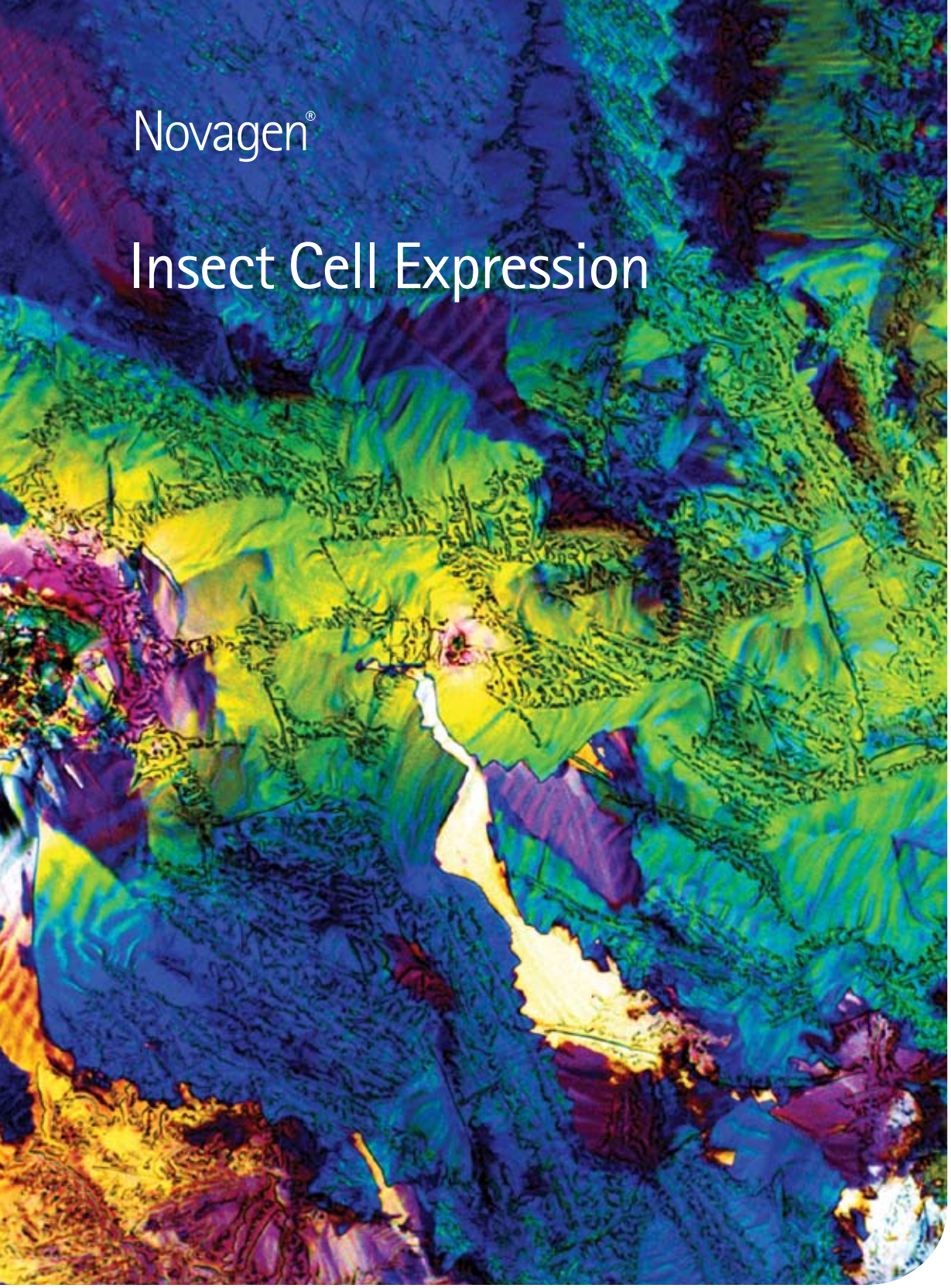

Novagen®

Insect Cell Expression

What's Inside:

MERCK

Insect Cell Expression Vector Selection Guide

	Vectors	Vector Size	Cat. No.	Enhancer/ Promoter(s)	Signal Seq.	Fusion Tags N-terminal	Fusion Tags C-terminal	Protease Cleavage Sites	Bacterial Expression	Insect Cell Expression InsectDirect	Insect Cell Expression BacMagic/ BacVector	Mammalian Expression
piEx	pIEx™-1	3897	71241	hr5/ie1		His•Tag®/S•Tag™	HSV•Tag®	Tb/Ek		✓		
	pIEx-1 Ek/LIC	3895	71237	hr5/ie1		His•Tag/S•Tag	HSV•Tag			✓		
	pIEx-2	4563	71238	hr5/ie1		GST•Tag™/His•Tag/S•Tag	HSV•Tag	Tb/Ek		✓		
	pIEx-2 Ek/LIC	4561	71240	hr5/ie1		GST•Tag/His•Tag/S•Tag	HSV•Tag	Tb/Ek		✓		
	pIEx-3	4629	71243	hr5/ie1	✓	GST•Tag/His•Tag/S•Tag	HSV•Tag	Tb/Ek		✓		
	pIEx-3 Ek/LIC	4627	71245	hr5/ie1	✓	GST•Tag/His•Tag/S•Tag	HSV•Tag	Tb/Ek		✓		
	pIEx-4	3780	71235	hr5/ie1			S•Tag/His•Tag			✓		
	pIEx-5	3843	71242	hr5/ie1	✓		S•Tag/His•Tag			✓		
	pIEx-6	3783	71333	hr5/ie1		His•Tag	S•Tag	Ek		✓		
	pIEx-7 Ek/LIC	3723	71339	hr5/ie1		His•Tag	S•Tag	Ek		✓		
	pIEx-8	3738	71555	hr5/ie1		Strep•Tag® II	His•Tag	Ek		✓		
	pIEx-8 Ek/LIC	3738	71339	hr5/ie1		Strep•Tag II	His•Tag	Ek		✓		
	pIEx-9	3747	71572	hr5/ie1		Strep•Tag II	His•Tag	3C/Tb		✓		
	pIEx-9 3C/LIC	3747	71573	hr5/ie1		Strep•Tag II	His•Tag	3C/Tb		✓		
	pIEx-10	3801	71557	hr5/ie1	✓	Strep•Tag II	His•Tag	Ek		✓		
	pIEx-10 Ek/LIC	3801	71574	hr5/ie1	✓	Strep•Tag II	His•Tag	Ek		✓		
pBiEx	pBiEx™-1	5475	71234	hr5/ie1, T7*lac*		His•Tag/S•Tag	HSV•Tag	Tb/Ek	✓	✓		
	pBiEx-2	6162	71233	hr5/ie1, T7*lac*		GST•Tag/His•Tag/S•Tag	HSV•Tag	Tb/Ek	✓	✓		
	pBiEx-3	5373	71232	hr5/ie1, T7*lac*			S•Tag/His•Tag		✓	✓		
NEW pIEx/Bac	pIEx/Bac™-1	6796	71724	hr5/ie1, p10		Strep•Tag II	His•Tag	Ek		✓	✓	
	pIEx/Bac-1 Ek/LIC	6751	71729	hr5/ie1, p10		Strep•Tag II	His•Tag	Ek		✓	✓	
	pIEx/Bac-2	6805	71725	hr5/ie1, p10		Strep•Tag II	His•Tag	3C/Tb		✓	✓	
	pIEx/Bac-2 3C/LIC	6766	71730	hr5/ie1, p10		Strep•Tag II	His•Tag	3C/Tb		✓	✓	
	pIEx/Bac-3	6802	71726	hr5/ie1, p10		His•Tag	Strep•Tag II	3C/Tb		✓	✓	
	pIEx/Bac-3 3C/LIC	6763	71731	hr5/ie1, p10		His•Tag	Strep•Tag II	3C/Tb		✓	✓	
pTriEx	pTriEx™-1.1	5301	70840	T7*lac*, p10, β-actin			HSV•Tag/His•Tag		✓		✓	✓
	pTriEx-2	5457	70826	T7*lac*, p10, β-actin		His•Tag/S•Tag	HSV•Tag/His•Tag	Tb/Ek	✓		✓	✓
	pTriEx-3	5082	70823	T7*lac*, p10, CMV			HSV•Tag/His•Tag		✓		✓	✓
	pTriEx-4	5238	70824	T7*lac*, p10, CMV		His•Tag/S•Tag	HSV•Tag/His•Tag	Tb/Ek	✓		✓	✓
	pTriEx-4 Ek/LIC	5238	70905	T7*lac*, p10, CMV		His•Tag/S•Tag	HSV•Tag/His•Tag	Tb/Ek	✓		✓	✓
	pTriEx-5	5061	71558	T7*lac*, p10, CMV		Strep•Tag II	His•Tag	Ek	✓		✓	✓
	pTriEx-5 Ek/LIC	5061	71575	T7*lac*, p10, CMV		Strep•Tag II	His•Tag	Ek	✓		✓	✓
	pTriEx-6	5070	71559	T7*lac*, p10, CMV		Strep•Tag II	His•Tag	3C/Tb	✓		✓	✓
	pTriEx-6 3C/LIC	5070	71577	T7*lac*, p10, CMV		Strep•Tag II	His•Tag	3C/Tb	✓		✓	✓
	pTriEx-7	5124	71560	T7*lac*, p10, CMV	✓	Strep•Tag II	His•Tag	Ek	✓		✓	✓
	pTriEx-7 Ek/LIC	5124	71576	T7*lac*, p10, CMV	✓	Strep•Tag II	His•Tag	Ek	✓		✓	✓
pBAC	pBAC™-1	5259	70003	*polh*			His•Tag				✓	
	pBACgus-1	7408	70054	*polh*			His•Tag				✓	
	pBAC-2cp	5411	70004	*polh*		His•Tag/S•Tag	His•Tag	Tb/Ek			✓	
	pBAC-2cp Ek/LIC	5409	70021	*polh*		His•Tag/S•Tag	His•Tag	Tb/Ek			✓	
	pBACgus-2cp	7560	70049	*polh*		His•Tag/S•Tag	His•Tag	Tb/Ek			✓	
	pBACgus-2cp Ek/LIC	7558	70051	*polh*		His•Tag/S•Tag	His•Tag	Tb/Ek			✓	
	pBAC-3	5474	70088	*polh*	✓	His•Tag/S•Tag	His•Tag	Tb/Ek			✓	
	pBACgus	7623	70089	*polh*	✓	His•Tag/S•Tag	His•Tag	Tb/Ek			✓	
	pBAC-5	5917	70222	gp64		His•Tag/S•Tag	His•Tag	Tb/Ek			✓	
	pBACgus-5	8066	70223	gp64		His•Tag/S•Tag	His•Tag	Tb/Ek			✓	
	pBAC-6	5517	70224	gp64	✓	His•Tag/S•Tag	His•Tag	Tb/Ek			✓	
	pBACgus-6	7666	70225	gp64	✓	His•Tag/S•Tag	His•Tag	Tb/Ek			✓	
	pBAC4x-1	5580	70045	*polh*, p10							✓	
	pBACgus4x-1	7729	70060	*polh*, p10							✓	
	pBACsurf-1	9429	70055	*polh*	✓		gp64				✓	

Complete vector sequences and maps are available at www.novagen.com/vector

For more information or to place an order, contact your local office (see back cover).

Table of Contents

Abbreviations:

AcNPV: *Autographa californica* nuclear polyhedrosis virus
3C: HRV 3C protease from human rhinovirus
BAC: bacterial artificial chromosome, except for pBAC, which is a baculovirus plasmid
CMV: cytomegalovirus promoter
DTT: dithiothreitol
Ek: catalytic subunit of enterokinase
hr5: transcriptional enhancer, AcNPV homologous region 5
HT: high throughput
ie1: AcNPV immediate early promoter
LIC: ligation-independent cloning
MOI: multiplicity of infection
ORF: open reading frame
p10: an AcNPV very late promoter
polh: AcNPV polyhedrin gene and very late promoter
T7*lac:* promoter and operator used for protein expression in *E. coli*
Tb: thrombin endoprotease

On the cover: Crystallized carnitine viewed with polarized light. Carnitine is an unusual amino acid derivative that acts to transport long-chain fatty acids across the inner mitochondrial membrane. Carnitine is a quaternary amine synthesized in the human liver from methionine and lysine, and is not utilized for protein synthesis. For more information, visit http://micro.magnet.fsu.edu/aminoacids/pages/carnitine.html.

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Insect Cell Expression Overview

InsectDirect™ System *(page 7)*

Baculovirus-mediated expression of recombinant proteins in insect cells is a valuable tool for producing soluble, active proteins. However, the traditional technique of generating recombinant baculovirus can be time consuming and laborious. To accelerate the process of protein expression in insect cells, the InsectDirect™ System provides rapid, plasmid-mediated expression to generate small to moderate amounts of recombinant protein without creating recombinant baculovirus. This system is ideal for high throughput (HT) expression screening in insect cells.

InsectDirect System components are sold separately:

- **pIEx/Bac™ series of dual-purpose vectors** *(page 9)*
  For both plasmid-mediated expression and robust baculovirus expression
- **pIEx™ series of early expression vectors** *(page 8)*
  For rapid, high-yield protein expression in insect cells, without creating recombinant baculovirus
- **pBiEx™ series of multisystem expression vectors** *(page 8)*
  For rapid plasmid-based characterization/expression of target genes in both *E. coli* and insect cells
- **Insect GeneJuice® Transfection Reagent** *(page 14)*
  For high-efficiency transfection of insect cells with minimal toxicity
- **Sf9 Insect Cells and BacVector® Insect Cell Medium** *(pages 14,15)*
  For optimized serum-free insect cell growth
- **Insect RoboPop™ Ni-NTA His•Bind® Purification Kit** *(page 17)*
  For HT His•Tag® fusion protein purification

BacMagic™ System *(page 10)*

For situations requiring a baculovirus approach, BacMagic™ DNA provides faster baculovirus production by eliminating the time-consuming and labor-intensive plaque purification steps. BacMagic DNA is an engineered AcNPV genome that prevents nonrecombinant parental virus from replicating in insect cells, saving up to 2 weeks for virus production. BacMagic is compatible with pIEx/Bac, pBAC™, pTriEx™, and other transfer plasmids using the *lef2*/603 and ORF1629 sites for recombination into the baculovirus genome. The BacMagic Transfection Kit contains all necessary components, except the transfer vector.

BacMagic System is available as:

- BacMagic DNA Kit: includes BacMagic DNA, Insect GeneJuice Transfection Reagent, and a Transfection Control Plasmid
- BacMagic Transfection Kit: includes, in addition to the components in the DNA kit, Sf9 Insect Cells and BacVector Insect Cell Medium

NEW pIEx/Bac™ Vectors *(page 9)*

Previously, InsectDirect™ and BacMagic™ methods required different vectors for high-yield protein expression. The new pIEx/Bac™ series of dual-purpose vectors is compatible with both methods, providing flexibility and greater optimization for insect cell expression. The pIEx/Bac vectors can be used with the InsectDirect System to rapidly screen clones and also can be used to produce recombinant baculovirus for robust protein expression.

pIEx/Bac dual-purpose vectors for:

- High throughput target protein screening in the InsectDirect System
- Producing recombinant baculovirus in the BacMagic or BacVector® System

BacVector® System *(page 13)*

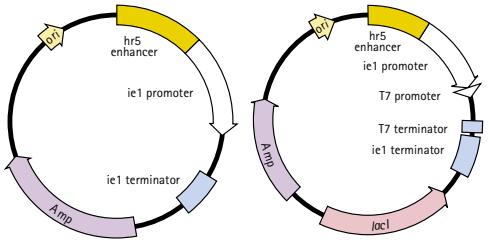
For traditional baculovirus expression of high levels of recombinant protein, the BacVector System offers a complete set of reagents and kits based on optimally engineered vectors. For the BacVector-3000, seven nonessential viral genes have been removed to increase target expression and reduce proteolysis and degradation of the target protein.

The BacVector System components available separately:

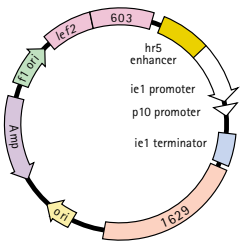
- BacVector Transfection Kits with a choice of BacVector Triple Cut Virus DNA, Insect GeneJuice® Transfection Reagent, and Sf9 Insect Cells *(page 14)*
- pIEx/Bac, pTriEx™, pBAC™, and pBACgus transfer plasmids and ligation-independent cloning (LIC) kits *(page 9,11,12, and 12, respectively)*
- Sf9 Insect Cells and BacVector Insect Cell Medium *(pages 14 ,15)*

Insect Cell Expression Systems

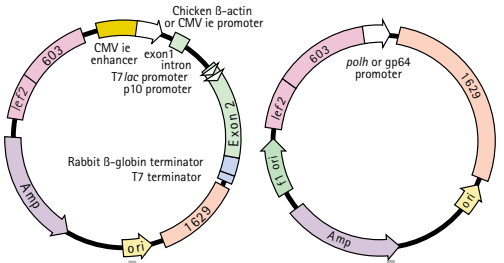
pIEx or pBiEx



NEW pIEx/Bac



pTriEx or pBAC



Plasmid-based (transfection)

InsectDirect™ System

Day 1	Transfect Sf9 Insect Cells with pIEx™ or pIEx/Bac™ recombinant plasmid for protein expression
Day 3	Proceed with purification

48 hours

Recombinant Baculovirus (infection)

BacMagic™ System

Day 1	Co-transfect insect cells with recombinant transfer plasmid plus AcNPV BacMagic™ DNA
Day 5	Harvest recombinant baculovirus; screen for expression; amplify viral stock (titer stock, optional)
Day 8	Infect insect cells and express protein
Day 11	Proceed with purification

10 days

BacVector® System

Day 1	Co-transfect insect cells with recombinant transfer plasmid plus linearized AcNPV BacVector® DNA
Day 4	Choose plaque and replate
Days 8	Choose plaque and amplify
Day 11	Screen for expression; amplify
Day 14	Titer viral stock; optimize MOI (optional)
Day 18	Infect insect cells and express protein
Day 21	Proceed with purification

20 days

For more information or to place an order, contact your local office (see back cover).

InsectDirect™ System

For high throughput (HT) expression screening, researchers have traditionally used *E. coli* systems for target protein expression. Insect cell systems offer advantages, such as improved target protein solubility and important post-translational modifications for increased activity, but previously were too time consuming for HT applications. The InsectDirect™ System uses plasmid-based target protein expression with optimal promoter/enhancer combinations in insect cells to provide rapid, high protein yields that are ideal for HT applications.

Features

- pIEx/Bac™, pIEx™, and pBiEx™ vectors contain optimal hr5/ie1 enhancer/promoter for plasmid-mediated target protein expression
- Insect GeneJuice® Transfection Reagent for efficient, non-toxic transfection
- Sf9 Insect Cells and serum-free BacVector® Insect Cell Medium for optimal cell growth
- Insect PopCulture® Reagent for total culture lysis without centrifugation
- Insect RoboPop™ Ni-NTA His•Bind® Purification Kit for HT His•Tag® fusion protein purification

For additional information and data, please visit www.novagen.com/insectdirect

FAQ

Which cell lines have been tested in the InsectDirect System?

Sf9, Sf21, S2, and High Five™ cells have all been successfully used with the InsectDirect System. Best results are obtained with Sf9 and Sf21, in passages 5 through 20.

Which media are compatible with the InsectDirect System?

We recommend using BacVector Insect Cell Medium for optimal results, however the following media have been successfully used with the InsectDirect System:

- TriEx™ Insect Cell Medium (page 15)
- ESF 921
- Grace's Insect Medium
- HyQ® SFX-Insect™
- Gibco® Sf-900 II SFM
- EX-CELL® 420 Insect Serum-Free Media
- Hink's TNM-FH Insect Medium

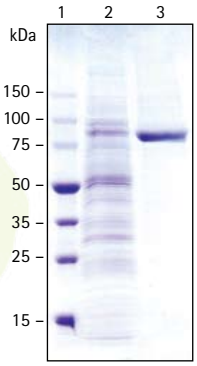
Do I need to generate recombinant virus using the InsectDirect System?

No. The InsectDirect System is a virus-free approach. Cells are transiently transfected with pIEx/Bac, pIEx, or pBiEx constructs carrying your gene of interest using Insect GeneJuice Transfection Reagent. Plasmid-based expression typically peaks between 48-72 hours after transfection.

What types of proteins have been expressed using the InsectDirect System?

We have expressed more than 40 proteins, including cytoplasmic protein kinases or regions of a receptor with kinase activity, kinase interacting proteins, phospolipases, nuclear transport proteins, phosphatases, and heat shock proteins. Yields were as high as 8 mg from 100 ml culture.

Protein kinase, 1-liter scale



Lane	Sample
1	Perfect Protein™ Markers, 15–150 kDa
2	Protein kinase, total cell protein
3	Protein kinase, eluate (13.3 mg yield)

Four 250-ml suspension cultures of Sf9 cells at 1 x 10^6 cells/ml (final volume and cell density) were transfected with a pIEx/protein kinase construct using Insect GeneJuice Transfection Reagent. After 48 h incubation at 28°C, Insect PopCulture Reagent and Benzonase® Nuclease were added directly to each flask and incubated to lyse the cells. The His•Tag fusion protein was purified on Ni-NTA His•Bind Resin according to the standard protocol. Protein yield, 13.3 mg, was quantified by a modified Bradford assay.

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pIEx™ and pBiEx™ Expression Vectors

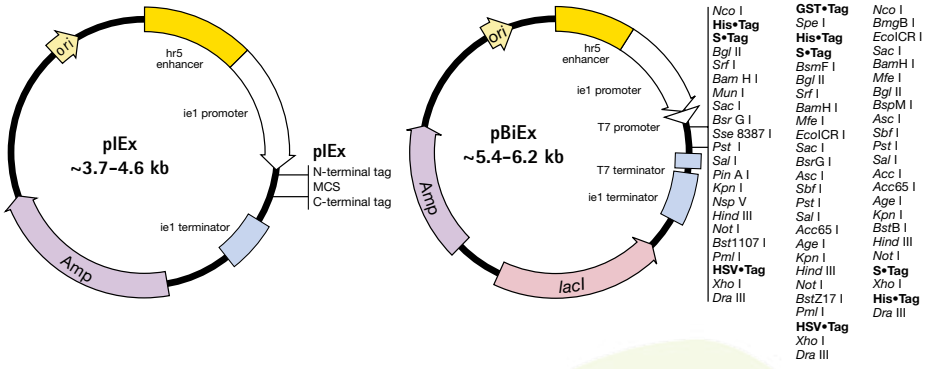
pIEx™ Vectors

The pIEx™ vectors are designed for rapid, high-yield protein expression in insect cells, without the time-consuming process of creating recombinant baculovirus. The vectors feature the hr5 enhancer and the ie1 (immediate early) promoter to direct expression in insect cells using endogenous insect cell transcription machinery. Ten vectors offer a variety of N-terminal and C-terminal fusion tags and secretion signals (Vector Selection Guide, page 2). Choice of cloning sites also provides the option of expressing proteins without fusion tags. pIEx-1, -2 , -3, -7, -8, -9, and -10 are available as LIC-prepared vectors. LIC-prepared vectors provide efficient directional cloning of PCR products for subsequent expression.

pBiEx™ Vectors

The pBiEx™ vectors are designed for protein expression in both *E. coli* and insect cells. The vectors feature the hr5 enhancer and the ie1 promoter, to direct expression in insect cells, and the tightly controlled T7*lac* promoter, for expression in *E. coli*. In insect cells, the level of target protein expressed in pBiEx vectors is suitable for expression screening, however for higher-level expression for protein production we recommend using a pIEx vector. Three pBiEx vectors offer different N-terminal and C-terminal fusion tags. Choice of cloning sites also provides the option of expressing proteins without fusion tags.

Complete vector sequences and maps are available at www.novagen.com/vector



Product	Size	Cat. No.	Price
pIEx™-1 DNA	20 µg	71241-3	
pIEx™-1 Ek/LIC Vector Kit	20 rxn	71237-3	
pIEx™-2 DNA	20 µg	71238-3	
pIEx™-2 Ek/LIC Vector Kit	20 rxn	71240-3	
pIEx™-3 DNA	20 µg	71243-3	
pIEx™-3 Ek/LIC Vector Kit	20 rxn	71245-3	
pIEx™-4 DNA	20 µg	71235-3	
pIEx™-5 DNA	20 µg	71242-3	
pIEx™-6 DNA	20 µg	71333-3	
pIEx™-7 Ek/LIC Vector Kit	20 rxn	71339-3	
pIEx™-8 DNA	20 µg	71555-3	
pIEx™-8 Ek/LIC Vector Kit	20 rxn	71572-3	
pIEx™-9 DNA	20 µg	71556-3	
pIEx™-9 3C/LIC Vector Kit	20 rxn	71573-3	
pIEx™-10 DNA	20 µg	71557-3	
pIEx™-10 Ek/LIC Vector Kit	20 rxn	71574-3	
pBiEx™-1 DNA	20 µg	71234-3	
pBiEx™-2 DNA	20 µg	71233-3	
pBiEx™-3 DNA	20 µg	71232-3	
pIE1-neo DNA	10 µg	70171-3	

Ek/LIC Vector Kit Components

Cat. Nos. 71237, 71240, 71245, 71339, 71572, 71573, 71574

• 1 µg	Ek/LIC Vector or 3C/LIC Vector
• 8 µl	β-Gal Control Insert
• 25 U	LIC-qualified T4 DNA Polymerase
• 50 µl	T4 DNA Polymerase Buffer
• 100 µl	100 mM DTT
• 40 µl	25 mM dATP
• 50 µl	25 mM EDTA
• 1.5 ml	Nuclease-free Water
• 22 × 50 µl	NovaBlue GigaSingles™ Competent Cells
• 5 × 2 ml	SOC Medium
• 10 µl	Test Plasmid

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Insect GeneJuice® Transfection Reagent
for optimal transfection efficiency

FAQ

How do expression levels compare between pIEx, pBiEx, and pIEx/Bac (page 9) vectors?

Expression in insect cells is slightly lower from pBiEx and pIEx/Bac vectors than from pIEx vectors. For the highest-level expression for protein production following transient transfection, we recommend using a pIEx vector.

Can pIEx, pBiEx, or pIEx/Bac vectors be used to create stable cell lines?

Yes, by co-transfecting pIE1-neo with pIEx, pBiEx, or pIEx/Bac containing your target gene, and selecting with G418, a stable cell line can be created.

For more information or to place an order, contact your local office (see back cover).

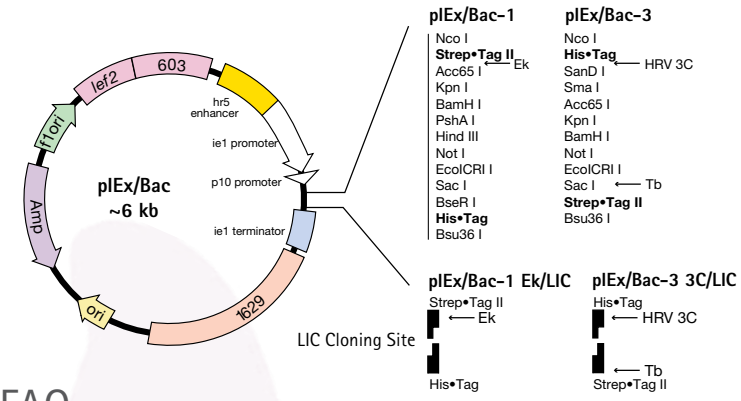
pIEx/Bac™ Expression Vectors

pIEx/Bac™ dual-purpose vectors contain 2 *Autographa californica* nuclear polyhedrosis virus (AcNPV) promoters. The hr5 enhancer/ie1 promoter combination is used for plasmid-mediated and early baculovirus-mediated expression. Following the generation of recombinant baculovirus, the AcNPV p10 very late promoter is used for robust baculovirus-mediated late/very late expression. The unique pIEx/Bac vectors are compatible with both InsectDirect™ transfection for plasmid-mediated expression (page 7) and with the BacMagic™ System (page 10) or the BacVector® System (page 13) for baculovirus infection and expression. Because the new pIEx/Bac vectors are compatible with both plasmid-mediated and baculovirus expression, it is easy to use a single construct in both expression systems for HT screening and optimized high-yield protein expression.

Features

- AcNPV hr5/ie1 promoter/enhancer for plasmid-mediated and early baculovirus expression
- AcNPV p10 very late promoter for late baculovirus expression
- Use in InsectDirect System for HT screening
- Use in BacMagic or BacVector System for robust protein expression
- N-terminal and C-terminal fusion tags for dual purification strategies
- 2 pIEx/Bac versions for optimal cloning, purification, and fusion tag removal
- Available as LIC Vector Kits for efficient, flexible ligation-independent cloning

Complete vector sequences and maps are available at www.novagen.com/vector



Product	Size	Cat. No.	Price
pIEx/Bac™-1 DNA	20 µg	71724-3	
pIEx/Bac™-1 Ek/LIC Vector Kit	20 rxn	71729-3	
pIEx/Bac™-3 DNA	20 µg	71726-3	
pIEx/Bac™-3 3C/LIC Vector Kit	20 rxn	71731-3	

Components

Cat. Nos. 71729, 71731

• 1 µg	Ek/LIC Vector or 3C/LIC Vector
• 8 µl	β-Gal Control Insert
• 25 U	LIC-qualified T4 DNA Polymerase
• 50 µl	T4 DNA Polymerase Buffer
• 100 µl	100 mM DTT
• 40 µl	25 mM dATP
• 50 µl	25 mM EDTA
• 1.5 ml	Nuclease-free Water
• 22 × 50 µl	NovaBlue GigaSingles™ Competent Cells
• 5 × 2 ml	SOC Medium
• 10 µl	Test Plasmid

page 14

Insect GeneJuice® Transfection Reagent
for optimal transfection efficiency

Dual-purpose pIEx/Bac vector for plasmid-mediated or baculovirus-mediated protein expression in insect cells

All pIEx/Bac vectors carry AcNPV-derived virus regions to mediate recombination. The recombination region upstream of the hr5/ie1/p10 enhancer/promoter combination is *lef2* and ORF603, while the recombination region downstream of the ie1 terminator is a partial ORF1629. The presence of "gentle elution" fusion tags at both the N-terminus and C-terminus of proteins expressed from these vectors is ideal for dual purification strategies designed to isolate full-length fusion proteins (Fiedler). The HRV 3C protease is highly specific for cleavage of the sequence LEVLFQ↓GP (Cordingley), and is active at low temperatures (Wang).

References:

Cordingley, M.G. et al. 1989. *J. Virol.* **63,** 5037–5045.
Fiedler, M. et al. 2002. *Protein Eng.* **15,** 931–941.
Wang, Q.M. et al. 1997. *Anal. Biochem.* **252,** 238–245.

FAQ

How is target protein expression from pIEx/Bac-derived baculovirus different than from traditional p10 or polyhedrin (*polh*) promoter-based transfer vectors?

Transfer vectors that only carry the p10 or *polh* promoter only direct expression in the late/very late phase of baculovirus infection. In contrast, baculovirus created with the unique pIEx/Bac vectors express target protein throughout the infection process. Expression in the early phase is directed by the hr5 enhancer/ie1 promoter combination and expression in the late/very late phase is directed by the p10 promoter. When expressing a sensitive target protein or a protein requiring uniform post-translational modification, harvesting earlier in the infection process may be optimal.

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BacMagic™ System

The BacMagic™ System improves on the traditional method for generating recombinant baculoviruses by eliminating time-consuming plaque purification. BacMagic DNA is an AcNPV genome with a portion of the essential open reading frame (ORF) 1629 deleted and a bacterial artificial chromosome (BAC) in place of the polyhedrin (*polh*) coding region. This combination prevents nonrecombinant virus from replicating in insect cells, yet allows the viral DNA to be propagated as circular DNA in bacterial cells. A compatible recombinant transfer plasmid, such as a pIEx/Bac™, pBAC™, or pTriEx™ vector containing the target coding sequence, is cotransfected with BacMagic DNA into insect cells. Homologous recombination within the cells restores the function of the viral ORF1629 and replaces the BAC sequence in the BacMagic DNA with the promoter and target coding sequence. Only this recombinant baculovirus can replicate in insect cells, producing a homogeneous population of recombinant baculovirus. The BacMagic DNA Kit includes BacMagic DNA, Insect GeneJuice® Transfection Reagent, and a positive control plasmid. The BacMagic Transfection Kit contains the same components as the DNA kit and adds Sf9 Insect Cells and BacVector® Insect Cell Medium, all of which have been qualified for optimal transfection performance.

Product	Size	Cat. No.	Price
BacMagic™ DNA Kit	5 rxn	71545-3	
BacMagic™ Transfection Kit	5 rxn	71546-3	

Components

Cat. No. 71545
- 0.5 µg BacMagic DNA
- 50 µl Insect GeneJuice Transfection Reagent
- 2 µg Transfection Control Plasmid

Cat. No. 71546
- 0.5 µg BacMagic DNA
- 50 µl Insect GeneJuice Transfection Reagent
- 2 × 1 ml Sf9 Insect Cells
- 1 L BacVector Insect Cell Medium
- 2 µg Transfection Control Plasmid

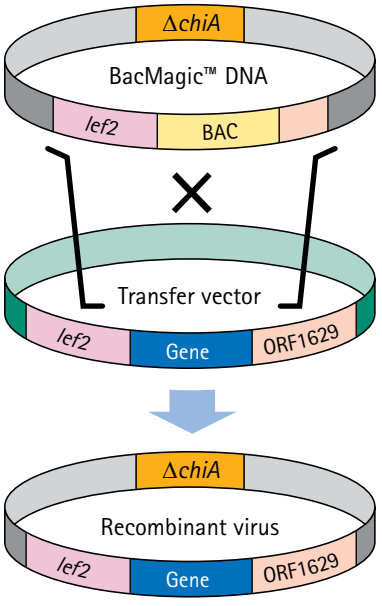
Features

- Faster baculovirus production without tedious plaque purification
- Compatible with pIEx/Bac, pBAC, pTriEx, and other transfer plasmids using the *lef2*/603 and ORF1629 recombination sites
- Deletion of chitinase to maximize secreted and membrane-targeted protein production
- Includes Insect GeneJuice Transfection Reagent for high efficiency transfection

Complete vector sequences and maps are available at www.novagen.com/vector

Protocol on page 25

BacMagic™ homologous recombination scheme



FAQ

With the pIEx/Bac vectors, how does plasmid-based expression compare with baculovirus expression for protein yields?

In some cases, the amount of purified protein obtained from each system (InsectDirect™ System for plasmid-based or BacMagic/BacVector System for baculovirus) was similar, while in other cases a significant enhancement in yield was obtained by conversion to a baculovirus system.

pTriEx™ Multisystem Expression Vectors

Unique pTriEx™ multisystem expression vectors enable optimal protein expression in bacterial, insect, and mammalian cells from a single plasmid. Proven features of pET, baculovirus, and mammalian expression systems are combined in the pTriEx series of vectors to provide the benefits of each system in one versatile vector. These vectors have been extensively tested with various target genes in all 3 expression systems. In each case, expression was similar to that obtained with system-specific vectors.

For inducible expression in *E. coli*, the pTriEx vectors contain the tightly controlled T7*lac* promoter, a ribosome binding site, and the pUC replication origin for high plasmid yield. For high-level expression in baculovirus-infected insect cells, the vectors contain the *lef2*/603 and ORF1629 sites for recombination into the baculovirus genome and use the p10 baculovirus promoter. Expression in mammalian cells is driven by either a chicken β-actin promoter or a CMV ie promoter, along with the CMV ie enhancer and rabbit β-globin polyadenylation signals. RNA generated from these vectors contains an intron designed to facilitate mRNA processing and export in mammalian cells, and an optimal translation initiation consensus sequence for mammalian cell expression.

Other TriEx vector features include:

- Blunt-end restriction sites at both ends of the MCS for all 3 reading frames to facilitate in-frame cloning
- Choice of 7 vectors with different N-terminal and C-terminal fusion tag sequences for convenient detection and purification
- Protease cleavage sites for effective removal of N-terminal fusion tags
- For stable expression in mammalian cells, pTriEx vectors encoding hygromycin- or neomycin-resistant genes are available

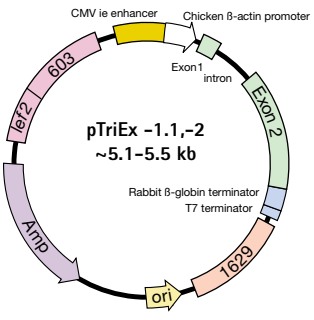
Complete vector sequences and maps are available at www.novagen.com/vector

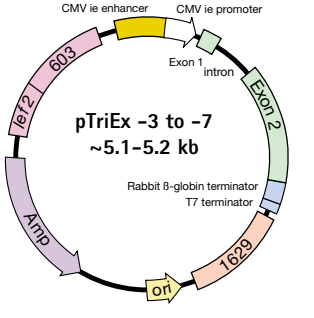
Product	Size	Cat. No.	Price
pTriEx™-1.1 DNA	20 µg	70840-3	
pTriEx™-2 DNA	20 µg	70826-3	
pTriEx™-3 DNA	20 µg	70823-3	
pTriEx™-4 DNA	20 µg	70824-3	
pTriEx™-4 Ek/LIC Vector Kit	20 rxn	70905-3	
pTriEx™-5 DNA	20 µg	71558-3	
pTriEx™-5 Ek/LIC Vector Kit	20 rxn	71575-3	
pTriEx™-6 DNA	20 µg	71559-3	
pTriEx™-6 3C/LIC Vector Kit	20 rxn	71576-3	
pTriEx™-7 DNA	20 µg	71560-3	
pTriEx™-7 Ek/LIC Vector Kit	20 rxn	71577-3	
pTriEx™-1.1 Hygro DNA	20 µg	70928-3	
pTriEx™-1.1 Neo DNA	20 µg	70927-3	
pTriEx™-2 Hygro DNA	20 µg	70930-3	
pTriEx™-2 Neo DNA	20 µg	70929-3	
pTriEx™-3 Hygro DNA	20 µg	70932-3	
pTriEx™-3 Neo DNA	20 µg	70931-3	
pTriEx™-4 Hygro DNA	20 µg	70934-3	
pTriEx™-4 Neo DNA	20 µg	70933-3	
TriEx™UP Primer	500 pmol	70846-3	
TriEx™ Down Primer	500 pmol	70847-3	

Components

Cat. No. 70905, 71575, 71576, 71577

• 1 µg	Ek/LIC Vector or 3C/LIC Vector
• 8 µl	β-Gal Control Insert
• 25 U	T4 DNA Polymerase, LIC-qualified
• 50 µl	10X T4 DNA Polymerase Buffer
• 100 µl	100 mM DTT
• 50 µl	25 mM EDTA
• 40 µl	25 mM dATP
• 1.5 ml	Nuclease-free Water
• 22 × 50 µl	NovaBlue GigaSingles™ Competent Cells
• 0.2 ml	Origami™ B(DE3)pLacI Competent Cells
• 5 × 2 ml	SOC Medium
• 10 µl	Test Plasmid





pTriEx-1.1
pTriEx-3

T7*lac*
p10
Nco I
Eco R V
Sma I
Ecl 136 I
Bam H I
Eco R I
Bgl II
Asc I
Bss H I*
Pst I
Sse 8387 I
Kpn I
Pin A I
Nsp V
Hin d III
Not I
Eag I*
Pvu II
Bst 1107 I
Pml I
HSV•Tag
Xho I
His•Tag
Dra III
Bsu 36 I

*not unique in pTriEx-1.1. or pTriEx-2

pTriEx-2
pTriEx-4

T7*lac*
p10
Nco I
His•Tag
Xcm I
S•Tag
thrombin site
Sma I ← Ek
Psh A I
Bse R I
Eco R V
Ecl 136 I
Bam H I
Eco R I
Bgl II
Asc I
Bss H I*
Pst I
Sse 8387 I
Kpn I
Pin A I
Nsp V
Hin d III
Not I
Eag I*
Pvu II
Bst 1107 I
Pml I
HSV•Tag
Xho I
His•Tag
Dra III
Bsu 36 I

pTriEx-5

T7*lac*
p10
Nco I
Strep•Tag II
*Bst*B I ← Ek
*Acc*65 I
Kpn I
*Bam*H I
*Psh*A I
*Hin*d III
Not I
Eag I
*Eco*CRI I
Sac I
*Bse*R I
His•Tag
Dra III
*Bsu*36 I

pTriEx-6

T7*lac*
p10
Nco I
Strep•Tag II
*Bst*B I ← HRV 3C
*Eco*N I
*San*D I
Xma I
Sma I
*Acc*65 I
Kpn I
*Bam*H I
Not I
Eag I
*Eco*CRI I
Sac I ← thrombin
His•Tag
Avr II
Dra III
*Bsu*36 I

pTriEx-7

T7*lac*
p10
IgM signal sequence
Strep•Tag II
*Acc*65 I ← Ek
Kpn I
*Bam*H I
*Psh*A I
*Hin*d III
Not I
Eag I
Sac I
*Eco*CRI I
*Bse*R I
His•Tag
Dra III
*Bsu*36 I

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pBAC™ Baculovirus Transfer Plasmids

The pBAC™ series of baculovirus transfer plasmids are designed to facilitate cloning of target genes for cotransfection with baculovirus genomic DNA (BacMagic™ DNA page 10, BacVector® Kits page 13) and subsequent recombination at the *lef2*/603 and ORF1629 sites and expression in insect cells. Most pBAC plasmids are also available as gus versions containing the β-glucuronidase marker gene to screen for recombinant plaques.

Complete vector sequences and maps are available at www.novagen.com/vector

High-level expression from *polh* promoter

The pBAC-1, -2cp, and -3 transfer plasmids are designed for high-yield protein expression directed by the *polh* promoter during the very late phase of infection. The pBAC-3 plasmid encodes the 21–amino acid gp64 signal peptide sequence to direct target proteins into the secretory pathway in infected insect cells. For additional vector information please refer to the Vector Selection Guide on page 2.

Coexpression from 1 recombinant virus

The pBAC4x plasmids contain 2 copies of the *polh* promoter and 2 copies of the p10 promoter and are designed for coexpression of up to 4 genes during the very late phase of infection. These vectors are extremely useful for the expression of multisubunit proteins, multiple copies of a gene, multiprotein complexes, and for protein:protein interactions studies (Weyer, Belyaev 1993, 1995).

Early expression for glycoproteins, secreted proteins, and highly processed proteins

The pBAC-5 and pBAC-6 transfer plasmids contain a modified gp64 tandem promoter that acts as both an immediate early promoter, for expression soon after infection, and a late promoter, for continued expression in the late phase of infection. With early expression, higher levels of biologically active target proteins can be produced before virus-induced cytopathic effects compromise the function of protein processing pathways, particularly glycosylation and secretion pathways (Jarvis).

Baculovirus surface display of target proteins

The pBACsurf-1 transfer plasmid is designed for in-frame insertion of target genes between the gp64 signal peptide sequence and the mature gp64 protein coding sequence. If the in-frame insert lacks a stop codon, target proteins can be displayed on virus or cell surfaces. Directed by the *polh* promoter, expressed fusion proteins, including glycoproteins, are secreted and anchored by the transmembrane domain of gp64 onto the virion surface.

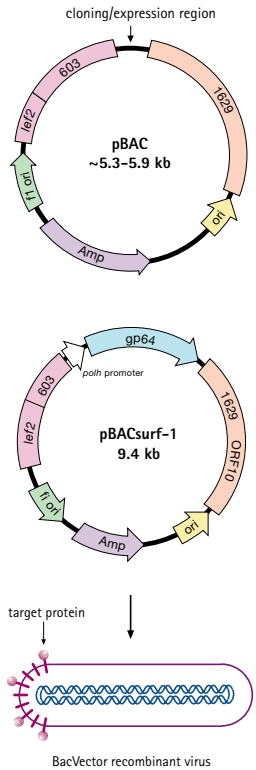
Features

- Use *lef2*/603 and ORF1629 sites for recombination
- Compatible with BacVector-1000, -2000, and -3000 Triple Cut DNA and BacMagic DNA
- High-copy plasmid origin of replication
- pBACgus versions to color screen for recombinant baculovirus plaques using X-Gluc *(page 15)*

Product	Size	Cat. No.	Price
pBAC™-1 DNA	10 µg	70003-3	
pBACgus-1 DNA	10 µg	70054-3	
pBAC™-2cp DNA	10 µg	70004-3	
pBACgus-2cp DNA	10 µg	70049-3	
pBAC™-3 DNA	10 µg	70088-3	
pBACgus-3 DNA	10 µg	70089-3	
pBAC™-5 DNA	10 µg	70222-3	
pBACgus-5 DNA	10 µg	70223-3	
pBAC™-6 DNA	10 µg	70224-3	
pBACgus-6 DNA	10 µg	70225-3	
pBAC™4x-1 DNA	10 µg	70045-3	
pBACgus4x-1 DNA	10 µg	70060-3	
pBACsurf-1 DNA	10 µg	70055-3	

References

Belyaev, A.S. and Roy, P. 1993. *Nucleic Acids Res.* **21,** 1219.
Belyaev, A.S. et al. 1995. *Gene* **156,** 229.
Boublik, Y. et al. 1995. *Bio/Technology* **13,** 1079.
Jarvis, D.L. et al. 1996. *inNovations* **5,** 1.
Weyer, U. and Possee, R.D. 1991. *J. Gen. Virol.* **72,** 2967.



BacVector® System

The BacVector® System is a complete set of reagents and kits designed for efficient and reliable construction of recombinant baculovirus and expression of target proteins in insect cells. This system is based on optimally engineered vectors used in a traditional baculovirus method.

BacVector Transfection Kits include a choice of BacVector-1000, BacVector-2000, or BacVector-3000 Triple Cut Virus DNA and are compatible with pIEx/Bac™, pBAC™, pTriEx™, and other transfer plasmids using the *lef2*/603 and ORF1629 recombination sites. In BacVector-1000 the AcNPV polyhedrin gene is replaced with the *lacZ* gene. During recombination the target gene replaces the *lacZ* gene and clear recombinant plaques are easy to distinguish from any blue parental virus plaques after staining with X-Gal (page 15). BacVector-2000 was additionally modified by removing 5 nonessential viral genes to improve target protein expression. BacVector-3000 has an additional 2 genes removed, *v-cath* to reduce proteolysis and degradation of expressed target proteins, and *chiA* to enhance secreted and membrane-targeted protein production.

To reduce non-recombinant background to < 5%, BacVector Triple Cut Virus DNA has been digested with a restriction enzyme that cuts within the essential ORF1629, upstream of the *lacZ* gene and *polh* promoter, and within the *lacZ* gene (Kitts). BacVector Transfection Kits also contain Insect GeneJuice® Transfection Reagent, Sf9 Insect Cells, BacPlaque™ Agarose, and a Transfection Control Plasmid, all of which have been qualified for optimal transfection performance.

For a complete system description and instructions please refer to the User Protocol TB216 available at www.novagen.com/product/TB216

Product	Size	Cat. No.	Price
BacVector®-1000 Transfection Kit	1 kit	70059-3	
BacVector®-2000 Transfection Kit	1 kit	70030-3	
BacVector®-3000 Transfection Kit	1 kit	70077-3	
BacVector®-1000 DNA Kit	12 rxn	70057-3	
BacVector®-2000 DNA Kit	12 rxn	70058-3	
BacVector®-3000 DNA Kit	12 rxn	70078-3	

Components

Cat. Nos. 70059, 70030, 70077

- 2 × 0.6 µg — BacVector-1000 Triple Cut Virus DNA (Cat. No. 70059) *or* BacVector-2000 Triple Cut Virus DNA (Cat. No. 70030) *or* BacVector-3000 Triple Cut Virus DNA (Cat. No. 70077)
- 2 µg — Transfection Control Plasmid
- 3 vials — Sf9 Insect Cells
- 2 × 3 g — BacPlaque Agarose
- 2 × 50 µl — Insect GeneJuice Transfection Reagent
- 2 × 500 µl — X-Gluc Solution
- 1.5 ml — Nuclease-free Water

Cat. Nos. 70057, 70058, 70078

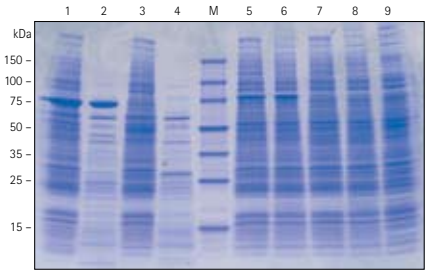
- 2 × 0.6 µg — BacVector Triple Cut Virus DNA
- 2 × 50 µl — Insect GeneJuice Transfection Reagent
- 2 µg — Transfection Control Plasmid

References

Kitts, P.A. and Possee, R.D. 1993. *BioTechniques* **14,** 810.

Features

- >95% recombinants
- BacVector-2000 DNA engineered to increase target protein expression
- BacVector-3000 DNA engineered to increase expression and reduce proteolysis and degradation of the target protein
- Presence of *lacZ* gene for blue/white screening
- Compatible with pIEx/Bac, pBAC, pTriEx, and transfer vectors using the *lef2*/603 and ORF1629 recombination sites



Lane	Sample
1	BacVector-1000, fresh
2	BacVector-1000, 4 wks
3	BacVector-2000, fresh
4	BacVector-2000, 4 wks
M	Perfect Protein™ Markers
5	BacVector-3000, fresh
6	BacVector-3000, 4 wks
7	BacVector-3000, fresh
8	BacVector-3000, 4 wks
9	Sf9 cells, 4 wks

Effect of *v-cath* protease gene deletion in BacVector-3000 on infected Sf9 cell lysates

Sf9 cells were infected with BacVector-1000, BacVector-2000, or two BacVector-3000 recombinant viruses expressing various recombinant fusion proteins. At 72 h post infection, soluble crude cell lysates were prepared and samples were immediately taken and mixed with SDS sample buffer (fresh). The remainder of the soluble lysates were stored at 4°C for 4 weeks and then samples were taken (4 wks). Samples containing equivalent amounts of proteins were run on an SDS-PAGE gel (4-20% gradient).

Additional Products for Insect Cell Expression

Insect GeneJuice® Transfection Reagent *(protocol page 22)*

Insect GeneJuice® Transfection Reagent is a proprietary liposome formulation optimized for maximal transfection efficiency of Sf9 insect cells. The reagent features extremely low toxicity to the cells and can be used for both transient and stable transfections in serum-containing or serum-free media, and for cotransfection of transfer plasmids with linearized virus DNA for the production of recombinant baculoviruses. Insect GeneJuice is ideal for large-scale protein expression in suspension culture transfections of Sf9 Insect Cells with pIEx™ vectors. Insect GeneJuice Transfection Reagent is provided as a 2 mg/ml suspension in 20 mM MES, 150 mM NaCl, pH 6.2 buffer. One ml is sufficient for 12 or 125 transfections in 10-ml suspension culture flasks or 35-mm plates, respectively.

Product	Size	Cat. No.	Price
Insect GeneJuice® Transfection Reagent	0.3 ml	71259-3	
	1 ml	71259-4	
	10 × 1 ml	71259-5	
Sf9 Insect Cells	3 vials	71104-3	
TriEx™ Sf9 Cells	3 vials	71023-3	

FAQ

How much DNA and Insect GeneJuice Transfection Reagent are required for a transient transfection?

For a 10 ml culture, use 15 µg DNA and 75 µl Insect GeneJuice Reagent. A ratio of 5 µl Insect GeneJuice Reagent per 1 µg DNA is optimal for Sf9 cells grown in BacVector® Insect Cell Medium.

Sf9 Insect Cells *(protocol page 18)*

Sf9 Insect Cells are provided as frozen stocks of *Spodoptera frugiperda* Sf9 cells for establishment of cultures suitable for any application. These cells plus BacVector Insect Cell Medium are recommended for cotransfection of transfer plasmids with BacMagic™ DNA or BacVector Triple Cut Virus DNA and for transfection of pIEx/Bac™, pIEx, and pBiEx™ vector constructs for the InsectDirect™ method. After recovery, the cells can be grown as semi-adherent cultures in tissue culture flasks or in suspension as shaker cultures. Each vial contains 2 x 10^6 cells.

TriEx™ Sf9 Cells *(protocol page 18)*

The unique TriEx™ Sf9 Cells are derived from high-yielding Sf9 cells. Pre-adapted for growth in serum-free TriEx Insect Cell Medium, these cells are recommended for superior growth and protein yields by either baculovirus infection or transfection with appropriate vectors. For cotransfection of plasmids with linearized baculovirus DNA, use Sf9 Insect Cells and BacVector Insect Cell Medium. After recovery from the frozen stock, cells can be grown as semi-adherent cultures in tissue culture flasks or in suspension as shaker cultures. Each vial contains 2 x 10^6 cells.

Sf9 and TriEx™ Sf9 Insect Cell Selection Guide

Application	Sf9 Insect Cells and BacVector Insect Cell Medium	TriEx Sf9 Cells and TriEx Insect Cell Medium
Transfection (plasmid only)	++	+
Cotransfection (BacMagic DNA or BacVector DNA with pIEx/Bac, pIEx, or pBAC™ transfer plasmids)	++	–
Protein production	+	++
Preparation of high titer virus stocks	+	++
Plaque assay, conventional	+	–
Plaque assay, FastPlax™ Titer Kit	+	+

Key: (++) highly recommended, (+) recommended, (–) not recommended

For more information or to place an order, contact your local office (see back cover).

BacVector® Insect Cell Medium

BacVector® Insect Cell Medium is optimized for serum-free growth of Sf9 Insect Cells and is compatible with Insect GeneJuice® Transfection Reagent. Sf9 cells grown in BacVector Medium are recommended for cotransfection of BacMagic™ DNA or BacVector Triple Cut Virus DNA with transfer plasmids to construct recombinant baculoviruses. This medium is also recommended for plasmid-mediated expression from pIEx/Bac™, pIEx™, and pBiEx™ vector recombinants and for plaque assays using agarose overlays.

TriEx™ Insect Cell Medium

TriEx™ Insect Cell Medium is a serum-free medium optimized for TriEx Sf9 cell growth and protein expression. This matched cell/medium combination has been developed to provide rapid, vigorous cell growth and high protein expression levels.

FAQ

Can I use antibiotics and fungicides in my cultures?

Antibiotics and fungicides may be added to culture media, however, these may mask low level contaminations which can adversely affect growth and expression, so it is not recommended. Antibiotics must not be present during the plaquing step for baculovirus generation and titering.

BacPlaque™ Agarose *(protocol page 27)*

Agarose overlays are commonly used in baculovirus plaque assays. Many commercial "DNA grade" agaroses contain contaminants that are toxic to insect cells or lack the physical characteristics required for good overlays. BacPlaque™ Agarose is prequalified in plaque assays and cotransfections to ensure consistent performance.

X-Gal Solution

The β-galactosidase substrate X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) is a chromogenic stain for β-gal activity, and is commonly used to distinguish between recombinants and nonrecombinants. X-Gal Solution is provided as a convenient 40 mg/ml concentrate in DMSO, ready for dilution into culture medium or appropriate buffers.

X-Gluc Solution

The β-glucuronidase substrate X-Gluc (5-bromo-4-chloro-3-indolyl-β-D-glucuronide) is a chromogenic stain for GUS activity, and is used to identify recombinants from pBACgus vectors and the Ek/LIC GUS Control Insert. X-Gluc Solution is provided as a 20 mg/ml stock solution, ready for dilution and staining plaques for GUS activity. Each vial contains enough substrate to stain 15 100-mm plates.

Product	Size	Cat. No.	Price
BacVector® Insect Cell Medium	1 L	70590-3	
TriEx™ Insect Cell Medium	1 L	71022-3	
BacPlaque™ Agarose	30 g	70034-3	
X-Gal Solution	3 ml	71077-3	
X-Gluc Solution	500 µl	70036-3	

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Additional Products for Insect Cell Expression *continued*

FastPlax™ Titer Kit and Antibody *(protocol page 30)*

The FastPlax™ Titer Kit is designed to titer baculovirus in just 24 to 48 hours, rather than the 3 to 4 days required for traditional methods, which relied on the appearance of plaques. The FastPlax method uses a high affinity monoclonal antibody to detect the AcNPV gp64 glycoprotein on the cell surface as early as 8 to 24 hours postinfection. The antibody is added directly to fixed cells and is detected with Goat Anti-Mouse IgG β-Galactosidase Conjugate followed by enhanced color development with X-Gal/NBT substrates. Plaques are clearly distinguished by their dark blue color (see photo below). The kit contains sufficient reagents to perform five 6-well plate assays. The FastPlax Antibody is also available separately and can be used to detect gp64 on Western blots.

Insect PopCulture® Reagent *(protocol page 34)*

Insect PopCulture® Reagent is a detergent-based lysis reagent specifically formulated for total insect cell culture extraction without the need for centrifugation. This method recovers both protein released into the medium and intracellular protein, increasing processing efficiency and target protein yields. Insect PopCulture reagent can be used for protein extraction from insect cells grown in suspension and adherent cells grown on tissue culture plates, and is compatible with automated expression-level screening and with Ni-NTA His•Bind® affinity purification.

Benzonase® Nuclease *(protocol page 34)*

Benzonase® Nuclease is a genetically engineered endonuclease from *Serratia marcescens* that degrades all forms of DNA and RNA (single-stranded, double-stranded, linear, and circular), but has no proteolytic activity. The enzyme has an exceptionally high specific activity and is effective over a wide range of conditions. Benzonase completely digests nucleic acids to 5′-monophosphate terminated oligonucleotides that are 2-5 bases long (below the hybridization limit), for compliance with FDA nucleic acid contamination guidelines. The ability of Benzonase to rapidly hydrolyze nucleic acids makes the enzyme an excellent choice for viscosity reduction to reduce processing time and increase protein yields. Benzonase Nuclease is available in ultrapure (>99% by SDS-PAGE) and pure (>90%) grades at a standard concentration of 25 U/µl and at a high concentration (HC) of 250 U/µl. Both preparations are free of detectable protease activity and have a specific activity >1 x 10^6 U/mg protein. The >99% purity grade is tested for endotoxins and contains <0.25 EU/1000 units. The product is supplied as a 0.2 µm filtered solution in 50% glycerol.

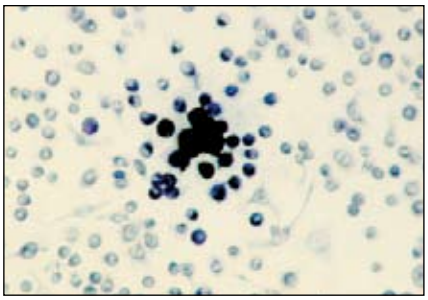
For additional product information please visit www.novagen.com/product/70664

Product	Size	Cat. No.	Price
FastPlax™ Titer Kit	5 assays	70850-3	
FastPlax™ Antibody	10 µl	70814-3	
Insect PopCulture® Reagent	50 ml 250 ml	71187-3 71187-4	
Benzonase® Nuclease, Purity >99%	10 KU	70664-3	
Benzonase® Nuclease HC, Purity >99%	25 KU	71206-3	
Benzonase® Nuclease, Purity >90%	2.5 KU 10 KU	70746-4 70746-3	
Benzonase® Nuclease HC, Purity >90%	25 KU	71205-3	

Components

Cat. No. 70850

- 2 × 50 ml — 10X TBST (100 mM Tris-HCl, pH 8.0, 1.5 M NaCl, 1% TWEEN® 20)
- 6 ml — 10% Gelatin (contains 0.02% azide)
- 10 µl — FastPlax Antibody
- 300 µl — Goat-Anti-Mouse β-Galactosidase Conjugate
- 300 µl — NBT (83 mg/ml in 70% dimethylformamide)
- 300 µl — X-Gal (50 mg/ml in dimethylformamide)



Detection of baculovirus-infected cells 24 hours post infection using the FastPlax Kit

Phosphatase Inhibitor Cocktail Set I

Phosphatase Inhibitor Cocktail Set I is a mixture of three inhibitors that will inhibit alkaline phosphatase as well as serine/ threonine protein phosphatases such as PP1 and PP2A. The inhibitor cocktail is solubilized in DMSO and provided in 5 x 1-ml vials with the following components: Bromotetramisole Oxalate (2.5 mM), Cantharidin (500 µM), and Microcystin-LR (500 nM). Dilute 1:100 immediately before use.

Not available for sale outside of the United States.

Phosphatase Inhibitor Cocktail Set II

Phosphatase Inhibitor Cocktail Set II is an aqueous solution of five phosphatase inhibitors for the inhibition of acid and alkaline phosphatases as well as protein tyrosine phosphatases (PTPs). It is suitable for use with tissue and cell extracts, including extracts containing detergents. The inhibitor cocktail is solubilized in DMSO and provided in 5 x 1-ml vials with the following components: Imidazole (200 mM), Sodium Fluoride (100 mM), Sodium Molybdate (115 mM), Sodium Orthovanadate (100 mM), and Sodium Tartrate Dihydrate (400 mM). Dilute 1:100 immediately before use.

Insect RoboPop™ Ni-NTA His•Bind® Purification Kit

The Insect RoboPop™ Ni-NTA His•Bind® Purification Kit is designed for filtration-based, 96-well purification of His•Tag® fusion proteins directly from transfected insect cell cultures. This kit includes Insect PopCulture® Reagent for protein extraction from total cultures, Benzonase® Nuclease for viscosity reduction, Ni-NTA His•Bind Resin and buffers, a 2-ml 96-well Filter Plate, and a Collection Plate with Sealer. The kit is configured for robotic processing of transfected 10-ml suspension cultures and purifies up to 400 µg His•Tag fusion protein per well based on binding capacity of the resin and characteristics of individual proteins. The Insect RoboPop Ni-NTA His•Bind Purification Kit can be used with centrifugation if a vacuum manifold is not available. Ni-NTA His•Bind Resin is also available separately.

For complete product information please refer to User Protocol TB368, available at www.novagen.com/product/TB368

Co-MAC™ and Ni-MAC™ Purification Kits and Cartridges

The Co-MAC™ and Ni-MAC™ Purification Kits are designed for rapid affinity purification of His•Tag fusion proteins by metal affinity chromatography on Co^{2+}-charged or Ni^{2+}-charged resins. The Co-MAC kit contains a set of concentrated Tris-based buffers and 5 ready-to-use cartridges. The Ni-MAC kit contains a set of concentrated phosphate-based buffers and 5 ready-to-use cartridges. Cartridges are also available separately. Each MAC cartridge is packed with 1 ml His•Bind Fractogel® Resin, precharged with either Co^{2+} or Ni^{2+}. The cartridges can be used manually with a syringe or with liquid chromatography instruments at flow rates up to 4 ml/min and pressures up to 20 bar. Each cartridge binds up to 30 mg protein and can be reused at least 10 times. Fractogel is a very stable and durable synthetic methacrylate-based resin that can be used at high flow rates, has low non-specific binding, and is readily regenerated for reuse.

Product	Size	Cat. No.	Price
Phosphatase Inhibitor Cocktail Set I	1 set	524624	
Phosphatase Inhibitor Cocktail Set II	1 set	524625	
Insect RoboPop™ Ni-NTA His•Bind® Purification Kit	1 kit	71257-3	
Ni-NTA His•Bind® Resin	10 ml 25 ml 100 ml	70666-3 70666-4 70666-5	
Co-MAC™ Purification Kit	1 kit	71659-3	
Co-MAC Cartridges	2 cartridges	71650-3	
Co-MAC Cartridges	5 cartridges	71650-4	
Ni-MAC™ Purification Kit	1 kit	71658-3	
Ni-MAC Cartridges	2 cartridges	71649-3	
Ni-MAC Cartridges	5 cartridges	71649-4	

Components

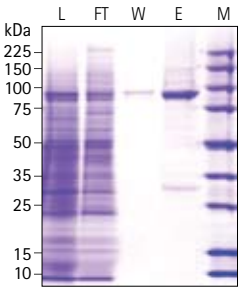
Cat. No. 71659

• 5	Co-MAC Cartridges
• 2 × 80 ml	8X Bind Buffer
• 3 × 25 ml	8X Wash Buffer
• 3 × 25 ml	4X Elute Buffer

Cat. No. 71658

• 5	Ni-MAC Cartridges
• 2 × 100 ml	4X MAC Wash Buffer, Phosphate
• 2 × 75 ml	4X MAC Bind Buffer, Phosphate
• 75 ml	4X MAC Elute Buffer, Phosphate

Hsp90β affinity purification



Lane	Sample
L	Total cellular protein load
FT	Flowthrough
W	Column wash (30 µl loaded)
E	Eluate (2 µg total protein loaded)
M	Perfect Protein™ Markers 10-225 kDa

Automated purification of His•Tag® Sf9 Hsp90ββ with Ni-MAC™ Cartridge

For protein expression, 50 ml TriEx™ Sf9 insect suspension cells (1.5 x 10^6 cells/ml) were infected with recombinant Heat Shock Protein 90 beta (Hsp90β) encoding baculovirus at MOI of 5. 72 hours after infection, cells were lysed using Insect PopCulture® Reagent (page 16) and Benzonase® Nuclease (page 16), according to the recommended protocols. Clarified lysates were applied to 2 Ni-MAC columns, joined together, resulting in a 2.0-ml bed volume for automated liquid chromotography. Crude extract, flowthrough, and eluate fractions were analyzed by SDS-PAGE and Coomassie blue staining.

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Storage and Growth of Sf9 and TriEx™ Sf9 Insect Cells *(page 14)*

from User Protocols TB329 and TB314

Storage

Remove Sf9 **(or TriEx™ Sf9)** Insect Cells from the foil pack. Recover immediately, or place at –70°C if using the cells within two weeks. To store longer than 2 weeks, cells should be stored in liquid N_2 as follows:

1. Thaw Sf9 **(or TriEx Sf9)** cells and prepare exponentially growing monolayer or suspension cultures, as described below.
2. Count cells using the Trypan blue exclusion method (below) to ensure >90% viability for Sf9 cells **(or >80% viability for TriEx Sf9 cells)**.
3. Adjust cell density to 4 x 10^6 cells/ml with BacVector® **(or TriEx)** Insect Cell Medium.
4. Prepare an equal volume of freezing medium [BacVector **(or TriEx)** Insect Cell Medium containing 20% (v/v) DMSO and 5% fetal bovine serum (v/v)].

Important: It is important to use high-grade DMSO.

5. Add an equal volume of freezing medium drop-wise to the cells.
6. Gently pipet the cell suspension to ensure complete mixing.
7. Aliquot 1 ml cell suspension into each cryogenic vial and close caps tightly. Place vials at –20°C for 2 h.
8. Transfer vials to –70°C as rapidly as possible for 12–16 h.
9. Transfer vials to a liquid nitrogen tank as rapidly as possible for long-term storage.

Note: In order to prevent contamination, use sterile techniques when working with uninfected Sf9 insect cells and medium. Whenever possible, dedicate one laminar flow hood to culturing and passaging uninfected insect cells, and a second for all virus work.

Trypan Blue Exclusion Method

1. Add 100 µl cells to 100 µl Trypan blue solution (0.4% Trypan blue in 0.85% saline) and pipet up and down 5 times to mix.
2. Immediately add a small amount of mixture to both sides of a hemocytometer.
3. Following the hemocytometer instructions, count the cells. For improved accuracy, repeat the count using several samples.

Note: Trypan blue dye stains only dead cells. However, it is important to perform cell counts soon after the dye is added, because living cells lose their capacity to exclude the dye with time.

Thawing Sf9 Insect Cells

Medium used here is BacVector® Insect Cell Medium (page 15), and serum is 5% (v/v) fetal bovine serum. Different lots of medium can vary in performance. Other sources of medium may also be suitable. Do not use untested lots of antibiotics, as they may significantly inhibit cell growth.

1. Place bottle of medium in a 28°C water bath.
2. Retrieve vial of Sf9 Insect Cells from the freezer and immediately immerse halfway in a 28°C water bath. Swirl gently until cells are fully thawed (approximately 2 min).
3. Sterilize outside of vial with 70% ethanol and move to a laminar flow hood.
4. Carefully open the vial and slowly pipet the cells into a sterile 50-ml polypropylene centrifuge tube (e.g., Falcon).
5. Add 5 ml pre-warmed 28°C medium drop-wise to the cells. Do not include antibiotics.

Note: The medium must be added drop-wise to the cells. Do not add cells directly to medium.

6. Gently pipet cell suspension 3–5 times. Transfer entire contents into one T-25 flask and close the cap.
7. Gently rock the flask to evenly disperse the cells.
8. Incubate the T-25 flask at 28°C for 30–60 min. Cells will attach to the bottom of the flask during this time.

Important: Do not allow this incubation to continue for more than 2 h because residual DMSO from frozen stock is harmful during prolonged exposure.

9. After attachment, tip the flask at 45° angle and gently remove medium with a pipet.
10. Immediately replace with 5 ml fresh pre-warmed 28°C medium by pipetting at the side of the flask and allowing medium to flow gently across cells.
11. Gently rock T-25 flask to ensure even distribution of medium.
12. Incubation at 28°C until the monolayer becomes 85–95% confluent. Check flask every other day to monitor viability and confluency. Cells can also be assayed by Trypan blue exclusion (page 18).

Passaging Monolayer Sf9 Cultures

1. Under a laminar flow hood, examine the monolayer using a microscope to assure cells are healthy and confluent (85–95%).

Note: Healthy Sf9 cells appear rounded with distinct cell boundaries, as compared to unhealthy, granular Sf9 cells. A large number of floating cells generally signify unhealthy cells. However, Sf9 cells grown beyond confluency will also start to float and divide as they are not subject to contact inhibition.

2. Gently aspirate medium from the T-25 flask.
3. Pipet 5 ml fresh medium, pre-warmed to 28°C, into the flask.
4. Using a sterile scraper, or by pipetting repeatedly, gently dislodge cells.
5. Transfer dislodged cells to a sterile 50-ml polypropylene centrifuge tube.

6. Count cells using the Trypan blue exclusion method (page 18).
7. Based on the cell count, seed cells to new flasks according to the application.

Typically, cells grown at 28°C in a monolayer are split 1:8 every 3–4 days. Depending on needs, nearly confluent monolayer cells can be split at any ratio between 1:2 and 1:20. As more cells are needed, additional flasks can be seeded. Typical seeding densities are listed in the table below.

Flask Size	Cell Number	Medum Volume
25-cm^2 flask	1.0 x 10^6	5 ml
75-cm^2 flask	3.0 x 10^6	10 ml
150-cm^2 flask	6.0 x 10^6	30 ml

Passaging Suspension Sf9 Cultures

Sf9 Insect Cells can be grown and maintained in suspension as shaker cultures. Exponentially growing cells are incubated in a temperature-controlled orbital shaker at 28°C, 150 rpm. To ensure proper aeration, the total culture volume should not be more than 20% of the flask volume, and threaded caps should be kept slightly loose.

1. Follow steps 1–5 from Passaging Monolayer Cultures (page 19).
2. Under a laminar flow hood, examine an aliquot (1 ml) of Sf9 Insect Cells from a dislodged monolayer or previously prepared suspension culture using a microscope to determine if the cells are healthy and confluent (85–95%).
3. Count cells using the Trypan blue exclusion method (page 18).
4. Based on the cell count, seed cells to new flasks according to the application.

Suspension cells are usually seeded at 0.5 x 10^6 cells/ml in a total volume of 50 ml in a 250-ml disposable plastic Erlenmeyer flask. To maximize viability and virus or protein production, maintain cells at a concentration between 2 x 10^5 and 5 x 10^6 cells/ml. Cells are split when the density reaches 4 x 10^6 cells/ml.

Important: Overdilution will result in cell death; avoid densities less than 2 x 10^5 cells/ml.

Thawing TriEx™ Sf9 Cells

Important: TriEx™ Insect Cell Medium (page 15) is recommended in the following protocols. Antibiotics may be used except where noted in transfection procedures. It is important to note that certain lots of antibiotics may severely inhibit cell growth. We therefore recommend testing new lots for compatibility with the cells prior to routine use.

1. Place bottle of medium in 28°C water bath.
2. Retrieve vial of TriEx Sf9 Cells from the freezer and immediately immerse halfway in a 37°C water bath. Swirl gently until cells are fully thawed (approximately 2 min).
3. Sterilize outside of vial with 70% ethanol and move to a laminar flow hood.
4. Carefully open vial and slowly pipet the cells into a sterile 50-ml polypropylene centrifuge tube (e.g. Falcon).

5. Add 5 ml pre-warmed 28°C medium drop-wise to cells.

Note: The medium must be added drop-wise to the cells. Do not add the cells directly to the medium.

6. Gently pipet cell suspension 3–5 times. Transfer entire contents into one T-25 flask and close the cap.
7. Gently rock the flask to evenly disperse the cells.
8. Incubate the T-25 flask at 28°C for 30–60 min. Cells will attach to the bottom of the flask during this time.

Important: Do not allow this incubation to continue for more than 2 h because residual DMSO from frozen stock is harmful during prolonged exposure.

9. After attachment, tip the flask at 45° angle and gently remove medium with a pipet.
10. Immediately replace with 5 ml fresh pre-warmed 28°C medium by pipetting at the side of the flask and allowing medium to flow gently across cells.
11. Gently rock T-25 flask to ensure even distribution of medium.
12. Incubate at 28°C until monolayer becomes 80–90% confluent. Check flask every other day to monitor viability and confluency.
13. At this point, initiate shake culture. To passage cells, dislodge by pipetting medium from flask over cells. Count cells and dilute with 28°C medium to final concentration of 0.5 x 10^6 cells/ml. Transfer to suspension culture.

TriEx™ Sf9 Cell Growth and Maintenance of Suspension (Shake) Cultures

TriEx™ Sf9 Cells are most conveniently grown and maintained in exponential growth using serum-free medium and shake flasks in a temperature-controlled orbital shaker operating at 28°C, 150 rpm. We recommend 28°C as a standard temperature, although lower temperatures may be used with corresponding increases in times for cell and virus replication and protein production. CO_2 is not required due to the composition of the TriEx Insect Cell medium. To ensure proper aeration, the liquid culture should be no more than 20% of the vessel volume, and threaded caps should be slightly loose.

We routinely grow cells (20–200 ml scale) in disposable plastic or glass Erlenmeyer flasks and infect or passage them when they reach exponential growth at 4 x 10^6 cells/ml. Shaker cultures are routinely passaged every 2–3 days with doubling times of 24 h under the conditions described here. For passaging, count the cells and dilute to 0.5 x 10^6 cells/ml using fresh prewarmed 28°C medium. *Overdilution will result in cell death.*

In general, maximum densities attained by shake cultures are 5–6 x 10^6 cells/ml. However, at these densities the cells stop growing and rapidly die due to the accumulation of toxic by-products and lack of sufficient oxygen. For maximum viability and successful virus and protein production, maintain the cell concentration between 2 x 10^5 and 5 x 10^6 cells/ml.

Detailed protocols for pTriEx virus and protein production can be found in User Protocol TB250 at:
www.novagen.com/product/TB250

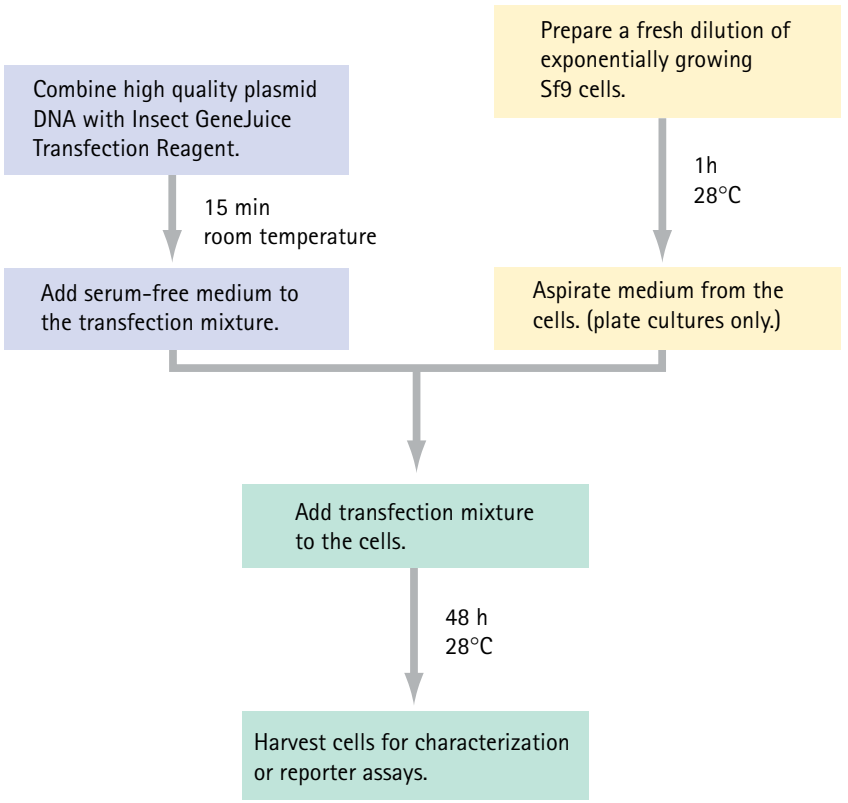
Insect GeneJuice® Transfection Reagent *(page 14)*

from User Protocol TB359

General Considerations

- Use only high quality DNA. Insect GeneJuice® has been optimized for transfection of DNA purified using the Novagen® Mobius™ or UltraMobius™ Plasmid Kits (Cat. Nos. 70853 and 70906). Final DNA preparation should be suspended in TE (10mM Tris-HCl, 1 mM EDTA, pH 8.0) or TlowE (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) at a concentration of 1 µg/µl.
- Passage cells regularly (e.g., every 2-3 days) and avoid confluent growth. For transfection, use only rapidly proliferating cells. Conditions for cell growth and density should be consistent for optimum reproducibility.
- Sf9 Insect Cells plus BacVector® Insect Cell Medium are recommended for transfection and for baculovirus plaque assays. TriEx™ Sf9 Cells can be used for transient or stable transfections, but they may give lower transfection efficiencies than Sf9 Insect Cells.
- Insect GeneJuice is compatible with both serum-containing and serum-free media. Note that serum must **not** be present during formation of the DNA/Insect GeneJuice complex.
- The ratio of Insect GeneJuice to DNA is a crucial factor for transfection optimization. We recommend 5 µl Insect GeneJuice Reagent per 1 µg DNA as a starting point. For optimization, vary between 4–8 µl Insect GeneJuice per 1 µg DNA.

Insect GeneJuice transfection method



Plasmid Transfection

The following procedures describe methods for introducing plasmid DNA into Sf9 cell cultures in 24-well monoculture and 10-ml suspension culture formats. Alternative formats require adjusting the amount of Insect GeneJuice® Transfection Reagent and cell seeding densities.

The following table lists the amount of cells and reagents for 3 different tissue culture formats.

	Tissue Culture Format		
	Plate format (well/plate)		Flask format
Transfection of Plasmid DNA	24-well	6-well	10 ml (125 ml flask)
Number of cells	2×10^5	1×10^6	1×10^7
Culture volume prior to transfection	0.5 ml	2.5 ml	8 ml
Amount of plasmid DNA	0.4 µg	2 µg	20 µg
Volume of Insect GeneJuice Transfection Reagent	2 µl	10 µl	100 µl
Volume of serum-free medium added to both the plasmid DNA *AND* the Insect GeneJuice Transfection Reagent for dilution	20 µl	100 µl	1 ml
Volume of serum-free medium added to Insect GeneJuice transfection mixture after 15 min incubation	160 µl	0.8 ml	N/A

24-well plate culture

Cell Preparation

1. Make 15 ml fresh dilution of Sf9 cells from an exponentially growing shake culture. Dilute the cells in prewarmed 28°C serum-free medium to 4×10^5 cells/ml.
2. One hour prior to transfection add 0.5 ml (2×10^5 cells) to each well of a 24-well plate.

Transfection

1. For each well to be transfected, dilute 0.4 µg DNA with 20 µl serum free medium. Also, dilute 2 µl Insect GeneJuice Transfection Reagent with 20 µl serum free medium.
2. Slowly add the diluted DNA dropwise to the diluted Insect GeneJuice Transfection Reagent. Mix immediately by gentle vortexing to avoid precipitation.
3. Incubate the Insect GeneJuice/DNA mixture at room temperature 15 min.
4. After the 15 min incubation, add 160 µl serum-free medium to the Insect GeneJuice/DNA transfection mixture.
5. Aspirate medium from the cells and add the transfection mixture to the cells.
6. Add the cover to the plate and carefully transfer to a flat-bottomed covered storage container containing a damp paper towel for moisture. Incubate the cells at 28°C for 48 h.
   Optional: Remove transfection mixture after 4 hours and replace with complete growth medium.
7. Harvest cells for characterization or reporter assays.

10-ml Suspension Culture

1. Seed 1 x 10^7 Sf9 cells in 8 ml serum-free medium per 125-ml Erlenmeyer flask.
2. In a sterile tube, dilute 20 µg plasmid DNA with 1 ml serum-free medium. Also, dilute 100 µl Insect GeneJuice Transfection Reagent with 1 ml serum-free medium.
3. Add the DNA dropwise to the Insect GeneJuice® Transfection Reagent and mix immediately by gentle vortexing to avoid precipitation.
4. Incubate at room temperature for 15 min.
5. Add the transfection mixture to the cells.
6. Incubate the cells at 28°C, shaking at 150 rpm, for 48 h.
7. Harvest cells for characterization or reporter assays.

See page 25 for the BacMagic™ cotransfection protocol.

Troubleshooting

Symptom	Possible cause	Solution
Low transfection efficiency	Serum present during formation of Insect GeneJuice/DNA complex	Use only serum-free medium during formation of complex. If cells were grown in presence of serum, wash the cells once before adding transfection mix.
	Cell density suboptimal at time of transfection	Use cell densities outlined in the Table on page 23.
	Poor quality DNA.	Prepare fresh plasmid DNA using an endotoxin removal step, such as with the UltraMobius™ 1000 Plasmid Kit. Alternatively, prepare supercoiled plasmid DNA by using a CsCl/EtBr protocol.
	Inhibitor present during transfection	In addition to endotoxin, transfection can be inhibited by the presence of polyanions such as heparin or dextran sulfate. Be sure that the DNA and the transfection medium are free of polyanions.

Preparation of Transfection Quality Plasmid DNA

For efficient transfection, use high quality, very pure transfer plasmid DNA. For consistent transfection-quality DNA, we use plasmid DNA purified from Novagen® Mobius™ or UltraMobius™ Plasmid Kits. Alternatively, transfection-quality DNA may be prepared by CsCl gradient using standard methods. Purified DNA should be suspended in TlowE (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) at 0.1 µg/µl.

Production of Recombinant Baculoviruses

Preparation of Cell Cultures for Transfection

For each cotransfection, prepare one 35-mm plate. We also recommend including plates for positive and negative transfection controls.

1. Seed dishes with insect cells at least 1 h before use. For Sf9 cells, use 1 x 10^6 cells/dish in 2 ml BacVector® Insect Cell Medium. Gently rock plates side-to-side and back-and-forth to ensure an even monolayer. Do not swirl plates because cells will cluster in the center. Incubate at 28°C for 1 h to allow cells to attach.
2. During incubation, prepare cotransfection mix of DNA and Insect GeneJuice® Transfection Reagent.

Preparation of Transfection Mixture

For each transfection, add the following components in the order listed to a sterile 6-ml polystyrene tube. Do NOT use polypropylene or polycarbonate.

1 ml	BacVector Insect Cell Medium
5 µl	Insect GeneJuice Transfection Reagent
5 µl	BacMagic™ DNA (100 ng total)
5 µl	transfer vector DNA (500 ng total)
1.015 ml	Total volume

The following reactions are optional, but highly recommended:

Negative transfection control:
Instead of recombinant transfer plasmid, use a corresponding amount of medium or TlowE.

Positive transfection control:
Instead of the recombinant transfer plasmid, use 500 ng of the supplied Transfection Control Plasmid.

3. Mix with gentle agitation or vortexing.
4. Incubate at room temperature for 15–30 min to allow complexes to form.
5. Just prior to the end of the transfection mixture incubation period, remove culture medium from 35-mm plate by tipping the dish to a 30–60° angle and aspirating the medium with a sterile pipet. Do not disturb cell monolayer. Do not let monolayer dry out.

Note: For cells maintained in serum-supplemented medium, wash the monolayer two times, each with 1 ml serum-free medium before proceeding with cotransfection.

6. Immediately after medium has been removed from cells, add 1 ml transfection mixture dropwise to center of dish. Incubate in humidified container at 28°C overnight (minimum 5 h).

7. After initial incubation period, add 1 ml BacVector® Insect Cell Medium to each dish. Continue incubation 5 days total. Serum can be added to medium at this point, if desired.

8. After 5 days incubation, harvest medium containing seed stock of recombinant baculovirus. The expected titer is generally 1 x 10^7 pfu/ml. Negative control cells will have formed a confluent monolayer. Virus-infected cells will appear grainy with enlarged nuclei and will not have formed a confluent monolayer.

Note: If the Transfection Control Plasmid was used, expression of β-glucuronidase can be evaluated. 3-5 days post-transfection, remove 100 µl medium from the dish and combine with 5 µl X-Gluc (20 mg/ml, page 15). Incubate overnight at room temperature for the X-Gluc staining. Recombinant pBACgus-containing viruses express β-glucuronidase (gus) and medium will stain blue.

9. Amplify virus.

Amplification of Recombinant Virus

Recombinant virus amplification is necessary before proceeding with experimental work. The following protocol is for amplification of virus in a suspension culture.

1. Prepare 100–200 ml culture of Sf9 cells at an appropriate cell density in log phase growth (e.g., 2 x 10^6 cells/ml for Sf9 cells). Cells should be infected at a low multiplicity of infection (MOI) of <1 pfu/cell.

Note: Surface area to volume in shake flasks should be as large as possible (not more than 20% of flask volume). Shake flasks should be shaken at speeds to maximize aeration (e.g., 150 rpm for Sf9 cells).

2. Add 0.5 ml recombinant virus seed stock to cell culture. Incubate with shaking until cells are well infected (usually 4–5 days).

Note: Under a phase-contrast inverted microscope, cells infected with virus appear grainy when compared to healthy cells. The infected cells become uniformly rounded and enlarged, with distinct enlarged nuclei.

3. When cells appear to be well infected with virus, harvest cell culture medium by centrifugation at 1000 x g for 20 min at 4°C. Remove supernatant aseptically. Store supernatant (recombinant virus) in dark at 4°C.

Note: Virus inoculum can be stored in the dark at 4°C for 6–12 months, although the titer will begin to drop after 3–4 months. Titer virus before use and reamplify if necessary. The addition of 2–5% serum when using serum-free medium can help avoid a drop in titer. Virus may be frozen at –80°C for longer periods of time. Avoid multiple freeze thaw cycles.

4. A plaque assay to determine accurate titer is strongly recommended before using the virus in subsequent experiments.

Plaque Assay to Titer Recombinant Virus

The following protocol is for a plaque assay to accurately determine the titer of the virus. An alternative method for quickly determining titer is the FastPlax™ Titer Kit (page 16, protocol page 30).

Preparation of BacPlaque™ Agarose Stock

BacPlaque™ Agarose (page 15) is used for all agarose overlays including the Direct Plaquing Transfection, and virus titration. To prepare 10 ml stock of 3% BacPlaque Agarose, add 0.3 g agarose to 10 ml sterile deionized water in a 100 ml Pyrex media bottle, cap loosely, and autoclave. Mix well as soon as the autoclave cycle has finished (handle the hot liquid carefully). Allow the bottle to cool, tighten the cap, and store at room temperature.

Plaque Assay

For most applications, a titer of 5 x 10^7 pfu/ml or higher is adequate. A titer of less than 1 x 10^7 pfu/ml will generally not be sufficient for expression studies.

1. Label ten 35-mm plates in duplicates of "-4", "-5", "-6", "-7", and "control". Add Sf9 cells (0.9 x 10^6cells/dish). Leave plates at room temperature for 1 h on a level surface.
2. During incubation period, label tubes from 10^{-1} to 10^{-7}. Prepare 0.5 ml dilutions, using 0.45 ml room temperature BacVector® Insect Cell Medium (no antibiotics; if using cells grown in serum-supplemented medium, serum must be added) in 7 sterile polystyrene tubes. Add 0.5 ml BacVector Insect Cell Medium to the tube negative control tube.
3. Add 50 µl undiluted recombinant virus to the tube labeled 10^{-1}. Mix thoroughly by inversion.
4. Using a fresh pipet tip, transfer 50 µl from this tube to the next (10^{-2}). Mix thoroughly by inversion. Continue this process diluting the virus until 10^{-7} is reached.
5. Once cells have formed even sub-confluent monolayer, remove the media and add 100 µl virus dilutions (10^{-4} to 10^{-7}), in duplicate, to cells and add 100 µl BacVector Insect Cell Medium to the negative control dishes.
6. Incubate plates at room temperature for 1 h, gently rocking the liquid across them twice during the hour to prevent cells from drying out. Rock stacked plates by tipping them just enough for the liquid to pool to the side of dish. Hold the position for 15–30 s, then tip the liquid across the dish. Rotate stack 90° and repeat process, ensuring the entire surface is covered with the mixture.
7. Approximately 15 min prior to completion of incubation period, prepare agarose overlay. Loosen cap of 10 ml 3% BacPlaque Agarose stock. Microwave until agarose is completely melted and no lumps remain. Place bottle in 37°C water bath, cool to less than 50°C and add prewarmed (37°C) BacVector Insect Cell Medium containing 5% fetal bovine serum to a total volume of 30 ml. The final agarose concentration is 1% (w/v) agarose at 2/3 strength medium containing serum. The overlay can be used immediately, or kept at 37°C for at least 1 day without solidifying.

Note: If agarose sets before use, do not remelt. Prepare fresh batch.

8. After incubation, remove virus inoculum from the cells.
9. Add 2 ml BacPlaque Agarose/medium/serum mixture to each plate, by pipetting it slowly down side of plate. Do not move plate again until agarose has solidified (usually 20 min at room temperature is sufficient). Keep plates covered.
10. When agarose overlay has set, add 1 ml BacVector Insect Cell Medium to each dish. The additional medium equilibrates medium in the agarose that is at 2/3 strength in order to promote optimal cell and virus growth.

11. Carefully transfer 35-mm dishes to a flat-bottomed covered storage container containing a damp paper towel for moisture. Incubate at 28°C for 3–4 days (72–96 h), by which time cell monolayer should be confluent.

Plaque Staining

After incubating for 3–4 days, transfection can be monitored by staining plaques in the monolayer. Successful plaque identification depends on careful evaluation of several dilutions of virus with appropriate staining techniques. Neutral Red stains live cells red, leaving clear plaques visible in the monolayer. X-Gluc staining can be used only with pBACgus vectors and Transfection Control Plasmid transfections. These staining methods do not harm the virus. During the staining process, always remove the medium from the plates or wells by tipping slightly so the agarose and cell monolayer are not disturbed. Do not pour off medium because this may cause monolayer to slip. Stain with one of the following methods.

1. **Neutral Red staining**
   Dilute a 0.33% (w/v) Neutral Red stock solution 1:13 with sterile phosphate-buffered saline (PBS: 43 mM Na_2HPO_4, 15 mM KH_2PO_4, 137 mM NaCl, 27 mM KCl, pH 7.4) just before use. To avoid precipitation, do not store diluted stain for long periods in the light at room temperature (>8 h). Carefully remove liquid overlay from plates, and pipet 1 ml freshly diluted staining solution onto center of each plate. Incubate plates at 28°C for 2 h. Carefully remove stain and store plates in dark at room temperature for 3 h or more. During this time cells take up stain, making the plaques more visible. Leaving plates at room temperature overnight may make plaques more distinct.

2. **Gus activity and Neutral Red staining**
   Prior to staining, thaw the X-Gluc solution (20 mg/ml, page 15) at room temperature (do not allow contact with polystyrene surfaces). Unused solution should be stored at –20°C. To visualize gus-producing plaques, carefully aspirate the liquid overlay from the wells or dishes. Return the plates or dishes to a flat surface and replace the medium with 1.0 ml freshly diluted Neutral Red solution in PBS (see above) and 15 µl/ml X-Gluc Solution; incubate at 28°C for 2 h. Carefully remove the staining solution and incubate at room temperature an additional 3 h to overnight. The gus gene is under the control of the late basic promoter (P6.9). Due to the low level expression with this promoter, pBACgus and Transfection Control Plasmid recombinants could take overnight to develop blue plaques.

Plaque Identification

Hold plate up to a light source and observe monolayer from underneath. Alternatively, place plate on a light box. Do not disturb monolayer by jarring or inclining the plate. Plaques appear cloudy against a more transparent background. Plaques may become more clearly defined with increasing incubation time and form large areas of cleared cells; however, because the monolayer will overgrow and plaques will overlap, it is best to identify plaques early. In addition, large plaques may obscure and be contaminated with small plaques, and reduce the numbers of plaques that can be counted per plate. It is useful to compare several plates representing different virus dilutions. Plaques at a high density are too numerous to count and start to overlap very quickly.

Calculation of Virus Titer

1. To count plaques, remove lid from plate and invert plate on a light box. It is helpful to touch one edge of the plate on a paper towel (while inverted) to catch any remaining dye that drains off the plate. The plate should then be kept in a level position on the light box while plaques are marked. Use a marker pen to circle the locations of well-isolated plaques on the underside of plate. Turn plates upright, replace lid, and let them remain at room temperature overnight. Since small plaques will be more visible after an overnight incubation, previously unmarked plaques should be counted the following day to obtain a more accurate titer.

2. The total number of plaques that could be obtained from any one transfection can be calculated by multiplying the number of plaques observed on each plate by the dilution factor. The dilution factor is the inverse of the dilution used. This number is then multiplied by 10, because 0.1 ml was applied to the plate for plaquing (i.e., 20 plaques on the 10^{-6} dilution gives a titer of 2.0×10^{8} pfu/ml).

Expression

For expression, cells should be infected at a high MOI to ensure all cells are infected simultaneously and the culture is synchronous. An optimal MOI is usually 5–10 pfu/cell, but should be optimized for each particular virus. To optimize the MOI, use various MOIs to infect prepared plates or shake cultures. Examples of recommended MOIs to test are 2, 5, and 10. Evaluate protein expression at different times after infection (24, 48, 72, and 96 h). Use Insect PopCulture® Reagent with Benzonase® Nuclease (page 16, protocol on page 34) or harvest cells and/or culture medium to evaluate protein expression using SDS-PAGE or Western analysis.

FastPlax™ Titer Kit *(page 16)*

from User Protocol TB272

Additional reagents/supplies needed

- BacVector® Insect Cell Medium, TriEx™ Insect Cell Medium (page 15), or equivalent
- 6-well tissue culture plates (e.g. Costar #3516)
- Sf9 Insect Cells, TriEx Sf9 Cells (page 14), or equivalent
- 37% formaldehyde solution
- PBS (43 mM Na_2HPO_4, 15 mM KH_2PO_4, 137 mM NaCl, 27 mM KCl, pH 7.4)
- PBS plus 5 mM $MgCl_2$

The following protocol specifies the use of Novagen® Sf9 or TriEx Sf9 Insect Cells and BacVector or TriEx Insect Cell Medium. However, any healthy Sf9 cell culture in logarithmic growth can be used. The assay should not be sensitive to medium type; other sources of serum-free or complete medium [such as TNMFH or TC100 (O'Reilly)] can be used provided the Sf9 cells have already been adapted.

The accuracy of titers determined by the FastPlax™ method, or by any method, will be affected by the accuracy of the dilution series. Carefully pipet small quantities of virus stocks, and mix each dilution well before removing an aliquot for the serial dilution series. To increase the accuracy of the titer determination, two wells are infected with each virus dilution.

It is convenient to infect cells the morning of one day, and to detect infected cells the afternoon of the following day. This allows the infection to proceed for 24–30 h, causing the infected foci to be easily visualized. The protocol uses a formaldehyde solution to fix the infected cells; however, other aqueous fixatives such as 0.5% (v/v) glutaraldehyde in PBS may also be used. Organic solvents such as methanol, ethanol, or acetone are not recommended for fixation because infected cells may be lost during the fixation and rehydration steps.

Cell Plating

1. Prepare an exponentially growing culture of Sf9 or TriEx Sf9 Cells.
2. Label three pairs of wells "-5", "-6", and "-7". Seed each well in a 6-well plate with 2 ml containing a total of 1 x 10^6 cells and mix gently, side-to-side and back-and-forth, to ensure an even monolayer. Do not swirl the plates because the cells will cluster in the center of the well. Allow the cells to attach to the plates (about 30 min at 28°C).

Virus Dilution

While the cells are attaching, prepare the virus dilutions. To maintain accuracy, try to avoid pipetting volumes less than 10 µl. Use sterile 1.5 ml eppendorf tubes, preferably with screw caps.

1. For each virus stock to be titered, place 5 sterile 1.5 ml eppendorf tubes in a rack. Label them "-2", "-4", "-5", "-6", and "-7".
2. Add 990 µl medium to the "-2" and "-4" tubes. Add 900 µl medium to the "-5", "-6", and "-7" tubes.

Caution: Measure accurately, since all other dilutions will be derived from the "-2" tube. Watch for droplets of virus stock that may cling to the outside of the pipet tip, and rinse the inside of the tip several times by pipetting the dilution up and down.

3. Begin the dilution series by adding 10 µl of virus stock to the tube labeled "-2" (this is the 10^{-2} dilution), close the cap and invert or vortex gently to mix.
4. Add 10 µl of the "-2" dilution to the tube labeled "-4". Cap and mix as above.
5. Add 100 µl of the "-4" dilution to the "-5" tube; cap and mix.
6. Add 100 µl of the "-5" dilution to the "-6" tube; cap and mix.
7. Add 100 µl of the "-6" dilution to the "-7" tube; cap and mix.

Infection

1. To remove the 2 ml medium from the wells, slowly tip the plate to about a 10° angle, and carefully aspirate from the edge of the well to avoid disturbing the monolayer.
2. Slowly add 100 µl of each virus dilution to each of 2 duplicate wells, drop by drop in the center of the well. Start with the "-7" dilution, then "-6" then "-5". The same pipet tip may be used for adding all dilutions if this order is followed.
3. Incubate the plate at room temperature for 60 min. During this incubation, tip the plate at 10 min intervals to keep the cell monolayer wet. Tip the plate to one side and allow the medium to pool at the lower corner. Then, quickly switch to the opposite direction to allow the pooled medium to flow across the cell monolayer.
4. At the end of the incubation, carefully add 2 ml of medium to each well. Incubate the plate at 28°C for 24–30 h in a sealed container with moist paper towels to prevent the plate from drying out.

Detection

Subsequent steps may be performed at room temperature on an open bench. A tilting platform or slow orbital shaker may be used during blocking, antibody incubations, and washes.

1. At 20–30 h post-infection, remove the medium, and dispose of as biological waste; it contains infectious baculovirus.
2. Wash the cells twice by adding 2 ml/well PBS.
3. Fix the cells by adding 2 ml 3.7% formaldehyde solution (1:10 dilution of 37% formaldehyde in PBS). Incubate for 10–15 min.
4. Remove the formaldehyde solution and wash twice with 2 ml/well PBS.
5. Prepare 1% Gelatin in TBST by placing the 10% Gelatin bottle in a beaker filled with hot tap water for a few minutes to melt; dilute 10% Gelatin 1:10 with 1X TBST.
6. Block cells by adding 2 ml/well 1% Gelatin in TBST. Incubate for 30 min with gentle rocking.
7. Remove the Gelatin solution and wash cells twice with 2 ml/well PBS.
8. Add 1 ml/well FastPlax™ Antibody diluted 1:10,000 in 1X TBST. Incubate for 60 min with gentle rocking.
9. Wash three times with 2 ml/well 1X TBST. Incubate each wash for 10 min.

10. Add 1 ml/well Goat Anti-Mouse β-gal Conjugate diluted 1:100 in 1X TBST. Incubate for 60 min with gentle rocking or agitation.
11. Wash three times with 2 ml/well 1X TBST. Incubate each wash for 10 min.
12. During the last wash, mix the developing solution by adding 60 µl X-Gal and 60 µl NBT per 15 ml PBS plus 5 mM $MgCl_2$.
13. Remove the last wash and add 2 ml/well developing solution. Incubate at 37°C for 15–60 min.

Important: Check the plate every 10 min under a microscope to avoid overdeveloping. Infected cells will appear medium blue to dark purple (the color completely fills the area of the cell) or as dark purple foci of staining.

14. Wash twice with 2 ml/well 1X TBST to stop color development.

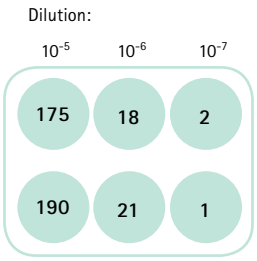
Note: The color may continue to develop and darken with time, even after washing. Therefore it is important to count stained cells immediately after washing. Uninfected cells can appear lightly stained if over developed.

15. Count the infected cells and foci immediately using a dissecting microscope (invert the plate so foci can be marked) or a low power objective on an inverted microscope. Single cells that are strongly stained should be counted, as well as small clusters of stained cells. The most accurate counts will come from wells with 10–100 infected cells/foci. If too many cells are infected, the stained foci will overlap and be difficult to count.

Calculating the Titer

Calculate the titer by the following formula: pfu/ml = 10 x (# infected cells/foci) x (dilution factor). Use the average of the 6 wells to determine the final titer. For example, a 6 well dish may have the following counts:

Plate



Infected cells/foci per well

Example Calculations

	x	foci/ well	x	dilution factor	=	
10	x	175	x	10^5	=	1.75×10^8
10	x	190	x	10^5	=	1.90×10^8
10	x	18	x	10^6	=	1.80×10^8
10	x	21	x	10^6	=	2.10×10^8
10	x	2	x	10^7	=	2.00×10^8
10	x	1	x	10^7	=	1.00×10^8
				Final titer (average)	=	1.76×10^8 pfu/ml

Troubleshooting

Symptom	Possible cause	Solution
No infected cells detected	Failure of cells	Check the viability of the cells before plating. The viability should be >85%. Plate any excess cells in a T-25 flask with 5 ml of medium and monitor their growth.
	Failure of infection	The titer of the virus stock may be very low. Repeat the assay, infecting one well with the 10^{-2} dilution and one well with the 10^{-4} dilution. Be sure the virus dilution has spread across the center of the well during the infection.
	Failure of antibody binding	The FastPlax™ antibody will not bind to native gp64; the cells must be fixed in order for antibody binding to occur. Try using a fresh bottle of 37% formaldehyde solution.
	Failure of staining	No staining will occur if the X-Gal is omitted from the staining solution. Failure to include $MgCl_2$ in the staining solution will greatly decrease the activity of the β-galactosidase, resulting in poor staining. Failure to include NBT in the staining solution will result in a much weaker color reaction, and diffusion of the reaction product.
Monolayer is damaged in center of well	Monolayer has dried out during the infection	Tip the plate frequently during the infection (e.g., every 10 min) and keep the plate in humid chamber. Make sure that the virus dilution is spreading across the center of the well; if necessary, hold the plate at an angle for a longer time to allow the virus dilution to pool in the lower corner of the wells.
Low titer	Infected cells were lost from monolayer	Aspirate medium and solutions gently, by tipping the plate and aspirating from the lower corner of the well. Add solutions slowly into the lower corner of the tipped well.
	Recombinant virus has altered growth rate	A recombinant virus may exhibit altered growth kinetics, delaying expression of detectable quantities of gp64. Incubate the infected plate 6–28 h longer before fixing (e.g., fix at 32–48 h post-infection).
	Single infected cells were not counted	It is important to count all strongly stained cells, even if no staining is detectable on neighboring cells. The FastPlax method relies on the early detection of infection, before the virus has had much opportunity to spread and infect neighboring cells.
	Error in dilution series	Repeat the assay taking extra care while setting up the dilution series of the virus stock. Be sure each dilution is well mixed before removing the aliquot for the next dilution.
High background staining in monolayer	Incomplete blocking	Rinse the wells twice with TBST after fixation to remove excess formaldehyde, before adding blocking solution. Extend the blocking incubation to 1 h.
	Incomplete washing	The wells must be washed thoroughly after each antibody incubation. Washes can be extended to 15–20 min each.
	Excessive length of staining incubation	Monitor the staining reaction more frequently. Look at the plate using a dissecting microscope every 5 min.

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Insect PopCulture Cell® Extract Preparation

1. Add 50 µl Insect PopCulture® Reagent per 1 ml culture volume, followed by 0.4 µl (10 U) Benzonase® Nuclease per 1 ml of the original culture volume.

Note: Benzonase Nuclease can be pre-mixed with Insect PopCulture Reagent prior to treating the cultures. Pre-mixed reagents should be stored at 4° C and used the same day.

2. Mix treated cell culture by inverting gently several times and incubate 15 min at room temperature.

Note: Highly expressed proteins may be analyzed directly by SDS-PAGE with Coomassie blue staining. Use a maximum load volume for the well with 0.25 volume of 4X SDS Sample Buffer.

Benzonase® Nuclease

Cell extracts are generally quite viscous due to the release of nucleic acids, which can also interfere with effective protein purification. Benzonase Nuclease degrades all forms of DNA and RNA (single-stranded, double-stranded, linear, and circular) to 5'-monophosphate terminated oligonucleotides 2–5 bases long (Nestle, Molin). The nuclease activity is sufficient for effective viscosity reduction.

Benzonase Nuclease retains its activity under a wide range of conditions.

Condition	Optimal	Effective
Mg^{2+} concentration (see note below)	1–2 mM	1–10 mM
pH	8.0-9.0	6.0-10.0
Temperature	37°C	0-42° C
Dithiothreitol (DTT)	0-100 mM	>100 mM
2-mercaptoethanol	0-100 mM	>100 mM
Monovalent cation concentration (i.e., Na^+, K^+)	0-20 mM	0-150 mM

Although Benzonase Nuclease requires Mg^{2+} for activation, it does not appear to require additional Mg^{2+} under many conditions.

Benzonase Nuclease is inhibited (approximately 50% reduction in relative activity) by concentrations of: monovalent cation >50 mM, phosphate >20 mM, and ammonium sulfate >25 mM.

For ease of handling, Benzonase Nuclease can be diluted with 50 mM Tris-HCl, 20 mM NaCl, 2 mM $MgCl_2$, pH 8.0. Diluted samples can be stored at 4°C for several days without loss of activity.

Treatment with Benzonase Nuclease is not generally recommended for purification of proteins that must be nuclease free. However, Benzonase Nuclease may be removed during purification by anion exchange chromatography. Residual nuclease activity can be checked by incubating purified protein with RNA or DNA markers followed by gel analysis.

Additional Guidelines for Insect PopCulture Reagent Optimization and Compatibility

Medium

TriEx™ and BacVector® Insect Cell Medium (page 15) have been used successfully with Insect PopCulture. Other serum-free media are likely to be compatible. Serum may interfere with downstream applications such as protein assays and purification.

Cell Lines

TriEx Sf9 and Sf9 Insect Cells (page 14) have been used successfully with Insect PopCulture® Reagent. Other insect lines should be compatible, though cell line-dependent differences could occur.

Temperature of Extraction

Insect PopCulture (with Benzonase® Nuclease) extraction can be performed at room temperature or at 4°C. However, incubation times may need to be increased because Benzonase activity decreases at lower temperatures.

pH of Extraction

Acidic pH (<5.0) can degrade components of Insect PopCulture.

Reducing Agents

Insect PopCulture is compatible with reducing agents such as 2-mercaptoethanol and DTT. However, reducing agents may activate proteases and can interfere with protein binding to some purification resins.

EDTA

Insect PopCulture is compatible with EDTA. However, Benzonase Nuclease is inhibited by EDTA at concentrations >1 mM because it chelates Mg^{+2} ions. EDTA can also interfer with some protein purification resins.

Protein Assays

Because proteins generally retain their activity and conformation, protein specific activity and immunoassays are likely to be compatible with Insect PopCulture extraction.

Protease Inhibitors

Protease inhibitors may be added to the culture with Insect PopCulture Reagent. Serine protease inhibitors should be avoided if the target protein will be treated with the serine proteases Thrombin, Factor Xa, or Recombinant Enterokinase because active inhibitor may be carried through the purification process and affect cleavage. Protease inhibitor cocktails resulting in a final concentration of >1 mM EDTA are not compatible with Ni-NTA His•Bind® Resin or Benzonase Nuclease treatment.

Purification Resins

The Insect PopCulture Reagent contains Tris buffer and is compatible with Ni-NTA His•Bind purification systems at a neutral pH (Loomis) and with His•Bind Fractogel® Resin. His•Bind (IDA agarose) and GST•Bind Resins are NOT compatible with Insect PopCulture extracts for protein purification. Insect PopCulture is expected to be compatible with many other affinity purification resins that are not affected by components in the culture medium.

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