B, and C, Continued

Multiple Cloning Site of pcDNA™3.1/V5-His A

Below is the multiple cloning site for pcDNA™3.1/V5-His A. Restriction sites are labeled to indicate the cleavage site. **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 is available for downloading from www.invitrogen.com or by contacting Technical Support (see page 14).



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

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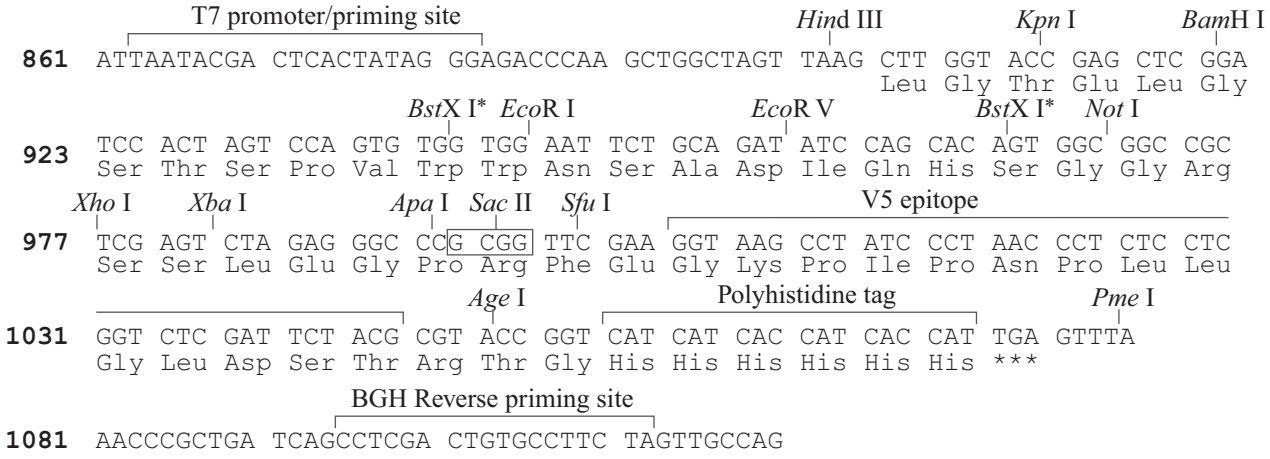
Cloning into pcDNA™3.1/V5-His A, B, and C, Continued

Multiple Cloning Site of pcDNA™3.1/V5-His B

Below is the multiple cloning site for pcDNA™3.1/V5-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 is available for downloading from www.invitrogen.com or by contacting Technical Support (see page 14).



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

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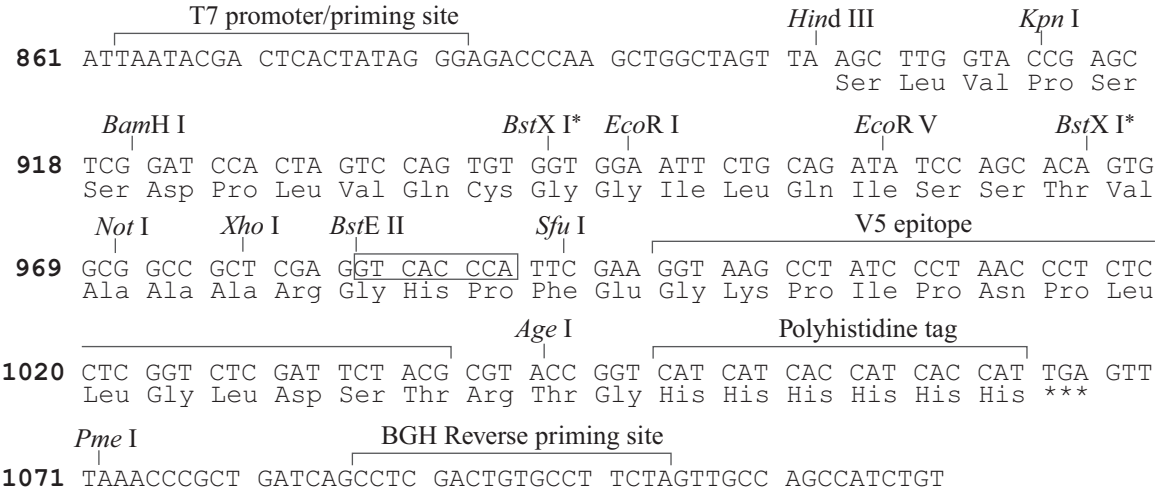
Cloning into pcDNA™3.1/V5-His A, B, and C, Continued

Multiple Cloning Site of pcDNA™3.1/V5-His C

Below is the multiple cloning site for pcDNA™3.1/V5-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 is available for downloading from www.invitrogen.com or by contacting Technical Support (see page 14).



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

Transformation and Transfection

E. coli Transformation

Transform your ligation mixtures into a competent *rec*A, *end*A *E. coli* strain (e.g., TOP10, TOP10F', DH5α™-T1^R, DH10B™, page 12) and select on LB plates containing 50–100 µg/ml ampicillin. Select 10–20 clones and analyze for the presence and orientation of your insert.

Applying Selective Pressure

We recommend taking 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, thus preserving your clones longer.
- **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.

We recommend that you sequence your construct with the T7 Promoter and BGH Reverse primers (see page 12 for ordering information) to confirm that your gene is fused in frame with the V5 epitope and the C-terminal polyhistidine tag. Refer to the diagrams on pages 3–5 for the sequence and location of the priming sites.

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

For your convenience, Invitrogen offers a custom primer service. For more information, visit www.invitrogen.com or call Technical Support (see page 14).

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 or the PureLink™ HiPure Midiprep Kit (see page 12 for ordering information) or CsCl gradient centrifugation.

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Transformation and Transfection, Continued

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, paying 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, use Lipofectamine™ 2000 Reagent available from Invitrogen (see page 12). For more information on Lipofectamine™ 2000 and other transfection reagents, visit our web site at www.invitrogen.com or contact Technical Support (see page 14).

Positive Control

pcDNA™3.1/V5-His/*lac*Z is provided as a positive control vector for mammalian transfection and expression (see page 11). pcDNA™3.1/V5-His/*lac*Z may be used to optimize transfection conditions for your cell line. The gene encoding β-galactosidase is expressed in mammalian cells as a fusion protein (MW 121 kDa). A successful transfection results 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 (see page 12 for ordering information) for fast, easy detection of β-galactosidase expression.

Detection of Fusion Proteins

A number of antibodies are available from Invitrogen that can be used to detect expression of your fusion protein from pcDNA™3.1/V5-His (see page 13 for ordering information).

Note

The C-terminal tag adds about 3 kDa to the size of your protein. Additional amino acids may be added to your protein depending on the sites used to clone the gene of interest.

Geneticin® Selective Antibiotic

For stable transfection, pcDNA™3.1/V5-His A, B, and C contain the resistance factor to Geneticin®. Geneticin® blocks protein synthesis in mammalian cells by interfering with ribosomal function. It is an aminoglycoside, similar in structure to neomycin, gentamycin, and kanamycin. Expression in mammalian cells of the bacterial aminoglycoside phosphotransferase gene (APH), derived from Tn*5*, results in detoxification of Geneticin® Selective Antibiotic (Southern and Berg, 1982).

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Geneticin® Selection Guidelines

Geneticin® is available from Invitrogen (see page 12 for ordering information). Use as follows:

1. Prepare Geneticin® in a buffered solution (e.g. 100 mM HEPES, pH 7.3).
2. Use 100 to 1,000 µg/ml of Geneticin® in complete medium.
3. Calculate concentration based on the amount of active drug (check the lot label).
4. Test varying concentrations of Geneticin® on your cell line to determine the concentration that kills your cells (kill curve). Cells differ in their susceptibility to Geneticin®.

Cells will divide once or twice in the presence of lethal doses of Geneticin® Selective Antibiotic, so the effects of the drug take several days to become apparent. Complete selection can take from 2 to 4 weeks of growth in selective medium.

Preparing Cells for Lysis

Use the procedure below to prepare cells for lysis prior to purification of your protein on ProBond™ (see page 13). 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 five T-75 flasks or 2 to 3 T-150 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, if necessary, and transfer the cells to a sterile microcentrifuge tube.
5. Centrifuge the cells at approximately $250 \times g$ for 5 minutes. You may lyse the cells immediately or freeze in liquid nitrogen and store at –80°C until needed.

Lysing of Cells

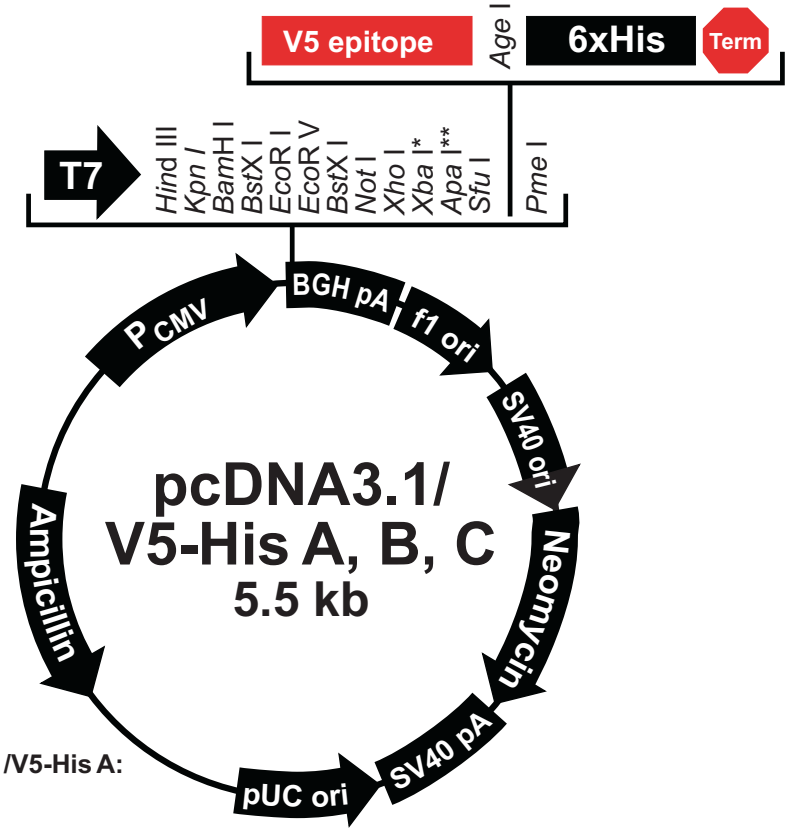
If you are using ProBond™ resin, refer to the ProBond™ Protein Purification manual for details about sample preparation for chromatography. If you are using other metal-chelating resin, refer to the manufacturer's instruction.

Appendix

pcDNA™3.1/V5-His A, B, and C Vectors

Map of pcDNA™3.1/V5-His

The figure below summarizes the features of the pcDNA™3.1/V5-His vectors. The sequences for pcDNA™3.1/V5-His A, B, and C are available for downloading from www.invitrogen.com or by contacting Technical Support (see page 14). Details of the multiple cloning sites are shown on page 3 for pcDNA™3.1/V5-His A, page 4 for pcDNA™3.1/V5-His B, and page 5 for pcDNA™3.1/V5-His C.



Comments for pcDNA3.1/V5-His A: 5502 nucleotides

CMV promoter: bases 209-863
T7 promoter/priming site: bases 863-882
Multiple cloning site: bases 902-999
V5 epitope: bases 1000-1041
Polyhistidine (6xHis) tag: bases 1051-1068
BGH reverse priming site: bases 1091-1108
BGH polyadenylation signal: bases 1090-1304
f1 origin of replication: bases 1357-1780
SV40 promoter and origin: bases 1845-2169
Neomycin resistance gene: bases 2205-2999
SV40 polyadenylation signal: bases 3018-3256
pUC origin: bases 3688-4361 (C)
Ampicillin resistance gene: bases 4506-5366 (C)
(C) = complementary strand

* After the *Xho* I site, 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 *Sfu* I site in version B only.

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Features of pcDNA™3.1/ V5-His

pcDNA™3.1/V5-His A (5,502 bp), pcDNA™3.1/V5-His B (5,506 bp), and pcDNA™3.1/ V5-His C (5,498 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 V5 epitope and polyhistidine C-terminal tag.
V5 epitope (Gly-Lys-Pro-Ile-Pro-Asn-Pro-Leu-Leu-Gly-Leu-Asp-Ser-Thr)	Allows detection of your recombinant protein with the Anti-V5 Antibody (Catalog no. R960-25) and the Anti-V5-HRP Antibody (Catalog no. R961-25) (Southern *et al.*, 1991).
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 (Catalog no. R930-25) and the Anti-His (C-term)-HRP Antibody (Catalog no. R931-25).
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 neomycin resistance gene and episomal replication in cells expressing the SV40 large T antigen.
Neomycin resistance gene	Selection of stable transfectants in mammalian cells (Southern and Berg, 1982).
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 vector in *E. coli.*

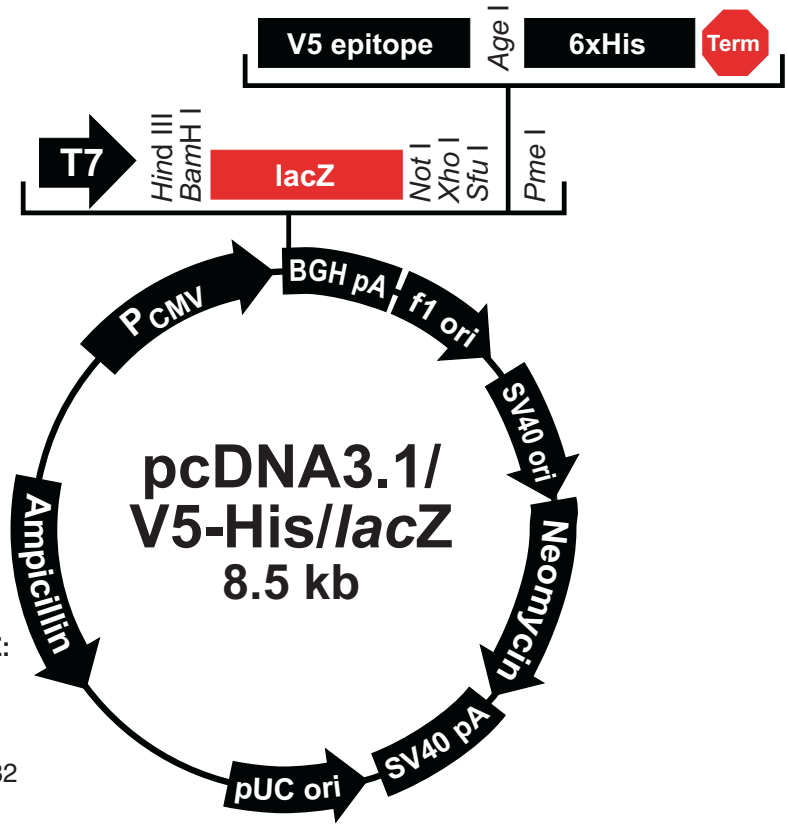
pcDNA™3.1/V5-His/*lacZ*

Description

pcDNA™3.1/V5-His/*lacZ* is a 8,549 bp control vector containing the gene for β-galactosidase. pcDNA™3.1/V5-His C was digested with *Eco*R V and *Not* I. A 3.2 kb blunt-*Not* I fragment containing the β-galactosidase gene was then ligated into pcDNA™3.1/V5-His C in frame with the C-terminal peptide.

Map of pcDNA™3.1/ V5-His/*lacZ*

The figure below summarizes the features of the pcDNA™3.1/V5-His/*lacZ* vector. The nucleotide sequence for pcDNA™3.1/V5-His/*lacZ* is available for downloading from www.invitrogen.com or by contacting Technical Support (see page 14).



Comments for pcDNA3.1/V5-His/*lacZ*: 8549 nucleotides

CMV promoter: bases 209-863
T7 promoter/priming site: bases 863-882
lacZ with C-terminal tag: 963-4115
  lacZ ORF: bases 963-4019
  V5 epitope: bases 4047-4088
  Polyhistidine (6xHis) tag: bases 4098-4115
BGH reverse priming site: bases 4138-4155
BGH polyadenylation signal: bases 4137-4351
f1 origin of replication: bases 4414-4827
SV40 promoter and origin: bases 4892-5216
Neomycin resistance gene: bases 5252-6046
SV40 polyadenylation signal: bases 6065-6303
pUC origin: bases 6735-7408 (C)
Ampicillin resistance gene: bases 7553-8413 (C)
(C) = complementary strand

Accessory Products

Additional Products

The following additional products may be used with the pcDNA™3.1/V5-His vectors. For more information, visit www.invitrogen.com or contact Technical Support (see page 14).

Item	Quantity	Cat. no.
One Shot® TOP10 Chemically Competent *E. coli*	10 reactions	C4040-10
One Shot® TOP10F´ Chemically Competent *E. coli*	20 × 50 µl	C3030-03
One Shot® Max Efficiency® DH5α™ T1^R Competent Cells	20 × 50 µl	12297-016
Max Efficiency® DH10B™ Competent Cells	5 × 0.2 ml	18297-010
Electrocomp™ Kit	2 × 20 reactions	C66511
Ampicillin	200 mg	11593-027
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

Geneticin® Selective Antibiotic

For stable transfection, the pcDNA™3.1/V5-His vectors contain the resistance factor to Geneticin®. Geneticin® is available from Invitrogen. For more information, visit www.invitrogen.com or contact Technical Support (see page 14).

Item	Quantity	Cat. no.
Geneticin® Selective Antibiotic	1 g	11811-023
	5 g	11811-031
	25 g	11811-098
	20 ml (50 mg/ml)	10131-035
	100 ml (50 mg/ml)	10131-027

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Accessory Products, Continued

Detecting Fusion Proteins

A number of antibodies are available from Invitrogen that can be used to detect expression of your fusion protein from pcDNA™3.1/V5-His. The table below describes the antibodies available and ordering information. The amount of antibody supplied is sufficient for 25 western blots.

Antibody	Purpose	Cat. no.
Anti-V5	Detects 14 amino acid epitope derived from the P and V proteins of the paramyxovirus, SV5 (Southern *et al.*, 1991)	R960-25
Anti-V5-HRP		R961-25
Anti-V5-AP		R962-25
Anti-His(C-term)	Detects the C-terminal polyhistidine tag (requires the free carboxyl group for detection)	R930-25
Anti-His(C-term)-HRP		R931-25
Anti-His(C-term)-AP		R932-25

Purifying Fusion Proteins

The following products can be used in conjunction with pcDNA™3.1/V5-His vectors to purify recombinant protein.

Item	Quantity	Cat. no.
ProBond™ Purification System	6 purifications	K850-01
ProBond™ Nickel-Binding Resin (Precharged resin provided as a 50% slurry in 20% ethanol)	50 ml	R801-01
	150 ml	R801-15

Technical Support

Web Resources

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

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

Contact Us

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

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MSDS

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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 (New York: Greene Publishing Associates and Wiley-Interscience).

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.

Chen, C., and Okayama, H. (1987). High-Efficiency Transformation of Mammalian Cells by Plasmid DNA. Mol. Cell. Biol. *7*, 2745-2752.

Chu, G., Hayakawa, H., and Berg, P. (1987). Electroporation for the Efficient Transfection of Mammalian Cells with DNA. Nuc. Acids Res. *15*, 1311-1326.

Felgner, P. L., Holm, M., and Chan, H. (1989). Cationic Liposome Mediated Transfection. Proc. West. Pharmacol. Soc. *32*, 115-121.

Felgner, P. L., and Ringold, G. M. (1989). Cationic Liposome-Mediated Transfection. Nature *337*, 387-388.

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.

Kozak, M. (1987). An Analysis of 5´-Noncoding Sequences from 699 Vertebrate Messenger RNAs. Nuc. 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.

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

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. Mol. Cell. Biol. *7*, 4125-4129.

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

Shigekawa, K., and Dower, W. J. (1988). Electroporation of Eukaryotes and Prokaryotes: A General Approach to the Introduction of Macromolecules into Cells. BioTechniques *6*, 742-751.

Southern, J. A., Young, D. F., Heaney, F., Baumgartner, W., and Randall, R. E. (1991). Identification of an Epitope on the P and V Proteins of Simian Virus 5 That Distinguishes Between Two Isolates with Different Biological Characteristics. J. Gen. Virol. *72*, 1551-1557.

Southern, P. J., and Berg, P. (1982). Transformation of Mammalian Cells to Antibiotic Resistance with a Bacterial Gene Under Control of the SV40 Early Region Promoter. J. Molec. Appl. Gen. *1*, 327-339.

Wigler, M., Silverstein, S., Lee, L.-S., Pellicer, A., Cheng, Y.-C., and Axel, R. (1977). Transfer of Purified Herpes Virus Thymidine Kinase Gene to Cultured Mouse Cells. Cell *11*, 223-232.

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