invitrogen

pMT/BiP/V5-His A, B, and C

USER GUIDE

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Life Technologies Corporation | 5781 Van Allen Way | Carlsbad, CA 92008
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Revision	Date	Description
A.0	22 October 2018	Corrections of the vector sequences
2.0	29 March 2012	Baseline for revisions

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1 Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product overview

Introduction

pMT/BiP/V5-His is a 3.6 kb expression vector designed for use with the *Drosophila* Inducible/Secreted Expression System (DES™; "Accessory products" on page 18 for ordering). Upon transfection, the vector allows transient, inducible, secreted expression of your protein of interest in *Drosophila* cells. When cotransfected with the selection vector, pCoHygro or pCoBlast, included with the appropriate DES™ Inducible/Secreted Kit, pMT/BiP/V5-His allows selection of stable cell lines exhibiting inducible, secreted expression of the protein of interest. The vector contains the following elements:

- The *Drosophila* metallothionein (MT) promoter for high-level, metal-inducible expression of the gene of interest in S2 cells (Angelichio *et al.*, 1991; Bunch *et al.*, 1988; Maroni *et al.*, 1986; Olsen, 1992)
- *Drosophila* BiP secretion signal for secreted expression of the gene of interest (Kirkpatrick *et al.*, 1995)
- Multiple cloning site to facilitate cloning the gene of interest
- C-terminal peptide containing the V5 epitope and polyhistidine (6xHis) tag for detection and purification of your protein of interest (if desired)
- Three reading frames to facilitate in-frame cloning with the C-terminal peptide
- Ampicillin resistance gene for selection of transformants in *E. coli*

The control plasmid, pMT/BiP/V5-His/GFP, is included for use as a positive control for transfection and expression.

For more information about the DES™ Inducible/Secreted Kits, pCoHygro, and pCoBlast, refer to the *Drosophila* Expression System manual. The manual is supplied with each DES™ Inducible/Secreted Kit, but is also available for downloading from **thermofisher.com** or by contacting Technical Support.

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.

BiP secretion signal

In addition to the MT promoter, the pMT/BiP/V5-His vector contains the *Drosophila* BiP secretion signal upstream of the multiple cloning site. The *Drosophila* BiP protein encodes an immunoglobulin binding chaperone protein (Kirkpatrick *et al.*, 1995). The secretion signal of the BiP protein was chosen because it efficiently targets high levels of BiP to the endoplasmic reticulum in the S2 cell line (Kirkpatrick and Shatzman, 1997). Its efficiency is comparable to that of the tPA secretion signal.

Experimental outline

The table below describes the general steps needed to clone and express your gene of interest. For more details, refer to the manual and pages indicated.

Step	Action	Source
1	Develop a cloning strategy to ligate your gene of interest into pMT/BiP/V5-His A, B, or C in frame with the C-terminal peptide encoding the V5 epitope and the polyhistidine tag (if desired).	"Cloning into pMT/BiP/V5-His A, B, and C" on page 8, this manual
2	Transform your ligation reactions into a *rec*A, *end*A *E. coli* strain (e.g. TOP10). Select on LB agar plates containing 50–100 µg/mL ampicillin.	"E. coli transformation" on page 13, this manual
3	Analyze your transformants for the presence of insert by restriction digest.	"Preparing a glycerol stock" on page 13, this manual
4	Select a transformant with the correct restriction pattern and sequence it to confirm that your gene is cloned in frame with the C-terminal peptide.	"Preparing a glycerol stock" on page 13, this manual
5	Transfect your pMT/BiP/V5-His construct into S2 cells and induce expression of the gene of interest with copper sulfate.	"Transfection and analysis" on page 13, this manual and DES™ manual

Step	Action	Source
6	Assay for transient expression of your recombinant protein.	"Detection and purification of recombinant Fusion™ proteins" on page 15, this manual and DES™ manual
7	To generate stable cell lines, cotransfect your pMT/BiP/V5-His construct and pCoHygro or pCoBlast into S2 cells and select for hygromycin resistant clones.	DES™ manual
8	Scale up expression for purification.	DES™ manual
9	Purify your recombinant protein by chromatography on metal-chelating resin (i.e. ProBond™).	DES™ manual

Kit contents and storage

Kit contents

20 µg of pMT/BiP/V5-His A, B, and C are supplied at 0.5 µg/µL in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 in a total volume of 40 µL.

20 µg of pMT/BiP/V5-His/GFP is supplied at 0.5 µg/µL in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 in a total volume of 40 µL.

Shipping/Storage

Vectors are shipped at room temperature. Upon receipt, store the plasmid at -20°C.

2 Methods

Cloning into pMT/BiP/V5-His A, B, and C

Introduction

See "Multiple cloning site of pMT/BiP/V5-His A" on page 10, "Multiple cloning site of pMT/BiP/V5-His B" on page 11, or "Multiple cloning site of pMT/BiP/V5-His C" on page 12 to help you clone your gene of interest into pMT/BiP/V5-His. General considerations for cloning and transformation are discussed below.

General molecular biology techniques

For help with DNA ligations, *E. coli* transformations, restriction enzyme analysis, DNA sequencing, and DNA biochemistry, refer to *Molecular Cloning: A Laboratory Manual* (Sambrook *et al.*, 1989) or *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994).

E. coli strain

Many *E. coli* strains are suitable for the propagation and maintenance of the pMT/BiP/V5-His vectors including TOP10, DH5α™-T1^R, and JM109. We recommend that you propagate the vectors in *E. coli* strains that are recombination deficient (*rec*A) and endonuclease A deficient (*end*A).

For your convenience, TOP10 and DH5α™-T1^R *E. coli* are available as chemically competent or electrocompetent (TOP10 only) cells in a One Shot™ format (see "Accessory products" on page 18 for ordering).

Transformation method

You may use any method of your choice for transformation. Chemical transformation is the most convenient method for many researchers. Electroporation is the most efficient and the method of choice for large plasmids.

Maintenance of plasmids

To propagate and maintain the pMT/BiP/V5-His and pMT/BiP/V5-His/GFP vectors, we recommend using the supplied 0.5 µg/µL stock solution in TE buffer, pH 8.0 to transform a *rec*A, *end*A *E. coli* strain like TOP10, DH5α™-T1^R, or equivalent. Select transformants on LB agar plates containing 50–100 µg/mL ampicillin. Be sure to prepare a glycerol stock of each plasmid for long-term storage (see "Preparing a glycerol stock" on page 13).

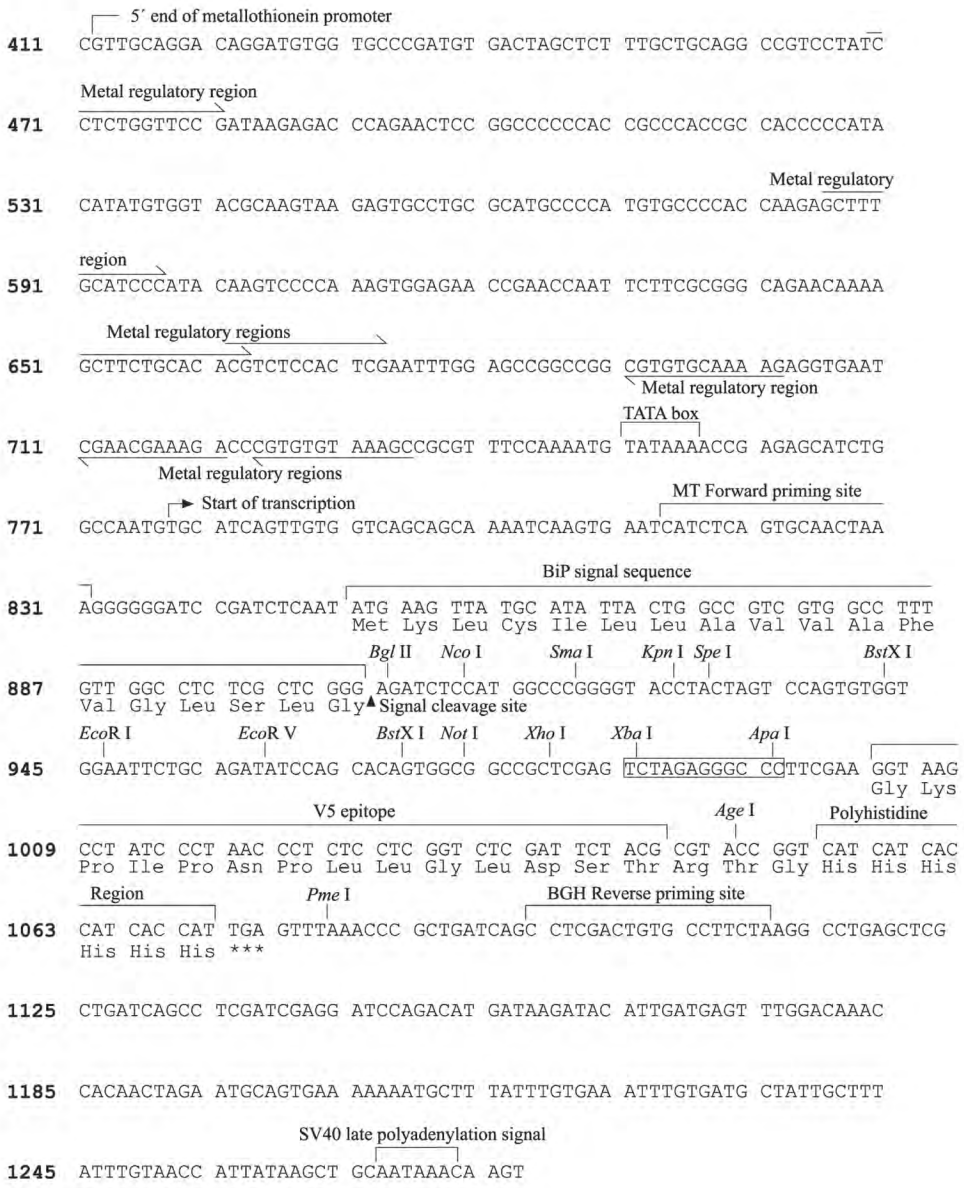
Cloning considerations

Consider the following points when designing a strategy to clone your gene of interest into pMT/BiP/V5-His.

- pMT/BiP/V5-His is a terminal fusion vector. To express your gene as a recombinant fusion protein, you **must** clone your gene in frame with the N-terminal BiP secretion signal. If you use the *Bgl* II site, only two amino acids (arginine and serine) will be fused to your protein. Be sure to remove the native signal sequence, if present, before fusing your gene to the BiP signal sequence.
- If you wish to wish to use the V5 epitope and the polyhistidine (6xHis) tag for detection and purification of your recombinant protein, you must clone your gene in frame with the C-terminal peptide. The vector is supplied in three reading frames to facilitate cloning. See "Multiple cloning site of pMT/BiP/V5-His A" on page 10, "Multiple cloning site of pMT/BiP/V5-His B" on page 11, or "Multiple cloning site of pMT/BiP/V5-His C" on page 12 to develop a cloning strategy. **Be sure that your gene does not contain a stop codon upstream of the C-terminal peptide.**
- If you do not wish to include the C-terminal peptide, include the native stop codon for your gene of interest.

Multiple cloning site of pMT/BiP/V5-His A

Below is the multiple cloning site for pMT/BiP/V5-His A. The metal regulatory regions are marked as per Maroni, *et al.,* 1986. The start of transcription is at nucleotide 778. The BiP signal cleavage site is also indicated (Rubin *et al.,* 1993). Restriction sites are labeled to indicate the actual cleavage site. The boxed nucleotides indicate the variable region. The nucleotide sequence of pMT/BiP/V5-His A is available for downloading from **thermofisher.com** or by contacting Technical Support. For a map and a description of the features of pMT/BiP/V5-His A, refer to "pMT/BiP/V5-His vector" on page 16.



Multiple cloning site of pMT/BiP/V5-His B

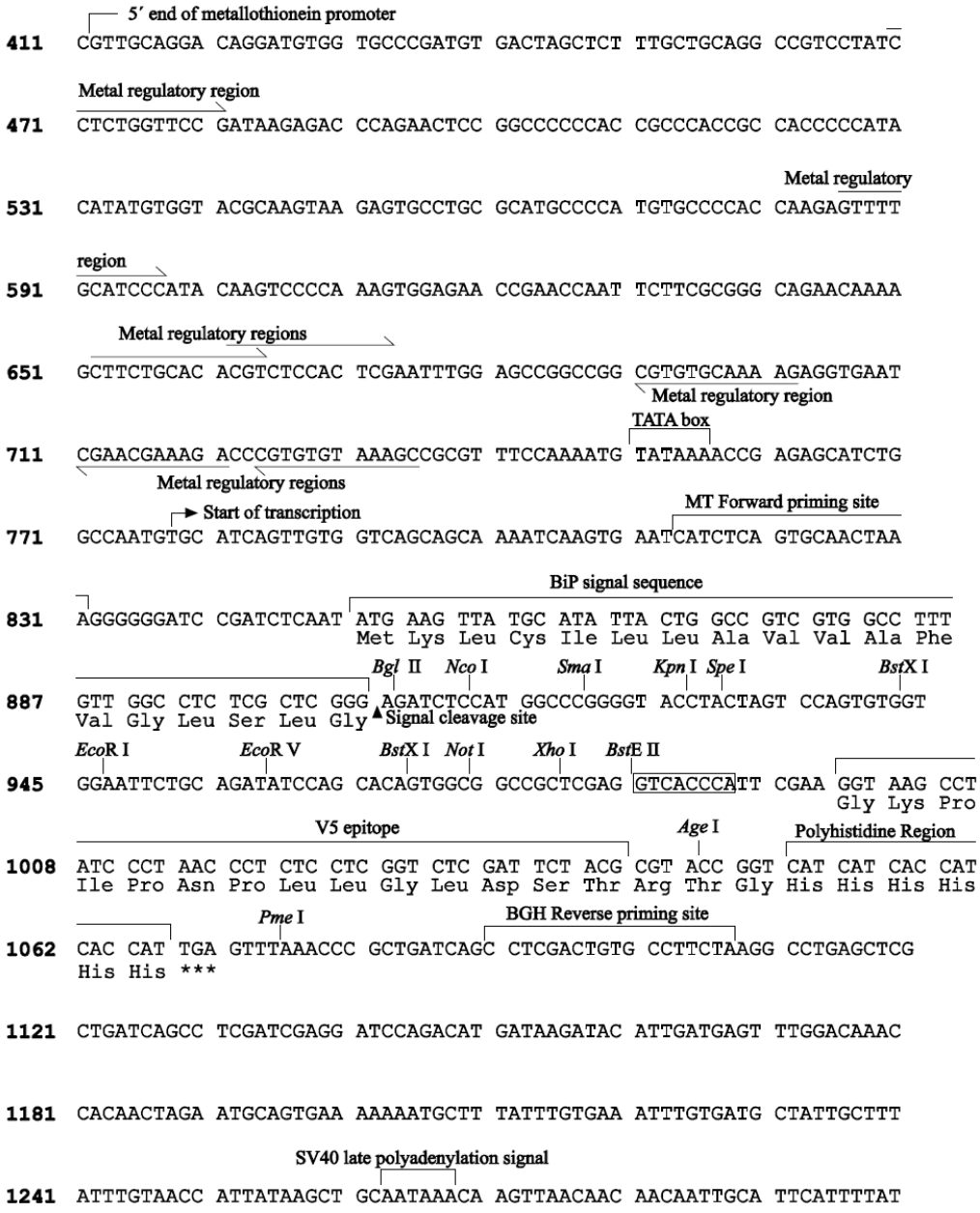
Below is the multiple cloning site for pMT/BiP/V5-His B. The metal regulatory regions are marked as per Maroni, *et al.*, 1986. The start of transcription is at nucleotide 778. The BiP signal cleavage site is also indicated (Rubin *et al.*, 1993). Restriction sites are labeled to indicate the actual cleavage site. The boxed nucleotides indicate the variable region. The nucleotide sequence of pMT/BiP/V5-His B is available for downloading from **thermofisher.com** or by contacting Technical Support. For a map and a description of the features of pMT/BiP/V5-His B, refer to "pMT/BiP/V5-His vector" on page 16.

```
                5′ end of metallothionein promoter
411   CGTTGCAGGA CAGGATGTGG TGCCCGATGT GACTAGCTCT TTGCTGCAGG CCGTCCTATC

      Metal regulatory region
471   CTCTGGTTCC GATAAGAGAC CCAGAACTCC GGCCCCCCAC CGCCCACCGC CACCCCCATA

                                                          Metal regulatory
531   CATATGTGGT ACGCAAGTAA GAGTGCCTGC GCATGCCCCA TGTGCCCCAC CAAGAGTTTT

      region
591   GCATCCCATA CAAGTCCCCA AAGTGGAGAA CCGAACCAAT TCTTCGCGGG CAGAACAAAA

          Metal regulatory regions
651   GCTTCTGCAC ACGTCTCCAC TCGAATTTGG AGCCGGCCGG CGTGTGCAAA AGAGGTGAAT
                                                  Metal regulatory region
                                                TATA box
711   CGAACGAAAG ACCCGTGTGT AAAGCCGCGT TTCCAAAATG TATAAAACCG AGAGCATCTG
      Metal regulatory regions
                 Start of transcription                MT Forward priming site
771   GCCAATGTGC ATCAGTTGTG GTCAGCAGCA AAATCAAGTG AATCATCTCA GTGCAACTAA
                                          BiP signal sequence
831   AGGGGGGATC CGATCTCAAT ATG AAG TTA TGC ATA TTA CTG GCC GTC GTG GCC TTT
                            Met Lys Leu Cys Ile Leu Leu Ala Val Val Ala Phe
                                  Bgl II  Nco I      Sma I     Kpn I Spe I        BstX I
887   GTT GGC CTC TCG CTC GGG AGATCTCCAT GGCCCGGGGT ACCTACTAGT CCAGTGTGGT
      Val Gly Leu Ser Leu Gly▲Signal cleavage site
      EcoR I        EcoR V      BstX I Not I    Xho I    Xba I       Apa I Sac II
945   GGAATTCTGC AGATATCCAG CACAGTGGCG GCCGCTCGAG TCTAGAGGGC CCGCGGTTCG AA GGT
                                                                            Gly
                                        V5 epitope                  Age I     Polyhistidine
1010  AAG CCT ATC CCT AAC CCT CTC CTC GGT CTC GAT TCT ACG CGT ACC GGT CAT CAT
      Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser Thr Arg Thr Gly His His
      Region                  Pme I                  BGH Reverse priming site
1064  CAC CAT CAC CAT TGA GTTTAAACCC GCTGATCAGC CTCGACTGTG CCTTCTAAGG
      His His His His ***

1119  CCTGAGCTCG CTGATCAGCC TCGATCGAGG ATCCAGACAT GATAAGATAC ATTGATGAGT

1179  TTGGACAAAC CACAACTAGA ATGCAGTGAA AAAAATGCTT TATTTGTGAA ATTTGTGATG
                                  SV40 late polyadenylation signal
1239  CTATTGCTTT ATTTGTAACC ATTATAAGCT GCAATAAACA AGTTAACAAC AACAATTGCA
```

*Note that there are two *Bst*X I sites in the polylinker.

Multiple cloning site of pMT/BiP/V5-His C

Below is the multiple cloning site for pMT/BiP/V5-His C. The metal regulatory regions are marked as per Maroni, *et al.*, 1986. The start of transcription is at nucleotide 778. The BiP signal cleavage site is also indicated (Rubin *et al.*, 1993). Restriction sites are labeled to indicate the actual cleavage site. The boxed nucleotides indicate the variable region. The nucleotide sequence of pMT/BiP/V5-His C is available for downloading from **thermofisher.com** or by contacting Technical Support. For a map and a description of the features of pMT/BiP/V5-His C, refer to "pMT/BiP/V5-His vector" on page 16.



*Note that there are two *Bst*X I sites in the polylinker.

E. coli transformation

Transform your ligation mixtures into a competent *rec*A, *end*A *E. coli* strain (e.g. TOP10, DH5α™-T1^R) and select on LB agar plates containing 50 to 100 µg/mL ampicillin. Select 10–20 clones and analyze for the presence and orientation of your insert.

Note: We recommend that you sequence your construct with the MT Forward and BGH Reverse primers (see "Accessory products" on page 18 for ordering) to confirm that your gene is in the correct orientation for expression and is cloned in frame with the BiP secretion signal and the C-terminal peptide. The MT Forward and BGH Reverse primers are included in each DES™ Inducible/Secreted Kit. Refer to the diagram on "Multiple cloning site of pMT/BiP/V5-His A" on page 10–"Multiple cloning site of pMT/BiP/V5-His C" on page 12 for the sequences and location of the priming sites.

Preparing a glycerol stock

Once you have identified the correct clone, purify the colony and make a glycerol stock for long-term storage. You should keep a DNA stock of your plasmid at -20°C.

1. Streak the original colony out on an LB plate containing 50 µg/mL ampicillin. Incubate the plate at 37°C overnight.
2. Isolate a single colony and inoculate into 1–2 mL of LB containing 50 µg/mL ampicillin.
3. Grow the culture to mid-log phase (OD_{600} = 0.5–0.7).
4. Mix 0.85 mL of culture with 0.15 mL of sterile glycerol and transfer to a cryovial.
5. Store at –80°C.

Transfection and analysis

Introduction

Once you have cloned your gene of interest into pMT/BiP/V5-His and have prepared purified plasmid DNA, you are ready to transfect your construct into S2 cells. If you are assaying for transient, inducible expression of your gene of interest, you may transfect your pMT/BiP/V5-His construct alone into S2 cells.

If you wish to generate stable cell lines, you **must** cotransfect your pMT/BiP/V5-His construct and pCoHygro or pCoBlast into S2 cells. Note that the pMT/BiP/V5-His vector does not contain a resistance marker for selection in *Drosophila* cells.

We recommend that you include the pMT/BiP/V5-His/GFP positive control vector and a mock transfection (negative control) in your experiments to evaluate your results. Specific guidelines and protocols for transient transfection and generation of stable cell lines can be found in the DES™ manual.

Note: Either pCoHygro or pCoBlast is supplied with the appropriate DES™ Inducible/Secreted Kit. For detailed information about each vector, refer to the DES™ manual.

Plasmid preparation

Plasmid DNA for transfection into eukaryotic cells must be clean and free from phenol and sodium chloride. Contaminants will kill the cells, decreasing transfection efficiency. We recommend isolating plasmid DNA using the PureLink™ HiPure MiniPrep™ Kit (up to 30 µg DNA), the PureLink™ HiPure MidiPrep Kit (up to 150 µg DNA) (see "Accessory products" on page 18 for ordering), or CsCl gradient centrifugation.

Positive control

pMT/BiP/V5-His/GFP is provided as a positive control vector for *Drosophila* cell transfection and expression (see "Map of pMT/BiP/V5-His/GFP" on page 18 for a map) and may be used to optimize transfection conditions for S2 cells. Transfection of pMT/BiP/V5-His/GFP results in induction of GFP expression upon addition of copper sulfate. A successful transfection will result in GFP expression that can be easily assayed (see "Assay for cycle 3-GFP" on page 14).

Note: The GFP gene in pMT/BiP/V5-His/GFP is a mutated form of GFP and is known as cycle 3-GFP (Crameri *et al.*, 1996) (see below).

GFP gene used in pMT/BiP/V5-His/GFP

The GFP gene used in pMT/BiP/V5-His is described in Crameri *et al.*, 1996. The codon usage was optimized for expression in *E. coli* and three cycles of DNA shuffling were used to generate a collection of mutants. The GFP mutant that exhibited the greatest fluorescence in mammalian cells is used in pMT/BiP/V5-His/GFP and is known as cycle 3-GFP. Cycle 3-GFP has the following characteristics:

- Excitation and emission maxima that are the same as wild-type GFP (395 nm and 478 nm for primary and secondary excitation, respectively, and 507 nm for emission)
- High solubility in *E. coli* for visual detection of transformed cells (if expressed from a promoter recognized by *E. coli*)
- >40-fold increase in fluorescent yield over wild-type GFP

Assay for cycle 3-GFP

You may assay for GFP expression in the following ways:

- Use fluorescence microscopy to visualize GFP-expressing cells

  To detect fluorescent cells, it is important to pick the best filter set to optimize detection. The primary excitation peak of cycle 3-GFP is at 395 nm. There is a secondary excitation peak at 478 nm. Excitation at these wavelengths yield a fluorescent emission peak with a maximum at 507 nm.
- Use fluorescence spectroscopy to assay the medium

  You can detect cycle 3-GFP fluorescence in the medium using fluorescence spectroscopy. Be sure to run a mock sample (medium alone) as the Schneider's *Drosophila* Medium has some autofluorescence (Zylka and Schnapp, 1996) and will interfere with detection of cycle 3-GFP fluorescence.
- Use western blot analysis to assay for GFP protein

  GFP Antiserum is available for purchase (see "Accessory products" on page 18)

After transfection, allow the cells to recover for 24 to 48 hours before inducing expression of cycle 3-GFP with copper sulfate. Induce for ~20 hours before assaying for fluorescence.

Induction of recombinant protein expression

Once you have transfected your pMT/BiP/V5-His construct into S2 cells, you will induce expression of recombinant protein using copper sulfate. 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 protein of interest. Refer to the DES™ manual for more details. Copper sulfate is provided in each DES™ Inducible/Secreted Kit.

Detection and purification of recombinant Fusion™ proteins

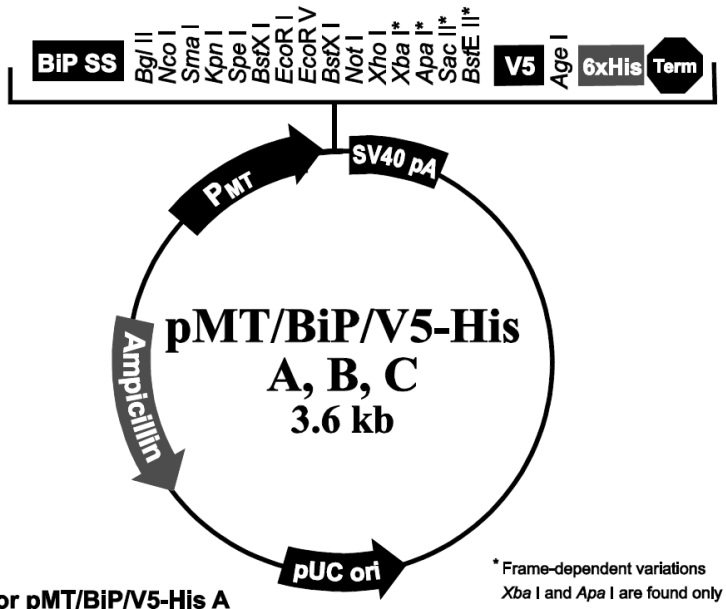
If you have cloned your gene of interest in frame with the C-terminal peptide containing the V5 epitope and the polyhistidine (6xHis) tag, you may use the Anti-V5 antibodies or Anti-His(C-term) antibodies available to detect secreted expression of your recombinant fusion protein by western blot analysis (see "Accessory products" on page 18 for ordering). The 6xHis tag also allows purification of recombinant protein using metal-chelating resins including ProBond™. Refer to the DES™ manual for more detailed guidelines and instructions to detect and purify your recombinant fusion protein.

Appendix

pMT/BiP/V5-His vector

Map of pMT/BiP/V5-His

The figure below summarizes the features of the pMT/BiP/V5-His A, B, and C vectors. For a more detailed description of each feature, see "Features of pMT/BiP/V5-His" on page 17. The nucleotide sequences of pMT/BiP/V5-His A, B, and C are available for downloading from **thermofisher.com** or by contacting Technical Support.



Comments for pMT/BiP/V5-His A
3642 nucleotides

* Frame-dependent variations
Xba I and *Apa* I are found only in versions A and B.
Sac II is found only in version B.
*Bst*E II is found only in version C.

Metallothionein promoter: bases 412-778
Start of transcription: base 778
MT Forward priming site: bases 814-831
BiP signal sequence: bases 851-904
Multiple cloning site: bases 906-999
V5 epitope tag: bases 1003-1044
Polyhistidine region: bases 1054-1074
BGH Reverse priming site: bases 1094-1111
SV40 late polyadenylation signal: bases 1267-1272
pUC origin: bases 1765-2438 (complementary strand)
bla promoter: bases 3444-3542 (complementary strand)
Ampicillin (*bla*) resistance gene ORF: bases 2583-3443 (complementary strand)

Features of pMT/BiP/V5-His

The features of pMT/BiP/V5-His A (3642 bp), pMT/BiP/V5-His B (3646 bp), and pMT/BiP/V5-His C (3638 bp) are described below. All features have been functionally tested. The multiple cloning site has been tested by restriction enzyme analysis.

Feature	Benefit
Drosophila metallothionein (MT) promoter	Permits high-level, inducible expression of heterologous proteins (Bunch *et al.*, 1988; Maroni *et al.*, 1986)
MT Forward priming site	Allows sequencing in the sense orientation
Drosophila BiP secretion signal	Permits secreted expression of the gene of interest (Kirkpatrick *et al.*, 1995)
Multiple cloning site	Allows insertion of your gene of interest
V5 epitope (Gly-Lys-Pro-Ile-Pro-Asn-Pro-Leu-Leu-Gly-Leu-Asp-Ser-Thr)	Allows detection of your recombinant protein with the Anti-V5 Antibodies available (see "Accessory products" on page 18) (Southern *et al.*, 1991)
Polyhistidine (6xHis) tag	Permits purification of your recombinant protein on metal-chelating resin such as ProBond™. In addition, the C-terminal 6xHis tag is the epitope for the Anti-His(C-term) Antibodies available (see "Accessory products" on page 18) (Lindner *et al.*, 1997)
BGH Reverse priming site	Permits sequencing of the non-coding strand
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 (*bla*) resistance gene (ß-lactamase)	Permits selection of transformants in *E. coli*

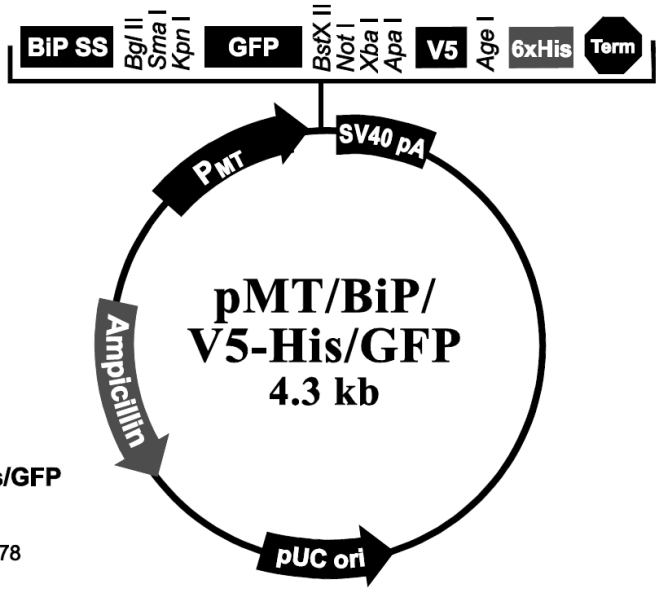
pMT/BiP/V5-His/GFP vector

Description

pMT/BiP/V5-His/GFP is a 4327 bp control vector expressing Green Fluorescent Protein (GFP). The plasmid was constructed by digesting pMT/BiP/V5-His A with *Kpn* I and *Eco*R V and ligating a 721 bp *Kpn* I–*Sac* I (Klenow) fragment containing the cycle 3-GFP gene in frame with the C-terminal peptide containing the V5 epitope and the polyhistidine tag.

Map of pMT/BiP/V5-His/GFP

The figure below summarizes the features of the pMT/BiP/V5-His/GFP vector. The nucleotide sequence for pMT/BiP/V5-His/GFP is available for downloading from **thermofisher.com** or by contacting Technical Support.



Comments for pMT/BiP/V5-His/GFP
4327 nucleotides

Metallothionein promoter: bases 412-778
Start of transcription: base 778
MT Forward priming site: bases 814-831
BiP signal sequence: bases 851-904
GFP portion of fusion: bases 938-1687
V5 epitope tag: bases 1688-1729
Polyhistidine region: bases 1739-1759
BGH Reverse priming site: bases 1779-1796
SV40 late polyadenylation signal: bases 1952-1957
pUC origin: bases 2450-3123 (complementary strand)
bla promoter: bases 4129-4227 (complementary strand)
Ampicillin (*bla*) resistance gene ORF: bases 3268-4128 (complementary strand)

Accessory products

Additional products

Reagents suitable for use with the products are available separately. Ordering information for these reagents is provided below. For more information, refer to **thermofisher.com** or by contacting Technical Support.

Item	Quantity	Catalog no.
Drosophila Inducible Expression System (DES™)	1 kit	K4120-01
	1 kit	K5120-01
One Shot™ TOP10 Chemically Competent *E. coli*	21 × 50 µL	C4040-03
One Shot™ TOP10 Electrocomp™ *E. coli*	21 × 50 µL	C4040-52
One Shot™ DH5α™-T1^R MAX Efficiency™ Chemically Competent *E. coli*	10 × 50 µL	12297-016

Item	Quantity	Catalog no.
PureLink™ HiPure MiniPrep™ Kit	25 preps	K2100-02
PureLink™ HiPure MidiPrep Kit	25 preps	K2100-04
BGH Reverse Primer	2 µg, lyophilized in TE	N575-02
Blasticidin S HCl	50 mg	R210-01
Schneider (S2) Cells	1 mL vial, 1 × 10^7 cells/mL	R690-07
Schneider's Drosophila Medium	500 mL	11720-034
Calcium Phosphate Transfection Kit	75 reactions	K2780-01

Detection of recombinant proteins

Expression of your recombinant fusion protein can be detected using an antibody to the appropriate epitope. The table below describes the antibodies available for detection of C-terminal fusion proteins expressed using pMT/BiP/V5-His. Horseradish peroxidase (HRP) or alkaline phosphate (AP)-conjugated antibodies allow one-step detection using colorimetric or chemiluminescent detection methods.

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 protein

The metal binding domain encoded by the polyhistidine tag allows simple, easy purification of your recombinant protein by Immobilized Metal Affinity Chromatography (IMAC) using the ProBond™ Resin (see below). To purify proteins expressed from pMT/BiP/V5-His, the ProBond™ Purification System or the ProBond™ resin in bulk are available separately. See the table below for ordering information.

Product	Quantity	Catalog no.
ProBond™ Metal-Binding Resin (precharged resin provided as a 50% slurry in 20% ethanol)	50 mL	R801-01
	150 mL	R801-15
ProBond™ Purification System (includes six 2 mL precharged, prepacked ProBond™ resin columns and buffers for native and denaturing purification)	6 purifications	K850-01
ProBond™ Purification System with Anti-V5-HRP Antibody	1 kit	K854-01
ProBond™ Purification System with Anti-His(C-term)-HRP Antibody	1 kit	K853-01
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References

Angelichio, P. L., Beck, J. A., Johansen, H., and Ivey-Hoyle, M. (1991). Comparison of Several Promoters and Polyadenylation Signals for Use in Heterologous Gene Expression in Cultured *Drosophila* Cells. Nucleic Acids Res. *19*, 5037-5043.

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1994). Current Protocols in Molecular Biology (New York: Greene Publishing Associates and Wiley-Interscience).

Bunch, T. A., Grinblat, Y., and Goldstein, L. S. (1988). Characterization and Use of the *Drosophila* Metallothionein Promoter in Cultured *Drosophila melanogaster* Cells. Nucleic Acids Res. *16*, 1043-1061.

Crameri, A., Whitehorn, E. A., Tate, E., and Stemmer, W. P. C. (1996). Improved Green Fluorescent Protein by Molecular Evolution Using DNA Shuffling. Nature Biotechnology *14*, 315-319.

Johansen, H., van der Straten, A., Sweet, R., Otto™, E., Maroni, G., and Rosenberg, M. (1989). Regulated Expression at High Copy Number Allows Production of a Growth Inhibitory Oncogene Product in *Drosophila* Schneider Cells. Genes and Development *3*, 882-889.

Kirkpatrick, R., and Shatzman, A. (1997). Personal communication.

Kirkpatrick, R. B., Ganguly, S., Angelichio, M., Griego, S., Shatzman, A., Silverman, C., and Rosenberg, M. (1995). Heavy Chain Dimers as well as Complete Antibodies are Efficiently Formed and Secreted from *Drosophila* Via a BiP-Mediated Pathway. J. Biol. Chem. *270*, 19800-19805.

Lindner, P., Bauer, K., Krebber, A., Nieba, L., Kremmer, E., Krebber, C., Honegger, A., Klinger, B., Mocikat, R., and Pluckthun, A. (1997). Specific Detection of His-tagged Proteins With Recombinant Anti-His Tag scFv-Phosphatase or scFv-Phage Fusion™s. BioTechniques *22*, 140-149.

Maroni, G., Otto™, E., and Lastowski-Perry, D. (1986). Molecular and Cytogenetic Characterization of a Metallothionein Gene of *Drosophila*. Genetics *112*, 493-504.

Olsen, M. K. (1992). Stable Production of an Analog of Human Tissue Plasminogen Activator from Cultured *Drosophila* Cells. Cytotechnology *10*, 157-167.

Rubin, D. M., Mehta, A. D., Zhu, J., Shoham, S., Chen, X., Wells, Q. R., and Palter, K. B. (1993). Genomic Structure and Sequence Analysis of *Drosophila* melanogaster *HSC70* Genes. Gene *128*, 155-163.

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, Second Edition (Plainview, New York: Cold Spring Harbor Laboratory Press).

Southern, J. A., Young, D. F., Heaney, F., Baumgartner, W., and Randall, R. E. (1991). Identification of an Epitope on the P and V Proteins of Simian Virus 5 That Distinguishes Between Two Isolates with Different Biological Characteristics. J. Gen. Virol. *72*, 1551-1557.

Zylka, M. J., and Schnapp, B. J. (1996). Optimized Filter Set and Viewing Conditions for the S65T Mutant of GFP in Living Cells. BioTechniques *21*, 220-226.

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- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
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- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

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