

pET-DEST42 Gateway[™] Vector

**A destination vector for high-level, inducible
expression in *E. coli***

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Important Information

Shipping and Storage

pET-DEST42 is shipped at room temperature. Upon receipt, store at -20°C. Product is guaranteed for six months from date of shipment when stored properly.

Contents

6 µg pET-DEST42 supplied at a concentration of 150 ng/µl in TE, pH 8.0

Accessory Products

Additional Products

Additional products that may be used with pET-DEST42 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
Ampicillin	5 g	Q100-16
Carbenicillin	5 g	10177-012
IPTG (isopropylthio-β-galactoside)	1 g	15529-019

Detection of Recombinant Proteins

Expression of your recombinant fusion protein can be detected using an antibody to the appropriate epitope. Horseradish peroxidase (HRP) or alkaline phosphatase (AP)-conjugated antibodies allow one-step detection using colorimetric or chemiluminescent detection methods. The amount of antibody supplied is sufficient for 25 Western blots.

Product	Epitope	Catalog no.
Anti-V5 Antibody	Detects 14 amino acid epitope derived from the P and V proteins of the paramyxovirus, SV5 (Southern <i>et al.</i> , 1991) GKPIP NPLLGLDST	R960-25
Anti-V5-HRP Antibody		R961-25
Anti-V5-AP Antibody		R962-25
Anti-His (C-term) Antibody	Detects the C-terminal polyhistidine (6xHis) tag, requires the free carboxyl group for detection (Lindner <i>et al.</i> , 1997) HHHHHHH-COOH	R930-25
Anti-His(C-term)-HRP Antibody		R931-25
Anti-His(C-term)-AP Antibody		R932-25

continued on next page

Accessory Products, continued

Purification of Recombinant Fusion Protein

If your gene of interest is in frame with the C-terminal peptide containing the V5 epitope and the polyhistidine (6xHis) tag, you may use Immobilized Metal Affinity Chromatography (IMAC) to purify your recombinant fusion protein. The ProBond™ Purification System or bulk ProBond™ resin are available separately from Invitrogen. See the table below for ordering information.

Product	Quantity	Catalog no.
ProBond™ Nickel-chelating Resin	50 ml	R801-01
	150 ml	R801-15
ProBond™ Purification System	6 purifications	K850-01
ProBond™ Purification System with Anti-His(C-term)-HRP Antibody	1 kit	K853-01
ProBond™ Purification System with Anti-V5-HRP Antibody	1 kit	K854-01
Purification Columns (10 ml polypropylene columns)	50	R640-50

Methods

Overview

Description

pET-DEST42 is a 7.4 kb vector adapted for use with the Gateway™ Technology. It is designed to allow high-level, inducible expression of recombinant fusion proteins in *E. coli* using the pET system.

The pET Expression System

The pET system was originally developed by Studier and colleagues and takes advantage of the high activity and specificity of the bacteriophage T7 RNA polymerase to allow regulated expression of heterologous genes in *E. coli* from the T7 promoter (Rosenberg *et al.*, 1987; Studier and Moffatt, 1986; Studier *et al.*, 1990). For more information about T7-regulated expression, see the next page.

Features

pET-DEST42 contains the following elements:

- *T7lac* promoter for high-level, IPTG-inducible expression of the gene of interest in *E. coli* (see next page for more information)
- Two recombination sites, *attR1* and *attR2*, downstream of the T7 promoter for recombinational cloning of the gene of interest from an entry clone
- Chloramphenicol resistance gene located between the two *attR* sites for counterselection
- The *ccdB* gene located between the two *attR* sites for negative selection
- V5 epitope and 6xHis tag for detection and purification (optional)
- Ampicillin resistance gene for selection in *E. coli*
- pBR322 origin for low-copy replication and maintenance of the plasmid in *E. coli*
- *lacI* gene encoding the lac repressor to reduce basal transcription from the *T7lac* promoter

For a map of pET-DEST42, see page 9.

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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 Gateway™ cloning 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. pET-DEST42).
3. Transform your expression clone into a BL21 strain of choice. Induce expression of your protein with IPTG.

For more information on the Gateway™ System, refer to the Gateway™ Technology Manual. This manual is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Service (page 11).

T7-Regulated Expression

pET-DEST42 uses elements from bacteriophage T7 to control expression of heterologous genes in *E. coli*. In the vector, expression of the gene of interest is controlled by a strong bacteriophage T7 promoter that has been modified to contain a *lac* operator sequence (see below). In bacteriophage T7, the T7 promoter drives expression of gene 10 (10). T7 RNA polymerase specifically recognizes this promoter. To express the gene of interest, it is necessary to deliver T7 RNA polymerase to the cells by inducing expression of the polymerase or infecting the cell with phage expressing the polymerase.

T7lac Promoter

pET-DEST42 has been designed to contain a T7lac promoter to drive expression of the gene of interest. The T7lac promoter consists of a *lac* operator sequence placed downstream of the T7 promoter. The *lac* operator serves as a binding site for the lac repressor (encoded by the *lacI* gene) and functions to further repress T7 RNA polymerase-induced basal transcription of the gene of interest in BL21 strains.

Using pET-DEST42



Important

The pET-DEST42 vector is supplied as a supercoiled plasmid. Although Invitrogen has previously recommended using a linearized destination vector for more efficient recombination, further testing has found that linearization of this vector is **NOT** required to obtain optimal results for any downstream application.

Propagating pET-DEST42

If you wish to propagate and maintain pET-DEST42, 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 *ccdB* gene.

DO NOT use general *E. coli* cloning strains including TOP10 or DH5 for propagation and maintenance as these strains are sensitive to CcdB effects.

Entry Clone

To recombine your gene of interest into pET-DEST42, 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 5-minute cloning of your gene of interest into an entry vector. For more information, refer to our Web site (www.invitrogen.com) or contact Technical Service (page 11).

For detailed information on constructing an entry clone, refer to the specific entry vector manual. For detailed information on performing the LR recombination reaction, refer to the Gateway[™] Technology Manual.

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Using pET-DEST42, continued

Points to Consider Before Recombining

- Your insert should contain a ribosome binding site [AGGAG(A/G)] approximately 9-10 base pairs upstream of the ATG initiation codon (Gold, 1988; Miller, 1992). This will ensure the optimal spacing for proper initiation of translation.
 - If you wish to include the V5 epitope and 6xHis tag, your gene in the entry clone **should not** contain a stop codon. The gene should also be designed to be in frame with the C-terminal epitope tag after recombination. Refer to the **Recombination Region** on the next page.
 - If you DO NOT wish to include the V5 epitope and 6xHis tag, your gene should contain a stop codon in the entry clone.
-

Recombining Your Gene of Interest

Each entry clone contains *attL* 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. The resulting recombination reaction is then transformed into *E. coli* and the expression clone selected. Recombination between the *attR* sites on the destination vector and the *attL* sites on the entry clone replaces the *ccdB* gene and the chloramphenicol (Cm^R) gene with the gene of interest and results in the formation of *attB* sites in the expression clone.

Follow the instructions in the Gateway™ Technology Manual to set up the LR Clonase™ reaction, transform a *recA endA* *E. coli* strain (e.g. TOP10 or DH5), and select for the expression clone.

Confirming the Expression Clone

The *ccdB* 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 *ccdB* 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.

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Using pET-DEST42, continued

Recombination Region The recombination region of the expression clone resulting from pET-DEST42 × entry clone is shown below.

Features of the Recombination Region:

- Shaded regions correspond to those DNA sequences transferred from the entry clone into pET-DEST42 by recombination. Non-shaded regions are derived from the pET-DEST42 vector.
- The underlined nucleotides flanking the shaded region correspond to bases 409 and 2092, respectively, of the pET-DEST42 vector sequence.

```

          T7 promoter                                lac operator
301  TCTCGATCCC GCGAAATTA TACGACTCAC TATAGGGGAA TTGTGAGCGG ATAACAATTC
    AGAGCTAGGG CGCTTTAATT ATGCTGAGTG ATATCCCCTT AACACTCGCC TATGTGTAAG
                                     409
361  CCCTCTAGAA ATAATTTTGT TTAACTTTAA GAAGGAATAT CACAAGTTTG TACAAAAAAG
    GGGAGATCTT TATTAAAACA AATTGAAATT CTTCCTTATA GTGTTCAAAC ATGTTTTTTC
                                     2092                                attB1
421  CAGGCTN--- ... Pro Ala Phe Leu Tyr Lys Val Val Ile Asn Ser Lys
    GTCCGAN--- GENE NAC CCA GCT TTC TTG TAC AAA GTG GTG ATC AAT TCG AAG
                                     attB2
          V5 epitope
2120 Leu Glu Gly Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser Thr
    CTT GAA GGT AAG CCT ATC CCT AAC CCT CTC CTC GGT CTC GAT TCT ACG
    GAA CTT CCA TTC GGA TAG GGA TTG GGA GAG GAG CCA GAG CTA AGA TGC

          6xHis tag
2168 Arg Thr Gly His His His His His His ***
    CGT ACC GGT CAT CAT CAC CAT CAC CAT TGA GTTTGATCCG GCTGCTAACA
    GCA TGG CCA GTA GTA GTG GTA GTG GTA ACT CAAACTAGGC CGACGATTGT
  
```

The Next Step Once you have generated your expression clone, you will need to transform it into a BL21 *E. coli* strain for expression studies. Proceed to the next section for guidelines on choosing a BL21 host strain.

BL21 *E. coli* Strains

Introduction

This section provides information on strains available from Invitrogen that are specifically designed for use with T7 promoter-based expression systems. **Note:** Other BL21 strains are suitable.

Recommended Strains

See the table below for recommended strains and their benefits. For more information on these strains and other BL21 strains available from Invitrogen, refer to our Web site (www.invitrogen.com) or contact Technical Service (page 11).

Product	Benefit	Catalog no.
BL21 Star™ (DE3) One Shot® Chemically Competent <i>E. coli</i>	Mutation in RNaseE improves stability of mRNA transcripts and increases protein yields.	C6010-03
BL21-AI™ One Shot® Chemically Competent <i>E. coli</i>	T7 RNA polymerase under the control of <i>araBAD</i> promoter for inducible expression and low basal levels. Especially useful for expression of toxic genes.	C6070-03

Expression and Analysis

Introduction

Once you have selected your *E. coli* host strain, you are ready to test for expression of your gene. This section provides general guidelines for expressing and analyzing your protein of interest. For detailed information on transforming your BL21 strain, inducing expression with IPTG, and analyzing samples, refer to your specific BL21 *E. coli* strain manual. If you are using a BL21 strain from Invitrogen, the manuals are available for downloading from our Web site or by contacting Technical Service (page 11).

Basic Strategy

The basic steps needed to induce expression of your gene in a BL21 *E. coli* strain are outlined below.

1. Isolate plasmid DNA using standard procedures and transform your construct into BL21 cells.
 2. Grow the transformants and induce expression with IPTG over several hours. Take several time points to determine the optimal time of expression.
-

Plasmid Preparation

You may prepare plasmid DNA using your method of choice. We recommend using the S.N.A.P.[™] MidiPrep Kit (Catalog no. K1910-01) for isolation of pure plasmid DNA. Note that since you are purifying a low-copy number plasmid, you may need to increase the amount of bacterial culture that you use to prepare your plasmid construct.

Choosing a Selection Agent

For most purposes, ampicillin works well for selection of transformants and expression experiments. However, if you find that your expression level is low, you may want to use carbenicillin instead. The resistance gene for ampicillin encodes a protein called β -lactamase. This protein is secreted into the medium where it hydrolyzes ampicillin, inactivating the antibiotic. Since β -lactamase is catalytic, ampicillin is rapidly removed from the medium, resulting in non-selective conditions. If your plasmid is unstable, this may result in the loss of plasmid and low expression levels.

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Expression and Analysis, continued

Using Carbenicillin

Carbenicillin is generally more stable than ampicillin, and studies have shown that using carbenicillin in place of ampicillin may help to increase expression levels by preventing loss of the pET-DEST42 expression plasmid. If you wish to use carbenicillin, perform your transformation and expression experiments in LB containing 50 µg/ml carbenicillin.

Note: If your gene of interest is highly toxic, increasing the concentration of carbenicillin used from 50 µg /ml to 200 µg /ml may help to increase expression levels.

Detection of Recombinant Fusion Proteins

To detect expression of your recombinant fusion protein by Western blot analysis, you may use antibodies against the appropriate epitope (see page v 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 or a polyhistidine (6xHis) tag. For more information, refer to our Web site (www.invitrogen.com) or contact Technical Service (page 11).



Note

The C-terminal peptide containing the V5 epitope and the polyhistidine tag will add approximately 4 kDa to your protein.

Purification of Recombinant Fusion Proteins

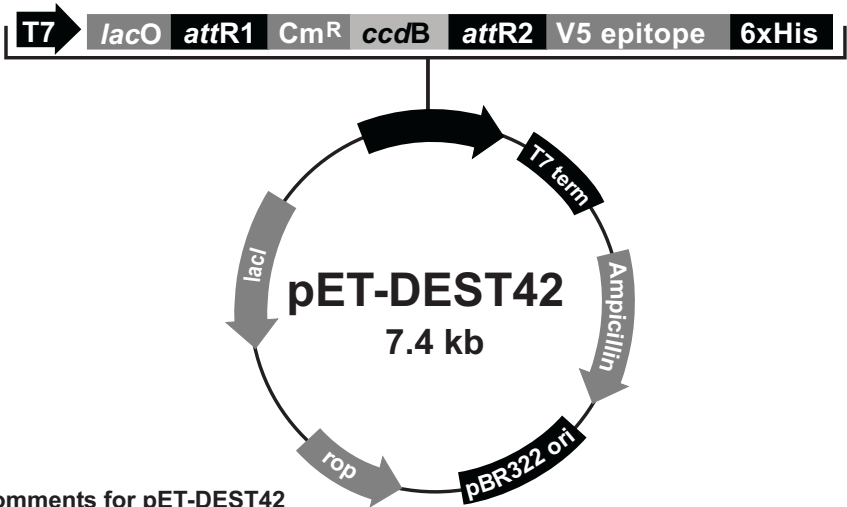
The presence of the C-terminal polyhistidine (6xHis) tag in your recombinant fusion protein allows use of a metal-chelating resin such as ProBond™ to purify your fusion protein. The ProBond™ Purification System and bulk ProBond™ resin are available from Invitrogen (see page vi for ordering information). Invitrogen also offers Ni-NTA Agarose (Catalog no. R901-01) for purification of proteins containing a polyhistidine (6xHis) tag. **Note:** Other metal-chelating resins and purification methods are suitable.

Appendix

Map and Features of pET-DEST42

Map of pET-DEST42

The map below shows the elements of pET-DEST42. DNA from the entry clone replaces the region between bases 409 and 2092. The complete sequence of pET-DEST42 is available from our Web site (www.invitrogen.com) or by contacting Technical Service (page 11).



Comments for pET-DEST42 7440 nucleotides

T7 promoter: bases 318-334

lac operator (*lacO*): bases 337-361

attR1 recombination site: bases 402-526

Chloramphenicol resistance gene: bases 635-1294

ccdB gene: bases 1636-1941

attR2 recombination site: bases 1982-2106

V5 epitope: bases 2126-2167

Polyhistidine (6xHis) region: bases 2177-2194

T7 transcription termination region: bases 2209-2337

bla promoter: bases 2638-2736

Ampicillin (*bla*) resistance gene (ORF): bases 2737-3597

pBR322 origin: bases 3742-4415

ROP ORF: bases 4786-4977 (complementary strand)

lacI ORF: bases 6289-7380 (complementary strand)

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Map and Features of pET-DEST42, continued

Features of pET-DEST42

pET-DEST42 (7440 bp) contains the following elements. All features have been functionally tested.

Feature	Benefit
T7 promoter	Allows high-level, IPTG-inducible expression of your recombinant protein in <i>E. coli</i> strains expressing the T7 RNA polymerase
<i>lac</i> operator (<i>lacO</i>)	Binding site for lac repressor that serves to reduce basal expression of the recombinant protein
<i>attR1</i> and <i>attR2</i> sites	Allows recombinational cloning of the gene of interest from an entry clone
Chloramphenicol resistance gene	Allows counterselection of expression clones
<i>ccdB</i> gene	Allows negative selection of expression clones
V5 epitope	Allows detection of the recombinant protein by the Anti-V5 antibodies (Southern <i>et al.</i> , 1991)
C-terminal polyhistidine tag	Allows purification of the recombinant protein on metal-chelating resin such as ProBond™ Allows detection of the recombinant protein by the Anti-His (C-term) antibodies (Lindner <i>et al.</i> , 1997)
T7 transcription termination region	Sequence from bacteriophage T7 which allows efficient transcription termination.
<i>bla</i> promoter	Allows expression of the ampicillin resistance gene
Ampicillin resistance gene	Allows selection of the plasmid in <i>E. coli</i>
pBR322 origin	Allows replication and maintenance in <i>E. coli</i>
<i>lacI</i> ORF	Encodes lac repressor which binds to the T7 <i>lac</i> promoter to block basal transcription of the gene of interest. Also binds the <i>lacUV5</i> promoter in BL21 strains containing the λDE3 lysogen to repress transcription of T7 RNA polymerase

Technical Service

Web Resources



Visit the Invitrogen Web site at www.invitrogen.com for:

- Technical resources, including manuals, vector maps and sequences, application notes, SDSs, FAQs, formulations, citations, handbooks, etc.
 - Complete technical service contact information
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SDS

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

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Technical Service, continued

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Introduction

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