pEF1/V5-His A, B, and C

Catalog no. V920-20

Rev. date: 7 June 2010

Manual part no. 28-0184

MAN0000664

User Manual

Table of Contents

Kit Contents and Storage iv

Introduction 1

Product Overview 1

Methods 2

Cloning into pEF1/V5-His A, B, and C 2

Transformation and Transfection 6

Appendix 9

Human EF-1α Promoter 9

pEF1/V5-His Vector 10

pEF1/V5-His/*lac*Z 12

Accessory Products 13

Technical Support 15

Purchaser Notification 16

References 17

Kit Contents and Storage

Shipping and Storage

pEF1/V5-His vectors are shipped on wet ice. Upon receipt, store vectors at -20°C.

Kit Contents

All vectors are supplied as detailed below. **Store the vectors at –20°C.**

Vector	Composition	Amount
pEF1/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
pEF1/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

pEF1/V5-His A, B, and C are 6.2 kb vectors derived from pcDNA™3.1/V5-His and designed for overproduction of recombinant proteins in mammalian cell lines. Features of the vectors allow purification and detection of expressed proteins (see pages 10–11 for more information). High-level stable and transient expression can be carried out in most mammalian cells. The vectors contain the following elements:

- Human elongation factor 1α-subunit promoter (hEF-1α) for high-level expression across a broad range of species and cell types (Goldman *et al.*, 1996; Mizushima and Nagata, 1990) (see page 9 for more information).
- Three reading frames to facilitate in-frame cloning with a C-terminal tag encoding the V5 epitope and a polyhistidine metal-binding peptide.
- 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, pEF1/V5-His/*lac*Z, is the pEF1/V5-His A vector with a 3.1 kb fragment containing the β-galactosidase gene cloned in frame with the C-terminal peptide (see page 12). It 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 pEF1/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.
3. Analyze your transformants for the presence of insert by restriction digestion.
4. Select a transformant with the correct restriction pattern and confirm that your gene is in frame with the C-terminal peptide by sequencing.
5. Transfect your construct into the cell line of choice.
6. 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 the Anti-V5 Antibody or the Anti-His(C-term) Antibody to detect your recombinant protein. See page 14 for more information.
7. To purify your recombinant protein, you may use metal-chelating resin such as ProBond™. ProBond™ resin is available separately.

Methods

Cloning into pEF1/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

Many *E. coli* strains are suitable for the growth of this vector including TOP10F´, DH5αF´, 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).

Maintaining pEF1/V5-His

To propagate and maintain the pEF1/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. Be sure to prepare a glycerol stock of each plasmid for long term storage.

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.

Continued on next page

Multiple Cloning Site of Version A

Below is the multiple cloning site for pEF1/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 *Spe* I site and the *BstX* I site.** The multiple cloning site has been confirmed by sequencing and functional testing. For more information on the hEF-1α promoter, see page 9.

```
                                                                        3´end of hEF-1α Intron 1
1579  GTTTGGATCT TGGTTCATTC TCAAGCCTCA GACAGTGGTT CAAAGTTTTT TTCTTCCATT TCAGGTGTCG TGAGGAATTA
                                                                           5´ end of hEF-1α Exon 2

                  T7 promoter/priming site                              Kpn I       BamH I    Spe I
1659  GCTTGGTACT AATACGACTC ACTATAGGGA GACCCAAGCT GGCTAGGTAA GCT TGG TAC CGA GCT CGG ATC CAC
                                                            Trp Tyr Arg Ala Arg Ile His

                BstX I  EcoR I        EcoR V        BstX I   Not I            Xba I
1733  TAG TCC AGT GTG GTG GAA TTC TGC AGA TAT CCA GCA CAG TGG CGG CCG CTC GAG TCT AGA GGG CCC
      *** Ser Ser Val Val Glu Phe Cys Arg Tyr Pro Ala Gln Trp Arg Pro Leu Glu Ser Arg Gly Pro

      BstB I                      V5 epitope                                       Polyhistidine
1799  TTC GAA GGT AAG CCT ATC CCT AAC CCT CTC CTC GGT CTC GAT TCT ACG CGT ACC GGT CAT CAT CAC
      Phe Glu Gly Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser Thr Arg Thr Gly His His His

      region            Pme I              BGH Reverse priming site
1865  CAT CAC CAT TGA GT TTAAACCCGC TGATCAGCCT CGACTGTGCC TTCTAGTTGC CAGCCATCTG TTGTTTGCCC
      His His His ***

                                                          BGH polyadenylation signal
1939  CTCCCCCGTG CCTTCCTTGA CCCTGGAAGG TGCCACTCCC ACTGTCCTTT CCTAATAAAA TGAGGAAATT GCATCGCATT

2019  GTCTGAGTAG GTGTCATTCT ATTCTGGGGG GTGGGGTGGG GCAGGACAGC AAGGGGGAGG ATTGGGAAGA CAATAGCAGG
```

Continued on next page

Multiple Cloning Site of Version B

Below is the multiple cloning site for pEF1/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. For more information on the hEF-1α promoter, see page 9.

```
                                                                          3´end of hEF-1α Intron 1
1579  GTTTGGATCT TGGTTCATTC TCAAGCCTCA GACAGTGGTT CAAAGTTTTT TTCTTCCATT TCAGGTGTCG TGAGGAATTA
                                                                          5´ end of hEF-1α Exon 2

              T7 promoter/priming site                                Kpn I      BamH I   Spe I
1659  GCTTGGTACT AATACGACTC ACTATAGGGA GACCCAAGCT GGCTAGGTAA G CTT GGT ACC GAG CTC GGA TCC ACT
                                                            Leu Gly Thr Glu Leu Gly Ser Thr

                  BstX I  EcoR I        EcoR V        BstX I   Not I          Xba I
1734  AGT CCA GTG TGG TGG AAT TCT GCA GAT ATC CAG CAC AGT GGC GGC CGC TCG AGT CTA GAG GGC CCG
      Ser Pro Val Trp Trp Asn Ser Ala Asp Ile Gln His Ser Gly Gly Arg Ser Ser Leu Glu Gly Pro

         BstB I                   V5 epitope                                         Polyhistidine
1800  CGG TTC GAA GGT AAG CCT ATC CCT AAC CCT CTC CTC GGT CTC GAT TCT ACG CGT ACC GGT CAT CAT
      Arg Phe Glu Gly Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser Thr Arg Thr Gly His His

      region                 Pme I             BGH Reverse priming site
1866  CAC CAT CAC CAT TGA GTTTAAAC CCGCTGATCA GCCTCGACTG TGCCTTCTAG TTGCCAGCCA TCTGTTGTTT
      His His His His ***

                                                                 BGH polyadenylation signal
1939  GCCCCTCCCC CGTGCCTTCC TTGACCCTGG AAGGTGCCAC TCCCACTGTC CTTTCCTAAT AAAATGAGGA AATTGCATCG

2019  CATTGTCTGA GTAGGTGTCA TTCTATTCTG GGGGGTGGGG TGGGGCAGGA CAGCAAGGGG GAGGATTGGG AAGACAATAG
```

Continued on next page

Multiple Cloning Site of Version C

Below is the multiple cloning site for pEF1/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. For more information on the hEF-1α promoter, see page 9.

```
                                                                   3´end of hEF-1α Intron 1
1579  GTTTGGATCT TGGTTCATTC TCAAGCCTCA GACAGTGGTT CAAAGTTTTT TTCTTCCATT TCAGGTGTCG TGAGGAATTA
                                                                      5´ end of hEF-1α Exon 2

                  T7 promoter/priming site                                  Kpn I       BamH I   Spe I
1659  GCTTGGTACT AATACGACTC ACTATAGGGA GACCCAAGCT GGCTAGGTAA GC TTG GTA CCG AGC TCG GAT CCA CTA
                                                                 Leu Val Pro Ser Ser Asp Pro Leu

                  BstX I EcoR I        EcoR V      BstX I Not I           BstE II     BstB I
1735  GTC CAG TGT GGT GGA ATT CTG CAG ATA TCC AGC ACA GTG GCG GCC GCT CGA GGT CAC CCA TTC GAA
      Val Gln Cys Gly Gly Ile Leu Gln Ile Ser Ser Thr Val Ala Ala Ala Arg Gly His Pro Phe Glu

                              V5 epitope                                  Polyhistidine region
1801  GGT AAG CCT ATC CCT AAC CCT CTC CTC GGT CTC GAT TCT ACG CGT ACC GGT CAT CAT CAC CAT CAC
      Gly Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser Thr Arg Thr Gly His His His His His

               Pme I                   BGH Reverse priming site
1867  CAT TGA GTTTAA ACCCGCTGAT CAGCCTCGAC TGTGCCTTCT AGTTGCCAGC CATCTGTTGT TTGCCCCTCC
      His ***

                                                       BGH polyadenylation signal
1939  CCCGTGCCTT CCTTGACCCT GGAAGGTGCC ACTCCCACTG TCCTTTCCTA ATAAAATGAG GAAATTGCAT CGCATTGTCT

2019  GAGTAGGTGT CATTCTATTC TGGGGGGTGG GGTGGGGCAG GACAGCAAGG GGGAGGATTG GGAAGACAAT AGCAGGCATG
```

Transformation and Transfection

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. Select 10–20 clones and analyze for the presence and orientation of your insert.

We recommend that you sequence your construct with the T7 Forward and BGH Reverse primers sequences 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 location and sequence of primer binding sites.

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 13 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.*, 1989; Felgner and Ringold, 1989), and electroporation (Chu *et al.*, 1987; Shigekawa and Dower, 1988). Invitrogen offers the Lipofectamine™ 2000 Reagent for mammalian transfection.

Positive Control

pEF1/V5-His/*lac*Z is provided as a positive control vector for mammalian transfection and expression (see page 12). It may be used to optimize transfection conditions for your cell line. The gene encoding β-galactosidase is expressed in mammalian cells under the hEF-1α promoter. A successful transfection will result in β-galactosidase expression that can be easily assayed (see next page).

Continued on next page

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.

Detecting Fusion Proteins

A number of antibodies are available from Invitrogen that can be used to detect expression of your fusion protein from pEF1/V5-His (see page 13).

Neomycin (Geneticin®) Activity

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 of the bacterial aminoglycoside phosphotransferase gene (APH), derived from Tn*5*, in mammalian cells results in detoxification of Geneticin® (Southern and Berg, 1982).

Geneticin® Selection Guidelines

Geneticin® is available from Invitrogen (see page 13). Use as follows:

- Prepare Geneticin® in a buffered solution (*e.g.*, 100 mM HEPES, pH 7.3).
- Use 100 to 1,000 μg/mL of Geneticin® in complete medium.
- Calculate concentration based on the amount of active drug (check the lot label).
- 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®, so the effects of the drug take several days to become apparent. Complete selection can take from 3 to 6 weeks of growth in selective medium.

Continued on next page

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 the ProBond™ 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 240 × *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™ Purification manual for details about sample preparation for chromatography. If you are using another metal-chelating resin, refer to the manufacturer's instruction for recommendations on sample preparation.

Appendix

Human EF-1α Promoter

Description

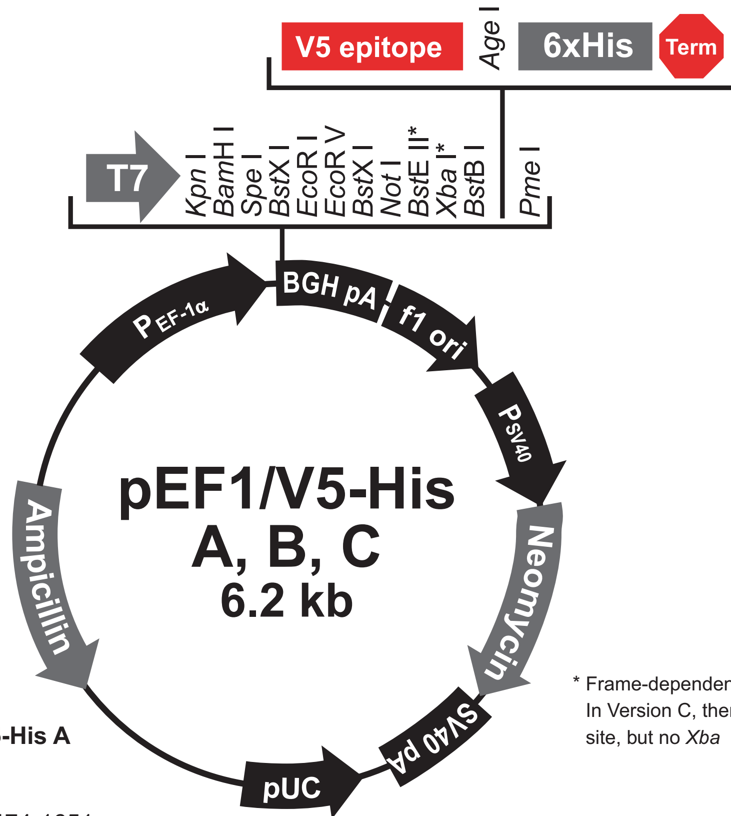
The diagram below shows all the features of the EF-1α promoter used in pEF1/V5-His vectors (Mizushima and Nagata, 1990) . Features are marked as per Uetsuki, *et al.*, 1989.

```
                       5´ end of human EF-1α promoter
461    GGAGTGCCTC GTGAGGCTCC GGTGCCCGTC AGTGGGCAGA GCGCACATCG CCCACAGTCC

521    CCGAGAAGTT GGGGGGAGGG GTCGGCAATT GAACCGGTGC CTAGAGAAGG TGGCGCGGGG

581    TAAACTGGGA AAGTGATGTC GTGTACTGGC TCCGCCTTTT TCCCGAGGGT GGGGGAGAAC
         TATA box                                Start of Transcription
641    CGTATATAAG TGCAGTAGTC GCCGTGAACG TTCTTTTTCG CAACGGGTTT GCCGCCAGAA
                                                             Exon I
                 5´ end of Intron 1
701    CACAGGTAAG TGCCGTGTGT GGTTCCCGCG GGCCTGGCCT CTTTACGGGT TATGGCCCTT

761    GCGTGCCTTG AATTACTTCC ACCTGGCTGC AGTACGTGAT TCTTGATCCC GAGCTTCGGG

821    TTGGAAGTGG GTGGGAGAGT TCGAGGCCTT GCGCTTAAGG AGCCCCTTCG CCTCGTGCTT

881    GAGTTGAGGC CTGGCCTGGG CGCTGGGGCC GCCGCGTGCG AATCTGGTGG CACCTTCGCG

941    CCTGTCTCGC TGCTTTCGAT AAGTCTCTAG CCATTTAAAA TTTTTGATGA CCTGCTGCGA

1001   CGCTTTTTTT CTGGCAAGAT AGTCTTGTAA ATGCGGGCCA AGATCTGCAC ACTGGTATTT
                        Sp 1
1061   CGGTTTTTGG GGCCGCGGGC GGCGACGGGG CCCGTGCGTC CCAGCGCACA TGTTCGGCGA
         Sp 1
1121   GGCGGGGCCT GCGAGCGCGG CCACCGAGAA TCGGACGGGG GTAGTCTCAA GCTGGCCGGC
                                             Sp 1        Sp 1
1181   CTGCTCTGGT GCCTGGCCTC GCGCCGCCGT GTATCGCCCC GCCCTGGGCG GCAAGGCTGG

1241   CCCGGTCGGC ACCAGTTGCG TGAGCGGAAA GATGGCCGCT TCCCGGCCCT GCTGCAGGGA
                                            Sp 1
1301   GCTCAAAATG GAGGACGCGG CGCTCGGGAG AGCGGGCGGG TGAGTCACCC ACACAAAGGA
                                             Ap 1
1361   AAAGGGCCTT TCCGTCCTCA GCCGTCGCTT CATGTGACTC CACGGAGTAC CGGGCGCCGT

1421   CCAGGCACCT CGATTAGTTC TCGAGCTTTT GGAGTACGTC GTCTTTAGGT TGGGGGGAGG

1481   GGTTTTATGC GATGGAGTTT CCCCACACTG AGTGGGTGGA GACTGAAGTT AGGCCAGCTT

1541   GGCACTTGAT GTAATTCTCC TTGGAATTTG CCCTTTTTGA GTTTGGATCT TGGTTCATTC
                                       3´ end of Intron 1
1601   TCAAGCCTCA GACAGTGGTT CAAAGTTTTT TTCTTCCATT TCAGGTGTCG TGA...
                                                     5´ end of Exon 2
```

pEF1/V5-His Vector

Map of pEF1/ V5-His

The figure below summarizes the features of the pEF1/V5-His vectors. The sequences for pEF1/V5-His A, B, and C are available for downloading from www.invitrogen.com or by contacting **Technical Support** (page 15).



* Frame-dependent variations. In Version C, there is a unique *Bst*E II site, but no *Xba* I site.

Comments for pEF1/V5-His A
6174 nucleotides

EF-1α promoter: bases 474-1651
T7 promoter/priming site: bases 1668-1687
Multiple cloning site: bases 1713-1881
V5 epitope: bases 1805-1846
Polyhistidine tag: bases 1856-1873
BGH reverse priming site: bases 1896-1913
BGH polyadenylation sequence: bases 1895-2122
f1 origin of replication: bases 2172-2600
SV40 promoter and origin: bases 2628-2935
Neomycin resistance gene (ORF): bases 3010-3804
SV40 polyadenylation sequence: bases 3980-4110
pUC origin: bases 4493-5155 (opposite strand)
Ampicillin resistance gene (ORF): bases 5300-6160 (opposite strand)

Continued on next page

Features of pEF1/V5-His

pEF1/V5-His A (6174 bp), pEF1/V5-His B (6178 bp), and pEF1/ V5-His C (6170 bp) contain the following elements. All features have been functionally tested.

Feature	Benefit
Human elongation factor 1α (hEF-1α) promoter	Allows overexpression of your recombinant protein in a broad range of mammalian cell types (Goldman *et al.*, 1996; Mizushima and Nagata, 1990).
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 or Anti-V5-HRP Antibody (Southern *et al.*, 1991) (see page 14 for ordering information).
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) Antibody and the Anti-His.(C-term)-HRP Antibody (see page 14 for ordering).
BGH reverse priming site	Allows 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 (Geneticin®) 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.*

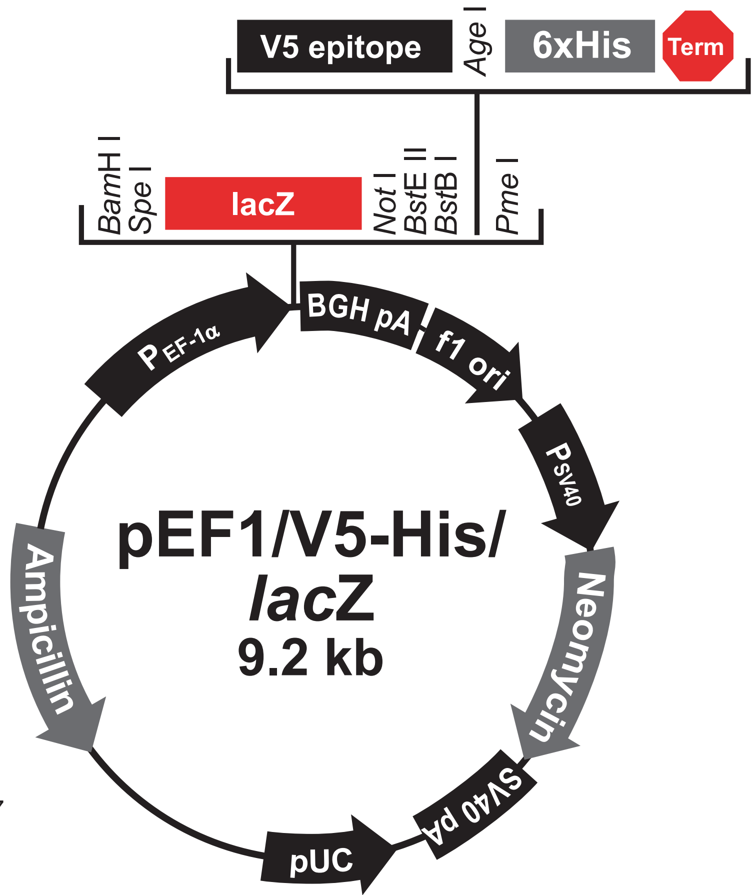
pEF1/V5-His/*lac*Z

Description

pEF1/V5-His/*lac*Z is a 9186 bp control vector containing the gene for β-galactosidase. pEF1/V5-His A was digested with *Kpn* I and *Pme* I. A 3.1 kb *Kpn* I-*Pme* I fragment containing the β-galactosidase gene was then ligated into the digested pEF1/ V5-His A vector in frame with the C-terminal peptide.

Map of Control Vector

The figure below summarizes the features of the pEF1/V5-His/*lac*Z vector. The nucleotide sequence for pEF1/V5-His/*lac*Z is available for downloading from www.invitrogen.com or by contacting **Technical Support** (page 15).



Comments for pEF1/V5-His/*lac*Z
9186 nucleotides

EF-1α promoter: bases 470-1653
LacZ portion of the fusion: 1721-4777
V5 epitope: bases 4805-4846
Polyhistidine tag: bases 4856-4873
BGH reverse priming site: bases 4896-4913
BGH polyadenylation sequence: bases 4899-5126
f1 origin of replication: bases 5172-5600
SV40 promoter and origin: bases 5654-5936
Neomycin resistance gene (ORF): bases 6011-6805
SV40 polyadenylation sequence: bases 6981-7111
pUC origin: bases 7494-8167 (opposite strand)
Ampicillin resistance gene (ORF): bases 8312-9172 (opposite strand)

Accessory Products

Introduction

The following products may be used with the pEF1/V5-His vectors. For details, visit www.invitrogen.com or contact **Technical Support** (page 15).

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
Electrocomp™ TOP10F´	5 × 80 µL	C665-55
One Shot® TOP10F´ Chemically Competent *E. coli*	20 × 50 µL	C3030-03
PureLink™ HiPure Plasmid Miniprep Kit	100 preps	K2100-03
PureLink™ HiPure Plasmid Midiprep Kit	25 preps	K2100-04
β-Gal Assay Kit	80 mL	K1455-01
β-Gal Staining Kit	1 kit	K1465-01
Geneticin®	1 gram	11811-023
	5 grams	11811-031
	25 grams	11811-098
Lipofectamine™ 2000 Reagent	0.75 mL	11668-027

Primers

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

Continued on next page

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

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

SDS

Safety Data Sheets (SDSs) are available on our website at www.invitrogen.com/sds.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to www.invitrogen.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

Limited Warranty

Invitrogen (a part of Life Technologies Corporation) is committed to providing our customers with high-quality goods and services. Our goal is to ensure that every customer is 100% satisfied with our products and our service. If you should have any questions or concerns about an Invitrogen product or service, contact our Technical Support Representatives.

All Invitrogen products are warranted to perform according to specifications stated on the certificate of analysis. The Company will replace, free of charge, any product that does not meet those specifications. This warranty limits the Company's liability to only the price of the product. No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. The Company reserves the right to select the method(s) used to analyze a product unless the Company agrees to a specified method in writing prior to acceptance of the order.

Invitrogen makes every effort to ensure the accuracy of its publications, but realizes that the occasional typographical or other error is inevitable. Therefore the Company makes no warranty of any kind regarding the contents of any publications or documentation. If you discover an error in any of our publications, please report it to our Technical Support Representatives.

Life Technologies Corporation shall have no responsibility or liability for any special, incidental, indirect or consequential loss or damage whatsoever. The above limited warranty is sole and exclusive. No other warranty is made, whether expressed or implied, including any warranty of merchantability or fitness for a particular purpose.

Purchaser Notification

Limited Use Label License No. 22: Vectors and Clones Encoding Histidine Hexamer

This product is licensed under U.S. Patent Nos. 5,284,933 and 5,310,663 and foreign equivalents from Hoffmann-LaRoche, Inc., Nutley, NJ and/or Hoffmann-LaRoche Ltd., Basel, Switzerland and is provided only for use in research. Information about licenses for commercial use is available from QIAGEN GmbH, Max-Volmer-Str. 4, D-40724 Hilden, Germany.

Limited Use Label License No. 60: EF-1α Promoter

EF-1alpha promoter products are sold under license for research purposes only. The use of this product for any commercial purpose, including but not limited to, use in any study for the purpose of a filing of a new drug application, requires a license from: Mochida Pharmaceutical Co., Ltd., 7, Yotsuya 1-Chome, Shinjuku-Ku, Tokyo 160, Japan. Tel: 81-3-3225-5451; Fax: 81-3-3225-6091.

References

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).

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

Chu, G., Hayakawa, H., and Berg, P. (1987). Electroporation for the Efficient Transfection of Mammalian Cells with DNA. Nucleic 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.

Goldman, L. A., Cutrone, E. C., Kotenko, S. V., Krause, C. D., and Langer, J. A. (1996). Modifications of Vectors pEF-BOS, pcDNA1, and pcDNA3 Result in Improved Convenience and Expression. BioTechniques *21*, 1013-1015.

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. 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.

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

Mizushima, S., and Nagata, S. (1990). pEF-BOS, a Powerful Mammalian Expression Vector. Nucleic Acids Res. *18*, 5322.

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.

Uetsuki, T., Naito, A., Nagata, S., and Kaziro, Y. (1989). Isolation and Characterization of the Human Chromosomal Gene for Polypeptide Chain Elongation Factor-1α. J. Biol. Chem. *264*, 5791-5798.

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.

©2009 Life Technologies Corporation. All rights reserved.

Notes

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**