Instruction Manual

pEXP1-DEST Gateway® Vector Kit
pEXP2-DEST Gateway® Vector Kit

Gateway®-adapted destination vectors for cloning and high-level, inducible expression in *E. coli*

Catalog nos. K9600-01, K9600-02, K9605-01, K9605-02, V960-01, V960-02

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Kit Contents and Storage

Shipping/Storage

The pEXP1-DEST and pEXP2-DEST vectors are shipped at room temperature. Upon receipt, store at -20°C.

Types of Kits

This manual is supplied with the products listed below.

All products except Catalog nos. V960-01 and V960-02 are also supplied with Expressway™ System reagents and an Expressway™ System manual. For a detailed description about the Expressway™ or Expressway™ Plus System, refer to the respective system manual.

Product	Amount	Catalog no.
Expressway™ Plus Expression System		
with pEXP1-DEST	20 reactions	K9900-20
with pEXP2-DEST	20 reaction	K9900-30
Expressway™ *In Vitro* Protein Synthesis System		
with pEXP1-DEST	20 reactions	K9600-01
	8 reactions	K9605-01
with pEXP2-DEST	20 reactions	K9600-02
	8 reactions	K9605-02
pEXP1-DEST Gateway® Vector Kit	6 µg	V960-01
pEXP2-DEST Gateway® Vector Kit	6 µg	V960-02

Contents

The following vectors are supplied. **Store at -20°C.**

Vector	Composition	Amount
pEXP-DEST Vector (pEXP1-DEST or pEXP2-DEST)	Lyophilized in TE Buffer, pH 8.0	6 µg
pEXP-GW/*lacZ* Control Plasmid (pEXP1-GW/*lacZ* or pEXP2-GW/*lacZ*)	Lyophilized in TE Buffer, pH 8.0	10 µg

Product Qualification

The structure of each vector is verified by restriction enzyme digestion. In addition, the functionality of the destination vectors is 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

The products below may be used with the pEXP1-DEST and pEXP2-DEST vectors. For more information, refer to Web site (www.invitrogen.com) or contact Technical Service (see page 20).

Item	Amount	Catalog no.
Expressway™ Plus Expression System	20 reactions	K9900-10
Gateway® LR Clonase™ Enzyme Mix	20 reactions	11791-019
T7 Promoter Primer	2 µg (328 pmoles)	N560-02
T7 Reverse Primer	2 µg (325 pmoles)	N590-02
Ampicillin	20 ml (10 mg/ml)	11593-019
Zeocin™	1 g	R250-01
	5 g	R250-05
Library Efficiency® DB3.1™ Competent Cells	1 ml (5 x 0.2 ml)	11782-018
One Shot® TOP10 Chemically Competent *E. coli*	20 x 50 µl	C4040-03
	40 x 50 µl	C4040-06
BL21 Star™(DE3)pLysS One Shot® Chemically Competent *E. coli*	20 x 50 µl	C6020-03
EKMax™	250 units	E180-01
	1000 units	E180-02
β-Gal Antiserum	50 µl*	R901-25
β-Gal Assay Kit	100 reactions	K4155-01

*The amount of antibody supplied is sufficient for 25 Western blots.

continued on next page

Accessory Products, continued

Detection of Recombinant Fusion Proteins

Expression of your recombinant fusion protein can be detected using an antibody to the appropriate epitope. The table below describes the antibodies available from Invitrogen for detection of fusion proteins expressed from the pEXP-DEST vectors, as appropriate. Horseradish peroxidase (HRP) or alkaline phosphatase (AP)-conjugated antibodies allow one-step detection using chemiluminescent or colorimetric detection methods.

The amount of antibody supplied is sufficient for 25 Western blots.

Item	Epitope	Catalog no.
Anti-Xpress™ Antibody	Detects the 8 amino acid Xpress™ epitope: DLYDDDDK	R910-25
Anti-Xpress™-HRP Antibody		R911-25
Anti-HisG Antibody	Detects the N-terminal poly-histidine (6xHis) tag followed by glycine: HHHHHHG	R940-25
Anti-HisG-HRP Antibody		R941-25
Anti-HisG-AP Antibody		R942-25
Anti-V5 Antibody	Detects 14 amino acid epitope derived from the P and V proteins of the paramyxo-virus, SV5 (Southern *et al.*, 1991): GKPIPNPLLGLDST	R960-25
Anti-V5-HRP Antibody		R961-25
Anti-V5-AP Antibody		R962-25
Anti-His(C-term) Antibody	Detects the C-terminal poly-histidine (6xHis) tag (requires the free carboxyl group for detection (Lindner *et al.*, 1997): HHHHHH-COOH	R930-25
Anti-His(C-term)-HRP Antibody		R931-25
Anti-His(C-term)-AP Antibody		R932-25

Purification of Recombinant Fusion Protein

If your gene of interest is in frame with the C-terminal or N-terminal peptide containing the polyhistidine (6xHis) tag, you may use a nickel-charged agarose resin such as ProBond™ or Ni-NTA to purify your recombinant fusion protein. 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
Ni-NTA Agarose	10 ml	R901-01
	25 ml	R901-15
	100 ml	R901-10
Ni-NTA Purification System	6 purifications	K950-01

Introduction

Overview

Introduction

pEXP1-DEST (4.6 kb) and pEXP2-DEST (4.4 kb) are vectors designed to allow T7-based, high-level expression of recombinant fusion proteins in the Expressway™ and Expressway™ Plus Systems or for inducible expression in *E. coli*. The vectors are derived from Invitrogen's pCR®T7/NT-TOPO® and pCR®T7/CT-TOPO® vectors, respectively, and adapted for use with the Gateway® Technology.

A choice of pEXP-DEST vectors allows you to fuse your gene of interest to an N-terminal or C-terminal peptide for production of recombinant fusion proteins that may be easily detected or purified (see table below).

pEXP-DEST Vector	Fusion Peptide	Fusion Tag	Benefit
pEXP1-DEST	N-terminal	Xpress™, 6xHis	Cleavable detection and purification tag
pEXP2-DEST	C-terminal	V5, 6xHis	Detection and purification tag

For more information about the Gateway® Technology, see the next page.

Features of the Vectors

The pEXP1-DEST and pEXP2-DEST vectors contain the following elements:

- Bacteriophage T7 promoter for high-level, inducible expression of the recombinant protein of interest in the Expressway™ Systems or in *E. coli*
- N-terminal or C-terminal fusion tags for detection and purification of recombinant fusion proteins (choice of tag varies depending on the particular vector; see above)
- Enterokinase (EK) recognition site for cleavage of the N-terminal peptide from your recombinant fusion protein (pEXP1-DEST only)
- Two recombination sites, *att*R1 and *att*R2, downstream of the T7 promoter for recombinational cloning of the gene of interest from an entry clone
- Chloramphenicol resistance gene (Cm^R) located between the two *att*R sites for counterselection
- *ccd*B gene located between the *att*R sites for negative selection
- Ampicillin resistance gene for selection in *E. coli*
- pUC origin for high-copy replication and maintenance of the plasmid in *E. coli*

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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 in *E. coli* using the Gateway® Technology, simply:

1. Clone your gene of interest into a Gateway® entry vector of choice 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.* pEXP1-DEST or pEXP2-DEST).
3. Introduce your expression clone into an *E. coli* expression strain for *in vivo* protein expression or use the Expressway™ or Expressway™ Plus System for *in vitro* protein synthesis (see below).

For more detailed information about Gateway® Technology and performing the LR recombination reaction, refer to the Gateway® Technology manual which is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Service (see page 20). For more information about the various entry vectors available from Invitrogen, see our Web site or call Technical Service.

Expressway™ Systems

The Expressway™ Systems are designed for T7-based, *in vitro* transcription and translation of target DNA to protein in a single tube. The Expressway™ *In Vitro* Protein Synthesis System uses extract from an *E. coli* strain to drive the reaction, and a transcription/translation mix that allows for optimal protein production (up to 50 µg protein per 50 µl reaction, depending on the protein of interest) in about 2 hours. In the Expressway™ Plus Expression System, these reagents have been enhanced to provide higher levels of functional protein over the original Expressway™ *In Vitro* Protein Synthesis System.

Once you have generated an expression clone containing your gene of interest in a pEXP-DEST vector (see above) and have purified the plasmid, you may use the DNA template in an Expressway™ reaction to synthesize your recombinant fusion protein. For more information about the Expressway™ Systems, refer to the respective Expressway™ System manual. The Expressway™ System manual is supplied with Catalog nos. K9600-01, K9600-02, K9605-01, and K9605-02. The Expressway™ Plus System manual is supplied with Catalog no. K9900-20 and K9900-30. These manuals are also available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Service (see page 20).

Methods

Generating an Entry Clone

Introduction

To recombine your gene of interest into pEXP1-DEST or pEXP2-DEST, you should have an entry clone containing your gene of interest. Refer to the Gateway® Technology manual for details to choose a Gateway ™ entry vector. This manual is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Service (see page 20).

If you plan to recombine your gene of interest into pEXP2-DEST, we recommend that you use an entry vector that supplies a ribosome binding site (RBS) (*e.g.* pENTR/SD/D-TOPO®). See the pENTR™ Directional TOPO® Cloning Kits manual on the Invitrogen Web site for more information.

Points to Consider Before Recombining into pEXP1-DEST

pEXP1-DEST 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 in *E. coli*. 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 diagram of the recombination region of pEXP1-DEST on page 5 for more information.

Points to Consider Before Recombining into pEXP2-DEST

pEXP2-DEST is a C-terminal fusion vector. Your gene of interest in the entry clone must:

- Contain an ATG initiation codon and a ribosome binding site (RBS) with optimal spacing for proper translation in *E. coli*

  Note: If you clone your gene of interest into an entry vector that supplies an RBS (*e.g.* pENTR/SD/D-TOPO®), then your gene of interest need only include an ATG initiation codon.
- Be in frame with the C-terminal tag after recombination.
- **NOT** contain a stop codon.

Refer to the diagram of the recombination region of pEXP2-DEST on page 6 for more information.

Creating an Expression Clone

Introduction

After you have generated an entry clone, you will perform the LR recombination reaction to transfer the gene of interest into the pEXP1-DEST or pEXP2-DEST vector to create your expression clone. To ensure that you obtain the best possible results, we recommend that you read this section and the next section entitled **Performing the LR Recombination Reaction** (pages 7-9) before beginning.

Experimental Outline

To generate an expression clone, you will:

1. Perform an LR recombination reaction using the *att*L-containing entry clone and the *att*R-containing pEXP1-DEST or pEXP2-DEST vector. **Note:** Both the entry clone and the destination vector should be supercoiled (see **Important Note** below).
2. Transform the reaction mixture into a suitable *E. coli* host.
3. Select for expression clones (refer to pages 5-6 for diagrams of the recombination region of the resulting expression clones).

Important

The pEXP1-DEST and pEXP2-DEST vectors are supplied as supercoiled plasmids. Although we have previously recommended using a linearized destination vector for more efficient recombination, further testing at Invitrogen has found that linearization of pEXP1-DEST or pEXP2-DEST is **not** required to obtain optimal results for any downstream application.

Resuspending the Vectors

The pEXP1-DEST and pEXP2-DEST vectors are supplied as 6 μg of plasmid, lyophilized in TE, pH 8.0. To use, simply resuspend the destination vector in 40 μl of sterile water to a final concentration of 150 ng/μl.

Propagating the Vectors

If you wish to propagate and maintain the pEXP1-DEST or pEXP2-DEST vectors, 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. To maintain the integrity of the vector, select for transformants in media containing 50-100 μg/ml ampicillin and 15-30 μg/ml chloramphenicol.

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.

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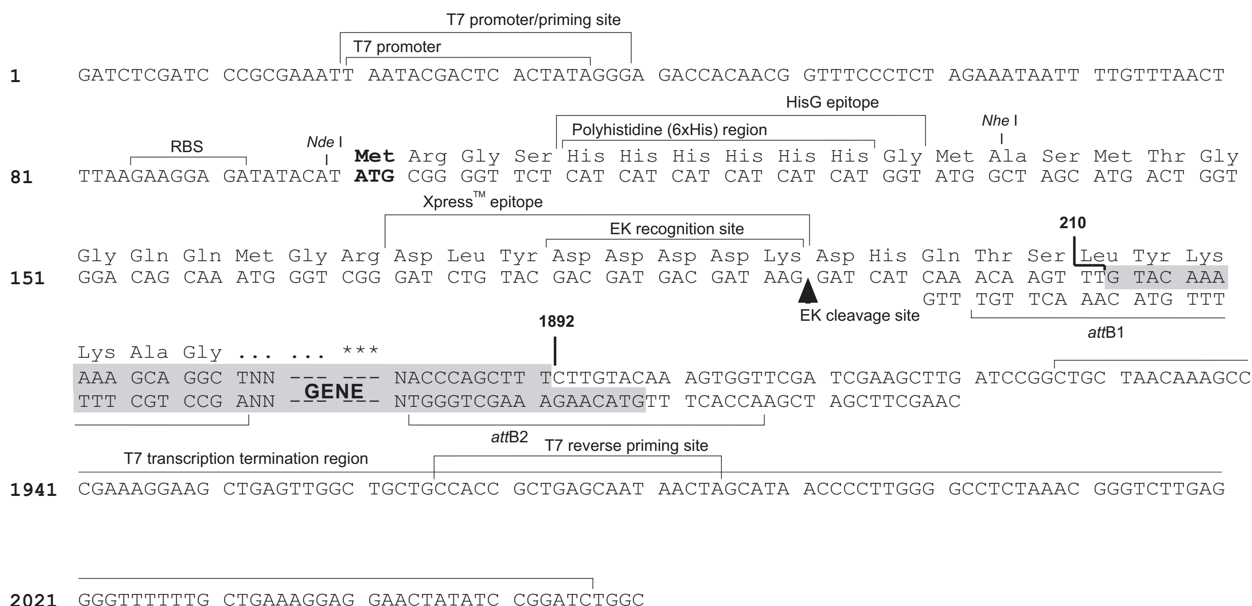
Recombination Region of pEXP1-DEST

The recombination region of the expression clone resulting from pEXP1-DEST x entry clone is shown below.

Features of the Recombination Region:

- Shaded regions correspond to those DNA sequences transferred from the entry clone into the pEXP1-DEST vector by recombination. Non-shaded regions are derived from the pEXP1-DEST vector.
- Bases 210 and 1892 of the pEXP1-DEST sequence are marked.
- Restrictions sites are labeled to indicate the actual cleavage site.



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Recombination Region of pEXP2-DEST

The recombination region of the expression clone resulting from pEXP2-DEST x entry clone is shown below.

Features of the Recombination Region:

- Shaded regions correspond to those DNA sequences transferred from the entry clone into the pEXP2-DEST vector by recombination. Non-shaded regions are derived from the pEXP2-DEST vector.
- Bases 106 and 1788 of the pEXP2-DEST sequence are marked.
- Restriction sites are labeled to indicate the actual cleavage site.

```
                                      T7 promoter/priming site
                               T7 promoter
1     GATCTCGATC CCGCGAAATT AATACGACTC ACTATAGGGA GACCACAACG GTTTCCCTCT AGAAATAATT TTGTTTAACT

                                     106                                              1788
                                                                   ... Pro Ala Phe Leu Tyr Lys Val
81    TTAAGAAGGA ATTATCAACA AGTTTGTACA AAAAAGCAGG CTN --- --- NAC CCA GCT TTC TTG TAC AAA GTG
                 TAATAGTTGT TCAAACATGT TTTTTCGTCC GAN -GENE- NTG GGT CGA AAG AAC ATG TTT CAC
                                       attB1                                     attB2
      Val Asp Asn Ser Lys Leu Glu Gly Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser Thr Arg
1801  GTT GAT AAT TCG AAG CTT GAA GGT AAG CCT ATC CCT AAC CCT CTC CTC GGT CTC GAT TCT ACG CGT
      CAA CTA TTA AGC TTC GAA CTT CCA TTC GGA TAG GGA TTG GGA GAG GAG CCA GAG CTA AGA TGC GCA
                                      V5 epitope
     Age I                                       V5 (C-term) reverse priming site
                                           Pme I
      Thr Gly His His His His His His ***
1867  ACC GGT CAT CAT CAC CAT CAC CAT TGA GTTTAAA CTATATAGAA TAAAAGAAGA AACCTTAGCT GAGCAATAAC
      TGG CCA GTA GTA GTG GTA GTG GTA ACT
                 Polyhistidine (6xHis) region
```

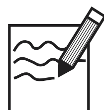
Performing the LR Recombination Reaction

Introduction

Once you have obtained an entry clone containing your gene of interest, you may perform an LR recombination reaction between the entry clone and pEXP1-DEST or pEXP2-DEST, and transform the reaction mixture into a suitable *E. coli* host (see below) to select for an expression clone. We recommend including a negative control (no entry vector) in your experiment to help you evaluate your results.

E. coli Host

You may use any *rec*A, *end*A *E. coli* strain including TOP10, DH5α™, or equivalent for transformation. **Do not** transform the LR reaction mixture into *E. coli* strains that contain the F′ episome (*e.g.* TOP10F′). These strains contain the *ccd*A gene and will prevent negative selection with the *ccd*B gene.



Note

The presence of the Zeocin™ resistance gene in pEXP2-DEST allows selection of *E. coli* transformants using Zeocin™ antibiotic. For selection, use Low Salt LB agar plates containing 25 μg/ml Zeocin™ (see page 19 for a recipe). Note that for Zeocin™ to be active, the salt concentration of the bacterial medium must remain low (<90 mM) and the pH must be 7.5.

Zeocin™ is available separately from Invitrogen (see page vi for ordering information). Instructions to prepare and handle Zeocin™ are supplied with the product. For more information, refer to our Web site (www.invitrogen.com) or contact Technical Service (see page 20).

Materials Needed

You should have the following materials on hand before beginning:

- Purified plasmid DNA of your entry clone (50-150 ng/μl in TE, pH 8.0)
- pEXP1-DEST or pEXP2-DEST vector (150 ng/μl in TE, pH 8.0)
- LR Clonase™ enzyme mix (Invitrogen, Catalog no. 11791-019; keep at -80°C until immediately before use)
- 5X LR Clonase™ Reaction Buffer (supplied with the LR Clonase™ enzyme mix)
- TE Buffer, pH 8.0 (10 mM Tris-HCl, pH 8.0, 1 mM EDTA)
- 2 μg/μl Proteinase K solution (supplied with the LR Clonase™ enzyme mix; thaw and keep on ice until use)
- Appropriate competent *E. coli* host and growth media for expression
- S.O.C. Medium
- LB agar plates containing the appropriate antibiotic to select for expression clones

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Setting Up the LR Recombination Reaction

1. Add the following components to 1.5 ml microcentrifuge tubes at room temperature and mix.

Component	Sample	Negative Control
Entry clone (100-300 ng/reaction)	1-10 µl	--
Destination vector (150 ng/µl)	2 µl	2 µl
5X LR Clonase™ Reaction Buffer	4 µl	4 µl
TE Buffer, pH 8.0	to 16 µl	10 µl

2. Remove the LR Clonase™ enzyme mix from -80°C and thaw on ice (~ 2 minutes).
3. Vortex the LR Clonase™ enzyme mix briefly twice (2 seconds each time).
4. To each sample above, add 4 µl of LR Clonase™ enzyme mix. Mix well by pipetting up and down.

   Reminder: Return LR Clonase™ enzyme mix to -80°C immediately after use.
5. Incubate reactions at 25°C for 1 hour.

   Note: Extending the incubation time to 18 hours typically yields more colonies.
6. Add 2 µl of the Proteinase K solution to each reaction. Incubate for 10 minutes at 37°C.
7. Transform 1 µl of the LR recombination reaction into a suitable *E. coli* host (follow the manufacturer's instructions) and select for expression clones.

   Note: You may store the LR reaction at -20°C for up to 1 week before transformation, if desired.

What You Should See

If you use *E. coli* cells with a transformation efficiency of 1 x 10^8 cfu/µg, the LR reaction should give >5000 colonies if the entire transformation is plated.

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 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 should not grow in the presence of chloramphenicol.

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Plasmid Preparation

Once you have generated your expression clone in the pEXP1-DEST or pEXP2-DEST backbone, you must isolate plasmid DNA before proceeding to express your recombinant fusion protein. We recommend isolating plasmid DNA using the S.N.A.P.™ MidiPrep Kit (Catalog no. K1910-01) or CsCl gradient centrifugation.

If you plan to express your recombinant fusion protein using the Expressway™ Systems, refer to the appropriate Expressway™ System manual for additional recommendations regarding purification of your plasmid DNA template.

Sequencing

To confirm that your gene of interest is in frame with the appropriate N-terminal or C-terminal peptide, you may sequence your expression construct using the recommended priming sites below, if desired. Refer to the diagrams on page 5 and page 6 for the location of the primer binding sites for each pEXP-DEST vector. The T7 forward and reverse primers are available from Invitrogen (see page vi for ordering information). Invitrogen also has a custom primer synthesis service (see our Web site for details).

Vector	Forward Primer	Reverse Primer
pEXP1-DEST	T7 Promoter	T7 Reverse
pEXP2-DEST	T7 Promoter	V5 (C-term) Reverse

Expression and Analysis

Introduction

Once you have obtained purified plasmid DNA of your pEXP-DEST expression construct, you may express your recombinant fusion protein by:

- Performing an *in vitro* protein synthesis reaction using the Expressway™ or Expressway™ Plus System
- Transforming an *E. coli* strain suitable for facilitating T7-controlled expression

General guidelines are provided in this section.

Positive Control

pEXP1-GW/*lacZ* and pEXP2-GW/*lacZ* are provided with pEXP1-DEST and pEXP2-DEST, respectively, for use as positive control vectors for protein synthesis in the Expressway™ Systems or for expression in a suitable *E. coli* host. The vectors allow expression of an N- or C-terminally tagged β-galactosidase fusion protein that may be easily detected by Western blot or functional assay. For details about each vector, see pages 17 and 18. To propagate and maintain each 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.

   Note: Transformants containing pEXP2-GW/*lacZ* may also be selected using Low Salt LB agar plates containing 25 µg/ml Zeocin™ (see page 19 for a recipe).
4. Prepare a glycerol stock of a transformant containing plasmid for long-term storage.

Using the Expressway™ Systems

To synthesize and analyze your recombinant fusion protein from pEXP-DEST using an Expressway™ System, refer to the appropriate Expressway™ System manual. The Expressway™ System and The Expressway™ Plus System manuals are supplied with the appropriate catalog numbers and are also available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Service (see page 20).

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BL21 *E. coli* Expression Strain

To facilitate expression of your recombinant fusion protein in *E. coli*, you must use a strain that permits expression of T7-regulated genes. We recommend using the BL21 Star™(DE3)pLysS strain available from Invitrogen (see page vi for ordering information) as a host for your pEXP-DEST construct. This strain:

- Contains the bacteriophage lambda DE3 lysogen which carries the gene for T7 RNA polymerase under the control of an inducible *lac*UV5 promoter and the *lac*I gene (encodes lac repressor) to repress basal expression of the T7 RNA polymerase
- Contains the pLysS plasmid which carries the gene for T7 lysozyme to further suppress T7 RNA polymerase and permit expression of toxic genes

Note: Other BL21-derived strains are suitable.

For more information about T7-regulated gene expression, refer to the pCR®T7 TOPO TA Expression Kits manual. For more information about the BL21 Star™(DE3)pLysS strain, refer to the BL21 Star™(DE3)pLysS manual. Both manuals are available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Service (see page 20).

Performing Expression in *E. coli*

To express your recombinant fusion protein in *E. coli*, you will transform a suitable BL21 *E. coli* strain (see above) and induce expression with IPTG. Protocols and guidelines to transform *E. coli* and perform expression studies are provided in the pCR®T7 TOPO® TA Expression Kits manual.

Detecting Recombinant Fusion Proteins

You may detect expression of your recombinant fusion protein by Western blot analysis using antibodies against the appropriate epitope available from Invitrogen (see page vii 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 an Xpress™, HisG, V5, or C-terminal 6xHis epitope. The ready-to-use WesternBreeze® Chromogenic Kits and WesternBreeze® Chemiluminescent Kits are available from Invitrogen to facilitate detection of antibodies by colorimetric or chemiluminescence methods. For more information, refer to our Web site (www.invitrogen.com) or contact Technical Service (see page 20).

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Note

Expression of your protein with the N- or C-terminal tags in pEXP1-DEST or pEXP2-DEST, respectively, will increase the size of your recombinant protein. The table below lists the increase in the molecular weight of your recombinant fusion protein that you should see from the particular tag (including the amino acids from the *att*B site) in each pEXP-DEST vector. Be sure to account for any additional amino acids between the tag and your fusion protein.

Vector	Peptide Tag	Expected Size Increase (kDa)
pEXP1-DEST	N-terminal	4.8 kDa
pEXP2-DEST	C-terminal	4.2 kDa

Assay for β-galactosidase

If you use the pEXP1-GW/*lacZ* or pEXP2-GW/*lacZ* plasmids as positive controls 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 the β-gal Antiserum (Catalog no. R901-25) and the β-Gal Assay Kit (Catalog no. K1455-01) for fast and easy detection of β-galactosidase expression.

Purifying Recombinant Fusion Proteins

The presence of the N-terminal or C-terminal polyhistidine (6xHis) tag in your recombinant fusion protein allows use of a metal-chelating resin such as ProBond™ or Ni-NTA to purify your fusion protein. ProBond™ and Ni-NTA are available from Invitrogen (see page vii for ordering information). Refer to the manual included with each product for protocols to purify your 6xHis-tagged fusion protein. **Note:** Other metal-chelating resins and purification methods are suitable.

Cleaving the N-terminal Tag in pEXP1-DEST

The pEXP1-DEST vector contains an enterokinase (EK) recognition site to allow removal of the N-terminal tag from your recombinant fusion protein, if desired. Note that after digestion with enterokinase, at least 12 amino acids will remain at the N-terminus of your protein (see diagram on page 5 for reference).

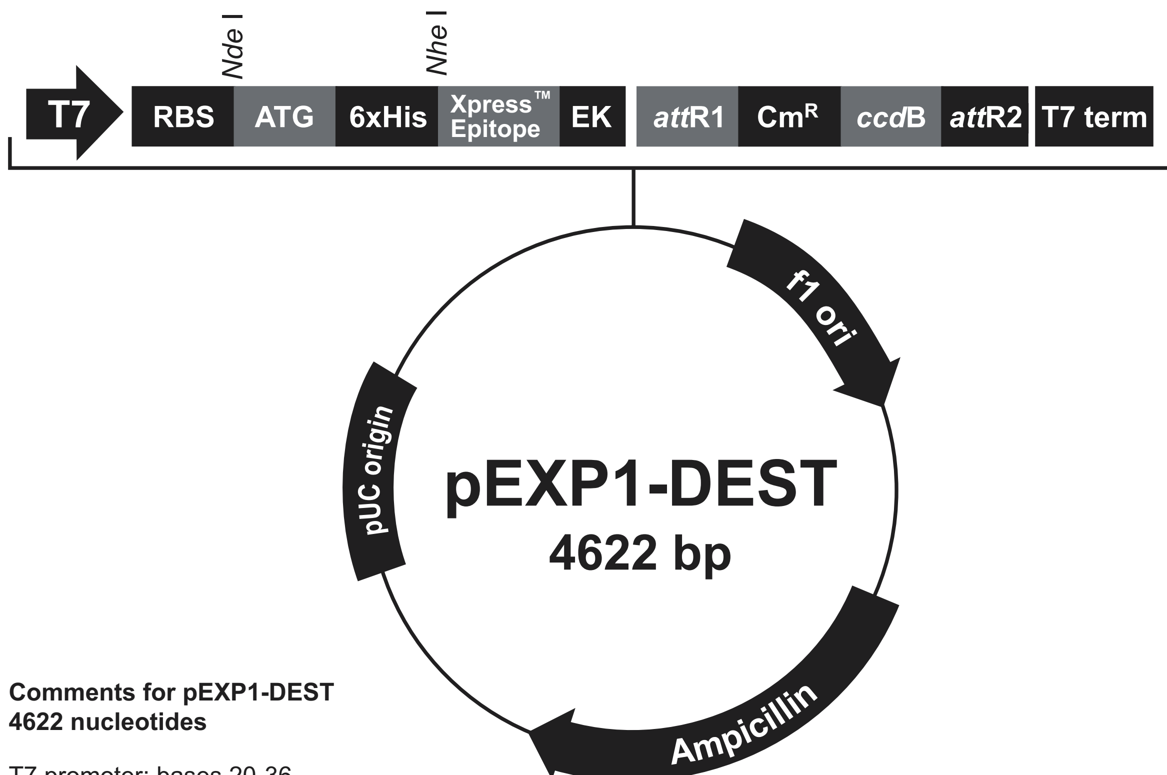
A recombinant preparation of the catalytic subunit of bovine enterokinase (EKMax™) is available from Invitrogen to remove the N-terminal tag from your recombinant fusion protein (see page vi for ordering information). Instructions for digestion are included with the product. For more information, see our Web site (www.invitrogen.com) or contact Technical Service (see page 20).

Appendix

Map and Features of pEXP1-DEST

Map of pEXP1-DEST

The map below shows the elements of pEXP1-DEST. DNA from the entry clone replaces the region between bases 210 and 1892. The complete sequence and restriction enzyme cleavage sites for pEXP1-DEST are available from our Web site (www.invitrogen.com) or by contacting Technical Service (see page 20).



Comments for pEXP1-DEST
4622 nucleotides

T7 promoter: bases 20-36
T7 promoter priming site: bases 20-39
Ribosome binding site: bases 85-92
Initiation ATG: bases 100-102
Polyhistidine (6xHis) region: bases 112-129
Xpress™ epitope: bases 169-192
Enterokinase (EK) recognition site: bases 178-192
*att*R1 site: bases 202-326
Chloramphenicol resistance gene (Cm^R): bases 435-1094
*ccd*B gene: bases 1436-1741
*att*R2 site: bases 1782-1906
T7 reverse priming site: bases 1966-1985
T7 transcription termination region: bases 1927-2056
f1 origin: bases 2127-2582
bla promoter: bases 2669-2767
Ampicillin resistance gene: bases 2768-3628
pUC origin: 3773-4446

continued on next page

Map and Features of pEXP1-DEST, continued

Features of pEXP1-DEST

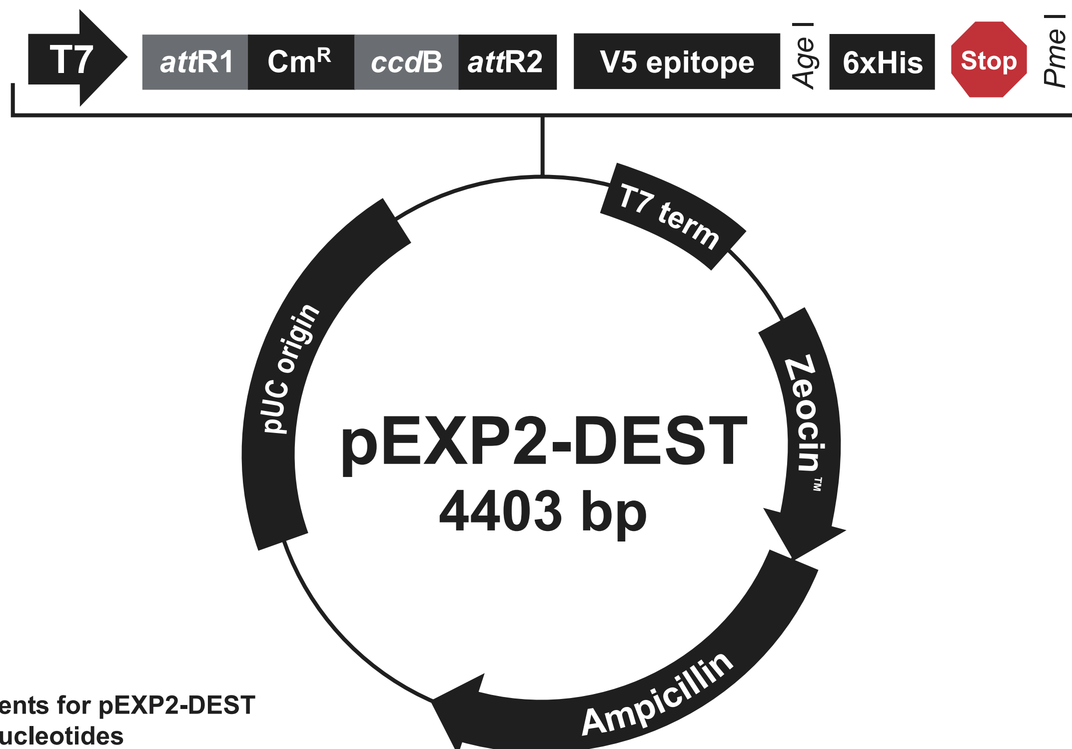
The pEXP1-DEST vector (4622 bp) contain the following elements. All features have been functionally tested.

Feature	Benefit
T7 promoter	Permits high-level, inducible expression of your recombinant protein in the Expressway™ Systems or in *E. coli* strains expressing the T7 RNA polymerase.
T7 promoter/priming site	Allows sequencing in the sense orientation.
Ribosome binding site	Optimally spaced from the initiation ATG for efficient translation of PCR product.
Initiation ATG	Allows translation initiation of the recombinant fusion protein.
N-terminal polyhistidine (6xHis) tag	Permits purification of recombinant fusion protein on metal-chelating resin (*e.g.* ProBond™ or Ni-NTA). In addition, it allows detection of recombinant protein with the Anti-HisG Antibodies.
Xpress™ epitope (Asp-Leu-Tyr-Asp-Asp-Asp-Asp-Lys)	Allows detection of the fusion protein by the Anti-Xpress™ Antibodies.
Enterokinase (EK) recognition site (Asp-Asp-Asp-Asp-Lys)	Allows removal of the N-terminal tag from your recombinant fusion protein using an enterokinase such as EKMax™ (Catalog no. E180-01).
*att*R1 and *att*R2 sites	Bacteriophage λ-derived DNA recombination sequences that permit recombinational cloning of the gene of interest from a Gateway® entry clone (Landy, 1989).
Chloramphenicol resistance gene (Cm^R)	Allows counterselection of the plasmid.
*ccd*B gene	Permits negative selection of the plasmid.
T7 transcription termination region	Sequence from bacteriophage T7 which permits efficient transcription termination.
T7 reverse priming site	Permits sequencing in the anti-sense orientation.
f1 origin	Allows rescue of single-stranded DNA.
bla promoter	Allows expression of the ampicillin resistance gene.
Ampicillin resistance gene (β-lactamase)	Allows selection of the plasmid in *E. coli*.
pUC origin	Permits high-copy replication and maintenance in *E. coli*.

Map and Features of pEXP2-DEST

Map of pEXP2-DEST

The map below shows the elements of pEXP2-DEST. DNA from the entry clone replaces the region between bases 106 and 1788. The complete sequence and restriction enzyme cleavage sites for pEXP2-DEST are available from our Web site (www.invitrogen.com) or by contacting Technical Service (see page 20).



Comments for pEXP2-DEST
4403 nucleotides

T7 promoter: bases 20-36
T7 promoter/priming site: bases 20-39
*att*R1 site: bases 98-222
Chloramphenicol resistance gene (Cm^R): bases 331-990
*ccd*B gene: bases 1332-1637
*att*R2 site: bases 1678-1802
V5 epitope: bases 1822-1863
V5 (C-term) reverse priming site: bases 1831-1851
Polyhistidine (6xHis) region: bases 1873-1890
T7 transcription termination region: bases 1928-2012
Zeocin™ resistance gene: bases 2156-2530
Ampicillin resistance gene: bases 2551-3411
pUC origin: bases 3556-4229

continued on next page

Map and Features of pEXP2-DEST, continued

Features of pEXP2-DEST

The pEXP2-DEST vector (4403 bp) contain the following elements. All features have been functionally tested.

Feature	Benefit
T7 promoter	Permits high-level, inducible expression of your recombinant protein in the Expressway™ Systems or in *E. coli* strains expressing the T7 RNA polymerase.
T7 promoter/priming site	Allows sequencing in the sense orientation.
*att*R1 and *att*R2 sites	Bacteriophage λ-derived DNA recombination sequences that permit recombinational cloning of the gene of interest from a Gateway® entry clone (Landy, 1989).
Chloramphenicol resistance gene (Cm^R)	Allows counterselection of the plasmid.
*ccd*B gene	Permits negative selection of the plasmid.
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).
V5 (C-term) reverse priming site	Permits sequencing in the anti-sense orientation.
Polyhistidine (6xHis) tag	Permits purification of recombinant fusion protein on metal-chelating resin (*e.g.* ProBond™ or Ni-NTA). In addition, it allows detection of recombinant fusion protein with the Anti-His(C-term) Antibodies.
T7 transcription termination region	Sequence from bacteriophage T7 which permits efficient transcription termination.
Zeocin™ resistance gene	Permits selection of the plasmid using Zeocin™ antibiotic. **Note:** A cryptic promoter is believed to control expression of the Zeocin™ resistance gene.
bla promoter	Allows expression of the ampicillin resistance gene.
Ampicillin resistance gene (β-lactamase)	Allows selection of the plasmid in *E. coli*.
pUC origin	Permits high-copy replication and maintenance in *E. coli*.

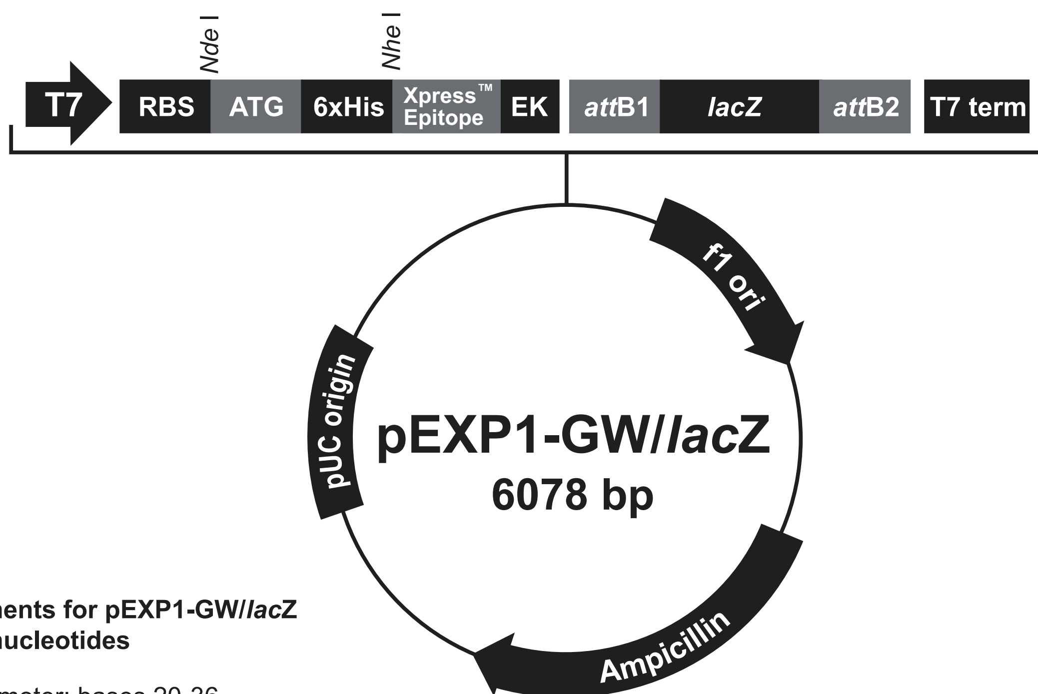
Map of pEXP1-GW/*lacZ*

Description

pEXP1-GW/*lacZ* is a 6078 bp control vector expressing β-galactosidase and was generated using the Gateway® LR recombination reaction between an entry clone containing the *lacZ* gene and pEXP1-DEST. β-galactosidase is expressed as an N-terminal fusion protein with a molecular weight of approximately 122 kDa.

Map of pEXP1-GW/*lacZ*

The map below shows the elements of pEXP1-GW/*lacZ*. The complete sequence of the vector is available from our Web site (www.invitrogen.com) or by contacting Technical Service (see page 20).



Comments for pEXP1-GW/*lac*Z
6078 nucleotides

T7 promoter: bases 20-36
T7 promoter priming site: bases 20-39
Ribosome binding site: bases 85-92
Initiation ATG: bases 100-102
Polyhistidine (6xHis) region: bases 112-129
Xpress™ epitope: bases 169-192
Enterokinase (EK) recognition site: bases 178-192
*att*B1 site: bases 202-226
*lac*Z gene: bases 247-3321
*att*B2 site: bases 3338-3362
T7 reverse priming site: bases 3422-3441
T7 transcription termination region: bases 3383-3512
f1 origin: bases 3583-4038
bla promoter: bases 4125-4223
Ampicillin resistance gene: bases 4224-5084
pUC origin: 5229-5902

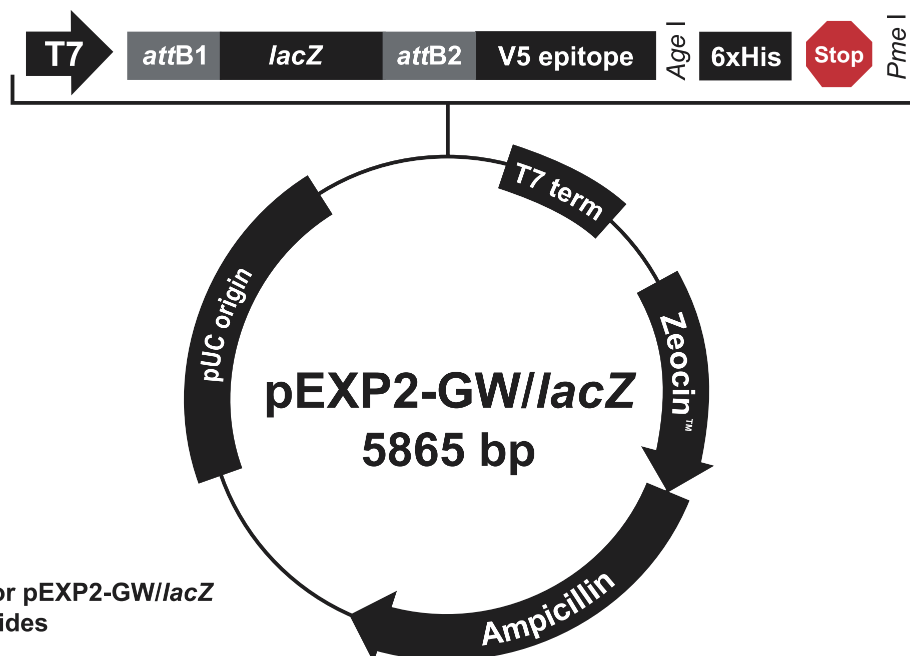
Map of pEXP2-GW/*lacZ*

Description

pEXP2-GW/*lacZ* is a 5865 bp control vector expressing β-galactosidase, and was generated using the Gateway® LR recombination reaction between an entry clone containing the *lacZ* gene and pEXP2-DEST. β-galactosidase is expressed as a C-terminal fusion protein with a molecular weight of approximately 121 kDa.

Map of pEXP2-GW/*lacZ*

The map below shows the elements of pEXP2-GW/*lacZ*. The complete sequence of the vector is available from our Web site (www.invitrogen.com) or by contacting Technical Service (see page 20).



Comments for pEXP2-GW/*lacZ*
5865 nucleotides

T7 promoter: bases 20-36
T7 promoter/priming site: bases 20-39
*att*B1 site: bases 98-122
lacZ gene: bases 164-3220
*att*B2 site: bases 3240-3264
V5 epitope: bases 3284-3325
V5 (C-term) reverse priming site: bases 3293-3313
Polyhistidine (6xHis) region: bases 3335-3352
T7 transcription termination region: bases 3390-3474
Zeocin™ resistance gene: bases 3618-3992
Ampicillin resistance gene: bases 4013-4873
pUC origin: bases 5018-5691

Recipes

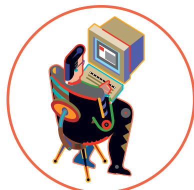
Low Salt LB Medium with Zeocin™

10 g Tryptone
5 g NaCl
5 g Yeast Extract

1. Combine the dry reagents above and add deionized, distilled water to 950 ml. Adjust pH to 7.5 with 5 M NaOH. Bring the volume up to 1 liter. For plates, add 15 g/L agar before autoclaving.
2. Autoclave on liquid cycle at 15 lbs/sq. in. and 121°C for 20 minutes.
3. Thaw Zeocin™ on ice and vortex before removing an aliquot.
4. Allow the medium to cool to at least 55°C before adding the Zeocin™ to 25 μg/ml final concentration.
5. Store plates at +4°C in the dark. Plates containing Zeocin™ are stable for 1-2 weeks.

Technical Service

World Wide Web



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