BD BaculoGold™ Baculovirus Expression System
Innovative Solutions for Proteomics

BD Biosciences
Clontech
Discovery Labware
Immunocytometry Systems
Pharmingen

BD

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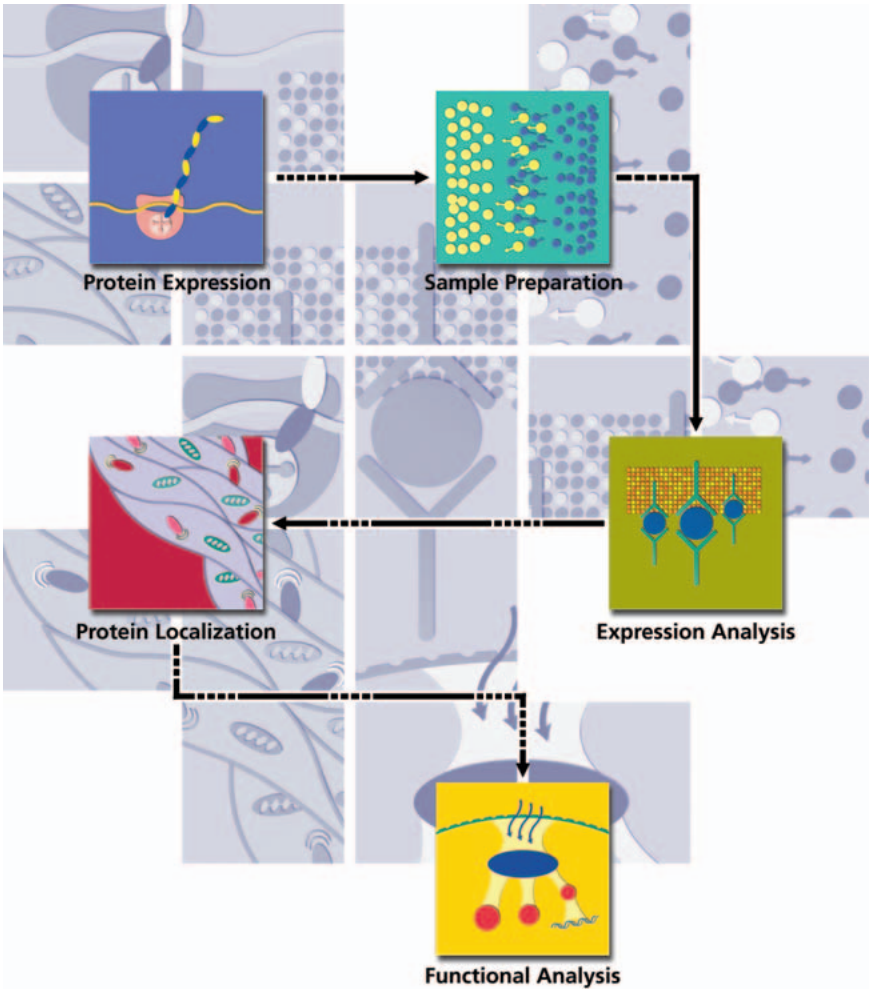
Innovative Solutions for Proteomics

BD Biosciences, comprised of Clontech, Discovery Labware, Immunocytometry Systems, and Pharmingen, is a leading provider of protein expression technologies for Proteomics.

No matter what the biological system—bacterial, insect, or mammalian—BD Biosciences provides reliable and innovative systems for cloning and protein expression. Proteins produced with these technologies are useful in generating antibodies for functional analysis as well as other applications.



Introduction

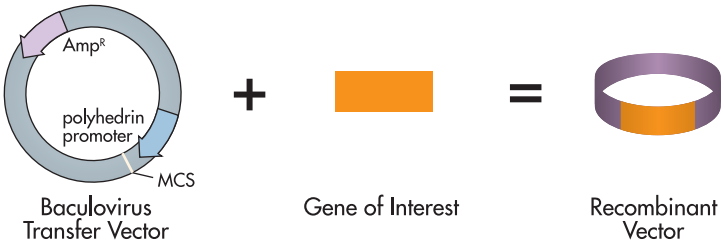
The Baculovirus Expression Vector System (BEVS) is a convenient and versatile eukaryotic system for heterologous gene expression. Baculovirus expression provides correct folding of recombinant protein as well as disulfide bond formation, oligomerization and other important post-translational modifications. Consequently, the overexpressed protein exhibits the proper biological activity and function.

The Baculovirus Expression Vector System is based on the introduction of a foreign gene into a nonessential region of the viral genome via homologous recombination with a transfer vector containing the cloned gene; an event that occurs in the co-transfected insect cells. The production of foreign protein is achieved by infection of additional insect cell cultures with the resultant recombinant virus. The Baculovirus Expression Vector System from BD Biosciences Pharmingen employs a modified *Autographa californica* nuclear polyhedrosis virus (AcNPV) genome—BD BaculoGold™ DNA, and an appropriate transfer vector. The diversity of AcNPV-based transfer vectors, combined with available *S. frugiperda Sf9* and *Sf21* cell lines, establish baculovirus expression as a preferred system for functional eukaryotic gene expression and the large-scale production of recombinant proteins.

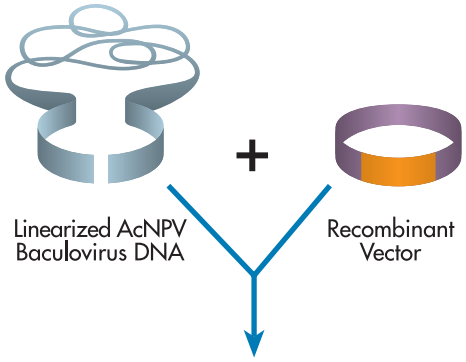
The baculovirus expression system offers the following advantages over prokaryotic and other eukaryotic systems:

- **High Level of Protein Expression**
  Yields of up to 100 mg of protein per 10^9 cells.
- **Post-Translational Modifications**
  Including disulfide bond formation, phosphorylation, glycosylation, oligomerization, and folding.
- **Relevant Cellular Compartmentalization of Proteins**
  Secreted, membrane-bound, cytoplasmic or nuclear.
- **Capacity of Large cDNA Inserts**
  Accommodates genes up to 8 kb.
- **Simultaneous Expression of Multiple Genes**
  With multiple promoter transfer vectors.

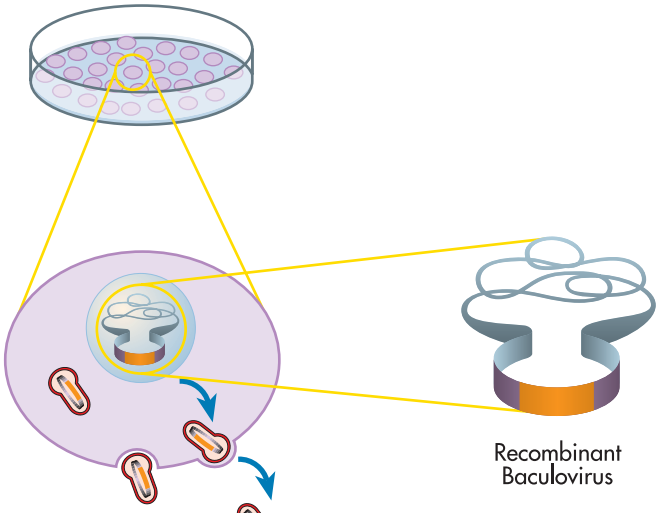
Generate recombinant BV transfer vector



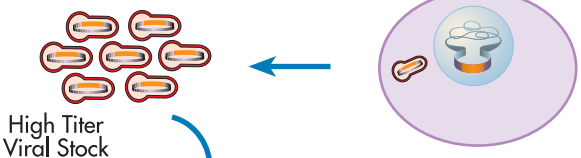
Co-transfect BD BaculoGold™ and recombinant transfer vector into Sf insect cells



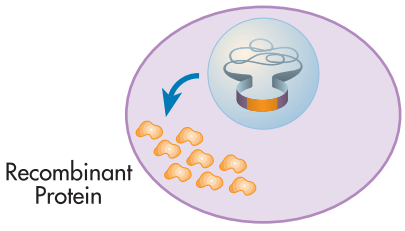
Recombination between the vector and the viral DNA occurs within the cell and recombinant baculovirus are produced



Harvest recombinant virus and amplify to produce high titer stock



Infect cells for recombinant protein expression and purification



BD BaculoGold™ Linearized Baculovirus DNA

Unlike the wild-type AcNPV virus, BD BaculoGold™ DNA is an engineered baculoviral DNA with a lethal deletion. Co-transfection of the BD BaculoGold DNA and a complementary baculovirus transfer vector restores viability by homologous recombination and rescues the virus along with the desired recombinant gene. Since only recombinant BD BaculoGold DNA produces viable virus, use of this improved viral DNA results in recombination efficiencies greater than 99%.

New BD BaculoGold Bright Linearized Baculovirus DNA

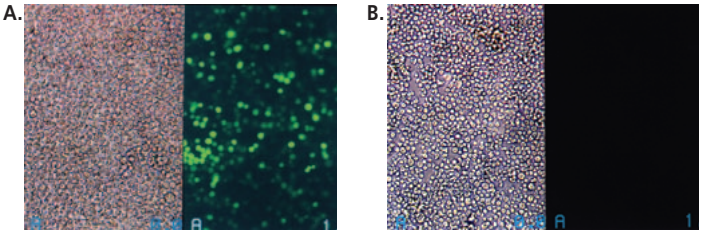
With the addition of the BD BaculoGold Bright Linearized DNA to our product line, we provide a new tool for fast and easy fluorescence detection and sorting of recombinant virus-infected insect cells. The new linearized baculovirus DNA contains the gene for GFP (green fluorescent protein) and cells infected with recombinant virus can be visualized by fluorescence microscopy or detected by flow cytometry. GFP-tagged recombinant viruses also allow studying the kinetics of infection and protein expression by fluorescence microscopy.

Recombinant virus can be purified 24h post infection (pi), by sorting single GFP-positive cells, using a BD FACSAria™ or a BD FACSVantage™ SE Cell Sorting System, replacing the plaque assay.

Subsequently the virus titer can be measured by flow cytometry or serial dilution assay. Since the BD BaculoGold Bright also contains a lacZ gene that is replaced by recombination with the plasmid containing the foreign gene, all recombinants will produce colorless plaques on X-gal plates. A small portion of non-recombinant virus plaques (usually less than 1%) will stain blue on X-gal plates. If preferred, the virus may be amplified from a single plaque using a plaque assay.

The BD BaculoGold Bright Advantage:

- Identify virus-infected cells 48h -72h after co-transfection or 24h post infection.
- Purify recombinant virus 24h post infection by sorting single, GFP-positive cells using flow cytometer (BD FACSAria and BD FACSVantage SE Cell Sorting Systems).
- Study virus infection kinetics and protein expression by fluorescence microscopy.
- Measure virus titer by flow cytometry or serial dilution assay.
- Express proteins in BEVS with high throughput.



Sf9 cells were co-transfected with BD BaculoGold™ Bright and hMip-1α (A) or BD BaculoGold Bright alone (B). 5 days after transfection cells were analyzed using light and fluorescence microscopy.

Quick recombinant BV purification, titration and protein expression using BD BaculoGold™ Bright

Day 1	Co-transfection
Day 3	Flow sorting
Day 4	Seeding 96-well plate with Sf9 Cells
Day 7	Harvest supernatant and infect 60 mm plate (1st amplification)
Day 9	Harvest supernatant and amplify virus (2nd amplification)
Day 11	Virus titration by flow cytometry
Day 12	Protein expression

XylE Baculovirus Control Vectors

BD BaculoGold™ XylE Baculovirus Control Vectors have a *Pseudomonas putrida* gene XylE, which encodes a 40 kDa protein. These are designed to be used as controls for co-transfection experiments. Co-transfection of BD BaculoGold DNA with any of these control vectors generates recombinant virus that expresses XylE or affinity-tagged XylE fusion proteins; infected insect cells, expressing XyIE, turn yellow in the presence of 500 mM catechol and 50 mM sodium bisulfate.

Baculovirus DNAs

DESCRIPTION	APPS	SIZE	CAT. NO.	NEW
BD BaculoGold Linearized Baculovirus DNA	BV	2.5 µg in 25 µl	**554739**	
BD BaculoGold Bright Linearized Baculovirus DNA	BV	2.5 µg in 25 µl	**552846**	■
BD BaculoGold Bright Linearized Baculovirus DNA	BV	2.5 ug in 25 ul	**inquire**	■

Baculovirus Control Vectors

DESCRIPTION	APPS	SIZE	CAT. NO.
pAcG2T-XylE Baculovirus Control Vector	BV	5 µg in 50 µl	**554788**
pAcGHLT-XylE Baculovirus Control Vector	BV	5 µg in 50 µl	**554798**
pAcHLT-XylE Baculovirus Control Vector	BV	5 µg in 50 µl	**554799**
pVL1392-XylE Baculovirus Control Vector	BV	5 µg in 50 µl	**554807**

BD Baculogold™ Starter Package and Transfection Kit

DESCRIPTION	APPS	SIZE	CAT. NO.
BD BaculoGold Starter Package	BV	5 transfections	**554738**
contains:			
Linearized BD BaculoGold Baculovirus DNA		2.5 µg each	
pVL1392/1393 Baculovirus Transfer Vector Set		5.0 µg	
pVL1392-XylE Control Vector		5.0 µg	
AcNPV Wild-Type High Titer Virus Stock (1 X 10^8)		1.0 ml	
Transfection Buffer A & B Set		5 ml each	
TNM-FH Insect Cell Medium		1 liter	
Live Sf9 Insect Cells (> 1 X 10^7)		1 flask	
Agarplaque Plus Agarose		50 g	
Baculovirus Procedures & Methods Manual		1	
BD BaculoGold Transfection Kit	BV	5 transfections	**554740**
contains:			
Linearized BD BaculoGold Baculovirus DNA		2.5 µg/25 ml	
pVL1392/1393 Baculovirus Transfer Vector Set		5 µg/50 ml each	
pVL1392-XylE Baculovirus Control Vector DNA		5 µg/5 ml	
Transfection Buffer A and B Set		5 ml each	
AcNPV Wild-Type High Titer Viral Stock		10^7 in 1 ml	
Baculovirus Procedures & Methods Manual		1	

BD BaculoGold™ Recombinant Virus Detection Kit

The NEW BD BaculoGold™ Recombinant Virus Detection Kit (Cat. No. 552153) was developed to rapidly screen for and verify recombinant virus generated using BD BaculoGold linearized DNA. Recombinant virus samples, treated briefly with a novel lysis buffer, are amplified by PCR* with primers specifically designed to amplify your gene of interest cloned into one of a variety of baculovirus transfer vectors *(Table 1)*. Baculovirus in a single plaque or in as little as 1 µl of virus stock is sufficient material to serve as template. Two forward PCR primers and two reverse primers are provided in the kit. Various combinations of these primers are used to amplify the cloned gene. Alternatively, a recombinant gene can be amplified using the virus lysis buffer and gene-specific primers. The PCR product is then analyzed by gel electrophoresis for the presence and predicted size of the gene of interest. The kit includes virus lysis buffer, PCR primers and recombinant virus supernatant for use as a positive control.

TRANSFER VECTOR	PRIMER				FLANKING REGION
	F1	F2	R1	R2	
pVL1392/1393	+		+		260 bp
pAcGP67-A, B, C	+		+		382 bp
pAcG1, G2T, G3X		+		+	180 bp
pAcGHLT-A, B, C		+		+	422 bp
pAcSecG2T		+		+	192 bp
pAcHLT-A, B, C	+			+	504 bp
pAcSG2	+			+	380 bp

Table 1. Primer Selection Table: *The expected fragment size = inserted gene (bp) + virus flanking region, based on the transfer vector used to generate the recombinant virus.*

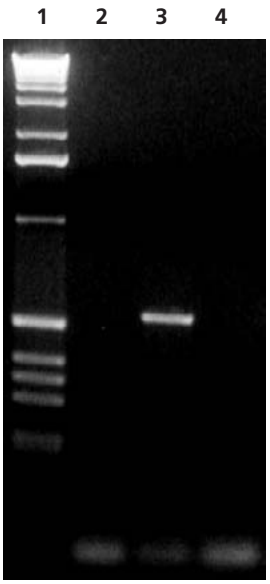


Figure 1. *PCR amplification of recombinant virus using BD BaculoGold™ Recombinant Virus Detection Kit. 1 Kb ladder (Lane 1), Negative Control, no virus (Lane 2), 1 µl Control Virus Supernatant in Lysis Buffer (Lane 3), 1 µl Control Virus Supernatant with no Lysis Buffer (Lane 4).*

Baculovirus Detection and Titer Kits

DESCRIPTION	APPS	SIZE	CAT. NO.	NEW
BD BaculoGold Recombinant Virus Detection Kit	BV	100 reactions	**552153**	■
contains:				
Virus Lysis Buffer				
Forward Primer 1				
Reverse Primer 1				
Forward Primer 2				
Reverse Primer 2				
Control Virus Supernatant				

** Some uses of this product may be claimed in patents. For example: U.S. Patent No. 4,683,195, "Process for amplifying, detecting, and/or-cloning nucleic acid sequences" and U.S. Patent No. 4,683,202, "Process for amplifying nucleic acid sequences" claim a method of Polymerase Chain Reaction (PCR). Both of these patents are assigned to Hoffman-LaRoche. Proper authorization or permission may be necessary to practice such patented methods. BD Biosciences Pharmingen will not be responsible for violations or patent infringements which may occur with the use of our products.*

BD BacPAK™ Baculovirus Rapid Titer Kit

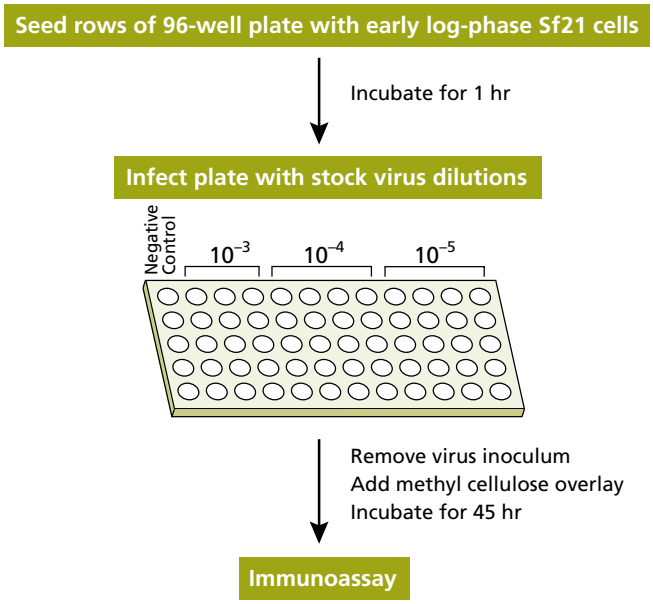
- Saves time by shortening baculovirus expression experiments up to six days
- Eliminates troublesome plaque assays
- Compatible with all AcNPV-based baculovirus expression systems

The BD BacPAK™ Baculovirus Rapid Titer Kit provides the quickest method for determining titers of baculovirus stocks, typically the most time consuming part of baculovirus expression protocols. The kit uses a standard immunological assay to accurately determine baculovirus titers within 48 hours, whereas other methods, such as plaque and end-point dilution assays, require 4–8 days.

In BD™ Baculovirus Expression Systems, infected cells express viral antigens long before plaques are formed. The BD BacPAK Rapid Titer assay allows titer determination after a much shorter incubation period. Furthermore, the titers obtained are comparable to those obtained with other methods. This kit is suitable for use with any virus stock with a titer of more than 104 pfu/ml and is compatible with all AcNPV-based baculovirus expression systems.

The BD BacPAK Rapid Titer immunoassay uses a primary monoclonal antibody raised against an AcNPV envelope glycoprotein (gp64) to accurately identify virally infected cells. An HRP-conjugated secondary antibody enables visualization of infected cells by light microscopy and determine viral titer.

The BD BacPAK Rapid Titer Kit includes a baculovirus control and all the necessary reagents, excluding organic solvents, to perform five titration assays.



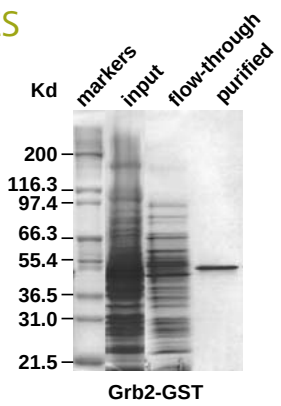
Flow chart of the BD BacPAK™ Baculovirus Rapid Titer Kit procedure.

Baculovirus Titer Kits

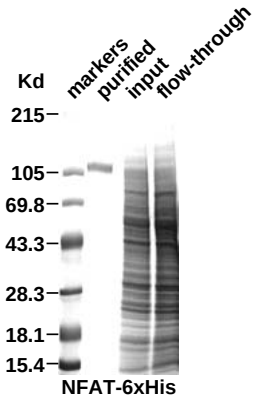
DESCRIPTION	APPS	SIZE	CAT. NO.	NEW
BD BacPAK Baculovirus Rapid Titer Kit	BV	5 assays	**631406**	■
contains:				
Mouse gp64 Antibody				
Goat Anti-Mouse Antibody/HRP Conjugate				
Normal Goat Serum				
Methyl Cellulose Overlay				
Resealable Plastic Bags				
Control Baculovirus				

Protein Expression and Purification Kits

In order to facilitate gene expression and protein purification, BD Biosciences Pharmingen has developed two baculovirus expression and purification kits utilizing affinity purification tags: the 6xHis Expression and Purification Kit and the GST Expression and Purification Kit. Both kits combine the advantages of rapid expression of functional and soluble recombinant proteins using BD BaculoGold™ baculovirus expression technology with the purification power of the 6xHis and GST affinity purification systems. Purifications to greater than 90% homogeneity are achieved in a single step under native conditions.



Purified Grb2-GST expressed with BD BaculoGold™



Purified NFAT-6xHis expressed with BD BaculoGold™

Baculovirus Expression and Purification Kits

DESCRIPTION	APPS	SIZE	CAT. NO.
6xHis Expression and Purification Kit	BV	1 Kit	**554802**
contains:			
Linearized BD BaculoGold Baculovirus DNA		2.5 µg	
pAcHLT-A,B,C Baculovirus Transfer Vector Set		20 µg each	
pAcHLT-XylE Baculovirus Control Vector		5.0 µg	
Thrombin Powder		20 mg (1000 U)	
Thrombin Dilution Buffer		1 ml	
Protease Inhibitor Cocktail		lyophilized	
1x Insect Cell Lysis Buffer		50 ml	
His Purification Resin		10 ml	
6xHis Elution Buffer		40 ml	
6xHis Wash Buffer		250 ml	
3M Imidazole Solution		125 ml	
Transfection Buffer A&B Set		5 ml each	
Baculovirus Procedures & Methods Manual		1	
GST Expression and Purification Kit	BV	1 Kit	**554803**
contains:			
Linearized BD BaculoGold Baculovirus DNA		2.5 µg	
pAcGHLT-A,B,C Baculovirus Transfer Vector Set		20 µg each	
pAcGHLT-XylE Baculovirus Control Vector		5.0 µg	
Thrombin Powder		20 mg (1000 U)	
Thrombin Dilution Buffer		1 ml	
Protease Inhibitor Cocktail		lyophilized	
1x Insect Cell Lysis Buffer		50 ml	
Glutathione Powder		62 mg	
Glutathione Agarose Beads		10 ml	
GST Elution Buffer		40 ml	
1x PBS Wash Buffer		375 ml	
Transfection Buffer A&B Set		5 ml each	
Baculovirus Procedures & Methods Manual		1	

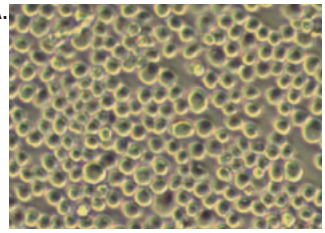
Antibodies to Recombinant Fusion Proteins

DESCRIPTION	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	NEW
Anti-Glutathione S-transferase (GST)	B19-2	Rat IgG_{2a},κ	BV	Purified	0.1 mg	**554824**	
	G172-1138	Mouse IgG_1	BV	Purified	0.1 mg	**554805**	
Anti-6xHis		Mouse IgG_{2a}	BV	Purified	200 µg	**631212**	■
		Mouse IgG_{2a}	BV	HRP	100 µg	**631210**	■

Insect Cell Lines and Cell Culture Media

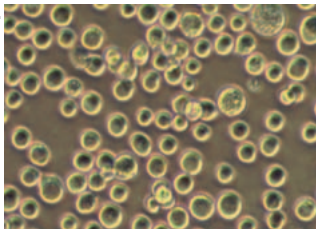
A variety of insect cell lines are susceptible to infection with the AcNPV baculovirus. The cell lines most frequently used are Sf9 and Sf21, originally established from ovarian tissues of *Spodoptera frugiperda* larvae. Sf9 and Sf21 cell lines are available in either our new BaculoGold™ Max-XP Serum Free Insect Cell Medium (please see below) or TNM-FH fully-supplemented Insect Cell Culture Medium (see Price List).

A.



Uninfected insect cells

B.



Insect cells infected with recombinant Baculovirus

BD BaculoGold™ Max-XP Serum-Free Insect Cell Medium

The BD BaculoGold™ Max-XP Serum-Free Insect Cell Medium is produced using proprietary systems for component analysis, nutrient delivery, and metabolic pathway feedback. The result is a metabolically enhanced medium which far outperforms the competition. Experiments indicate that the production of functional recombinant protein in insect cell lines grown in BD BaculoGold Max-XP increases three-fold as compared with insect cells grown in TNM-FH/10% FBS and other serum-free media. In addition, BD BaculoGold Max-XP is designed to be used in multiple cell lines and has been shown to optimize growth and recombinant protein production in *Drosophila melanogaster*, *Tricholplusia ni*, *Heliothis zea*, in the *Spodoptera frugiperda* cell lines *Sf9* and *Sf21*, and more. BD BaculoGold Max-XP is ideal for quick, direct adaptation of your insect cells cultured in serum-supplemented media or in other serum-free media.

BD BaculoGold Max-XP promotes high cell density and optimum protein production, making it ideal for both large scale industrial use and research-scale production. In addition, the BD BaculoGold Max-XP composition is precisely defined, which facilitates the purification of recombinant proteins and isolation of virus.

In summary, this metabolically designed serum-free medium enhances the growth of many types of insect cells, amplifies recombinant protein production, and augments baculovirus yield.

Insect Cell Lines and Insect Media

DESCRIPTION	APPS	SIZE	CAT. NO.
TNM-FH Insect Medium	BV, InsCC	1 liter	**554760**
BD BaculoGold Max-XP Insect Cell Medium	BV, InsCC	1 Liter	**551411**
Sf9 Insect Cells (Live) in TNM-FH Medium	BV, InsCC	10^7 cells	**554763**
Sf9 Insect Cells (Frozen) in TNM-FH Medium	BV, InsCC	10^7 cells	**554762**
Sf9 Insect Cells (Live) in Max-XP Medium	BV, InsCC	10^7 cells	**551408**
Sf9 Insect Cells (Frozen) in Max-XP Medium	BV, InsCC	10^7 cells	**551407**
Sf21 Insect Cells (Live) in Max-XP Medium	BV, InsCC	10^7 cells	**551410**
Sf21 Insect Cells (Frozen) in Max-XP Medium	BV, InsCC	10^7 cells	**551409**

Supplementary Baculovirus Reagents

DESCRIPTION	APPS	SIZE	CAT. NO.
3M Imidazole Solution	BV	125 ml	**554801**
6xHis Elution Buffer	BV	40 ml	**554804**
6xHis Wash Buffer (1X)	BV	2 bottles of 125 ml each	**554800**
AcNPV Wild Type Virus High Titer Stock Solution	BV	1 ml, $1x10^8$ pfu/ml	**554744**
AgarPlaque Plus™ Agarose	BV	50 grams	**554766**
Glutathione Agarose Beads	BV	10 ml	**554780**
Glutathione Powder	BV	62 mg	**554782**
GST Elution Buffer	BV	40 ml	**554787**
Insect Cell Lysis Buffer (1X)	BV	50 ml	**554778**
PBS Wash Buffer (1X)	BV	3 bottles of 125 ml each	**554781**
Protease Inhibitor Cocktail	BV	1 vial	**554779**
Thrombin Dilution Buffer	BV	1 ml	**554786**
Thrombin Powder	BV	20 mg	**554783**
Transfection Buffer A & B Set	BV	5 ml each	**554806**

Baculovirus Transfer Vectors

The system provides an array of versatile transfer vectors. Polyhedrin locus-based and p10 locus-based transfer vectors are available in the following conformations: with the multiple cloning site in opposite orientations, with single promoter or multiple promoters for expression of 2, 3, or 4 proteins simultaneously. Vectors are also available in three translational reading frames for expression of proteins which are singly or multiply-tagged with 6xHis, GST or a signal peptide for protein secretion. Additionally, vectors are available for easy visualization of GFP-, BFP-, or YFP-tagged proteins. Please find all currently available products listed below.

Transfer Vectors at-a-glance:

VECTOR	PROMOTER	TYPE	FUSION PROTEIN	FEATURES	CAT. NO.
SINGLE PROMOTER PLASMIDS					
pVL1392/3 (set)	Polyhedrin	very late	no	Standard polyhedrin locus vectors	**554745**
pAcSG2	Polyhedrin	very late	site dependent	Recommended for large inserts, has an ATG	**554769**
pAcMP2/3 (set)	Basic protein	late	no	Facilitates post-translational modifications	**554750**
pAcUW21	p10	very late	no	Allows for in-larval expression, F1 origin	**554748**
pAcGHLT-A, -B, -C (set)	Polyhedrin	very late	yes	GST-tag, 6xHis-tag thrombin cleavage site	**554792**
pAcHLT-A, -B, -C (set)	Polyhedrin	very late	yes	6xHis-tag, thrombin cleavage site	**554796**
pAcG1	Polyhedrin	very late	yes	GST-tag	**554771**
pAcG2T	Polyhedrin	very late	yes	GST-tag, thrombin cleavage site	**554772**
pAcG3X	Polyhedrin	very late	yes	GST-tag, factor Xa cleavage site	**554773**
pVL1393-GFP/BFP/YP	Polyhedrin	very late	yes	GFP tag	**554813**
pAcHLT-A-GFP/BFP/YP	Polyhedrin	very late	yes	GFP tag, 6xHis tag, thrombin cleavage site	**554817**
SECRETORY					
pAcGP67 A, B, C (set)	Polyhedrin	very late	yes	Signal sequence	**554759**
pAcSecG2T	Polyhedrin	very late	yes	Signal sequence, GST-tag	**554797**
MULTIPLE PROMOTER PLASMIDS					
pAcUW51	Polyhedrin, p10	very late	no	Simultaneous expression of 2 foreign genes; F1 origin	**554747**
pAcAB3	Polyhedrin, p10	very late	no	Simultaneous expression of 3 foreign genes	**554755**
pAcDB3	Polyhedrin, p10	very late	no	Simultaneous expression of 3 foreign genes; F1 origin	**554825**
pAcAB4	Polyhedrin, p10	very late	no	Simultaneous expression of 4 foreign genes	**554770**
BD CREATOR BACPAK SHUTTLE VECTORS					
pLP-BacPAK9 Acceptor Vector	Polyhedrin	very late	no	Cre-loxP recombination sites	**631407**
pLP-BacPAK9 -6xHN Acceptor Vector	Polyhedrin	very late	yes	Cre-loxP recombination sites, 6xHN-tag	**631408**

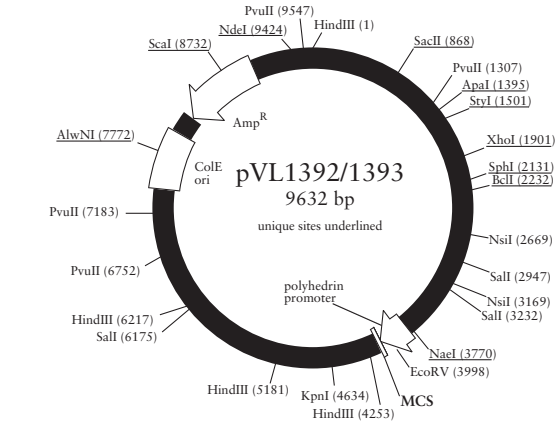
Baculovirus Transfer Vectors

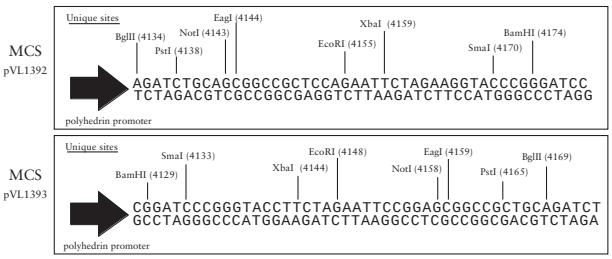
Single Polyhedrin Promoter Plasmids

pVL1392, pVL1393

DESCRIPTION	SIZE	CAT. NO.
pVL1392, pVL1393 Baculovirus Transfer Vector Set	5 µg in 50 µl each	**554745**

The pVL1392 and pVL1393 transfer vectors contain polyhedrin gene loci. A multiple cloning site (MCS) has been inserted 37 nucleotides downstream of the polyhedrin ATG start codon, which also has been changed to ATT. It is advisable that the ATG start codon of the cloned gene not be in-frame with the vector ATT due to translation initiation that has been reported using these vectors in some recombinant viruses. If the cloned gene contains a 5′ untranslated region longer than 100 nucleotides, this will cause poor protein expression. The multiple cloning site is in the opposite orientation in pVL1392 and pVL1393.

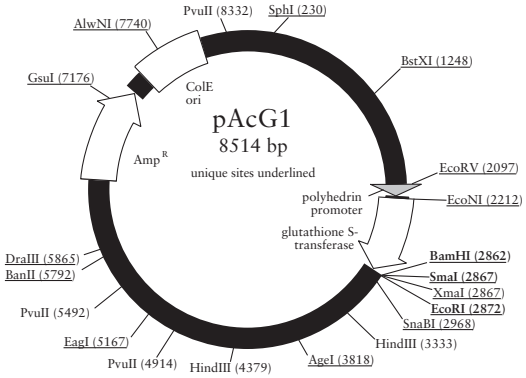




pAcG1

DESCRIPTION	SIZE	CAT. NO.
pAcG1 Baculovirus Transfer Vector	20 µg in 20 µl	**554771**

The pAcG1 transfer vector is a derivative of the pAcCL29 vector. Recombinant genes are expressed as GST fusion proteins when cloned in one of the available restriction enzyme sites (BamHI, SmaI or EcoRI). All inserts cloned must be in-frame with the GST gene.



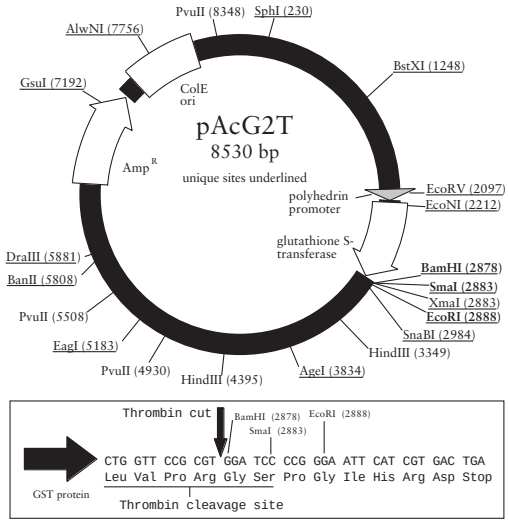
Baculovirus Transfer Vectors

Single Polyhedrin Promoter Plasmids *(continued)*

pAcG2T

DESCRIPTION	SIZE	CAT. NO.
pAcG2T Baculovirus Transfer Vector	20 µg in 20 µl	554772

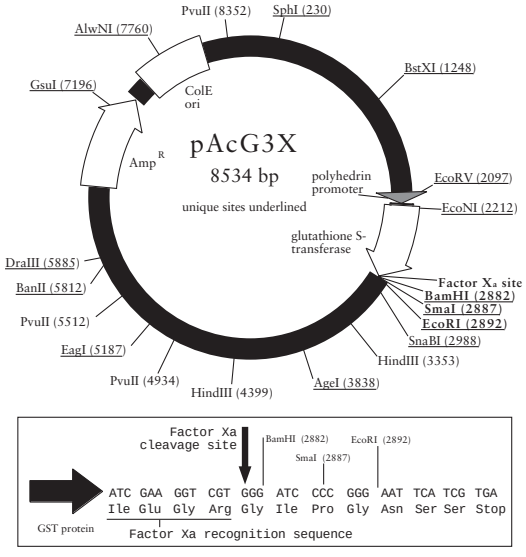
The pAcG2T transfer vector is a derivative of the pAcG1 vector. Recombinant genes are expressed as GST fusion proteins when cloned in one of the available restriction enzyme sites (BamHI, SmaI or EcoRI). All inserts cloned must be in-frame with the GST gene. A thrombin cleavage site is included between the GST gene and SmaI site.



pAcG3X

DESCRIPTION	SIZE	CAT. NO.
pAcG3X Baculovirus Transfer Vector	20 µg in 20 µl	554773

The pAcG3X transfer vector is a derivative of the pAcCL29 vector. Recombinant genes are expressed as GST fusion proteins when cloned in one of the available restriction enzyme sites (BamHI, SmaI or EcoRI). All inserts cloned must be in-frame with the GST gene. The GST tag can be removed by proteolytic factor Xa cleavage.



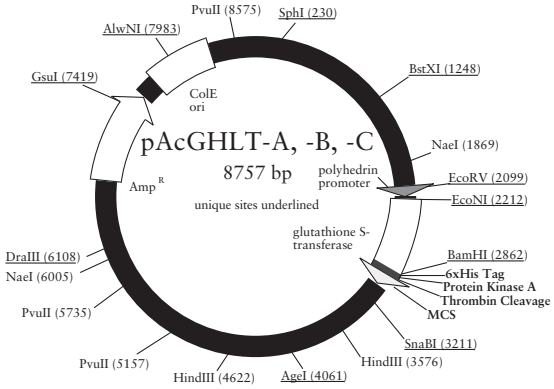
Baculovirus Transfer Vectors

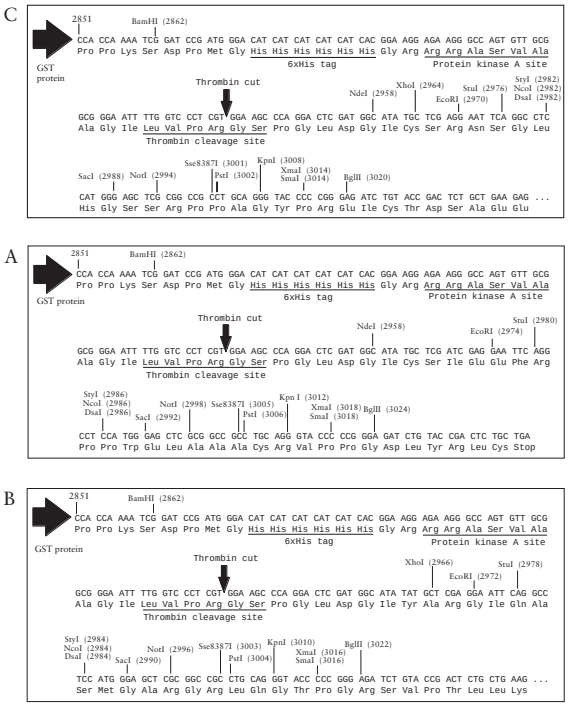
Single Polyhedrin Promoter Plasmids *(continued)*

pAcGHLT-A,B,C

DESCRIPTION	SIZE	CAT. NO.
pAcGHLT-A,B,C Baculovirus Transfer Vector Set	20 µg in 20 µl each	554792
pAcGHLT-A Baculovirus Transfer Vector	20 µg in 20 µl	554789
pAcGHLT-B Baculovirus Transfer Vector	20 µg in 20 µl	554790
pAcGHLT-C Baculovirus Transfer Vector	20 µg in 20 µl	554791

These transfer vectors both contain a GST tag and a 6xHis tag followed by multiple cloning sites in different reading frames for convenient cloning purposes. The recombinant fusion protein can be phosphorylated at the protein kinase A site which follows the 6xHis sequence. Both GST and 6xHis tags can be removed by proteolytic thrombin cleavage.





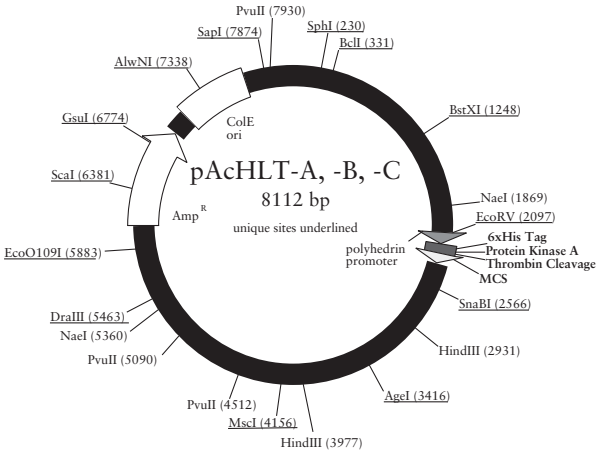
Baculovirus Transfer Vectors

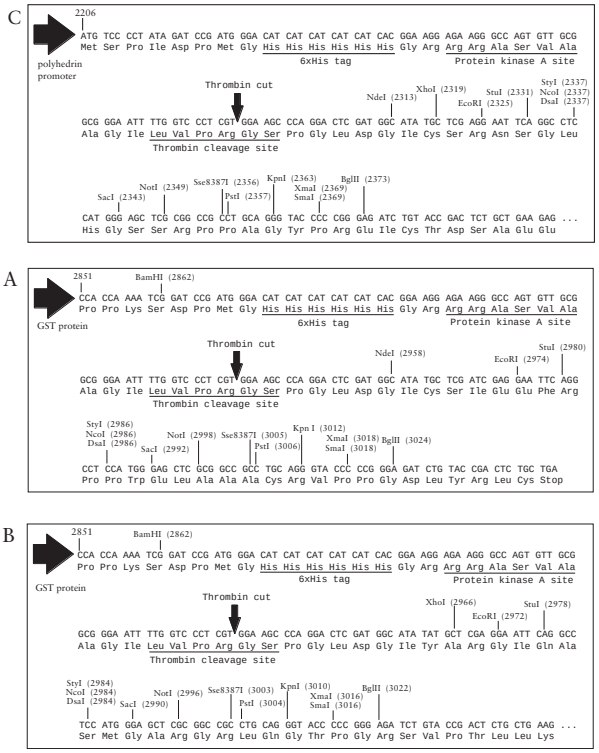
Single Polyhedrin Promoter Plasmids *(continued)*

pAcHLT-A,B,C

DESCRIPTION	SIZE	CAT. NO.
pAcHLT-A,B,C Baculovirus Transfer Vector Set	20 µg in 20 µl each	554796
pAcHLT-A Baculovirus Transfer Vector	20 µg in 20 µl	554793
pAcHLT-B Baculovirus Transfer Vector	20 µg in 20 µl	554794
pAcHLT-C Baculovirus Transfer Vector	20 µg in 20 µl	554795

These transfer vectors contain a 6xHis tag followed by multiple cloning sites in different reading frames for convenient cloning purposes. The 6xHis fusion recombinant protein can be phosphorylated at the protein kinase A site which follows the 6xHis sequence. The 6xHis tag can be removed by proteolytic thrombin cleavage.





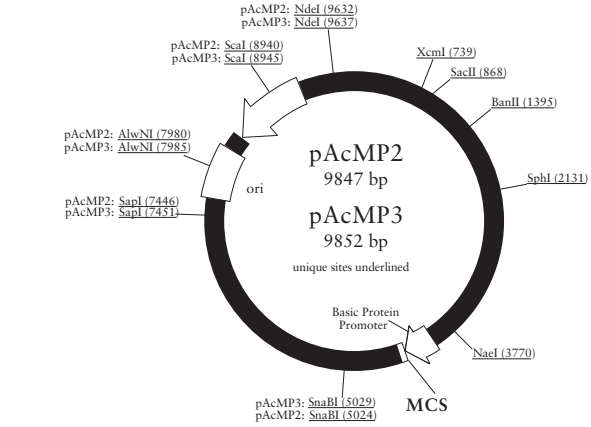
Baculovirus Transfer Vectors

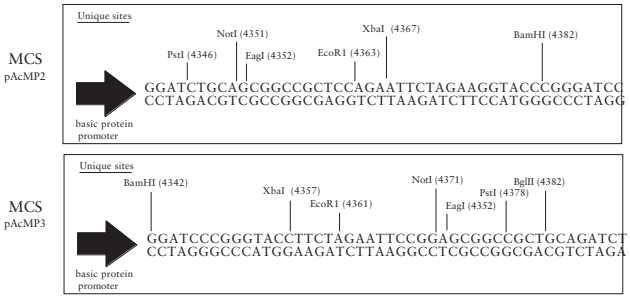
Single Polyhedrin Promoter Plasmids *(continued)*

pAcMP2, pAcMP3

DESCRIPTION	SIZE	CAT. NO.
pAcMP2, pAcMP3 Baculovirus Transfer Vector Set	5 µg in 50 µl each	**554750**
pAcMP2 Baculovirus Transfer Vector	5 µg in 50 µl	**554751**
pAcMP3 Baculovirus Transfer Vector	5 µg in 50 µl	**554752**

These transfer vectors contain a copy of the AcNPV basic protein promoter instead of the polyhedrin gene promoter. They permit heterologous gene expression in the late phase of viral infection vs. the very late phase which results with the use of polyhedrin or p10 promoters. Since these vectors have residual polyhedrin gene coding sequences and their flanking regions, the recombination will occur at the polyhedrin locus of the AcNPV. The pAcMP2 and pAcMP3 vectors have multiple cloning sites in opposite orientations that are inserted downstream of the basic promoter.

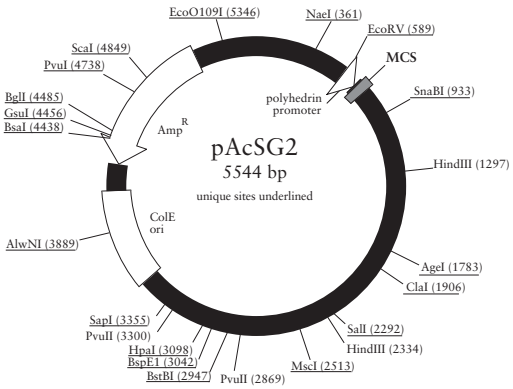




pAcSG2

DESCRIPTION	SIZE	CAT. NO.
pAcSG2 Baculovirus Transfer Vector	5 µg in 50 µl	**554769**

The pAcSG2 transfer vector is a smaller vector which contains only the essential polyhedrin gene locus portions of the AcNPV. The multiple cloning site immediately follows the polyhedrin promoter to improve recombinant protein expression levels. In the multiple cloning site, the Nco I site contains an ATG initiation codon and thus sequences and thus sequences cloned downstream of Nco I must not contain their own start codon or they should be in-frame with the ATG of the Nco I site. Because of its small size, inserts as large as 8 Kb may be cloned.



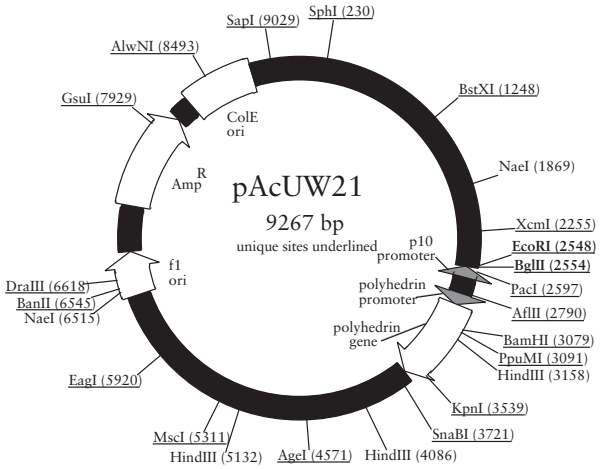
Baculovirus Transfer Vectors

Single Polyhedrin Promoter Plasmids *(continued)*

pAcUW21

DESCRIPTION	SIZE	CAT. NO.
pAcUW21 Baculovirus Transfer Vector	5 µg in 50 µl	**554748**

The pAcUW21 transfer vector is an AcNPV polyhedrin locus-based vector that contains the AcNPV p10 promoter and SV40 transcription termination signals inserted upstream of the complete AcNPV polyhedrin gene. Foreign genes may be cloned into the Bgl II or EcoR I site located downstream of the p10 promoter. The recombinant virus will be occlusion-body positive. This vector will be of use to those researchers interested in producing recombinant protein in insect larvae. pAcUW21 contains the f1 origin of replication and can produce, by helper phage mediation, single stranded DNA, useful in sequencing and mutagenesis.



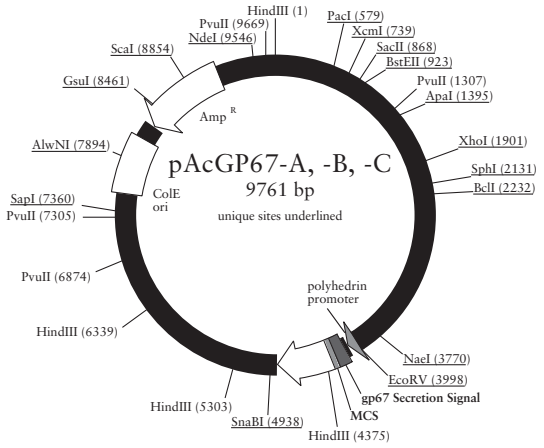
Baculovirus Transfer Vectors

Secretory

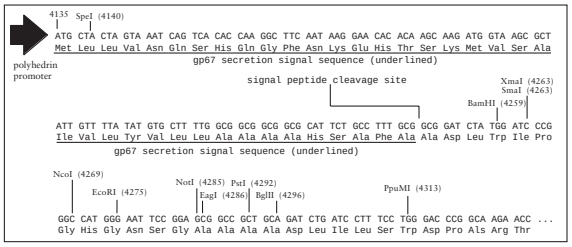
pAcGP67 A,B,C

DESCRIPTION	SIZE	CAT. NO.
pAcGP67 A,B,C Baculovirus Transfer Vector Set	5 µg in 50 µl each	554759
pAcGP67-A Baculovirus Transfer Vector	5 µg in 50 µl	554756
pAcGP67-B Baculovirus Transfer Vector	5 µg in 50 µl	554757
pAcGP67-C Baculovirus Transfer Vector	5 µg in 50 µl	554758

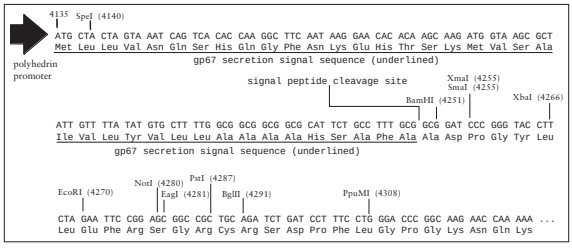
The acidic glycoprotein gp67 (syn.: gp64) is the most abundant envelope surface glycoprotein of the AcNPV and is essential for the entry of baculovirus particles into host insect cells. This gene contains the most effective baculovirus-encoded signal sequences for protein secretion. The pAcGP67-A, -B, -C transfer vectors harbor the gp67 signal sequence followed by multiple cloning sites in a different reading frame for each vector. The gp67 signal peptide mediates the forced secretion of the recombinant protein, even if it is normally not secreted. During transport across the cell membrane, the signal peptide is cleaved and the recombinant protein can be purified from the supernatants of infected cell cultures.



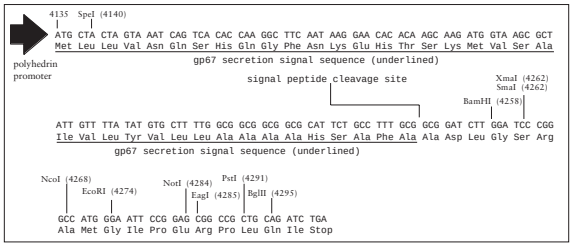
C



A



B



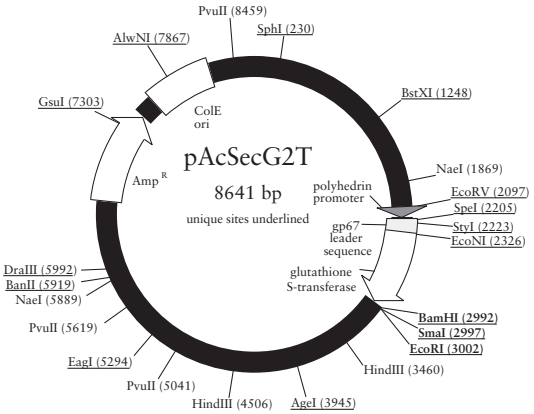
Baculovirus Transfer Vectors

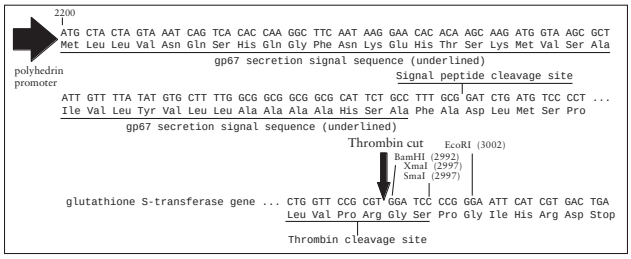
Secretory *(continued)*

pAcSecG2T

DESCRIPTION	SIZE	CAT. NO.
pAcSecG2T Baculovirus Transfer Vector	20 µg in 20 µl	554797

The pAcSecG2T transfer vector contains a gp67 signal sequence preceded by the polyhedrin promoter. Foreign genes are inserted downstream of the GST gene into one of the available restriction enzyme sites and in frame with the GST coding sequence. The gp67 signal sequence is cleaved from the fusion recombinant protein during its transport across the cell membrane, the recombinant GST fusion protein can be purified from the supernatant of infected insect cell cultures.



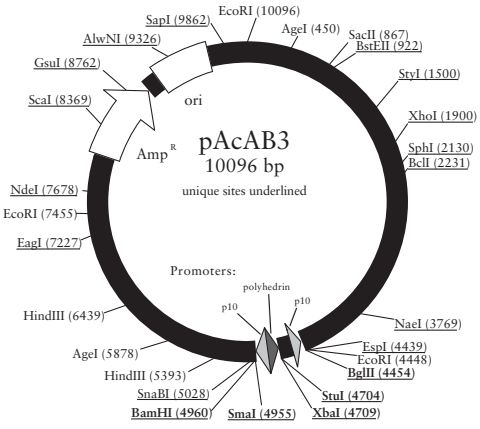


Multiple Polyhedrin Promoter Plasmids

pAcAB3

DESCRIPTION	SIZE	CAT. NO.
pAcAB3 Baculovirus Transfer Vector	5 µg in 50 µl	554755

pAcAB3 is a polyhedrin locus-based transfer vector that contains one copy of the polyhedrin promoter and two copies of p10 promoters. Downstream of the first p10 promoter are SmaI and BamHI cloning sites. Upstream of this, there is an inverted polyhedrin promoter with either an Xba I or Stu I cloning site and a Bgl II site, followed by a second p10 promoter and a BglII site. This transfer vector allows simultaneous expression of three foreign genes during the very late phase of the baculovirus infection cycle.



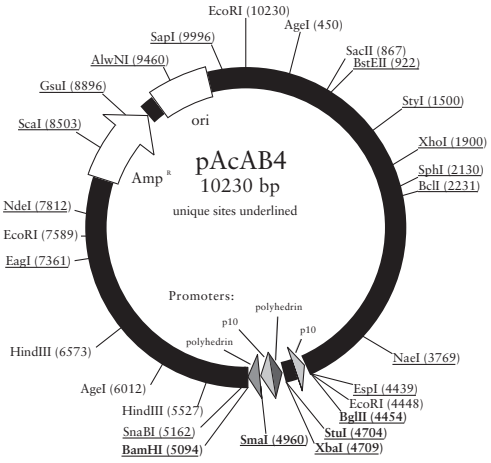
Baculovirus Transfer Vectors

Multiple Polyhedrin Promoter Plasmids *(continued)*

pAcAB4

DESCRIPTION	SIZE	CAT. NO.
pAcAB4 Baculovirus Transfer Vector	5 µg in 50 µl	554770

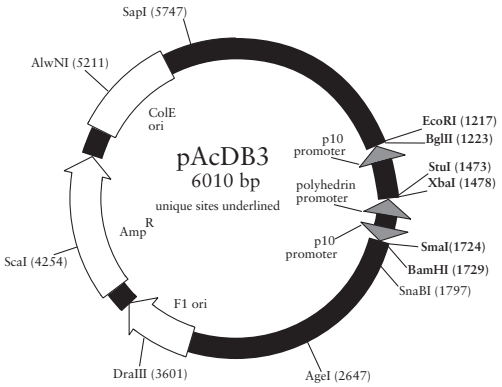
The pAcAB4, an AcNPV polyhedrin locus-based transfer vector, contains two copies each of p10 and polyhedrin promoters. One set of p10 and polyhedrin promoters is in tandem and downstream of but in opposite orientation to the other set of tandem polyhedrin and p10 promoters. The available cloning sites for each promoter are typed in boldface. Using the pAcAB4 transfer vector, four foreign genes can be simultaneously expressed in the very late phase of the virus infection cycle.



pAcDB3

DESCRIPTION	SIZE	CAT. NO.
pAcDB3 Baculovirus Transfer Vector	5 µg in 50 µl	554825

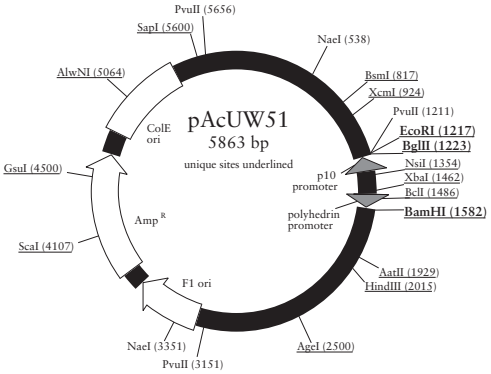
The pAcDB3 is a polyhedrin locus-based transfer vector derived from pAcAB3. The pAcDB3 has similar features as pAcAB3 with the exception of a smaller size and an additional EcoRI cloning site adjacent to Bgl II. This transfer vector allows simultaneous expression of three foreign genes during the very late phase of the baculovirus infection cycle.



pAcUW51

DESCRIPTION	SIZE	CAT. NO.
pAcUW51 Baculovirus Transfer Vector	5 µg in 50 µl	554747

pAcUW51 is a polyhedrin locus-based transfer vector that contains the p10 and polyhedrin promoters in opposite orientation. The BamH I cloning site is under the control of the polyhedrin promoter. The Bgl II and EcoR I sites are under the control of the p10 promoter. The restriction enzyme sites can be utilized for cloning and expression of heterologous genes. Recombinant viruses may simultaneously express two foreign proteins.



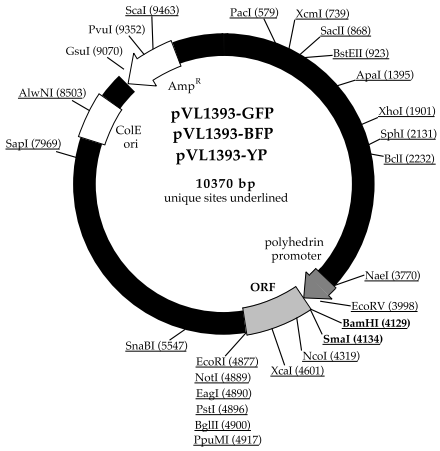
Baculovirus Transfer Vectors

GFP Baculovirus Vectors

pVL1393-GFP/BFP/YP

DESCRIPTION	SIZE	CAT. NO.
pVL1393-GFP/BFP/YP Baculovirus Control Vector Set	20 µg in 20 µl each	554813
pVL1393-GFP	20 µg in 20 µl	554810
pVL1393-BFP	20 µg in 20 µl	554811
pVL1393-YP	20 µg in 20 µl	554812

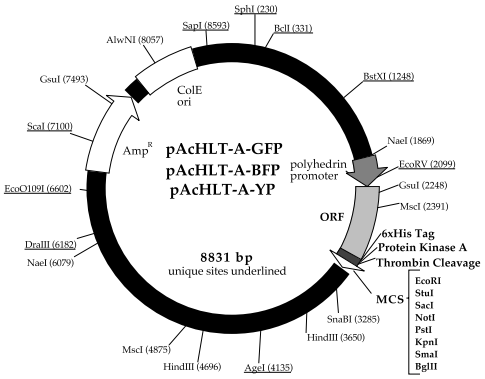
The set includes three vectors derived from pVL1393 containing GFP, BFP and YFP each. The GFP genes are expressed when cotransfected with BD BaculoGold™ Linearized Baculovirus DNA in insect cells. Heterologous genes can be inserted in-frame at the BamH I or Sma I sites, located at the N-termini of the GFP gene, and expressed as GFP fusion proteins. In addition, the GFP genes are flanked by several unique restriction enzyme sites, readily enabling their excision from the vector and allowing for easy cloning into other vectors as desired.



pAcHLT-A-GFP/BFP/YP

DESCRIPTION	SIZE	CAT. NO.
pAcHLT-A-GFP/BFP/YP Baculovirus Transfer Vector Set	20 µg in 20 µl each	554817
pAcHLT-A-GFP	20 µg in 20 µl	554814
pAcHLT-A-BFP	20 µg in 20 µl	554815
pAcHLT-A-YP	20 µg in 20 µl	554816

The set includes three vectors derived from pAcHLT-A plasmid containing GFP, BFP and YFP each and with a 6xHis tag followed by the multiple cloning site (MCS) downstream. The GFP genes are expressed when cotransfected with BD BaculoGold™ Linearized Baculovirus DNA in insect cells. Heterologous genes can be expressed as a GFP-6xHis tagged fusion protein when cloned in-frame with the GFP-6xHis tag.



BD Creator™ BacPAK9 Shuttle Vectors

Easy preparation of baculoviral shuttle constructs via Cre-loxP recombination

- BD Creator™ cloning is fast and efficient
- Vectors for native expression of proteins under optimal folding conditions
- Tagged expression vector provides easy purification with BD TALON™ Resins

Do you need to express your protein in a baculoviral system, use vectors that eliminate complicated subcloning procedures and let you proceed directly to expression in the shortest time possible? The new pLP-BacPAK9 and pLP-BacPAK9-6xHN Vectors from BD Biosciences Clontech do just that. These BacPAK9 Shuttle Vectors are BD Creator Acceptor Vectors that provide efficient subcloning and compatibility with our BD BaculoGold™ or BD BacPAK™ Baculovirus Expression Vector Systems from BD Biosciences.

BD Creator™ technology* ensures high-efficiency cloning

These vectors act as acceptor vectors for the BD Creator Gene Cloning and Expression System*. Both vectors contain the loxP sequence from the P1 bacteriophage, instead of a multiple cloning site. In BD Creator cloning, Cre Recombinase transfers a gene of interest from any BD Creator Donor Vector into any BD Creator Acceptor Vector in just 15 minutes without restriction digestion or ligation. This method of subcloning is extremely efficient.

For more information about the BD Creator™ Gene Cloning and Expression System or BD Talon™ Metal Affinity Resins, please call **877.232.8995 or visit **www.bdbiosciences.com/clontech***

Quickly focus on protein expression

After transferring your gene of interest to the expression cassette of the shuttle vector, you can express the protein as part of the baculoviral genome. The AcNPV sequences flanking the loxP site promote recombination with baculoviral DNA to transfer the expression cassette to the polyhedrin locus of the baculoviral genome.

Easy purification of 6xHN-tagged proteins with BD TALON™ Resins*

You can express a protein bearing a 6xHN tag with pLP-BacPAK9-6xHN. Once this protein is expressed, it can be easily purified using BD TALON™ Resin*, our patented cobalt-based, immobilized, metal affinity resin from BD Biosciences Clontech.

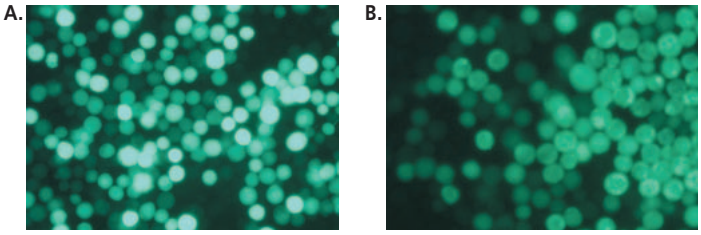
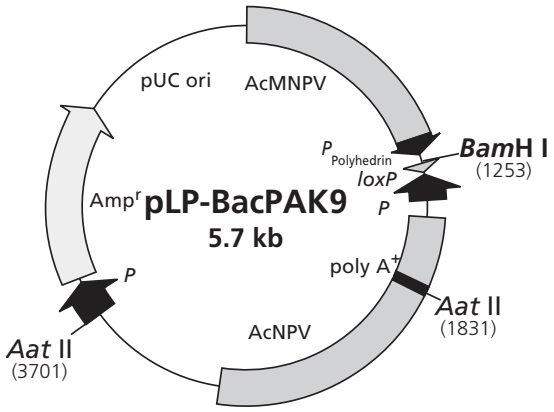


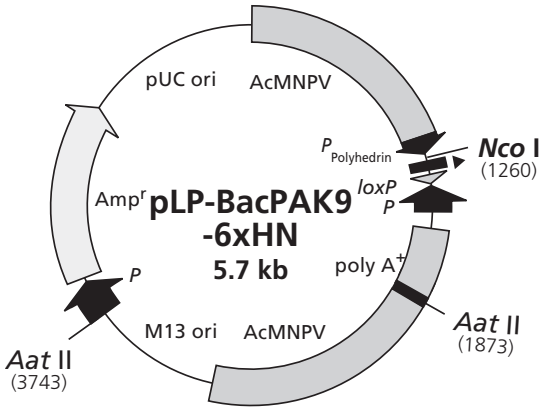
Figure 2. Expression of Enhanced Green Fluorescent Protein (EGFP) and 6xHN-tagged EGFP from BacPAK9 Shuttle Vector constructs in Spodoptera frugiperda (Sf21) cells.

pLP-BacPAK9 and pLP-BacPAK9-6xHN were used to generate pLP-BacPAK9-EGFP and pLP-BacPAK9-6xHN-EGFP respectively by rapid transfer of the EGFP gene from a Donor Vector. These recombinant vectors were then used to generate recombinant virus. Panel A. Shown above are Sf21 cells infected with recombinant virus. pLP-BacPAK9-EGFP. Panel B. pLP-BacPAK9-6xHN-EGFP.

pLP-BacPAK9



pLP-BacPAK9-6xHN



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BD Biosciences is pleased to offer an extended array of customized reagents and services to support your experiments from discovery research through to clinical trials. You are no longer restricted by the boundaries of regular products in our catalog. Simply choose the customized reagent or service you need.

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(Not all custom reagents suitable for clinical trial use – please inquire.)

Baculovirus Expression and Purification

1. Cloning of a gene of interest into a baculovirus transfer vector
   - Cutting the gene of interest out of a customer-supplied vector and subcloning it into a baculovirus transfer vector
   - Verification of the orientation of the subcloned insert by restriction mapping
   - Large-scale amplification of the recombinant baculovirus transfer vector

2. Generation, identification and purification of recombinant baculovirus
   - Co-transfection of the recombinant transfer vector with BD BaculoGold DNA
   - Isolation and plaque-purification of ten baculovirus recombinants

3. Amplification of recombinant baculovirus for high-titer stock
   - Low-titer stock solution from a single recombinant will be used to generate 500 ml of high-titer stock solution
   - Plaque assay to determine the titer

4. Large-scale expression of proteins from recombinant baculoviruses
   - One to ten liters of insect cells ($1x10^9$ cells/L) will be infected with the high titer virus stock solution

5. Protein purification (on request, only in combination with other services)
   - GST-fusion protein purification over glutathione affinity column
   - 6xHis-fusion protein purification over Ni-NTA affinity column
   - Immunoaffinity chromatography *(if antibody available)*

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