pcDNA3.1/nV5-DEST™ Gateway™ Vector Pack

A Gateway™-adapted expression vector (destination vector) for cloning and expression of N-terminal V5 fusion proteins in mammalian cells

Catalog no. 12290-010

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Important Information

Shipping and Storage

pcDNA3.1/nV5-DEST™ and pcDNA3.1/nV5-GW/*lac*Z™ are shipped at room temperature. Upon receipt, store at -20°C. Products are guaranteed for six months from date of shipment when stored properly.

Contents

pcDNA3.1/nV5-DEST™ Gateway™ Vector Pack components are listed below.

Item	Concentration	Volume
pcDNA3.1/nV5-DEST™ Vector	lyophilized in TE, pH 8.0	6 µg
pcDNA3.1/nV5-GW/*lac*Z™ Control Plasmid	lyophilized in TE, pH 8.0	10 µg

Quality Control

The pcDNA3.1/nV5-DEST™ and pcDNA3.1/nV5-GW/*lac*Z™ vectors are qualified by restriction endonuclease digestion. pcDNA3.1/nV5-DEST™ is further qualified in a recombination assay using Gateway™ LR Clonase™ enzyme mix. The *ccd*B gene is assayed by transformation using an appropriate *E. coli* strain.

Accessory Products

Additional Products

Additional products that may be used with pcDNA3.1/nV5-DEST™ are available from Invitrogen. Ordering information is provided below.

Product	Amount	Catalog no.
Gateway™ LR Clonase™ Enzyme Mix	20 reactions	11791-019
One Shot® TOP10 Chemically Competent Cells	10 reactions	C4040-10
	20 reactions	C4040-03
One Shot® TOP10 Electrocompetent Cells	10 reactions	C4040-50
	20 reactions	C4040-52
T7 Promoter Primer	2 µg (328 pmoles)	N560-02
Lipofectamine™ 2000 Reagent	1.5 ml	11668-019
	0.75 ml	11668-027
Geneticin®	1 g	11811-023
	5 g	11811-031
	20 ml (50 mg/ml)	10131-035
	100 ml (50 mg/ml)	10131-027
TEV Protease	1000 units	10127-017

Detection of Recombinant Proteins

Expression of your recombinant fusion protein can be detected using Anti-V5 antibodies available from Invitrogen. Horseradish peroxidase (HRP) or alkaline phosphatase (AP)-conjugated antibodies allow one-step detection using colorimetric or chemiluminescent detection methods. The fluorescein isothiocyanate (FITC)-conjugated antibody allows one-step detection in immunofluorescence experiments.

The amount of antibody supplied is sufficient for 25 Western blots or 25 immunostaining reactions (FITC-conjugated antibody only).

Product	Epitope	Catalog no.
Anti-V5 Antibody	Detects 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 Antibody		R961-25
Anti-V5-AP Antibody		R962-25
Anti-V5-FITC Antibody		R963-25

Methods

Overview

Description

pcDNA3.1/nV5-DEST™ is a 7.1 kb vector derived from pcDNA3.1™ and adapted for use with the Gateway™ Technology. It is designed to allow high-level, constitutive expression of the gene of interest in a variety of mammalian hosts. For more information on the Gateway™ Technology, see the next page.

Features

pcDNA3.1/nV5-DEST™ contains the following elements:

- Human cytomegalovirus immediate-early (CMV) promoter/enhancer for high-level expression in a wide range of mammalian cells
- The N-terminal V5 epitope tag for detection using Anti-V5 antibodies
- The TEV protease recognition site for removal of the N-terminal V5 epitope tag from your recombinant fusion protein
- Two recombination sites, *att*R1 and *att*R2, downstream of the CMV promoter for recombinational cloning of the gene of interest from an entry clone
- Chloramphenicol resistance gene located between the two *att*R sites for counterselection
- The *ccd*B gene located between the two *att*R sites for negative selection
- Bovine growth hormone (BGH) polyadenylation sequence for proper termination and processing of the recombinant transcript
- f1 intergenic region for production of single-strand DNA in F plasmid-containing *E. coli*
- SV40 early promoter and origin for expression of the neomycin resistance gene and stable propagation of the plasmid in mammalian hosts expressing the SV40 large T antigen
- Neomycin resistance gene for selection of stable cell lines using Geneticin®
- The pUC origin for high copy replication and maintenance of the plasmid in *E. coli*
- The ampicillin (*bla*) resistance gene for selection in *E. coli*

For a map of pcDNA3.1/nV5-DEST™, see page 12.

continued on next page

Overview, continued

The Gateway™ Technology

Gateway™ is a universal cloning technology that takes advantage of the site-specific recombination properties of bacteriophage lambda (Landy, 1989) to provide a rapid and highly efficient way to move your gene of interest into multiple vector systems. To express your gene of interest using the Gateway™ Technology, simply:

1. Clone your gene of interest into a Gateway™ entry vector to create an entry clone.
2. Generate an expression clone by performing an LR recombination reaction between the entry clone and a Gateway™ destination vector (*e.g.* pcDNA3.1/nV5-DEST™).
3. Transfect your expression clone into the cell line of choice for transient or stable expression of your gene of interest.

For more information on Gateway™, refer to the Gateway™ Technology Manual. For detailed information on constructing an entry clone, refer to the specific entry vector manual. Manuals are available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Service (page 15).

Using pcDNA3.1/nV5-DEST™

Important

The pcDNA3.1/nV5-DEST™ vector is supplied as a supercoiled plasmid. Although Invitrogen has previously recommended using a linearized destination vector for more efficient recombination, further testing at Invitrogen has found that linearization of pcDNA3.1/nV5-DEST™ is **NOT** required to obtain optimal results for any downstream application.

Propagating pcDNA3.1/nV5-DEST™

If you wish to propagate and maintain pcDNA3.1/nV5-DEST™, we recommend using Library Efficiency® DB3.1™ Competent Cells (Catalog no. 11782-018) from Invitrogen for transformation. The DB3.1™ *E. coli* strain is resistant to CcdB effects and can support the propagation of plasmids containing the *ccd*B gene.

Note: DO NOT use general *E. coli* cloning strains including TOP10 or DH5α for propagation and maintenance as these strains are sensitive to CcdB effects.

Entry Clone

To recombine your gene of interest into pcDNA3.1/nV5-DEST™, you should have an entry clone containing your gene of interest. For your convenience, Invitrogen offers the pENTR Directional TOPO® Cloning Kit (Catalog no. K2400-20) for highly efficient, 5-minute cloning of your gene of interest into an entry vector. Other entry vectors are available. For more information on entry vectors available from Invitrogen, refer to our Web site (www.invitrogen.com) or contact Technical Service (page 15). For detailed information on constructing an entry clone, refer to the specific entry vector manual.

Points to Consider Before Recombining

pcDNA3.1/nV5-DEST™ is an N-terminal fusion vector and contains an ATG codon within the context of a Kozak consensus sequence for proper initiation of translation (Kozak, 1987; Kozak, 1991; Kozak, 1990). Your gene of interest in the entry clone must:

- Be in frame with the N-terminal tag after recombination
- Contain a stop codon

Refer to the **Recombination Region** on the next page for more information.

Note

If your gene of interest in the entry clone contains its own Kozak consensus sequence with an ATG initiation codon, proper initiation of translation will still occur immediately upstream of the V5 epitope. Infrequently, initiation of translation may also occur at the second Kozak consensus sequence, resulting in expression of a small amount of native, untagged protein.

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Using pcDNA3.1/nV5-DEST™, continued

Recombining Your Gene of Interest

Each entry clone contains *att*L sites flanking the gene of interest. Genes in an entry clone are transferred to the destination vector backbone by mixing the DNAs with the Gateway™ LR Clonase™ enzyme mix (see page vi for ordering information). The resulting recombination reaction is then transformed into *E. coli* and the expression clone selected. Recombination between the *att*R sites on the destination vector and the *att*L sites on the entry clone replaces the chloramphenicol (Cm^R) gene and the *ccd*B gene with the gene of interest and results in the formation of *att*B sites in the expression clone.

Follow the instructions in the Gateway™ Technology Manual to perform the LR Clonase™ reaction, transform *E. coli*, and select for the expression clone.

Confirming the Expression Clone

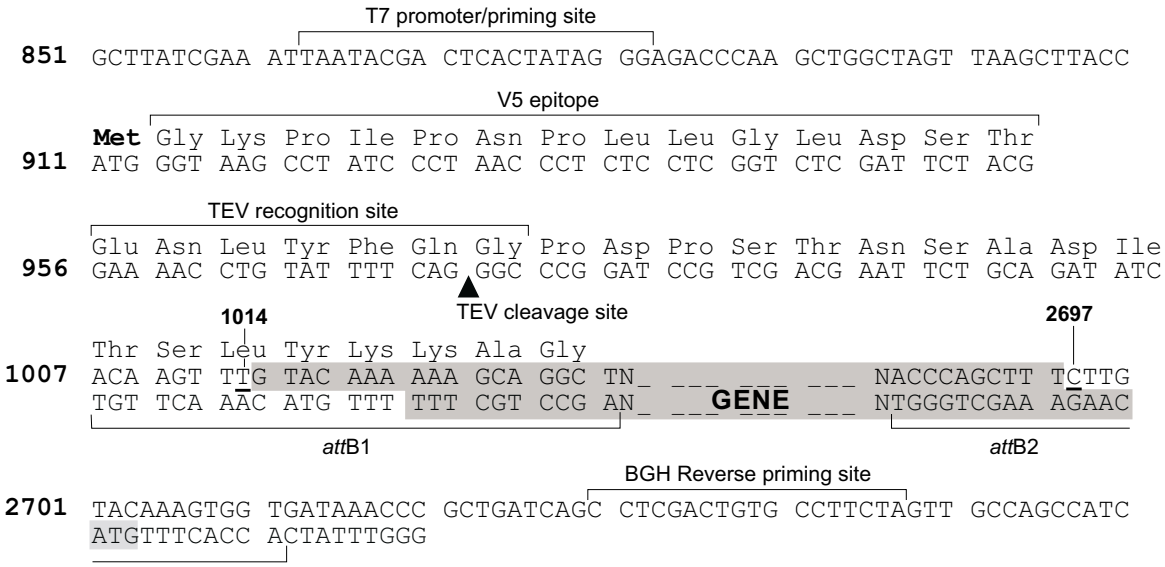
The *ccd*B gene mutates at a very low frequency, resulting in a very low number of false positives. True expression clones will be ampicillin-resistant and chloramphenicol-sensitive. Transformants containing a plasmid with a mutated *ccd*B gene will be both ampicillin- and chloramphenicol-resistant. To check your putative expression clone, test for growth on LB plates containing 30 µg/ml chloramphenicol. A true expression clone will not grow in the presence of chloramphenicol.

Recombination Region

The recombination region of the expression clone resulting from pcDNA3.1/nV5-DEST™ × entry clone is shown below.

Features of the Recombination Region:

- Shaded regions correspond to DNA sequences transferred from the entry clone into pcDNA3.1/nV5-DEST™ by recombination. Non-shaded regions are derived from the pcDNA3.1/nV5-DEST™ vector.
- The underlined nucleotides flanking the shaded region correspond to bases 1014 and 2697, respectively, of the pcDNA3.1/nV5-DEST™ vector sequence.



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Using pcDNA3.1/nV5-DEST™, continued

Sequencing

To confirm that your gene of interest is in frame with the N-terminal V5 epitope, you may sequence your expression construct, if desired. We suggest using the T7 Promoter and V5 Reverse primer sequences. Refer to the diagram on the previous page for the sequence and location of the primer binding sites.

For your convenience, Inivtrogen offers the T7 Promoter Primer (see page vi for ordering information) as well as a custom primer synthesis service. For more information, refer to our Web site (www.invitrogen.com) or contact Technical Service (page 15).

Plasmid Preparation

Once you have generated your expression clone, you must isolate plasmid DNA for transfection. 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 S.N.A.P.™ MiniPrep Kit (10-15 µg DNA, Catalog no. K1900-01), the S.N.A.P.™ MidiPrep Kit (10-200 µg DNA, Catalog no. K1910-01), or CsCl gradient centrifugation.

Transfection

Introduction

Once you have purified plasmid DNA of your expression clone, you are ready to transfect the plasmid into the mammalian cell line of choice. Note the following guidelines for transfection. We recommend that you include the pcDNA3.1/nV5-GW/*lac*Z™ positive control vector and a mock transfection (negative control) in your experiments to evaluate your results.

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. We recommend that you follow exactly the protocol for your cell line. Pay particular attention to medium requirements, when to pass the cells, and at what dilution to split the cells. Further information is provided in *Current Protocols in Molecular Biology* (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). If you wish to use a cationic lipid-based reagent for transfection, we recommend using Lipofectamine™ 2000 Reagent available from Invitrogen (Catalog no. 11668-027). For more information about Lipofectamine™ 2000 and other transfection reagents available from Invitrogen, refer to our Web site (www.invitrogen.com) or contact Technical Service (page 15).

Positive Control

pcDNA3.1/nV5-GW/*lac*Z™ is provided as a positive control vector for mammalian cell transfection and expression (see page 14 for a map) and may be used to optimize recombinant protein expression levels in your cell line. The vector allows expression of an N-terminally tagged β-galactosidase fusion protein that may be detected by Western blot or functional assay.

To propagate and maintain the plasmid:

1. Resuspend the vector in 10 µl sterile water to prepare a 1 µg/µl stock solution. Use the stock solution to transform a *rec*A, *end*A *E. coli* strain like TOP10, DH5α, JM109, or equivalent.
2. Select transformants on LB agar plates containing 50-100 µg/ml ampicillin.
3. Prepare a glycerol stock of a transformant containing plasmid for long-term storage.

Expression and Analysis

Introduction

Expression of your gene of interest from the expression clone can be performed in either transiently transfected cells or stable cell lines (see page 9 for guidelines to create stable cell lines). You may use a functional assay or a Western blot analysis to detect your recombinant protein (see below).

Preparation of Cell Lysates

To detect your fusion protein by Western blot, you will need to prepare a cell lysate from transfected cells. A sample protocol is provided below. Other protocols are suitable. To lyse cells:

1. Wash cell monolayers (~5 x 10^5 to 1 x 10^6 cells) once with phosphate-buffered saline (PBS, see the **Appendix**, page 11 for a recipe).
2. Scrape cells into 1 ml PBS and pellet the cells at 1500 x g for 5 minutes.
3. Resuspend in 50 µl Cell Lysis Buffer (see the **Appendix**, page 10 for a recipe). Other cell lysis buffers are suitable. Vortex.
4. Incubate cell suspension at 37°C for 10 minutes to lyse the cells. **Note:** You may prefer to lyse the cells at room temperature or on ice if degradation of your protein is a potential problem.
5. Centrifuge the cell lysate at 10,000 x g for 10 minutes at +4°C to pellet nuclei and transfer the supernatant to a fresh 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 (see the **Appendix,** page 11 for a recipe) 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 (see the next page). Use the appropriate percentage of acrylamide to resolve your fusion protein.

Polyacrylamide Gel Electrophoresis

To facilitate separation and visualization of your recombinant fusion protein by polyacrylamide gel electrophoresis, a wide range of pre-cast NuPAGE® and Novex® Tris-Glycine polyacrylamide gels and electrophoresis apparatus are available from Invitrogen. The NuPAGE® Gel System avoids the protein modifications associated with Laemmli-type SDS-PAGE, ensuring optimal separation for protein analysis. Invitrogen also carries a large selection of molecular weight protein standards and staining kits. For more information about the appropriate gels, standards, and stains to use to visualize your recombinant protein, refer to our Web site (www.invitrogen.com) or contact Technical Service (page 15).

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Expression and Analysis, continued

Detecting Recombinant Fusion Proteins

To detect expression of your recombinant fusion protein by Western blot analysis, you may use the Anti-V5 antibodies available from Invitrogen (see page vi for ordering information) or an antibody to your protein of interest. In addition, the Positope™ Control Protein (Catalog no. R900-50) is available from Invitrogen for use as a positive control for detection of fusion proteins containing a V5 epitope. The ready-to-use WesternBreeze® Chromogenic Kits and WesternBreeze® Chemiluminescent Kits are available from Invitrogen to facilitate detection of antibodies by colorimetric or chemiluminescent methods. For more information, refer to our Web site (www.invitrogen.com) or contact Technical Service (page 15).

Note

If your gene of interest contains its own Kozak consensus sequence with an ATG initiation codon, initiation of translation may infrequently occur at this consensus sequence. This will result in expression of a small amount of native, untagged protein which will appear as an additional, smaller band on a Western blot when detected with an antibody other than an anti-V5 antibody.

Assay for β-galactosidase Activity

If you use the pcDNA3.1/nV5-GW/*lac*Z™ plasmid as a positive control for your expression studies, you may assay for β-galactosidase expression by Western blot analysis or activity assay using cell-free lysates (Miller, 1972). Invitrogen offers β-Gal Antiserum (Catalog no. R901-25), the β-Gal Assay Kit (Catalog no. K1455-01), and the β-Gal Staining Kit (Catalog no. K1465-01) for fast and easy detection of β-galactosidase expression.

Note

The N-terminal peptide containing the V5 epitope and the TEV recognition site will add approximately 4 kDa to your protein.

Cleavage of the N-terminal Tag

The pcDNA3.1/nV5-DEST™ vector contains a TEV recognition site to allow removal of the N-terminal tag from your recombinant fusion protein, if desired. For your convenience, TEV Protease is available separately from Invitrogen (see page vi for ordering information).

Note that after digestion with TEV Protease, at least 19 amino acids will remain at the N-terminus of your protein (see diagram on page 4).

Creating Stable Cell Lines

Introduction

The pcDNA3.1/nV5-DEST™ vector contains the neomycin resistance gene to allow selection of stable cell lines using Geneticin®. General information and guidelines are provided below.

We recommend that you linearize your pcDNA3.1/nV5-DEST™ construct before transfection. While linearizing the vector may not improve the efficiency of transfection, it increases the chances that the vector does not integrate in a way that disrupts elements necessary for expression in mammalian cells. To linearize your construct, cut at a unique site that is not located within a critical element or within your gene of interest.

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® (Southern and Berg, 1982).

Determination of Antibiotic Sensitivity

To successfully generate a stable cell line expressing your protein of interest, you need to determine the minimum concentration of Geneticin® required to kill your untransfected host cell line. Test a range of concentrations (see below) to ensure that you determine the minimum concentration necessary for your cell line.

1. Plate or split a confluent plate so the cells will be approximately 25% confluent. Prepare a set of 7 plates and add the following concentrations of Geneticin® to each plate: 0, 50, 125, 250, 500, 750, and 1000 µg/ml Geneticin®.
2. Replenish the selective media every 3-4 days and observe the percentage of surviving cells.
3. Count the number of viable cells at regular intervals to determine the appropriate concentration of antibiotic that prevents growth within 1-3 weeks after addition of the antibiotic.

Geneticin® Selection Guidelines

Once you have determined the appropriate Geneticin® concentration to use for selection, you can generate a stable cell line expressing your pcDNA3.1/nV5-DEST™ construct. Geneticin® is available separately from Invitrogen (see page vi for ordering information). Use as follows:

1. Prepare Geneticin® in a buffered solution (*e.g.* 100 mM HEPES, pH 7.3).
2. Use the predetermined concentration of Geneticin® in complete medium.
3. Calculate concentration based on the amount of active drug.
4. 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 2 to 3 weeks of growth in selective medium.

Appendix

Recipes

LB (Luria-Bertani) Medium and Plates

Composition:

1.0% Tryptone
0.5% Yeast Extract
1.0% NaCl
pH 7.0

1. For 1 liter, dissolve 10 g tryptone, 5 g yeast extract, and 10 g NaCl in 950 ml deionized water.
2. Adjust the pH of the solution to 7.0 with NaOH and bring the volume up to 1 liter.
3. Autoclave on liquid cycle for 20 minutes at 15 psi. Allow solution to cool to 55°C and add antibiotic (100 µg/ml ampicillin) if needed.
4. Store at room temperature or at +4°C.

LB agar plates

1. Prepare LB medium as above, but add 15 g/L agar before autoclaving.
2. Autoclave on liquid cycle for 20 minutes at 15 psi.
3. After autoclaving, cool to ~55°C, add antibiotic (100 µg/ml of ampicillin), and pour into 10 cm plates.
4. Let harden, then invert and store at +4°C.

Cell Lysis Buffer

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

1. This solution can be prepared from the following common stock solutions. For 100 ml, combine

1 M Tris base	5 ml
5 M NaCl	3 ml
Nonidet P-40	1 ml

2. Bring the volume up to 90 ml with deionized water and adjust the pH to 7.8 with HCl.
3. Bring the volume up to 100 ml. Store at room temperature.

To prevent proteolysis, you may add 1 mM PMSF, 1 µM leupeptin, or 0.1 µM aprotinin before use.

continued on next page

Recipes, continued

Phosphate-Buffered Saline (PBS)

137 mM NaCl
2.7 mM KCl
10 mM Na_2HPO_4
1.8 mM KH_2PO_4

1. Dissolve: 8 g NaCl
   0.2 g KCl
   1.44 g Na_2HPO_4
   0.24 g KH_2PO_4
   in 800 ml deionized water.
2. Adjust pH to 7.4 with concentrated HCl.
3. Bring the volume to 1 liter. You may wish to filter-sterilize or autoclave the solution to increase shelf life.

4X SDS-PAGE Sample Buffer

1. Combine the following reagents:

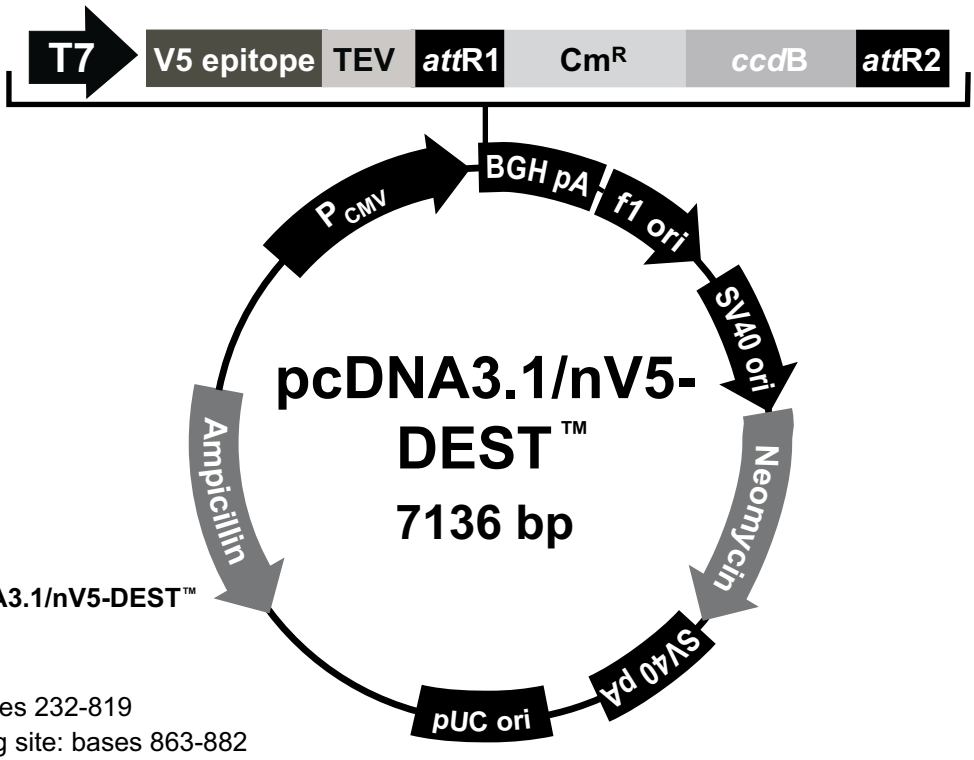
0.5 M Tris-HCl, pH 6.8	5 ml
Glycerol (100%)	4 ml
β-mercaptoethanol	0.8 ml
Bromophenol Blue	0.04 g
SDS	0.8 g

2. Bring the volume to 10 ml with sterile water.
3. Aliquot and freeze at -20°C until needed.

Map and Features of pcDNA3.1/nV5-DEST™

Map of pcDNA3.1/nV5-DEST™

The map below shows the elements of pcDNA3.1/nV5-DEST™. DNA from the entry clone replaces the region between bases 1014 and 2697. **The complete sequence of pcDNA3.1/nV5-DEST™ is available for downloading from our World Wide Web site (www.invitrogen.com) or by contacting Technical Service (page 15).**



Features of pcDNA3.1/nV5-DEST™ 7136 nucleotides

CMV promoter: bases 232-819
T7 promoter/priming site: bases 863-882
V5 epitope: bases 914-955
TEV recognition site: bases 956-976
*att*R1 recombination site: bases 1007-1131
Chloramphenicol resistance gene: bases 1240-1899
*ccd*B gene: bases 2241-2546
*att*R2 recombination site: bases 2587-2711
BGH Reverse priming site: bases 2730-2747
BGH polyadenylation region: bases 2733-2960
f1 origin: bases 3006-3434
SV40 early promoter and origin: bases 3461-3769
Neomycin resistance gene (ORF): bases 3844-4638
SV40 early polyadenylation region: bases 4812-4942
pUC origin: bases 5325-5995
Ampicillin resistance gene (ORF): bases 6140-7000 (complementary strand)

continued on next page

Map and Features of pcDNA3.1/nV5-DEST™, continued

Features of pcDNA3.1/nV5-DEST™

pcDNA3.1/nV5-DEST™ (7136 bp) contains the following elements. All features have been functionally tested.

Feature	Benefit
Human cytomegalovirus (CMV) immediate-early promoter/enhancer	Allows 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 *in vitro* transcription in the sense orientation and sequencing through the insert
V5 epitope (Gly-Lys-Pro-Ile-Pro-Asn-Pro-Leu-Leu-Gly-Leu-Asp-Ser-Thr)	Allows detection of the recombinant fusion protein by the Anti-V5 antibodies (Southern *et al.*, 1991)
TEV recognition site (Glu-Asn-Leu-Tyr-Phe-Gln-Gly)	Allows removal of the N-terminal tag from your recombinant protein using TEV protease (Carrington and Dougherty, 1988; Dougherty *et al.*, 1988)
*att*R1 and *att*R2 sites	Allows recombinational cloning of the gene of interest from an entry clone
Chloramphenicol resistance gene	Allows counterselection of expression clones
*ccd*B gene	Allows negative selection of expression clones
BGH Reverse priming site	Allows sequencing of the insert
Bovine growth hormone (BGH) polyadenylation signal	Allows 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	Allows selection of stable transfectants in mammalian cells (Southern and Berg, 1982)
SV40 early polyadenylation signal	Allows efficient transcription termination and polyadenylation of mRNA
pUC origin	Allows high-copy number replication and growth in *E. coli*
Ampicillin (*bla*) resistance gene (β-lactamase)	Allows selection of transformants in *E. coli*

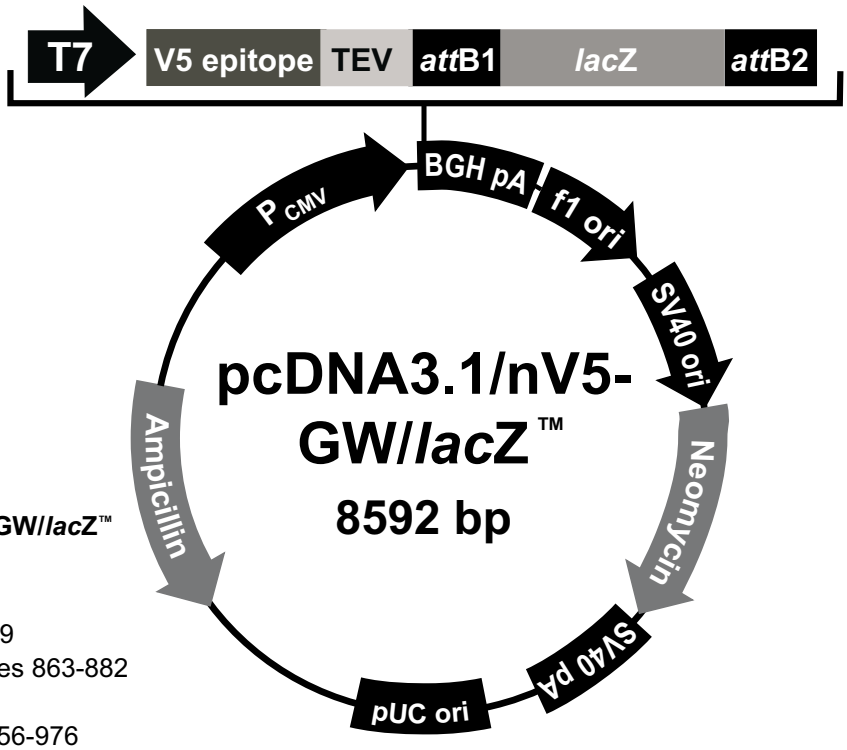
Map of pcDNA3.1/nV5-GW/*lacZ*™

Description

pcDNA3.1/nV5-GW/*lacZ*™ is an 8592 bp control vector containing the gene for β-galactosidase. pcDNA3.1/nV5-GW/*lacZ*™ was constructed using the Gateway™ LR recombination reaction between an entry clone containing the *lacZ* gene and pcDNA3.1/nV5-DEST™. β-galactosidase is expressed as a fusion to the N-terminal V5 epitope. The molecular weight of the fusion protein is approximately 120 kDa.

Map of pcDNA3.1/nV5-GW/*lacZ*™

The map below shows the elements of pcDNA3.1/nV5-GW/*lacZ*™. **The complete sequence of pcDNA3.1/nV5-GW/*lacZ*™ is available for downloading from our World Wide Web site (www.invitrogen.com) or by contacting Technical Service (page 15).**



Features of pcDNA3.1/nV5-GW/*lacZ*™
8592 nucleotides

CMV promoter: bases 232-819
T7 promoter/priming site: bases 863-882
V5 epitope: bases 914-955
TEV recognition site: bases 956-976
*att*B1 recombination site: bases 1007-1031
lacZ ORF: bases 1052-4126
*att*B2 recombination site: bases 4143-4167
BGH Reverse priming site: bases 4186-4203
BGH polyadenylation region: bases 4192-4416
f1 origin: bases 4462-4890
SV40 early promoter and origin: bases 4917-5225
Neomycin resistance gene (ORF): bases 5300-6094
SV40 early polyadenylation region: bases 6268-6398
pUC origin: bases 6781-7451
Ampicillin resistance gene (ORF): bases 7596-8456 (complementary strand)

Technical Service

World Wide Web

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

- Get the scoop on our hot new products and special product offers
- View and download vector maps and sequences
- Download manuals in Adobe® Acrobat® (PDF) format
- Explore our catalog with full color graphics
- Obtain citations for Invitrogen products
- Request catalog and product literature

Once connected to the Internet, launch your Web browser (Internet Explorer 5.0 or newer or Netscape 4.0 or newer), then enter the following location (or URL):

http://www.invitrogen.com

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

Contact Us

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

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