invitrogen™

pMT-DEST48 Gateway® Vector

A destination vector for cloning and inducible expression in *Drosophila* cells

Catalog no. 12282-018

Version D
23 December 2008
25-0414

User Manual

Table of Contents

Important Information

Shipping and Storage	pMT-DEST48 is shipped on wet ice. Upon receipt, store at –20°C. Product is guaranteed for six months from date of shipment when stored properly.
Contents	6 µg of pMT-DEST48 vector at 150 ng/µl in TE buffer, pH 8.0 (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Volume: 40 µl.
Quality Control	The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to www.invitrogen.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

Accessory Products

Additional Products

Additional products that may be used with pMT-DEST48 are available from Invitrogen. Ordering information is provided below.

Product	Amount	Catalog no.
DES® Inducible Kit with pCoHygro	1 kit	K4120-01
DES® Inducible Kit with pCoBlast	1 kit	K5120-01
DES®-Blasticidin Support Kit*	1 kit	K5150-01
Schneider (S2) Cells	1 ml vial, 1 x 10^7 cells/ml	R690-07
Schneider's *Drosophila* Medium	500 ml	11720-034
Fetal Bovine Serum	500 ml	16000-044
Penicillin-Streptomycin	100 ml	15070-063
Calcium Phosphate Transfection Kit	75 reactions	K2780-01
Hygromycin B	1 g	R220-05
Blasticidin S HCl	50 mg	R210-01
pENTR Directional TOPO® Cloning Kit	20 reactions	K2400-20
PureLink™ HiPure Plasmid Midiprep Kit	25 preps	K2100-04
One Shot® *ccd*B Survival™ 2 T1 Phage-Resistant Cells	10 reactions	A10460
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

*The DES®-Blasticidin Support Kit contains pCoBlast, the Calcium Phosphate Transfection Kit, frozen S2 cells, DES® Expression Medium, and blasticidin (50 mg).

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Accessory Products, continued

Detection of Recombinant Proteins

Expression of your recombinant fusion protein can be detected using an antibody to the appropriate epitope. The amount of antibody supplied is sufficient for 25 Westerns.

Product	Epitope	Catalog no.
Anti-V5 Antibody	Detects 14 amino acid epitope derived from the P and V proteins of the paramyxovirus, SV5 (Southern *et al.*, 1991) GKPIPNPLLGLDST	R960-25
Anti-V5-HRP Antibody		R961-25
Anti-V5-AP Antibody		R962-25
Anti-His (C-term) Antibody	Detects the C-terminal polyhistidine (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 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 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

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Accessory Products, continued

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 10).

Methods

Overview

Description

pMT-DEST48 is derived from pMT/V5-His and adapted for use with the Gateway® Technology. It is designed for use with the *Drosophila* Inducible Expression System (DES®; Catalog no. K4120-01) available from Invitrogen. Upon transfection, the vector allows transient, inducible expression of your protein of interest in *Drosophila* cells. Co-transfecting pMT-DEST48 with the selection vector, pCoHygro or pCoBlast, allows selection of stable cell lines exhibiting inducible expression of the protein of interest.

Note: The DES® Inducible Kit is available with either pCoHygro or pCoBlast. pCoBlast is also available in the DES®-Blasticidin Support Kit. For more information on these products, see page vi.

Features

pMT-DEST48 contains the following elements:

- *Drosophila* metallothionein (MT) promoter for high-level, metal-inducible expression of the gene of interest in S2 cells (see next page for more information)
- Two recombination sites, *att*R1 and *att*R2, downstream of the MT promoter for recombinational cloning of the gene of interest from an entry clone
- Chloramphenicol resistance gene located between the two *att*R sites for counterselection
- The *ccd*B gene located between the two *att*R sites for negative selection
- The V5 epitope and 6xHis tag for detection and purification (optional)
- SV40 late polyadenylation sequence for proper termination and processing of the recombinant transcript
- The pUC origin for replication and maintenance of the plasmid in *E. coli*
- The ampicillin (*bla*) resistance gene for selection in *E. coli*

For a map of pMT-DEST48, see page 8.

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Overview, continued

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.* pMT-DEST48).
3. Introduce your expression clone into *Drosophila* S2 cells.

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 10).

Description of MT Promoter

The *Drosophila* MT promoter allows high-level, inducible expression of the gene of interest in *Drosophila* S2 cells. When used to express heterologous proteins, the promoter is extremely efficient and tightly regulated, even at high copy number (Johansen *et al.*, 1989). The MT promoter is well characterized (Angelichio *et al.*, 1991; Bunch *et al.*, 1988; Maroni *et al.*, 1986; Olsen, 1992) with regulatory elements and the start of transcription well defined.

The MT promoter is inducible by addition of copper sulfate or cadmium chloride to the culture medium (Bunch *et al.*, 1988). Copper sulfate is generally the preferred inducer due to its reduced toxicity as compared to cadmium. While cadmium is an effective inducer, it also induces a heat-shock response in S2 cells.

Using pMT-DEST48

Important

The pMT-DEST48 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 pMT-DEST48

If you wish to propagate and maintain pMT-DEST48, we recommend using One Shot® *ccd*B Survival™ 2 T1 Phage-Resistant Cells for transformation (page vi). 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 pMT-DEST48, 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 on entry vectors available from Invitrogen, refer to our Web site (www.invitrogen.com) or contact Technical Support (page 10).

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 pMT-DEST48, continued

Points to Consider Before Recombining

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 contain an ATG initiation codon and 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 both an ATG initiation codon and a stop codon in the entry clone.

Recombining Your Gene of Interest

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

Confirming the Expression Clone

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

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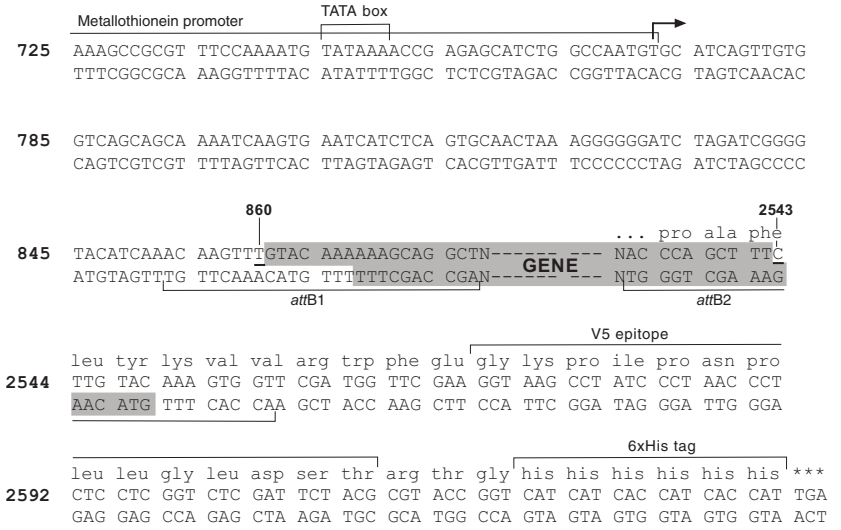
Using pMT-DEST48, continued

Recombination Region

The recombination region of the expression clone resulting from pMT-DEST48 × entry clone is shown below.

Features of the Recombination Region:

- Shaded regions correspond to those DNA sequences transferred from the entry clone into pMT-DEST48 by recombination. Non-shaded regions are derived from the pMT-DEST48 vector.
- The underlined nucleotides flanking the shaded region correspond to bases 866 and 2717, respectively, of the pMT-DEST48 vector sequence.



Transfection and Analysis

Introduction

The section provides general guidelines for transfecting *Drosophila* S2 cells. If you are assaying for transient, inducible expression of your gene of interest, you may transfect your pMT-DEST48 construct alone into S2 cells. If you wish to generate stable cell lines, you **must** cotransfect your pMT-DEST48 construct with pCoHygro or pCoBlast into S2 cells. We recommend that you include a mock transfection (negative control) in your experiments to evaluate your results.

For specific guidelines on culturing S2 cells, performing transient transfection, and generating stable cell lines, refer to the *Drosophila* Expression System (DES®) manual. This manual also contains detailed information about pCoHygro and pCoBlast and is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Support (page 10).

Note

Note that pMT-DEST48 does not contain a resistance marker for selection in *Drosophila* cells.

Plasmid Preparation

Plasmid DNA for transfection into S2 cells must be very clean and free from phenol and sodium chloride. Contaminants will kill the cells, and salt will interfere with lipid complexing, decreasing transfection efficiency. We recommend isolating plasmid DNA using the S.N.A.P.™ MiniPrep Kit (10-15 µg DNA, Catalog no. K1900-01), the S.N.A.P.™ MidiPrep Kit (10-200 µg DNA, Catalog no. K1910-01), or CsCl gradient centrifugation.

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Transfection and Analysis, continued

Induction of Recombinant Protein Expression

Once you have transfected your pMT-DEST48 construct into S2 cells, you will use copper sulfate to induce expression of your recombinant protein. In general, we recommend that you add copper sulfate directly to the culture medium to a final concentration of 500 µM and incubate the cells for 24 hours to obtain maximal induction of your recombinant protein of interest. Expression conditions may vary depending on the nature of the recombinant protein of interest. You may want to perform a time course to optimize expression of the recombinant protein. For more details, refer to the DES® manual. Copper sulfate is provided with the DES® Inducible Kit available from Invitrogen (see page vi for ordering information).

Detecting Recombinant Fusion Proteins

To detect expression of your recombinant fusion protein by Western blot analysis, you may use the Anti-V5 antibodies or the Anti-His(C-term) antibodies 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 a V5 epitope or a polyhistidine (6xHis) tag.

Note

The C-terminal peptide containing the V5 epitope and the 6xHis tag will add approximately 4 kDa to your protein.

Purification of Recombinant Fusion Proteins

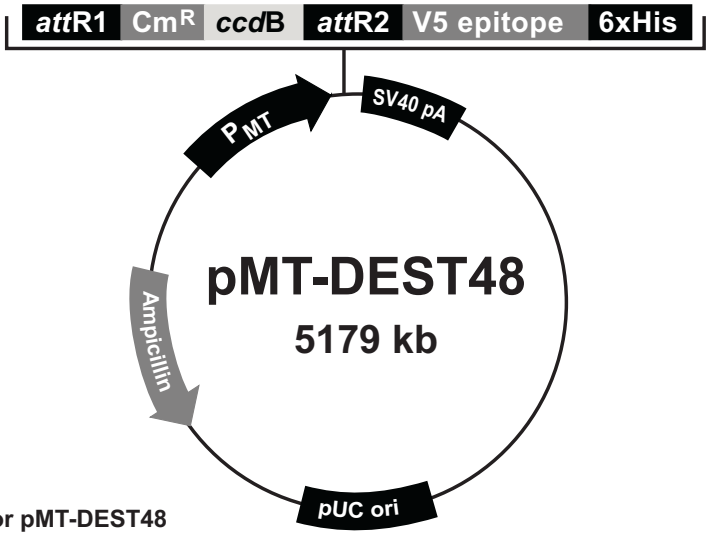
The presence of the C-terminal polyhistidine (6xHis) tag in your recombinant fusion protein allows use of a metal-chelating resin such as ProBond™ to purify your fusion protein. The ProBond™ Purification System and bulk ProBond™ resin are available from Invitrogen (see page vii for ordering information). Refer to the ProBond™ Purification System manual for protocols to purify your fusion protein. 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 pMT-DEST48

Map of pMT-DEST48

The map below shows the elements of pMT-DEST48. DNA from the entry clone replaces the region between bases 860 and 2543. **The complete sequence of pMT-DEST48 is available from our Web site (www.invitrogen.com) or by contacting Technical Support (page 10).**



Comments for pMT-DEST48
5179 nucleotides

Metallothionein promoter: bases 406-772
*att*R1 recombination site: bases 853-977
Chloramphenicol resistance gene: bases 1086-1745
*ccd*B gene: bases 2087-2392
*att*R2 recombination site: bases 2433-2557
V5 epitope: bases 2571-2612
6xHis tag: bases 2622-2639
SV40 late polyadenylation region: bases 2782-2922
pUC origin: bases 3242-3975
Ampicillin (*bla*) resistance ORF: bases 4120-4980 (complementary strand)
bla promoter: bases 4981-5079 (complementary strand)

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Map and Features of pMT-DEST48, continued

Features of pMT-DEST48

pMT-DEST48 (5179 bp) contains the following elements. All features have been functionally tested.

Feature	Benefit
Drosophila metallothionein (MT) promoter	Allows strong, inducible expression of your recombinant protein (Angelichio *et al.*, 1991; Bunch *et al.*, 1988; Maroni *et al.*, 1986; Olsen, 1992)
*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 recombinant fusion proteins by the Anti-V5 antibodies (Southern *et al.*, 1991)
C-terminal polyhistidine tag	Allows purification of recombinant fusion proteins on metal-chelating resin (*e.g.* ProBond™) Allows detection of the recombinant protein by the Anti-His (C-term) antibodies (Lindner *et al.*, 1997)
SV40 late polyadenylation signal	Allows efficient transcription termination and polyadenylation of mRNA (Angelichio *et al.*, 1991)
pUC origin	Allows high-copy number replication and growth in *E. coli*
bla promoter	Allows expression of the ampicillin (*bla*) resistance gene
Ampicillin resistance gene (β-lactamase)	Allows selection of transformants in *E. coli*

Technical Support

World Wide Web

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- Complete technical support contact information
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MSDS

Material Safety Data Sheets (MSDSs) are available on our website at www.invitrogen.com/msds.

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Technical Support, continued

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Purchaser Notification, continued

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