invitrogen™

pBAD-DEST49 Gateway® Destination Vector

A destination vector for cloning and regulated expression of HP-thioredoxin N-terminal fusions in E. coli

Catalog no. 12283-016

Version F
29 December 2010
25-0427

User Manual

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Kit Contents and Storage

Shipping and Storage

pBAD-DEST49 is shipped on wet ice. Upon receipt, store at –20°C.

pBAD-DEST49 is guaranteed for six months from the date of shipment when stored at –20°C.

Contents

6 µg of pBAD-DEST49 vector at 150 ng/µl in TE buffer, pH 8.0 (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).
Volume: 40 µl.

Accessory Products

Additional Products

The products below are also available from Invitrogen. For more details visit our Web site at www.invitrogen.com, or contact Technical Support (page 18).

Product	Quantity	Catalog no.
Gateway® LR Clonase™ Enzyme Mix	20 reactions	11791-019
One Shot® TOP10 Chemically Competent *E. Coli* Cells	10 reactions 20 reactions	C4040-10 C4040-03
One Shot® TOP10 Electrocompetent *E. Coli* Cells	10 reactions 20 reactions	C4040-50 C4040-52
One Shot® *ccd*B Survival™ 2 T1 Phage-Resistant Cells	10 reactions	A10460
pENTR Directional TOPO® Cloning Kit	20 reactions	K2400-20
PureLink™ HiPure Plasmid Midiprep Kit	25 preps	K2100-04
EKMax™ Enterokinase	250 units 1000 units	E180-01 E180-02
EK-Away™ Resin	7.5 ml	R180-01

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 antibody volume supplied is sufficient for 25 Western blots.

Product	Quantity	Catalog no.	Epitope
Anti-Thio™ Antibody	50 µl	R920-25	Detects His-Patch thioredoxin fusion proteins **Note:** The exact epitope detected by this antibody has not been mapped.
Anti-V5 Antibody	50 µl	R960-25	Detects 14 amino acid epitope derived from the P and V proteins of the paramyxovirus, SV5 (Southern *et al.*, 1991) GKPIPNPLLGLDST
Anti-V5-HRP Antibody	50 µl	R961-25	
Anti-V5-AP Antibody	125 µl	R962-25	
Anti-His (C-term) Antibody	50 µl	R930-25	Detects the C-terminal polyhistidine (6xHis) tag, requires the free carboxyl group for detection (Lindner *et al.*, 1997) HHHHHH-COOH
Anti-His (C-term)-HRP Antibody	50 µl	R931-25	
Anti-His (C-term)-AP Antibody	125 µl	R932-25	

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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. For more details on these products, visit our Web site at www.invitrogen.com or contact Technical Support (page 18).

Product	Quantity	Catalog no.
ProBond™ Nickel-chelating Resin	50 ml 150 ml	R801-01 R801-15
Purification Columns (10 ml polypropylene columns, empty)	50 each	R640-50
ProBond™ Purification System	6 purifications	K850-01
Positope™ Control Protein	5 µg	R900-50

Gel Electrophoresis

A wide range of pre-cast NuPAGE® and Novex® Tris-Glycine polyacrylamide gels and electrophoresis apparatuses are available to facilitate separation and visualization of your recombinant fusion protein.

For more details on these products, visit our Web site at www.invitrogen.com or contact Technical Support (page 18).

Introduction

Overview

Description

pBAD-DEST49 is a 6.2 kb vector derived from pBAD/Thio and adapted for use with the Gateway® Technology. pBAD-DEST49 is designed for regulated expression of N-terminal HP-thioredoxin fusion proteins in *E. coli*.

For a map of pBAD-DEST49, see page 16.

Features

pBAD-DEST49 contains the following features:

- *ara*BAD (pBAD) promoter: provides tight, dose-dependent regulation of heterologous gene expression
- His-Patch Thioredoxin (HP-thioredoxin) fusion partner for efficient translation of the protein of interest and, in some cases, increased solubility
- Two recombination sites, *att*R1 and *att*R2, downstream of the HP-thioredoxin gene 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
- The V5 epitope and 6xHis tag for detection and purification (optional)
- *rrn*B transcription terminator for strong transcription termination
- The pUC origin for replication and maintenance of the plasmid in *E. coli*.

  Note: Although pBAD-DEST49 contains a pUC origin, it acts as a low-copy number plasmid, resulting in lower yields of the vector.
- The ampicillin (*bla*) resistance gene for selection in *E. coli*
- The *ara*C gene for regulation of the *ara*BAD promoter (Lee, 1980; Schleif, 1992)

Continued on next page

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® 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.* pBAD-DEST49).
3. Transform your expression clone into *E. coli*. Induce expression of your gene of interest using L-arabinose.

For more information on the Gateway® Technology, refer to the Gateway® Technology Manual. This manual is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Support (page 18).

Regulation of Expression by L-Arabinose

Expression of pBAD is induced in the presence of L-arabinose. In the absence of L-arabinose, only very low levels of transcription are observed from pBAD (Lee, 1980; Lee *et al.*, 1987). Uninduced levels are further repressed by growth in the presence of glucose. Glucose reduces the levels of 3´, 5´-cyclic AMP, lowering expression from the catabolite-repressed pBAD promoter (Miyada *et al.*, 1984). By varying the concentration of L-arabinose, protein expression levels can be optimized to ensure maximum protein expression. In addition, the tight regulation of pBAD by AraC is useful for expression of potentially toxic or essential genes (Carson *et al.*, 1991; Dalbey and Wickner, 1985; Guzman *et al.*, 1992; Kuhn and Wickner, 1985; Russell *et al.*, 1989; San Millan *et al.*, 1989).

For additional information on the mechanism of expression and repression of the *ara* regulon, see page 15.

Continued on next page

Thioredoxin

The 11.7 kDa thioredoxin protein is found in yeast, plants, and mammals, as well as in bacteria. It was originally isolated from *E. coli* as a hydrogen donor for ribonuclease reductase (see Holmgren, 1985 for a review) . The gene has been completely sequenced (Wallace and Kushner, 1984), and the protein has been crystallized and its three-dimensional structure determined (Katti *et al.*, 1990).

When overexpressed in *E. coli*, thioredoxin is able to accumulate to approximately 40% of the total cellular protein and still remain soluble. When used as a fusion partner, thioredoxin can increase translation efficiency and, in some cases, solubility of eukaryotic proteins expressed in *E. coli*.

Examples of eukaryotic proteins that have been produced as soluble C-terminal fusions to the thioredoxin protein in *E. coli* (LaVallie *et al.*, 1993) include:

- Murine interleukin-2
- Human interleukin-3
- Murine interleukin-4
- Murine interleukin-5
- Human macrophage colony stimulating factor
- Murine steel factor
- Murine leukemia inhibitory factor
- Human bone morphogenetic protein-2

His-Patch Thioredoxin

To create a metal binding domain in the thioredoxin protein, the glutamate residue at position 32 and the glutamine residue at position 64 were mutated to create histidine residues. When His-Patch thioredoxin folds, the histidines at positions 32 and 64 interact with a native histidine at position 8 to form a "patch". This histidine patch has high affinity for divalent cations (Lu *et al.*, 1996). His-Patch thioredoxin (HP-thioredoxin) proteins can therefore be purified on metal-chelating resins (*e.g.* ProBond™, page vi).

Methods

Using pBAD-DEST49

Important

The pBAD-DEST49 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 pBAD-DEST49

If you wish to propagate and maintain pBAD-DEST49, we recommend using One Shot® *ccd*B Survival™ 2 T1 Phage-Resistant Cells for transformation (page v). The *ccd*B Survival™ 2 T1 Phage-Resistant *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 pBAD-DEST49, you should have an entry clone containing your gene of interest. For your convenience, Invitrogen offers the pENTR Directional TOPO® Cloning Kit (page v) for 5-minute cloning of your gene of interest into an entry vector.

For detailed information on constructing an entry clone, refer to the specific entry vector manual. See the next page for factors to consider when designing your entry clone.

Specific Features

pBAD-DEST49 is designed with the following features to facilitate expression:

- Initiation ATG is correctly spaced from the optimized ribosome binding site to ensure optimal translation of HP-thioredoxin fusions
- HP-thioredoxin acts as a translation leader to facilitate high-level expression and in some cases, solubility
- HP-thioredoxin can be removed after protein purification using enterokinase (*e.g.* EKMax™, page v).

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Points to Consider Before Recombining

It is very important to have a properly designed entry clone before recombining with pBAD-DEST49. Refer to the table below and the **Recombination Region** on page 6.

If you wish to…	Then your gene in the entry clone…
clone in frame with HP-thioredoxin	must be in frame with the HP-thioredoxin gene after recombination.
include the V5 epitope and 6xHis tag	must not contain a stop codon and must be in frame with the C-terminal tag after recombination.
exclude the V5 epitope and 6xHis tag	must contain a stop codon.

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. 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 *ccd*B gene and the chloramphenicol (Cm^R) 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 set up the LR Clonase™ reaction, transform a *rec*A *end*A *E. coli* strain (*e.g.* TOP10 or DH5α) , and select for the expression clone.

Resuspending pBAD-DEST49

Before you perform the LR Clonase™ reaction, resuspend pBAD-DEST49 to 50–150 ng/µl in sterile water.

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Confirming the Expression Clone

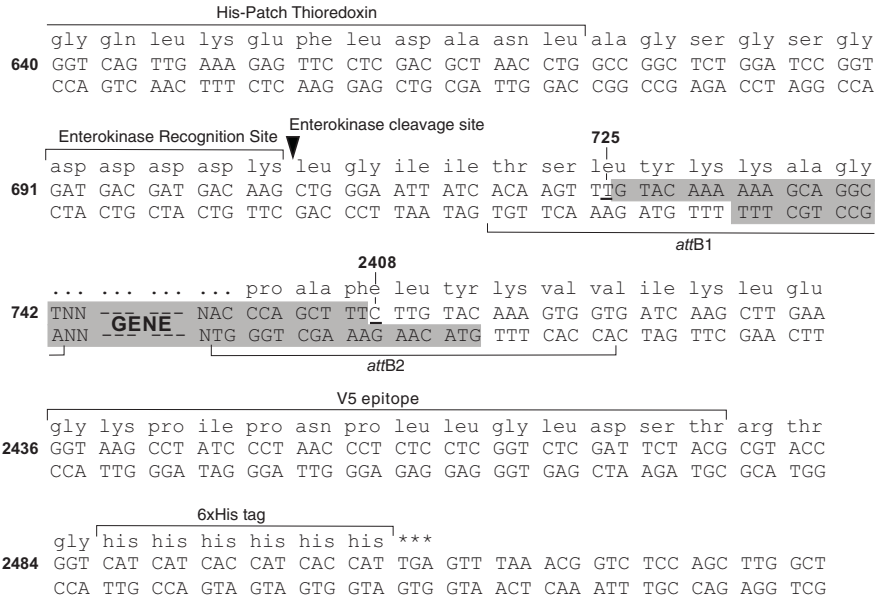
The *ccd*B gene mutates at a very low frequency, resulting in a very low number of false positives. True expression clones will be ampicillin-resistant and chloramphenicol-sensitive. Transformants containing a plasmid with a mutated *ccd*B gene will be both ampicillin- and chloramphenicol-resistant. 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 pBAD-DEST49 × entry clone is shown below.

Features of the Recombination Region:

- Shaded regions correspond to those DNA sequences transferred from the entry clone into pBAD-DEST49 by recombination. Non-shaded regions are derived from the pBAD-DEST49 vector.
- The underlined nucleotides flanking the shaded region correspond to bases 725 and 2408, respectively, of the pBAD-DEST49 vector sequence.



Expressing the Gene of Interest

Introduction

Once you have selected your expression clone, you are ready to transform it into *E. coli* and test for expression of your gene. Since each recombinant protein has different characteristics that may affect optimal expression, it is helpful to vary the L-arabinose concentration and/or run a time course of expression to determine the best conditions for optimal expression of your particular protein.

Plasmid Preparation

You may prepare plasmid DNA using your method of choice. We recommend using the PureLink™ HiPure Plasmid Midiprep Kit (page v) for isolation of pure plasmid DNA. Note that since you are purifying a vector that acts as a low-copy number plasmid, you may need to increase the amount of bacterial culture that you use to prepare your plasmid construct.

E. coli Strain

We recommend that you transform your construct into an *E. coli* strain that is deficient for the *ara* operon. TOP10 competent cells are deleted for *ara*BADC and are available from Invitrogen as chemically competent or electrocompetent cells (page v).

L-Arabinose

Prepare a 20% solution of L-arabinose in deionized water and filter sterilize. Store at room temperature.

To determine the optimal concentration for expression of your particular protein, see the next page.

D-arabinose will not induce expression and should not be used.

Note: L-arabinose can be ordered from Sigma (Catalog no. A3256).

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Expressing the Gene of Interest, Continued

Basic Strategy

Once you have clones that you wish to characterize, we recommend the following strategy to determine the optimal expression level.

1. Pilot Expression: In this expression experiment you will vary the amount of arabinose over a 10,000-fold range (0.00002% to 0.2%) to determine the approximate amount of arabinose needed for maximum expression of your protein. (See next page for protocol).
2. To optimize expression of your protein, you may wish to try arabinose concentrations spanning the amount determined in Step 1, or you may perform a time course.

Note: If your protein is insoluble, remember to analyze the supernatant and the pellet of lysed cells for expression of soluble protein (see page 10).

Materials Needed

Be sure to have the following solutions and equipment on hand **before starting** the experiment:

- SOB or LB medium containing 50–100 µg/ml ampicillin (see pages 13-14 for recipes)
- 37°C shaking incubator
- 20% L-arabinose
- 37°C heat block or water bath
- 42°C water bath
- Liquid nitrogen
- 1X and 2X SDS-PAGE sample buffer
- Reagents and apparatus for SDS-PAGE gel
- Boiling water bath
- Lysis Buffer (see page 14 for recipe)
- Sterile water

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Pilot Expression

In addition to testing your transformants, we recommend that you include cells without a vector as a negative control.

1. For each transformant or control, inoculate 2 ml of SOB or LB medium containing 50–100 µg/ml ampicillin with a single recombinant *E. coli* colony.
2. Grow cells overnight on a 37°C shaking incubator at 225–250 rpm. The cells should have an optical density at 600 (OD_{600}) of 1–2
3. Label five tubes 1 through 5 and add 10 ml of SOB or LB medium containing 50–100 µg/ml ampicillin.
4. Inoculate each tube with 0.1 ml of the overnight culture.
5. Grow the cultures in the tubes on a 37°C shaking incubator (use vigorous shaking) Grow cells to an OD_{600} of ~0.5 (the cells should be in mid-log phase).
6. While the cells are growing, prepare four 10-fold serial dilutions of 20% L-arabinose with sterile water using aseptic technique (*e.g.* 2%, 0.2%, 0.02%, and 0.002%).
7. Take a 1 ml aliquot of grown cells from each tube, and place each aliquot in separate microcentrifuge tubes. Centrifuge at maximum speed in a microcentrifuge for 30 seconds, and aspirate the supernatant.
8. Freeze the cell pellet at –20°C. This is the zero time point-sample.
9. Using the solutions prepared in Step 6, add the following volumes of L-arabinose to the five 9 ml cultures.
   Note: It is only necessary to test the highest concentration of L-arabinose.

Tube	Stock Solution	Volume (ml)	Final Concentration
1	0.002%	0.09	0.00002%
2	0.02%	0.09	0.0002%
3	0.2%	0.09	0.002%
4	2%	0.09	0.02%
5	20%	0.09	0.2%

10. Grow at 37°C with shaking for 4 hours.
11. Take 1 ml samples at 4 hours and treat samples as in Steps 7 and 8. You will have a total of 10 samples for each transformant and two samples for the negative control.
12. Proceed to **Analyzing Samples**, next page.

Analyzing Samples

Preparation of Samples

Before starting, prepare SDS-PAGE gels or use one of the pre-cast polyacrylamide gels available from Invitrogen (page vi) to analyze all the samples you collected.

Note: If you wish to analyze your samples for soluble protein, see the section below.

1. When all the samples have been collected from Steps 8 and 11 in the **Pilot Expression**, resuspend each pellet in 100 µl of 1X SDS-PAGE sample buffer.
2. Heat for 5 minutes at 70°C and centrifuge briefly.
3. Load 5–10 µl of each sample on an SDS-PAGE gel and electrophorese. Save your samples by storing at –20°C.

Preparing Samples for Soluble/ Insoluble Protein

1. Thaw and resuspend each pellet in 500 µl of Lysis Buffer (see page 14 for recipe).
2. Freeze sample in dry ice or liquid nitrogen and then thaw at 42°C. Repeat 2 to 3 times.

   Note: To facilitate lysis, you may need to add lysozyme or sonicate the cells.
3. Centrifuge samples at maximum speed in a microcentrifuge for 1 minute at +4°C to pellet insoluble proteins. Transfer supernatant to a fresh tube and store on ice.
4. Mix together equivalent amounts of supernatant and 2X SDS-PAGE sample buffer and boil for 5 minutes.
5. Add 500 µl of 1X SDS-PAGE sample buffer to the pellets from Step 3 and boil 5 minutes.
6. Load 10 µl of the supernatant sample and 5µl of the pellet sample onto an SDS-PAGE gel and electrophorese.

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Analyzing Samples

To determine the success of your expression experiment, you may want to perform the following types of analyses:

1. Stain the polyacrylamide gel with Coomassie blue and look for a band of increasing intensity in the expected size range for the recombinant protein. Use the uninduced culture as a negative control.
2. Perform a Western blot to confirm that the overexpressed band is your desired protein (see below).
3. Determine the approximate arabinose concentration for maximum expression.

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 (page v) or an antibody to your protein of interest. In addition, the Positope™ Control Protein (page vi) is available from Invitrogen for use as a positive control for detection of fusion proteins containing a thioredoxin, V5, or C-terminal 6xHis epitope.

Note

Expression of your protein with the N-terminal HP-thioredoxin peptide and the C-terminal tag will increase the size of your protein by approximately 14 kDa and 4 kDa, respectively.

Continued on next page

Optimization of Expression

Once you have detected expression of your protein of interest, you may wish to perform experiments to further optimize expression. Use the Pilot Expression protocol (page 9), but vary the L-arabinose concentration over a smaller range. For example, if you obtained the best expression at 0.002%, try 0.0004%, 0.0008%, 0.001%, 0.004%, and 0.008%.

You may also perform a time course of induction to determine if varying the time increases expression. Take time points every hour, over a 5 to 6 hour period.

If your protein is insoluble, you may wish to analyze the supernatant and pellet of lysed cells when you vary the L-arabinose concentration (see **Preparing Samples for Soluble/Insoluble Protein**, page 10).

Remember to store your cell lysates at –20°C.

Expression of Toxic Proteins

To ensure low levels of expression, you may want to utilize glucose to further repress the *ara*BAD promoter. Follow the Pilot Expression protocol (page 9) using SOB or LB containing 50–100 µg/ml ampicillin plus glucose at all steps (see pages 13-14 for recipes).

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™ (page vi) to purify your fusion protein.

Note: Other metal-chelating resins and purification methods are suitable.

Removal of the N-terminal Leader by Enterokinase

The enterokinase (EK) recognition site can be used to remove the N-terminal leader from your recombinant fusion protein after purification. Note that after digestion with enterokinase, there will be twelve vector-encoded amino acids remaining at the N-terminus of the protein (see diagram on page 6).

A recombinant preparation of the catalytic subunit of bovine enterokinase (EKMax™) is available from Invitrogen (see page v).

Appendix

Recipes

LB (Luria-Bertani) Medium and Plates

1.0% Tryptone
0.5% Yeast Extract
1.0% NaCl
0.1% glucose (optional)
pH 7.0

1. Dissolve 10 g tryptone, 5 g yeast extract, and 10 g NaCl in 950 ml deionized water.
2. Adjust solution to pH 7.0 with NaOH and add deionized water to 1 liter.
3. Autoclave on liquid cycle for 20 minutes at 15 psi. Allow solution to cool to 55°C. Add antibiotic if needed.
4. Add 5 ml of a 20% sterile glucose solution (optional).
5. 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 if needed.
4. Add 5 ml of a 20% sterile glucose solution (optional).
5. Pour into 10 cm plates and let harden. Invert plates and store at +4°C in the dark.

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SOB Medium

2% Tryptone
0.5% Yeast Extract
0.05% NaCl
2.5 mM KCl
10 mM $MgCl_2$
0.1% glucose (optional)

1. Dissolve 20 g tryptone, 5 g yeast extract, and 0.5 g NaCl in 950 ml deionized water.
2. Make a 250 mM KCl solution by dissolving 1.86 g of KCl in 100 ml of deionized water. Add 10 ml of this stock KCl solution to the solution in Step 1.
3. Adjust pH to 7.5 with 5 M NaOH and add deionized water to 1 liter.
4. Autoclave this solution and cool to ~55°C. Add 10 ml of sterile 1 M $MgCl_2$. Add antibiotic if needed.
5. Add 5 ml of a 20% sterile glucose solution (optional).
6. Store at +4°C. Medium is stable for only 1–2 weeks.

Lysis Buffer

50 mM potassium phosphate, pH 7.8
400 mM NaCl
100 mM KCl
10% glycerol
0.5% Triton X-100
10 mM imidazole

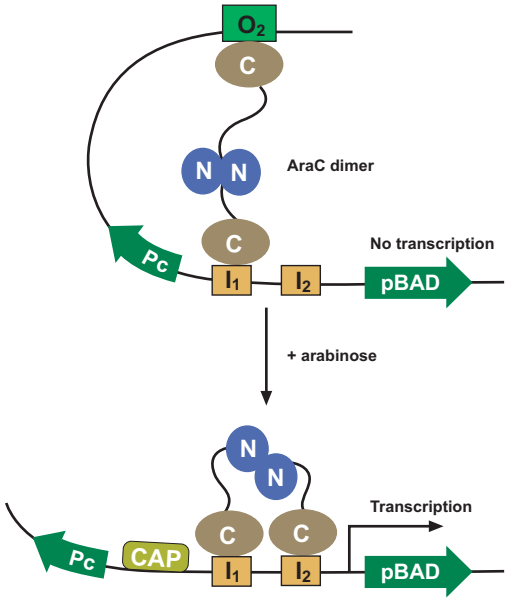
1. Prepare 1 M stock solutions of KH_2PO_4 and K_2HPO_4.
2. For 100 ml, dissolve the following reagents in 90 ml of deionized water:
   0.3 ml KH_2PO_4
   4.7 ml K_2HPO_4
   2.3 g NaCl
   0.75 g KCl
   10 ml glycerol
   0.5 ml Triton X-100
   68 mg imidazole
3. Mix thoroughly and adjust pH to 7.8 with HCl. Bring the volume to 100 ml.
4. Store at +4°C.

Regulation by L-Arabinose

Regulation of the P_{BAD} Promoter

The *ara*BAD promoter used in pBAD-DEST49 is both positively and negatively regulated by the product of the *ara*C gene (Ogden *et al.*, 1980; Schleif, 1992). The AraC protein is a transcriptional regulator that forms a complex with L-arabinose. In the absence of L-arabinose the AraC dimer contacts the O_2 and I_1 half sites of the *ara*BAD operon, forming a 210 bp DNA loop (see figure below). For maximum transcriptional activation two events are required.

- L-arabinose binds to AraC and causes the protein to release the O_2 site and bind the I_2 site, which is adjacent to the I_1 site. This releases the DNA loop and allows transcription to begin.
- The cAMP activator protein (CAP)-cAMP complex binds to the DNA and stimulates binding of AraC to I_1 and I_2.



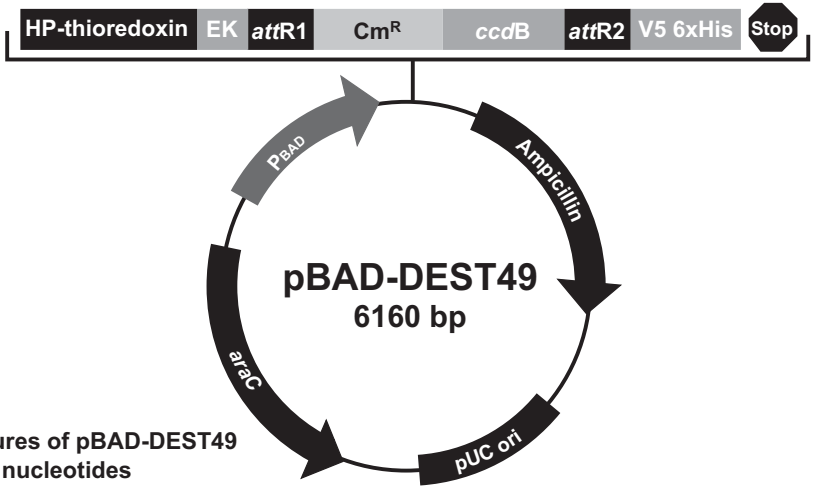
Glucose Repression

Glucose represses basal expression levels by lowering cAMP levels which in turn decrease the binding of CAP. As cAMP levels are lowered, transcriptional activation is decreased. Add glucose to the culture medium to repress basal expression levels.

Map of pBAD-DEST49

Map of pBAD-DEST49

The map below shows the elements of pBAD-DEST49. DNA from the entry clone replaces the region between bases 725 and 2408. The complete sequence of pBAD-DEST49 is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Support (page 18).



Features of pBAD-DEST49
6160 nucleotides

Arabinose O_2 operator: bases 4-19
Arabinose O_1 operator: bases 161-182
CAP binding site: bases 203-216
Arabinose I_1 and I_2 region: bases 213-251
Arabinose minimal promoter: bases 248-276
RBS: bases 329-332
His-Patch Thioredoxin: bases 346-674
Enterokinase recognition site: bases 691-705
*att*R1 recombination site: bases 718-842
Chloramphenicol resistance gene: bases 951-1610
*ccd*B gene: bases 1952-2257
*att*R2 recombination site: bases 2298-2422
V5 epitope: bases 2436-2477
6xHis tag: bases 2487-2507
*rrn*B transcription termination region: bases 2610-2767
bla promoter: bases 2948-3046
Ampicillin resistance gene (*bla*): bases 3047-3907
pUC origin: bases 4052-4725
*ara*C gene: bases 5256-6134 (complementary strand)

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Features of pBAD-DEST49

Features of pBAD-DEST49

pBAD-DEST49 (6160 bp) contains the following elements. Features have been functionally tested.

Feature	Benefit
*ara*BAD promoter (P_{BAD})	Provides tight, dose-dependent regulation of heterologous gene expression (Guzman *et al.*, 1995)
O_2 region	Binding site of AraC that represses transcription from P_{BAD}
O_1 region	Binding site of AraC that represses transcription of the *ara*C promoter (P_C) (transcribed on the opposite strand)
CAP binding site	Site where CAP (cAMP binding protein) binds to activate transcription from P_{BAD} and P_C
I_2 and I_1 regions	Binding sites of AraC that activate transcription from P_{BAD}
Ribosome binding site	Increases efficiency of recombinant fusion protein expression
HP-thioredoxin	Provides a highly efficient fusion partner for translation of the fusion protein
Enterokinase recognition site	Allows removal of the N-terminal tag from the recombinant fusion protein
*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
V5 epitope	Allows detection of the recombinant protein by the Anti-V5 antibodies (Southern *et al.*, 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 *et al.*, 1997)
*rrn*B transcription termination region	Strong transcription termination region
Ampicillin resistance gene	Allows selection of the plasmid in *E. coli*
pUC origin (see note on page 1)	Allows low-copy replication and growth in *E. coli*
*ara*C gene	Encodes the regulatory protein for tight regulation of the P_{BAD} promoter (Lee, 1980; Schleif, 1992)

Technical Support

Web Resources

Visit the Invitrogen website at www.invitrogen.com for:

- Technical resources including manuals, vector maps and sequences, application notes, MSDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical support contact information.
- Access to the Invitrogen Online Catalog.
- Additional product information and special offers.

Contact Us

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