



The *Drosophila* Expression System.

Great Features. Great Expression.



The *Drosophila* Expression System is an insect expression system that offers:

- Higher protein yields than mammalian systems
- Easy high-density cell culture
- Non-lytic expression for reduced degradation

Stable high-level insect expression

The *Drosophila* Expression System (DES[®]) combines the best features of mammalian and insect expression systems for simple, efficient production of recombinant protein.

DES[®] provides:

- Straightforward generation of insect cell lines that stably express high levels of your protein
- Vectors with an inducible promoter for expression of toxic proteins
- *Drosophila* S2 cells for easy high-density growth

A proven expression technology

A wide range of proteins have been expressed using DES[®]. The system is especially well-suited for expressing secreted proteins, receptors, enzymes, and toxic proteins. Table 1 lists a sampling of the many proteins produced with DES[®].

Table 1 - Proteins Expressed with DES[®]

Class	Product	Expression Level	Ref.
Enzyme	Dopamine β -hydroxylase	> 16 μ g/L	(1)
Toxic	H-ras	0.2 to 0.5% of cellular protein	(2)
Secreted	Soluble hIL-5	22 mg/L	(3)
Antibody	IgG ₁ mAb	> 1 mg/L (not optimized)	(4)
Receptor	hIL-5R α	1 x 10 ⁶ sites/cell	(3)
Glycoprotein	gp120	5-35 mg/L	(5)
Ion Channel	GABA Receptor	3.5 x 10 ⁴ sites/cell	(6)

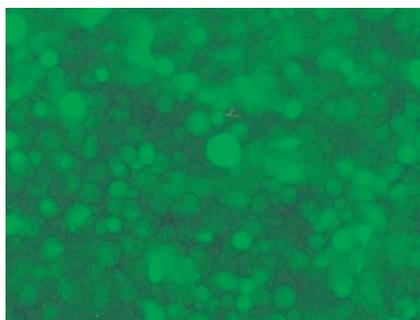
Ideal cell line for expression

Drosophila S2 cells (Figure 1) are perfectly suited for high-level, low-cost production of eukaryotic proteins (7). S2 cells are cost-effective and easy to use because they:

- Grow to high densities without CO₂ in serum-free medium, reducing laboratory costs
- Produce proteins with eukaryotic posttranslational modifications
- Integrate multiple copies of expression plasmids to allow isolation of high-producing polyclonal stable cell lines

In addition, endogenous *Drosophila* proteins generally do not interact with mammalian proteins, so S2 cells provide a “null background” for functional studies of proteins.

Figure 1 - S2 Cells Stably Expressing GFP



The gene encoding GFP was cloned into pAc5.1/V5-His to create pAc5.1/V5-His/GFP. The vector was cotransfected with the selection vector pCoHygro. Stable S2 cells were selected in 400 μ g/ml hygromycin.

Powerful vectors

DES[®] offers a variety of easy-to-use vectors with features such as inducible promoters, secretion signals, and tags. When a DES[®] expression vector is co-transfected with the pCoBlast or pCoHygro selection vector, blasticidin or hygromycin-resistant S2 cells can be selected for powerful stable expression.

Inducible expression with ease

DES[®] offers the *Drosophila* metallothionein (MT) promoter for high-level, inducible expression of your gene of interest from the vectors pMT/V5-His, pMT/BiP/V5-His (Figure 2), and pMT-DEST48 (Figure 5). Use of an inducible promoter allows you to control when a protein is produced. This is especially crucial when expressing proteins that may be deleterious to growing cells. The MT promoter is tightly regulated (2) and is easily induced by the addition of copper sulfate (CuSO₄) to the culture medium. Figure 3 demonstrates the tight regulation of β-galactosidase expression in S2 cells using the MT promoter.

Secretion of native proteins

Frequently, secretion of recombinant proteins into the culture medium improves their yield and quality. pMT/BiP/V5-His carries the *Drosophila* BiP signal sequence for secretion. The *Drosophila* BiP protein encodes an immunoglobulin-binding chaperone protein. This secretion signal efficiently targets high levels of BiP into the secretory pathway of S2 cells (4). By using the pMT/BiP/V5-His vector, you'll get secretion of native protein for improved yield and protein quality.

Fast cloning saves time

TOPO[®] Cloning is the fastest method for cloning an amplified gene of interest into a vector. We've combined this revolutionary cloning technology with pMT/V5-His so that you can get great expression results and save time cloning. With pMT/V5-His-TOPO[®] (Figure 4) you can clone PCR products with the highest efficiency in just 5 minutes on your benchtop. Ligase and overnight ligation have been eliminated. The TOPO[®] Cloning method saves an entire day compared to ligase-dependent cloning methods.

Figure 2 - Inducible DES[®] Vectors

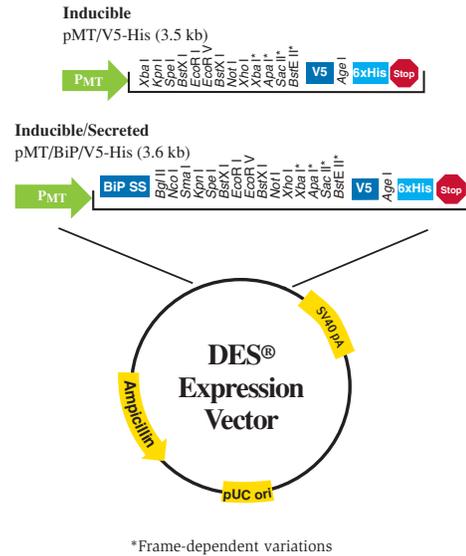


Figure 3 - Western Analysis of β-galactosidase Expression in S2 Cells

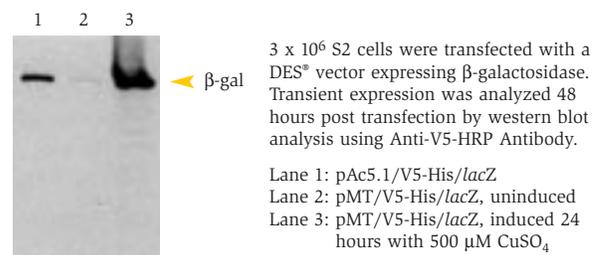
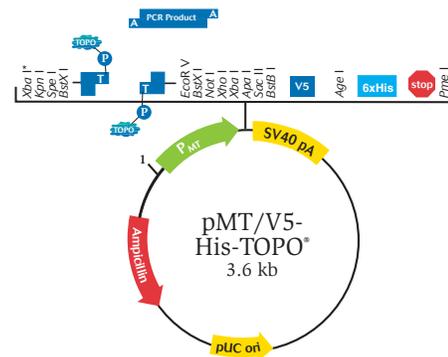


Figure 4 - pMT/V5-His-TOPO[®] Vector



Powerful vectors, continued

The power of Gateway™

Gateway™ Technology allows transfer of your gene of interest between different vectors by recombination, eliminating the need for restriction endonucleases and ligase. You simply clone your gene of interest into an entry vector and then move it into the destination vector of your choice for expression. The pMT-DEST48 destination vector (Figure 5) combines the features of the parental pMT/V5-His vector with the easy cloning of Gateway™. This offers you the option to express your gene of interest in the DES® system and then quickly and easily move the gene to another destination vector for expression in a different system.

Options for constitutive expression

DES® also offers a constitutive expression vector, pAc5.1/V5-His (Figure 6). The pAc5.1/V5-His vector uses the *Drosophila* gene promoter for high-level, constitutive expression of your protein.

Figure 3 (previous page) demonstrates the constitutive expression levels of β -galactosidase obtained from pAc5.1/V5-His.

Figure 5 - Gateway™-adapted DES® Vector

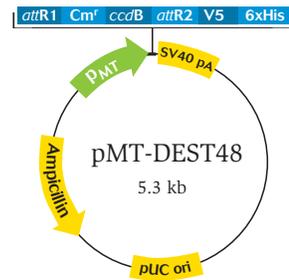
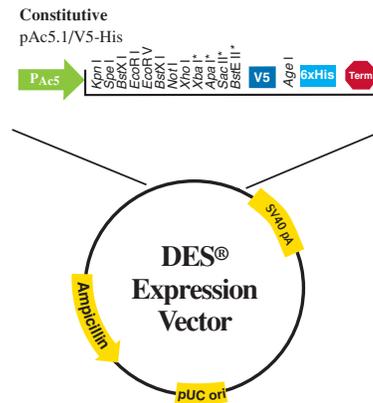


Figure 6 - Constitutive DES™ Vector



*Frame-dependent variations

Feature rich

The DES® vectors contain many features to simplify cloning, detection, and purification. Each DES® vector contains a C-terminal tag, which adds the V5 epitope for detection with Invitrogen's Anti-V5 Antibody and polyhistidine (6xHis) sequence for quick and easy purification using ProBond™ resin (Figures 2, 4, 5, and 6). The supercoiled vectors are provided with the

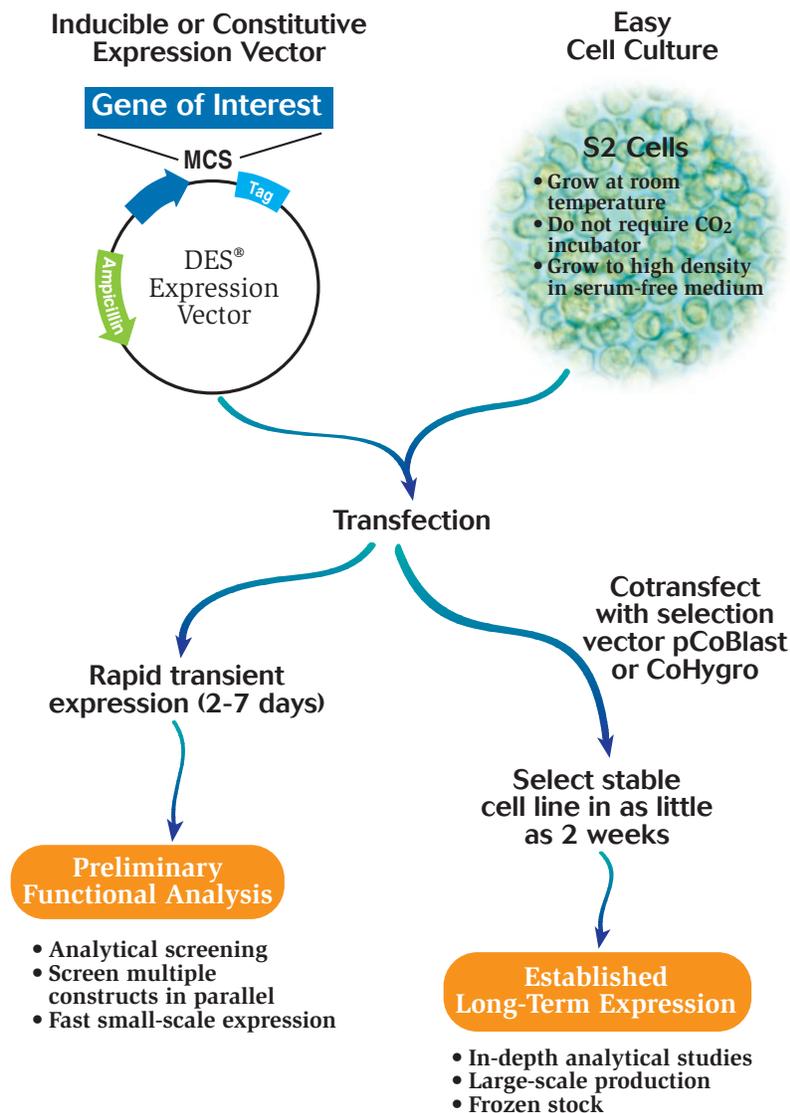
multiple cloning site in three reading frames relative to the C-terminal coding sequence to simplify in-frame cloning. In addition, pMT/V5-His is available Gateway™ adapted (pMT-DEST48) and topoisomerase-activated (pMT/V5-His TOPO®). You'll save hours of time and get great results.

Simple, yet powerful

The power of DES[®] is in its simplicity. Stable S2 cell lines are generated by cotransfection of a DES[®] expression vector with the selection vector pCoBlast or pCoHygro. Once your expression construct is inside the S2 cell, hundreds of copies of the expression plasmid containing your gene of interest will spontaneously integrate into the

genome. After just a few weeks of selection, a polyclonal cell line is established that stably expresses high levels of your protein. In mammalian systems this can take as long as two months. Producing your protein with DES[®] combines the simplicity of transfection and selection with the powerful expression of insect cells.

Figure 7 - Overview of Expression Using DES[®]



Custom services, too

If you don't have the time or resources for expression, let the Invitrogen experts do the work. Our experienced staff is highly trained to perform all the steps necessary for expression of your

protein in S2 cells. Call our Custom Services Sales Representative today at 1-800-955-6288, x67265 for more information.

Ready to start

Six complete DES[®] Kits are available to allow you to easily establish this powerful technology in your lab. Each kit comes with everything you'll need to produce your protein of interest in *Drosophila* S2 cells (Table 2). The DES[®] Blasticidin Support Kit is

also available to be paired with any DES[®] Expression vector for rapid selection of stable S2 cells with Blasticidin. With the proven power and simplicity of the DES[®] System, you'll be sure to get great results. Order a DES[®] Kit today.

Table 2 - DES[®] Kit Contents

Components - Complete Kit			Components – Blasticidin Support Kit
<ul style="list-style-type: none"> Your choice of DES[®] vector for inducible (pMT/V5-His), inducible/secreted (pMT/BiP/V5-His), or constitutive expression (pAc5.1/V5-His) A positive expression control pCoBlast or pCoHygro for stable selection Blasticidin or Hygromycin B Frozen S2 cells 1 L of Schneider's <i>Drosophila</i> Medium Prequalified reagents for calcium phosphate transfections Sequencing Primers 			<ul style="list-style-type: none"> pCoBlast for stable selection Prequalified reagents for calcium phosphate transfections Frozen S2 cells 1 L of Schneider's <i>Drosophila</i> Medium Blasticidin
Description	Quantity	Cat. no.	
DES [®] -Inducible Kit–with pCoBlast	1 kit	K5120-01	
with pCoHygro	1 kit	K4120-01	
DES [®] -Inducible/Secreted Kit–with pCoBlast	1 kit	K5130-01	
with pCoHygro	1 kit	K4130-01	
DES [®] -Constitutive Kit–with pCoBlast	1 kit	K5110-01	
with pCoHygro	1 kit	K4110-01	
DES [®] -Blasticidin Support Kit	1 kit	K5150-01	
DES [®] Expression Vectors			
DES [®] TOPO [®] TA Expression Kit	20 rxn	K4125-01	
pMT/V5-His A,B,C	20 µg each	V4120-20	
pMT/BiP/V5-His A,B,C	20 µg each	V4130-20	
pMT-DEST48	6µg	12282-018	
pAc5.1/V5-His A,B,C	20 µg each	V4110-20	

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Important Licensing Information

The *Drosophila* Expression System (DES[®]) and its use are the subject of U.S. Patent Nos. 5,550,043; 5,681,713; 5,705,359 and other pending patents licensed exclusively to Invitrogen. Purchase of DES[®] products grants you a limited, non-exclusive license to use the product for research purposes only. For more information, contact Invitrogen at 800 955 6288 or visit our web site at www.invitrogen.com.



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