invitrogen™
by life technologies™

Champion™ pET302/NT-His and pET303/CT-His Vectors

Vectors for high-level, inducible expression of N- and C-terminal 6x His-tagged protein in *E. coli*

Catalog no. K6302-03

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Manual part no. 25-0957

MAN0000576

User Manual

Table of Contents

Important Information

Shipping and Storage

Champion™ pET302/NT-His, pET303/CT-His and pET303/CT-Rac Kinase vectors are shipped at room temperature. Upon receipt, store lyophilized vectors at -20°C.

Contents

The Champion™ pET302/NT-His, pET303/CT-His vector kit contains the N- and C-terminal His vectors and an expression control plasmid as listed below:

Item	Concentration	Amount
pET302/NT-His	lyophilized in TE, pH 8.0	6 µg
pET303/CT-His	lyophilized in TE, pH 8.0	6 µg
pET303/CT-Rac Kinase	lyophilized in TE, pH 8.0	10 µg

Accessory Products

Additional Products

Additional products that may be used with pET302/NT-His and pET303/CT-His are available from Invitrogen. Ordering information is provided below.

Product	Amount	Catalog no.
BL21(DE3) Chem. Competent Cells	20 x 50 µl	C6000-03
One Shot® BL21 Star (DE3) Chem. Competent Cells	20 x 50 µl	C6010-03
One Shot® BL21(DE3) pLysS Chem. Competent Cells	20 x 50 µl	C6060-03
MagicMedia™ *E. coli* Expression Medium	1 L SoluPouch™ 5 x 1 L SoluPouch™ 1 L liquid	K6801 K6802 K6803
Ampicillin	5 g	Q100-16
Carbenicillin	5 g	10177-012

Purification and Detection of Recombinant Fusion Protein

If your gene of interest is in frame with the N- or C-terminal polyhistidine (6x His) tag, you may detect your fusion protein with an antibody to the polyhistidine tag. You may also purify your recombinant fusion protein using a metal chelating system.

Product	Quantity	Catalog no.
Mouse anti-His Tag monoclonal antibody	100 µg	37-2900
ProBond™ Nickel-chelating Resin	50 ml	R801-01
	150 ml	R801-15
ProBond™ Purification System	6 purifications	K850-01
Ni-NTA Agarose	10 ml 25 ml 100 ml	R901-15 R901-25 R901-10
Purification Columns (10 ml polypropylene columns)	50	R640-50

Overview

Description

Champion™ pET302/NT-His (5.7 kb) and pET303/CT-His (5.3 kb) vectors allow you to clone your gene of interest using restriction enzyme digestion and ligation. Both Champion™ pET302/NT-His and pET303/CT-His are included, allowing you to choose the best configuration (*i.e.* N- or C-terminal polyhistidine tag) and to optimize protein expression levels. The vectors are designed to allow high-level, inducible expression of recombinant fusion proteins in *E. coli* using the pET system. A control expression plasmid, pET303/CT-Rac Kinase, is included to optimize protein expression.

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 & Moffatt, 1986; Studier *et al.*, 1990). For more information about T7-regulated expression, see the next page.

Features

Champion™ pET302/NT-His and pET303/CT-His contain the following elements:

- T7*lac* promoter for high-level expression of the gene of interest in *E. coli* (see next page for more information)
- Multiple cloning site for restriction enzyme digestion and ligation of gene of interest
- N- or C-terminal 6x His tag for detection and purification of protein
- Ampicillin resistance gene for selection in *E. coli*
- pBR322 origin for low-copy replication and maintenance of the plasmid in *E. coli*
- *lac*I gene encoding the lac repressor to reduce basal transcription from the T7*lac* promoter

For maps of pET302/NT-His and pET303/CT-His, see pages 11-12.

Continued on next page

Overview, continued

T7-Regulated Expression

pET302/NT-His and pET303/CT-His contain 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. 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.

T7*lac* Promoter

pET302/NT-His and pET303/CT-His contain the T7*lac* promoter to drive expression of the gene of interest. The T7*lac* 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 *lac*I gene) and functions to further repress T7 RNA polymerase-induced basal transcription of the gene of interest in BL21 strains.

BL21 Strains

The BL21(DE3) *E. coli* strain is specifically designed for expression of genes regulated by the T7 promoter. Basal level expression of T7 polymerase, particularly in BL21(DE3) cells, may lead to plasmid instability if your gene of interest is toxic to *E. coli*. You may also use BL21 Star™(DE3) and BL21 Star™(DE3)pLysS strains if your protein is toxic to *E. coli*. See page v for ordering information.

Methods

Cloning

Introduction

The following information is provided to help you clone your gene of interest into pET302/NT-His and pET303/CT-His. For basic information on DNA ligations, *E. coli* transformations, restriction analysis, DNA sequencing and DNA biochemistry, see *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994).

Resuspending and Propagating the Vectors

Resuspend pET302/NT-His and pET303/CT-His to 150 ng/µl in sterile water. Store at -20°C.

To propagate the vectors, use this stock solution to transform a *rec*A, *end*A *E. coli* strain like DH5α, TOP10F′ or equivalent. Transformants are selected on LB plates containing 50-100 µg/ml ampicillin. Be sure to prepare a glycerol stock of a transformant containing plasmid for long-term storage (see page 10).

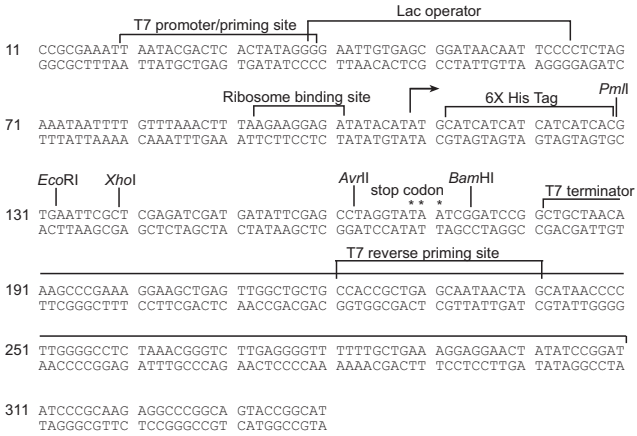
Cloning, continued

Cloning into pET302/NT-His

pET302/NT-His is an N-terminal fusion vector and contains an ATG initiation codon and a Shine-Dalgarno ribosome binding site (RBS) with optimal spacing for proper translation. Your gene of interest must:

- **Be in frame with the N-terminal tag (you may need to add additional nucleotides between your gene of interest and the restriction site)**
- **Contain a stop codon if you are cloning with *Bam*HI**

The multiple cloning region of pET302/NT-His is shown below:



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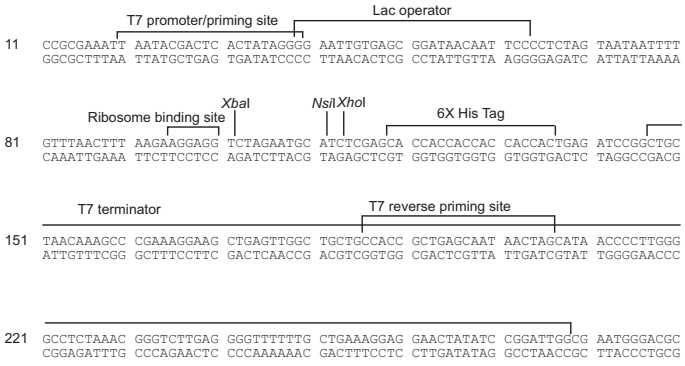
Cloning, continued

Cloning into pET303/CT-His

pET303/CT-His is an C-terminal fusion vector and contains a Shine-Dalgarno ribosome binding site (RBS) with optimal spacing for proper translation. Your gene of interest must include:

- An ATG initiation codon
- If you wish to include the 6x His tag, your gene **should not** contain a stop codon.
- If you do not wish to fuse your gene of interest to the 6xHis tag, your gene **should** contain a stop codon

The multiple cloning region of pET303/CT-His is shown below:



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Cloning, continued

Ligation

Once you have determined a cloning strategy, digest the vector with the selected restriction enzymes. Ligate your gene of interest into the vector using standard molecular biology techniques.

Transformation

After ligating your gene of interest into pET302/NT-His or pET303/CT-His, transform the ligation mixture into competent *E. coli*. Select 10-20 clones and analyze plasmid DNA for the presence and orientation of your insert by sequencing or appropriate restriction enzyme digestion.

Sequencing

To confirm that your gene of interest is in frame with the N- or C-terminal 6X His tag, you may sequence your expression construct, if desired. Refer to the diagrams on pages 4-5 for the location of the primer binding sites.

For your convenience, Invitrogen offers a custom primer synthesis service. For more information, go to www.invitrogen.com or contact Technical Service (page 15).

Primer	Sequence
T7 Promoter	5′-TAATACGACTCACTATAGGG-3′
T7 Reverse	5′-TAGTTATTGCTCAGCGGTGG-3′

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. See page 15 for recommended BL21 host strains and media, and proceed to **Expression and Analysis**, next section.

Expression and Analysis

Introduction

This section provides general guidelines for expressing and analyzing your protein of interest. For detailed information on transforming your BL21 strain, inducing expression, and analyzing samples, refer to your specific BL21 *E. coli* strain manual.

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. Use pET303/CT-GW/Rac Kinase included with the kit as a positive control for transformation and protein expression (see next page).
2. Grow the transformants and induce expression over several hours. Take several time points to determine the optimal time of expression. Alternatively, you can grow *E. coli* using MagicMedia™ (see Recommendation, below)

MagicMedia *E. coli* Expression Medium allows high yield of T7-regulated heterologous protein expression without time-consuming steps such as monitoring O.D. or adding inducing agents such as IPTG. MagicMedia™ is available separately from Invitrogen, see page v for ordering information or go to www.invitrogen.com for more details.

Plasmid Preparation

You may prepare plasmid DNA using your method of choice. We recommend using the PureLink™ HiPure Plasmid Midiprep Kit 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.

Continued on next page

Expression and Analysis, continued

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.

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 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.

Expression Control Vector pET303/CT-Rac Kinase

pET303/CT-Rac Kinase is provided for use as a positive control vector for protein synthesis in a suitable *E. coli* host. This vector allows expression of a 6x His C-terminally tagged fusion protein of 57.7 kDa (Jones *et al.*, 1991). For details about the vector, see page 14. To propagate and maintain the plasmid:

1. Resuspend the vector in 10 µl of sterile water to prepare a 1 µg/µl stock solution.
2. Use the stock solution to transform a *rec*A, *end*A *E. coli* strain like TOP10, DH5α™-T1^R, or equivalent. Use 10 ng of plasmid for transformation.
3. Select transformants on LB agar plates containing 50-100 µg/ml ampicillin.
4. Prepare a glycerol stock of a transformant containing plasmid for long-term storage (see page 10).

Continued on next page

Expression and Analysis, continued

Detection of Recombinant Fusion Proteins

To detect expression of your recombinant fusion protein by western blot analysis, you may use antibodies against the polyhistidine tag (such as mouse anti-His monoclonal antibody, available separately from Invitrogen) or an antibody to your protein of interest. For more information, see page v, go to www.invitrogen.com or contact Technical Service (page 15).

Note

The N-terminal peptide containing the 6x His tag will add approximately 1.5 kDa to your protein. The C-terminal peptide containing the 6x His tag will add approximately 0.9 kDa to your protein.

Purification of Recombinant Fusion Proteins

The presence of the N- or C-terminal 6x His 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 v for ordering information). Invitrogen also offers Ni-NTA Agarose for purification of proteins containing a 6x His tag.
Note: Other metal-chelating resins and purification methods are suitable.

Appendix

Recipes

Making Glycerol Stocks

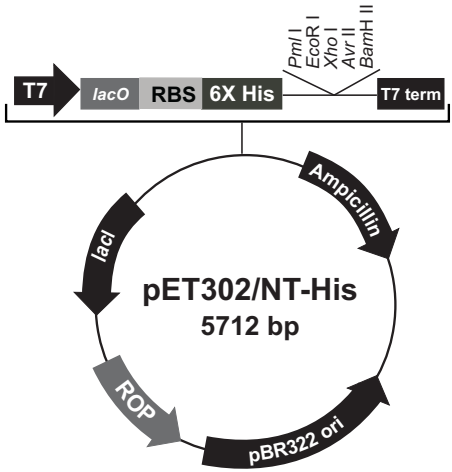
1. Grow 1-2 ml of the *E. coli* strain to be frozen in SOB medium overnight with antibiotic selection when appropriate.
2. Combine 0.85 ml of the overnight culture with 0.15 ml of sterile glycerol (sterilized by autoclaving).
3. Mix well by vortexing.
4. Transfer to an appropriate freezing vial (preferably a screw cap, air-tight gasket).

Freeze in an ethanol-dry ice bath or liquid nitrogen and then transfer to -80°C for long-term storage.

Map of pET302/NT-His

Map of pET302/NT-His

The map below shows the elements of pET302/NT-His. **The complete sequence of pET302/NT-His is available from www.invitrogen.com or by contacting Technical Service (page 15.)**



Comments for pET302 NT-His
5712 nucleotides

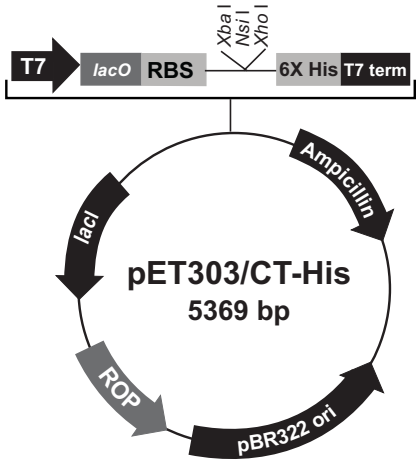
T7 promoter: bases 20-36
T7 promoter priming site: bases 20-39
lac operator (*lac*O): bases 39-63
Ribosome binding site (RBS): bases 93-101
6X His Tag: bases 112-129
T7 transcription termination region: bases 182-310
T7 reverse priming site: bases 221-241
bla promoter: bases 615-719
Ampicillin (*bla*) resistance gene: bases 714-1574
pBR322 origin: bases 1785-2516 (c)
ROP ORF: bases 2760-2951 (c)
lacI ORF: bases 4263-5375 (c)

(c) = complementary strand

Map of pET303/CT-His

Map of pET303/CT-His

The map below shows the elements of pET303/CT-His. **The complete sequence of pET303/CT-His is available from www.invitrogen.com or by contacting Technical Service (page 15).**



Comments for pET303 CT-His
5369 nucleotides

T7 promoter: bases 20-36
T7 promoter priming site: bases 20-39
lac operator (*lac*O): bases 39-63
Ribosome binding site (RBS): bases 95-100
6X His Tag: bases 119-136
T7 reverse priming site: bases 186-206
T7 transcription termination region: bases 147-277
F1 origin: bases 287-742
bla promoter: bases 775-879
Ampicillin (*bla*) resistance gene: bases 874-1734
pBR322 origin: bases 1945-2678 (c)
ROP ORF: bases 2920-3011 (c)
lacI ORF: bases 3914-5032 (c)

(c) = complementary strand

Features of pET302/NT-His and pET303/CT-His

Features of the Vectors

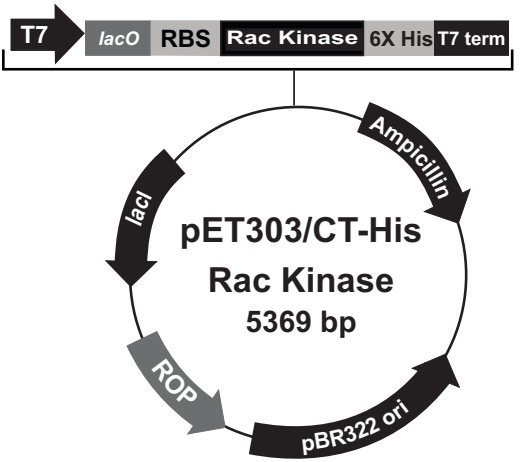
The pET302/NT-His and pET303/CT-His vectors contain the following elements. Features have been functionally tested.

Feature	Benefit
T7 promoter	Allows high-level, inducible expression of your recombinant protein in *E. coli* strains expressing the T7 RNA polymerase
T7 primer binding site	Allows sequencing of the insert
lac operator (*lac*O)	Binding site for lac repressor that serves to reduce basal expression of the recombinant protein
Ribosome binding site	Optimally spaced from the initiation site for efficient translation of insert
N-terminal or C-terminal 6x His tag	Allows purification of the recombinant protein on metal-chelating resin such as ProBond™
T7 reverse primer binding site	Allows sequencing of the insert
T7 transcription termination region	Sequence from bacteriophage T7 which allows efficient transcription termination.
bla promoter	Allows expression of the ampicillin resistance gene
Ampicillin resistance gene	Allows selection of transformants in *E. coli*
pBR322 origin	Allows replication and maintenance in *E. coli*
ROP ORF	Interacts with the pBR322 origin to facilitate low-copy replication in *E. coli.*
*lac*I ORF	Encodes lac repressor which binds to the T7*lac* promoter to block basal transcription of the gene of interest. Also binds the *lac*UV5 promoter in BL21 strains containing the λDE3 lysogen to repress transcription of T7 RNA polymerase

Map of pET303/CT-Rac Kinase

Map of pET303/CT-Rac Kinase

The map below shows the elements of pET303/CT-Rac Kinase. **The complete sequence of pET303/CT-Rac Kinase is available from www.invitrogen.com or by contacting Technical Service (page 15).**



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pBR322 origin: bases 1945-2678 (c)
ROP ORF: bases 2920-3011 (c)
lacI ORF: bases 3914-5032 (c)

(c) = complementary strand

Technical Service

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).

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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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Limited Use Label License No. 30: T7 Expression System

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Notes

Notes

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