invitrogen™

pUB6/V5-His A, B, and C

Catalog no. V250-01 and V250-20

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User Manual

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Kit Contents and Storage

Shipping and Storage

pUB6/V5-His A, B, and C vectors are shipped on wet ice. Upon receipt, store vectors at –20°C.

Kit Contents

The pUB6/V5-His A, B, and C vectors and the pUB6/V5-His */lacZ* control plasmid are supplied with each product (Cat. no. V250-01, V250-20). The pUB6/V5-His with Blasticidin Kit (Cat. no. V250-01) also includes blasticidin antibiotic. The amount of each reagent provided is listed below.

Cat. no.	Vector	Composition	Amount
V250-01	pUB6/V5-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
	pUB6/V5-His */lacZ*	40 µL of 0.5 µg/µL vector in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0	20 µg
	Blasticidin	powder	50 mg
V250-20	pUB6/V5-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
	pUB6/V5-His */lacZ*	40 µL of 0.5 µg/µL vector in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0	20 µg

Intended Use

For research use only. Not intended for human or animal diagnostic or therapeutic uses.

Introduction

Product Overview

Description of the System

pUB6/V5-His A, B, and C are 5.5 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 12–13 for more information). High-level stable and transient expression can be carried out in most mammalian cells. The vectors contain the following elements:

- Human ubiquitin C promoter (hUbC) for high-level expression across a broad range of species and cell types (Schorpp *et al.*, 1996; Wulff *et al.*, 1990) (see page 11 for more information).
- Three reading frames to facilitate in-frame cloning with a C-terminal peptide encoding the V5 epitope and a polyhistidine (6×His) metal-binding tag.
- Blasticidin resistance gene (*bsd*) for selection of stable cell lines (see page 15 for more information).
- Episomal replication in cell lines that are latently infected with SV40 or that express the SV40 large T antigen (*e.g.* COS-1, COS-7).

The control plasmid, pUB6/V5-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 pUB6/V5-His.

1. 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.
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 C-terminal peptide.
5. Transfect your construct into the cell line of choice. Generate a stable cell line, if desired.
6. Test for expression of your recombinant gene by western blot analysis or other functional assay. For antibodies to the V5 epitope or the polyhistidine, C-terminal tag, see the page 17.
7. To purify your recombinant protein, you may use metal-chelating resin such as ProBond™. ProBond™ resin is available separately (see page 17 for ordering information).

Methods

Cloning into pUB6/V5-His A, B, and C

General Molecular Biology Techniques

For help with DNA ligations, *E. coli* transformations, restriction enzyme analysis, purification of single-stranded DNA, DNA sequencing, and DNA biochemistry, refer to *Molecular Cloning: A Laboratory Manual* (Sambrook *et al.*, 1989) or *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994).

E. coli Strain for Transformation

Many *E. coli* strains are suitable for the propagation of this vector, including TOP10F´, JM109, and INVαF´. We recommend that you propagate vectors containing inserts in *E. coli* strains that are recombination deficient (*rec*A) and endonuclease A deficient (*end*A).

For your convenience, TOP10F´ is available as chemically competent or electrocompetent cells from Invitrogen (see page 17).

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.

Maintaining pUB6/V5-His

To propagate and maintain the pUB6/V5-His vectors, use a small amount of 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 plasmid-containing *E. coli* strain for long-term storage (see page 5).

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

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

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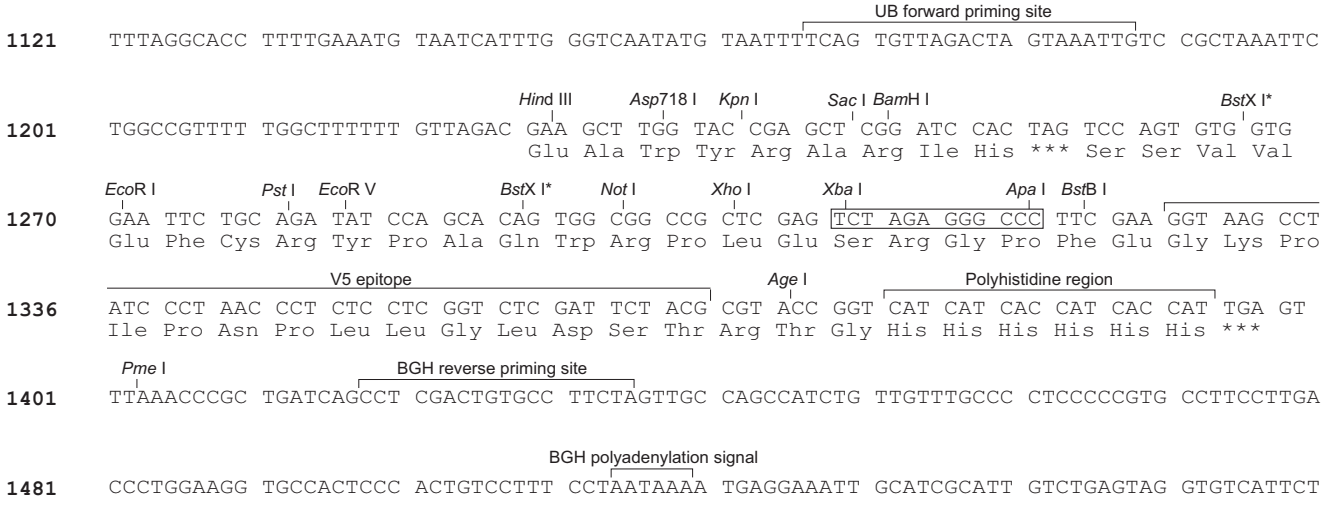
Cloning into pUB6/V5-His A, B, and C, Continued

Multiple Cloning Site of pUB6/V5-His A

Below is the multiple cloning site for pUB6/V5-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 vector sequence may be downloaded from www.invitrogen.com or from **Technical Support** (see page 18). For more information on the hUbC promoter, see page 11.



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

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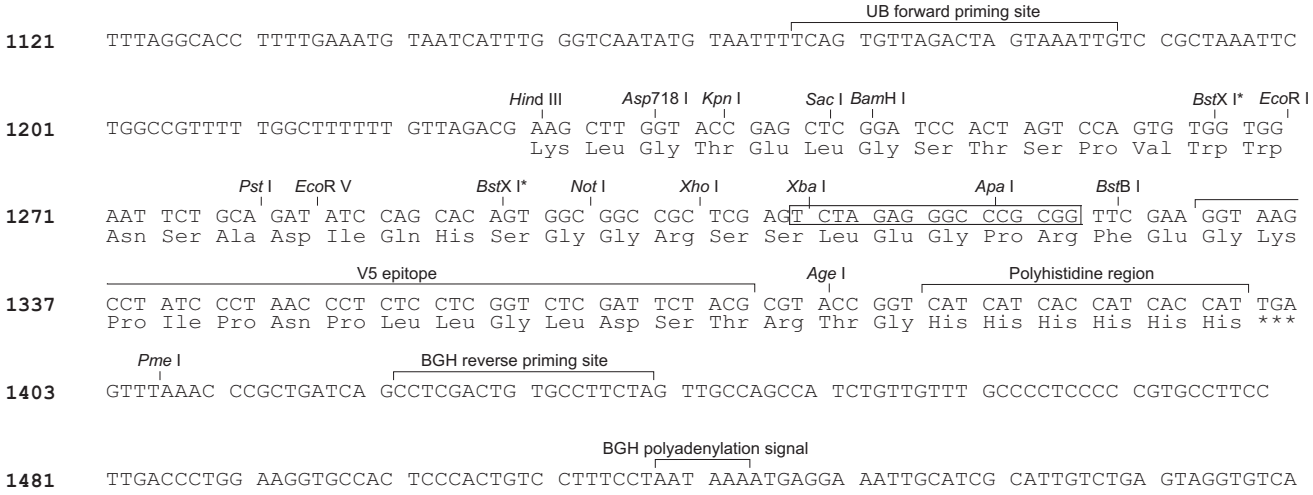
Cloning into pUB6/V5-His A, B, and C, Continued

Multiple Cloning Site of pUB6/V5-His B

Below is the multiple cloning site for pUB6/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 vector sequence may be downloaded from www.invitrogen.com or from **Technical Support** (see page 18). For more information on the hUbC promoter, see page 11.



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

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Cloning into pUB6/V5-His A, B, and C, Continued

Multiple Cloning Site of pUB6/V5-His C

Below is the multiple cloning site for pUB6/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 vector sequence may be downloaded from www.invitrogen.com or from **Technical Support** (see page 18).

```
                                                              UB forward priming site
1121  TTTAGGCACC TTTTGAAATG TAATCATTTG GGTCAATATG TAATTTTCAG TGTTAGACTA GTAAATTGTC CGCTAAATTC

                                        Hind III     Asp718 I  Kpn I    Sac I  BamH I                   BstX I*   EcoR I
1201  TGGCCGTTTT TGGCTTTTTT GTTAGACGA AGC TTG GTA CCG AGC TCG GAT CCA CTA GTC CAG TGT GGT GGA
                                      Ser Leu Val Pro Ser Ser Asp Pro Leu Val Gln Cys Gly Gly

              Pst I  EcoR V           BstX I*  Not I      Xho I    BstE II          BstB I         V5 epitope
1272  ATT CTG CAG ATA TCC AGC ACA GTG GCG GCC GCT CGA GGT CAC CCA TTC GAA GGT AAG CCT ATC CCT
      Ile Leu Gln Ile Ser Ser Thr Val Ala Ala Ala Arg Gly His Pro Phe Glu Gly Lys Pro Ile Pro

                                              Age I          Polyhistidine region            Pme I
1338  AAC CCT CTC CTC GGT CTC GAT TCT ACG CGT ACC GGT CAT CAT CAC CAT CAC CAT TGA GTTTAA
      Asn Pro Leu Leu Gly Leu Asp Ser Thr Arg Thr Gly His His His His His His ***

                  BGH Reverse priming site
1401  ACCCGCTGAT CAGCCTCGAC TGTGCCTTCT AGTTGCCAGC CATCTGTTGT TTGCCCCTCC CCCGTGCCTT CCTTGACCCT

                              BGH polyadenylation signal
1481  GGAAGGTGCC ACTCCCACTG TCCTTTCCTA ATAAAATGAG GAAATTGCAT CGCATTGTCT GAGTAGGTGT CATTCTATTC
```

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

Transforming Ligation Mixtures

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 UB Forward and BGH Reverse primer sequences to confirm that your gene is fused in frame with the V5 epitope and the C-terminal polyhistidine tag. Refer to the diagram on pages 3–5 for the sequence and location of the primer binding sites.

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.

1. Streak the original colony out for single colonies 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.

Transfection and Analysis

Introduction

Once you have confirmed that your construct is in the correct orientation and fused to the C-terminal peptide (if desired), then 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 17 for ordering information).

Methods of Transfection

For established cell lines (e.g., HeLa), consult original references or the supplier of your cell line for the optimal method of transfection. 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 for transfection include calcium phosphate (Chen and Okayama, 1987; Wigler et al., 1977), lipid-mediated (Felgner et al., 1987; Felgner et al., 1989), and electroporation (Chu et al., 1987; Shigekawa and Dower, 1988). Invitrogen offers the Lipofectamine™ 2000 Reagent for mammalian transfection (see page 17 for ordering).

Positive Control

pUB6/V5-His/*lac*Z is provided as a positive control vector for mammalian transfection and expression (see page 14). It 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 human ubiquitin C (hUbC) 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 17 for ordering information).

Continued on next page

Detecting Fusion Proteins

Several antibodies are available from Invitrogen that can be used to detect expression of your fusion protein from pUB6/V5-His (see page 17 for ordering information).

To detect the fusion protein by western blot, you will need to prepare a cell lysate from transfected cells. We recommend that you perform a time course to optimize expression of the fusion protein (*e.g.* 24, 48, 72 hours, etc. after transfection). To lyse cells:

1. Wash cell monolayers (~10^6 cells) once with phosphate-buffered saline (PBS).
2. Scrape cells into 1 mL PBS and pellet the cells at 1,500 × *g* for 5 minutes.
3. Resuspend in 50 µL Cell Lysis Buffer (see recipe below). Other lysis buffers are suitable.
4. Incubate cell suspension at 37°C for 10 minutes to lyse the cells.
5. Vortex the cell lysate and centrifuge at 10,000 × *g* for 10 minutes to pellet nuclei. 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.

Creating Stable Cell Lines

Introduction

The pUB6/V5-His vectors contain the blasticidin resistance gene (*bsd*) to allow for selection of stable cell lines using blasticidin (Kimura *et al.*, 1994). 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.

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 some unique sites that may be used to linearize your construct prior to transformation. **Other restriction sites are possible.** Note that for the enzymes listed below, the cleavage site is indicated for versions A, B, and C of pUB6/V5-His. 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 hUbC promoter	Many
*Bst*1107 I	End of SV40 polyA	AGS*, Fermentas, Takara
Sap I	Backbone	New England Biolabs
*Bsp*LU 11I	Backbone	Boehringer-Mannheim
*Alw*N I	pMB1 origin	Amersham, New England Biolabs, Life Technologies
*Eam*1105 I	Ampicillin gene	AGS*, Fermentas, Takara
Bgl I	Ampicillin gene	Many
Fsp I	Ampicillin gene	Many
Sca I	Ampicillin gene	Many
Ssp I	Backbone	Many

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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. See page 15 for details on handling and preparing Blasticidin solution.

1. Seed cells at 20–25% confluency for each time point (~6 time points) and allow the 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 blasticidin).
3. 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.
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 concentration of blasticidin is determined, you can generate a stable cell line with your construct.

1. Transfect your cells using the optimal protocol for your cell line. Include a sample of untransfected cells as a negative control.
2. 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.
3. Replenish selective medium every 3–4 days until blasticidin-resistant colonies are detected.
4. 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™ Protein Purification manual).

1. Seed cells (from a stable cell line) 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 1,500 × *g* for 5 minutes. Resuspend the cell pellet in PBS.
6. Centrifuge the cells at 1,500 × *g* 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™ Protein Purification manual for details about sample preparation for chromatography.

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

Appendix

Human UbC Promoter

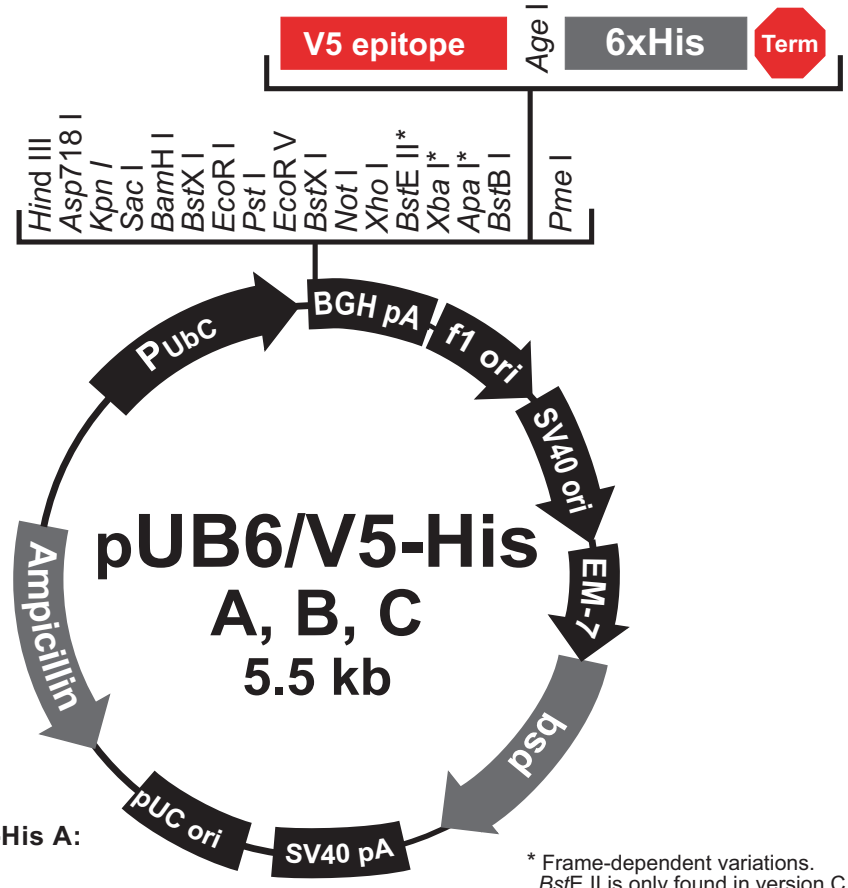
Description

The human UbC promoter allows high-level expression of recombinant protein in most mammalian cell lines (Wulff *et al.*, 1990) and in virtually all tissues tested in transgenic mice (Schorpp *et al.*, 1996). The diagram below shows the features of the UbC promoter used in pUB6/V5-His (Nenoi *et al.*, 1996). Features are marked as per Nenoi, *et al.*, 1996.

pUB6/V5-His Vector

Map of pUB6/V5-His

The figure below summarizes the features of the pUB6/V5-His vectors. The sequences for pUB6/V5-His A, B, and C are available for downloading from www.invitrogen.com or from **Technical Support** (see page 18).



* Frame-dependent variations. *Bst*E II is only found in version C. In addition, there are no *Xba* I or *Apa* I sites in version C.

Comments for pUB6/V5-His A: 5463 nucleotides

UbC promoter: bases 18-1227
UB forward priming site: bases 1167-1188
Multiple cloning site: bases 1229-1326
V5 epitope: bases 1327-1368
Polyhistidine tag: bases 1378-1395
BGH reverse priming site: bases 1418-1435
BGH polyadenylation signal: bases 1421-1648
f1 origin: bases 1694-2122
SV40 promoter and origin: bases 2149-2458
EM-7 promoter: bases 2506-2561
Blasticidin resistance gene: bases 2580-2978
SV40 polyadenylation signal: bases 3136-3266
pUC origin: bases 3649-4322
Ampicillin resistance gene: bases 4467-5327

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pUB6/V5-His Vector, Continued

Features of pUB6/V5-His

pUB6/V5-His A (5463 bp), pUB6/V5-His B (5467 bp), and pUB6/V5-His C (5459 bp) contain the following elements. All features have been functionally tested.

Feature	Benefit
Human ubiquitin C (hUbC) promoter	Allows overexpression of your recombinant protein in a broad range of mammalian cell types (Hershko and Ciechanover, 1982; Wulff *et al.*, 1990; Schorpp *et al.*, 1996).
Multiple cloning site in three reading frames	Allows insertion of your gene and facilitates cloning in frame with the V5 epitope and the C-terminal polyhistidine 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 Antibodies (Southern *et al.*, 1991).
C-terminal polyhistidine tag	Allows 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) Antibodies (Lindner *et al.*, 1997).
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	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 vector in *E. coli.*

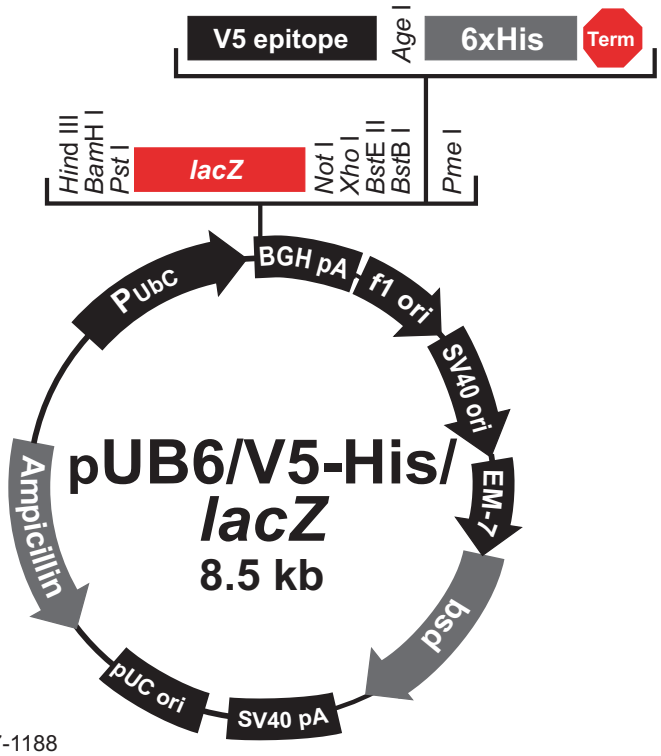
pUB6/V5-His/*lacZ*

Description

pUB6/V5-His/*lacZ* is a 8510 bp control vector containing the gene for β-galactosidase. This vector was constructed by ligating a 3190 bp *Hin*d III-*Age* I fragment containing the *lacZ* gene and the V5 epitope to a 5320 bp *Hin*d III-*Age* I fragment containing the hUbC promoter, polyhistidine tag and blasticidin resistance gene from pUB6/V5-His A.

Map of Control Vector

The figure below summarizes the features of the pUB6/V5-His/*lacZ* vector. The nucleotide sequence for pUB6/V5-His/*lacZ* is available for downloading from www.invitrogen.com or by contacting **Technical Support**. (see page 18).



Comments for pUB6/V5-His/*lacZ*: 8510 nucleotides

UbC promoter: bases 18-1227
UB forward priming site: bases 1167-1188
LacZ ORF: bases 1290-4346
V5 epitope: bases 4374-4415
Polyhistidine tag: bases 4425-4442
BGH reverse priming site: bases 4465-4482
BGH polyadenylation signal: bases 4468-4695
f1 origin: bases 4741-5169
SV40 promoter and origin: bases 5196-5505
EM-7 promoter: bases 5553-5608
Blasticidin resistance gene: bases 5627-6025
SV40 polyadenylation signal: bases 6183-6313
pUC origin: bases 6696-7369
Ampicillin resistance gene: bases 7514-8374

Blasticidin

Description

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).

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.

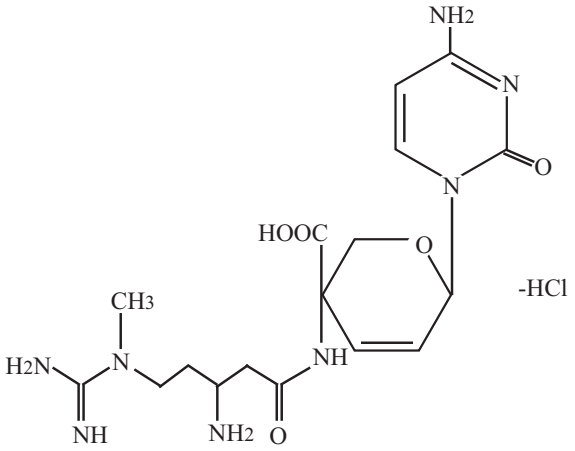
Preparing and Storing Stock Solutions

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.

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.



Recipe

Cell Lysis Buffer

50 mM Tris
150 mM NaCl
1% Nonidet P-40
pH 7.8

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 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

Accessory Products

Introduction

The following products may be used with the pUB6/V5-His vectors. For details, visit www.invitrogen.com or contact **Technical Support** (page 18).

Item	Amount	Catalog no.
ProBond™ Purification System	6 × 2 mL precharged, prepacked ProBond™ resin columns and buffers for native and denaturing purification	K850-01
ProBond™ Resin	50 mL	R801-01
	150 mL	R801-15
PureLink™ HiPure Plasmid Midiprep Kit	25 preps	K2100-04
Lipofectamine™ 2000 Reagent	0.75 mL	11668-027
Electrocomp™ TOP10F´	5 × 80 µL	C665-55
One Shot® TOP10F´ Chemically Competent *E. coli*	20×50 µL	C3030-03
β-Gal Assay Kit	80 mL	K1455-01
β-Gal Staining Kit	1 kit	K1465-01

Antibodies

If you do not have an antibody specific to your protein, Invitrogen offers the Anti-V5, or Anti-His(C-term) antibodies to detect your recombinant fusion protein. Horseradish peroxidase (HRP)- and alkaline phosphatase (AP)-conjugated antibodies are available for convenient one-step detection.

Antibody	Epitope	Catalog no.
Anti-V5	Detects a 14 amino acid epitope derived from the P and V proteins of the paramyxovirus, SV5 (Southern et al., 1991): GKPIPNPLLGLDST	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) (Lindner et al., 1997): HHHHHH-COOH	R930-25
Anti-His(C-term)-HRP		R931-25
Anti-His(C-term)-AP		R932-25

Primers

For your convenience, Invitrogen offers a custom primer synthesis service. Visit www.invitrogen.com for more details.

Technical Support

Web Resources

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

- Technical resources, including manuals, vector maps and sequences, application notes, SDSs, 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).

Corporate Headquarters:
5791 Van Allen Way
Carlsbad, CA 92008 USA
Tel: 1 760 603 7200
Tel (Toll Free): 1 800 955 6288
Fax: 1 760 602 6500
E-mail: tech_support@invitrogen.com

Japanese Headquarters:
LOOP-X Bldg. 6F
3-9-15, Kaigan
Minato-ku, Tokyo 108-0022
Tel: 81 3 5730 6509
Fax: 81 3 5730 6519
E-mail: jpinfo@invitrogen.com

European Headquarters:
Inchinnan Business Park
3 Fountain Drive
Paisley PA4 9RF, UK
Tel: +44 (0) 141 814 6100
Tech Fax: +44 (0) 141 814 6117
E-mail: eurotech@invitrogen.com

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Notes

invitrogen™

Corporate Headquarters
5791 Van Allen Way
Carlsbad, CA 92008
T: 1 760 603 7200
F: 1 760 602 6500
E: tech_support@invitrogen.com

For country-specific contact information, visit our web site at **www.invitrogen.com**