InsectDirect™

Protein Expression & Purification System

When should I use an insect cell expression system?

Researchers dealing with high-throughput (HT) expression screening face the fundamental challenge of trying to rapidly identify the appropriate expression system for many targets in parallel. The focus has traditionally not involved insect expression systems due to their complexity and time-consuming protocols. However, insect cell systems provide an efficient means for expressing proteins that require specific post-translational modifications to retain solubility and activity.

What does the InsectDirect System offer?

The InsectDirect System provides a rapid and powerful, virus-free method for heterologous protein expression in insect cells. The InsectDirect System consists of the following:

- The pIEx™ series of plasmid expression vectors for rapid, high-level protein expression in insect cells, which eliminates the need to create recombinant baculovirus for protein expression
- The pBiEx™ series of multisystem expression vectors designed to allow rapid characterization/expression of target genes in both *E. coli* and insect cells
- Insect GeneJuice® Transfection Reagent for high-efficiency transfection of insect cells with minimal toxicity
- Insect RoboPop™ Ni-NTA His•Bind® Purification Kit for automation-compatible His•Tag® fusion protein purification

MERCK

InsectDirect System

How does use of the InsectDirect™ System benefit me?

48 hours VERSUS 21 days

- Allows protein expression in 48 hours, not 2 to 3 weeks
- Provides an option for HT screening of multiple targets
- Produces up to 80 µg target protein per ml culture
- Includes a protocol that can be scaled for larger culture volumes and higher protein yields
- Offers vector choices for secretion signals, N-terminal and C-terminal fusion tags, and "tagless" options

Insect Cell Expression Vectors

The pIEx™ and pBiEx™ vectors allow rapid protein expression in insect cells, omitting the time-consuming production of recombinant baculovirus. Both the pIEx and pBiEx vectors feature an hr5 enhancer and ie1 (immediate early) promoter combination derived from AcNPV baculovirus. The hr5/ie1 combination utilizes endogenous insect cell transcription machinery for direct expression, thereby avoiding baculovirus infection and its associated cytopathic effects. The pBiEx vectors also allow expression in *E. coli* by using the tightly controlled T7*lac* promoter. Both vectors are suitable for expression screening in insect cells, but the pIEx vectors are capable of high-level expression, making them useful for protein production.

Vectors	Promoter(s)	Signal sequence	Fusion Tags N-terminal	Fusion Tags C-terminal	Protease cleavage sites†	Ek/LIC vector option	Size	Cat. No.
pIEx-1	hr5/ie1	No	His•Tag®/S•Tag™	HSV•Tag®	Tb/Ek	Yes††	20 µg	71241-3
pIEx-2	hr5/ie1	No	GST•Tag™/His•Tag/S•Tag	HSV•Tag	Tb/Ek	Yes††	20 µg	71238-3
pIEx-3	hr5/ie1	Yes	GST•Tag/His•Tag/S•Tag	HSV•Tag	Tb/Ek	Yes††	20 µg	71243-3
pIEx-4	hr5/ie1	No	None	S•Tag/His•Tag	None	No	20 µg	71235-3
pIEx-5	hr5/ie1	Yes	None	S•Tag/His•Tag	None	No	20 µg	71242-3
pIEx-6	hr5/ie1	No	His•Tag	S•Tag	Ek	No	20 µg	71333-3
pIEx-7 Ek/LIC*	hr5/ie1	No	His•Tag	S•Tag	Ek	Yes	20 rxn	71339-3
pBiEx-1	hr5/ie1, T7*lac*	No	His•Tag/S•Tag	HSV•Tag	Tb/Ek	No	20 µg	71234-3
pBiEx-2	hr5/ie1, T7*lac*	No	GST•Tag/His•Tag/S•Tag	HSV•Tag	Tb/Ek	No	20 µg	71233-3
pBiEx-3	hr5/ie1, T7*lac*	No	None	S•Tag/His•Tag	None	No	20 µg	71232-3

* pIEx-7 is available only as an Ek/LIC vector. The pIEx-7 Ek/LIC Vector Kit includes linearized Ek/LIC Vector; T4 DNA Polymerase, LIC-qualified; polymerase buffer; DTT, EDTA, and dATP solutions; Nuclease-free Water; competent cells; SOC Medium; and controls.

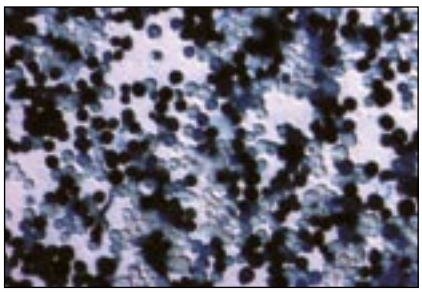
† Tb: thrombin; Ek: enterokinase; 3C: HRV 3C

†† pIEx-1, pIEx-2, pIEx-3, pIEx-8, pIEx-9, and pIEx-10 vectors are also available in an LIC Vector Kit. For more information visit our website address listed below.

Insect GeneJuice® Transfection Reagent

Insect GeneJuice Transfection Reagent is a liposomal formulation optimized for maximal transfection efficiency that offers extremely low toxicity to insect cells. It can be used for both transient and stable transfections in serum-containing or serum-free media. Insect GeneJuice is ideal for HT or large-scale protein expression when using the pIEx or pBiEx vectors for suspension culture transfection of Sf9 and other insect cells.

Product	Size	Cat. No.
Insect GeneJuice® Transfection Reagent	0.3 ml	71259-3
	1 ml	71259-4
	10 × 1 ml	71259-5



Sf9 cells transfected with pIEx-1/β-gal using Insect GeneJuice Transfection Reagent

Transfected cells were stained for β-galactosidase activity using the X-Gal Solution–based BetaBlue™ Staining Kit.

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

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. The kit configuration allows for robotic processing of transfected 10-ml suspension cultures and purification of up to 400 µg His•Tag fusion protein per culture based on binding capacity of the resin.

The kit includes:

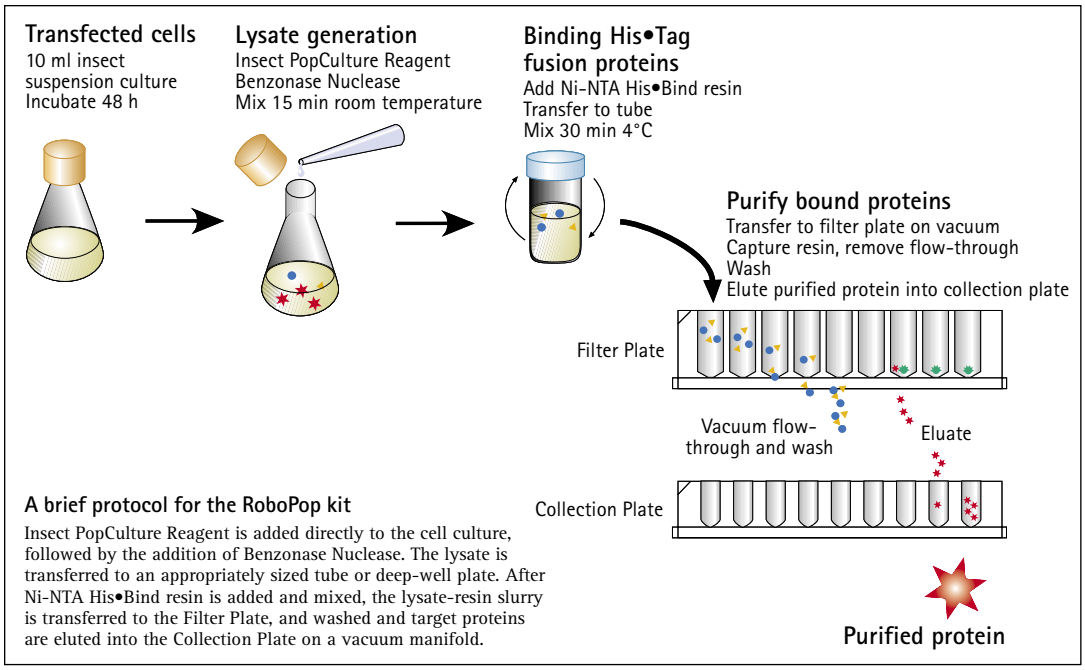
- Insect PopCulture® Reagent, for protein extraction directly from total cultures
- Benzonase® Nuclease, for viscosity reduction
- Ni-NTA His•Bind Resin and buffers
- 2-ml 96-well Filter Plate and Collection Plate with Sealer

Product	Cat. No.
Insect RoboPop™ Ni-NTA His•Bind® Purification Kit	71257-3

Components:

• 50 ml	Insect PopCulture® Reagent
• 10 KU	Benzonase® Nuclease, Purity > 90%
• 10 ml	Ni-NTA His•Bind Resin
• 125 ml	4X Ni-NTA Bind Buffer
• 2 × 125 ml	4X Ni-NTA Wash Buffer
• 50 ml	4X Ni-NTA Elute Buffer
• 1	2-ml 96-well Filter Plate
• 1	Collection Plate with Aluminum Plate Sealer

Note: 1 KU = 1000 units



A brief protocol for the RoboPop kit

Insect PopCulture Reagent is added directly to the cell culture, followed by the addition of Benzonase Nuclease. The lysate is transferred to an appropriately sized tube or deep-well plate. After Ni-NTA His•Bind resin is added and mixed, the lysate-resin slurry is transferred to the Filter Plate, and washed and target proteins are eluted into the Collection Plate on a vacuum manifold.

Overview of reagent requirements for various culture sizes

Culture size	Sf9 cells	Plasmid DNA dilution in BacVector® Medium	Insect GeneJuice Transfection Reagent dilution in BacVector Medium	Insect PopCulture Reagent	Benzonase Nuclease	His•Bind Ni-NTA Resin	Resin pool applied to column	His•Bind Ni-NTA 1X Wash Buffer	His•Bind Ni-NTA 1X Elute Buffer
10 ml	9 ml at 1.0×10^6/ml	20 µg + 0.5 ml	60 µl + 0.4 ml	0.5 ml	5 µl	0.25 ml	96-well filter plate	2 × 1.5 ml	0.5 ml
100 ml	90 ml at 1.1×10^6/ml	200 µg + 5 ml	1 ml + 4 ml	5 ml	50 µl	2.5 ml	30 mm × 20 cm	2 × 15 ml	7.5 ml
200 ml	180 ml at 1.1×10^6/ml	400 µg + 10 ml	2 ml + 8 ml	10 ml	100 µl	10 ml	30 mm × 20 cm	2 × 30 ml	15 ml
1 liter (4 × 250-ml cultures)	225 ml at 1.1×10^6/ml per flask	500 µg + 12 ml per flask	2.5 ml + 10 ml per flask	12.5 ml per flask	125 µl per flask	25 ml to pooled lysate	30 mm × 23 cm	2 × 100 ml	75 ml

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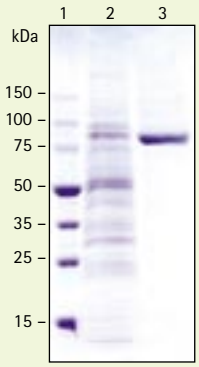
Summary of yields from small- and large-scale cultures using InsectDirect™

Target	Purified protein yield	
	Small-scale yield (µg/ml of culture)	Large-scale* yield (mg/L of culture)
β-galactosidase	13.7	30
p38MAP Kinase	9.1	14
cdc-2	6.3	16
Protein Kinase 1	4.4	13.3
Heat Shock Protein 1	48.3	80
Heat Shock Protein 2	31.4	38
Phospholipase	14.9	21
Secreted Kinase	1.0	7.6

*Large-scale = 100 ml - 1 L cultures

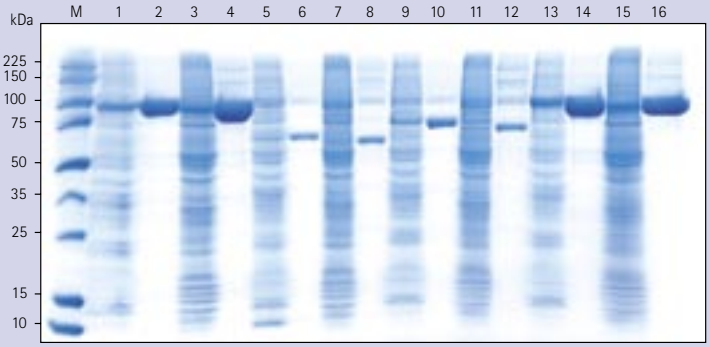
Protein kinase, 1-liter scale



Lane	Sample	Yield
1	Perfect Protein Markers, 15–150 kDa	
2	Protein Kinase, Total cell protein	
3	Protein Kinase, Eluate	13.3 mg

Four 250-ml suspension cultures were seeded with Sf9 cells (1×10^6 cells/ml) in serum-free BacVector® Insect Cell Medium. A pIEx/protein kinase isoform construct was added to the diluted Insect GeneJuice® Transfection Reagent, incubated for 15 min at room temperature, and then added to the flasks. After 48 h incubation at 28°C, Insect PopCulture® Reagent and Benzonase® Nuclease were added directly to each flask and incubated for 15 min at room temperature to lyse the cells. The His•Tag® fusion protein was manually purified on Ni-NTA His•Bind® Resin according to the standard protocol. Protein yield was quantified by a modified Bradford assay.

A comparison of the baculovirus and InsectDirect systems for expression and purification



Lane	Sample
M	Perfect Protein™ Markers, 10-225 kDa
1	vBAC-2cp - Heat Shock Protein A, crude
2	vBAC-2cp - Heat Shock Protein A, purified
3	pIEx-7 - Heat Shock Protein A, crude
4	pIEx-7 - Heat Shock Protein A, purified
5	vBAC-2cp - Protein Phosphatase, crude
6	vBAC-2cp - Protein Phosphatase, purified
7	pIEx-7 - Protein Phosphatase, crude
8	pIEx-7 - Protein Phosphatase, purified
9	vBAC-2cp - Protein Kinase, crude
10	vBAC-2cp - Protein Kinase, purified
11	pIEx-7 - Protein Kinase, crude
12	pIEx-7 - Protein Kinase, purified
13	vBAC-2cp - Heat Shock Protein B, crude
14	vBAC-2cp - Heat Shock Protein B, purified
15	pIEx-7 - Heat Shock Protein B, crude
16	pIEx-7 - Heat Shock Protein B, purified

For InsectDirect, Sf9 cells in 10-ml suspension cultures ($1x10^6$ cells/ml) grown in BacVector Insect Cell Medium were transfected with 15 µg recombinant plasmid using Insect GeneJuice Transfection Reagent. For baculovirus, 10-ml suspension cultures of TriEx™ Sf9 cells ($1x10^6$ cells/ml) grown in TriEx Insect Cell Medium were infected at an MOI of 5. Total culture extracts for both the InsectDirect–transfected and baculovirus-infected cultures were prepared 72 h later by adding Insect PopCulture Reagent followed by the addition of Benzonase Nuclease. Samples were removed at that point to assess total cell protein. Ni-NTA His•Bind Resin was then added to the extracts and the His•Tag fusion proteins were manually purified according to the standard protocol. Samples of the total cell protein and purified fractions were analyzed by SDS-page (10-20% gradient).

For more information or to order Novagen products, contact your local office.

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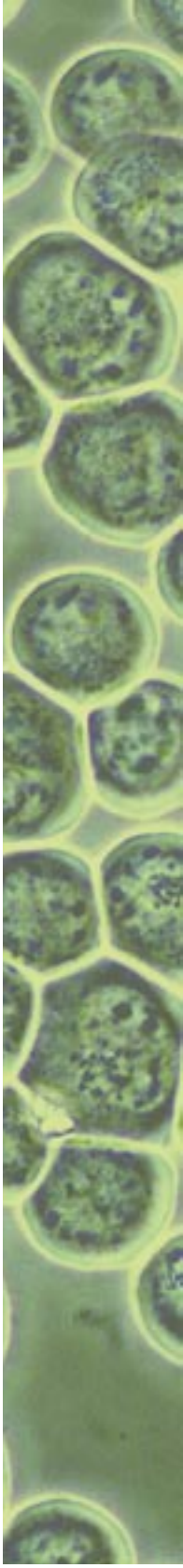

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