InsectDirect™ pIEx™ Vectors

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About the Kits

InsectDirect™ pIEx™-1 DNA	20 µg	71241-3
InsectDirect pIEx-2 DNA	20 µg	71238-3
InsectDirect pIEx-3 DNA	20 µg	71243-3
InsectDirect pIEx-4 DNA	20 µg	71235-3
InsectDirect pIEx-5 DNA	20 µg	71242-3
InsectDirect pIEx-6 DNA	20 µg	71333-3
InsectDirect pIEx-8 DNA	20 µg	71555-3
InsectDirect pIEx-9 DNA	20 µg	71556-3
InsectDirect pIEx-10 DNA	20 µg	71557-3

Description

The InsectDirect™ pIEx™ vectors are designed for protein expression by transient transfection of *Spodoptera*-derived insect cells. To drive target gene expression, the vectors employ an optimal combination of two transcription elements derived from AcNPV baculovirus, the hr5 enhancer and the ie1 immediate early promoter [1-4]. This promoter/enhancer combination recruits endogenous insect cell transcription machinery, thereby avoiding the need for using baculovirus and the cytopathic effects associated with infection. If desired, the vectors can also be used for the generation of drug-resistant stable cell lines by cotransfection with the plasmid pIE1-Neo (see User Protocol TB176). The vectors are designed with the option of producing unfused proteins or fusions with a variety of tags that aid in the detection, purification, and quantification of the fusion protein. The vectors also carry a high-copy pUC replicon to facilitate the preparation of high-quality plasmid DNA for transfection. Upon purification of InsectDirect™ pIEx plasmids and subsequent transfection with Insect GeneJuice® Transfection Reagent, optimal transfection efficiencies and expression levels can be achieved.

Components

- 20 µg InsectDirect pIEx DNA

Storage

Store DNA at –20°C.

Multiple Cloning Sites and Fusion Tags

Vector features

The InsectDirect™ pIEx™ vectors contain a wide array of restriction sites including an *Nco* I site (except for InsectDirect pIEx-10) that provides the option for production of unfused target proteins. The extensive multiple cloning site (MCS) regions in these vectors facilitate traditional subcloning, and many of the restriction enzyme sites are also found in Novagen pET, pBAC™, and pTriEx™ expression vectors. High-throughput (HT) compatible Ek/LIC or 3C/LIC cloning versions of some of the InsectDirect pIEx vectors are also available (InsectDirect pIEx-1, -2, -3, -7, -8, and -10 Ek/LIC and InsectDirect pIEx-9 3C/LIC; see User Protocols TB163 and TB450, respectively). InsectDirect pIEx-1, -2, and -3 encode N-terminal fusion tags followed by thrombin (Tb) and enterokinase (Ek) cleavage sites for fusion tag removal, an extensive MCS, and an optional C-terminal epitope tag (the HSV•Tag® sequence). InsectDirect pIEx-4 and InsectDirect pIEx-5 encode optional C-terminal His•Tag® and S•Tag™ sequences. InsectDirect pIEx-3 and InsectDirect pIEx-5 encode the adiopkinetic hormone (AKH) signal sequence, designed for highly efficient secretion of the target protein. InsectDirect pIEx-6 encodes an N-terminal His•Tag sequence followed by an EK cleavage site and an optional C-terminal S•Tag sequence. InsectDirect pIEx-8, -9, and -10 encode an N-terminal Strep•Tag® II coding sequence [5] just upstream of an Ek or human rhinovirus type 14 3C (3C) cleavage site and an optional C-terminal His•Tag sequence. In addition to these features, InsectDirect pIEx-10 also encodes a mouse IgM secretion signal sequence [6] to allow secretion of the expressed fusion protein into the cell culture medium. The complete vector map can be found in the User Protocol as indicated in the table below.

InsectDirect pIEx Vector Characteristics Table

Vector	Signal Sequence	Fusion Tags					Protease Cleavage Sites	User Protocol
		GST•Tag	Strep•Tag II	His•Tag	S•Tag	HSV•Tag		
InsectDirect pIEx-1				N	N	C	Tb, Ek	TB349
InsectDirect pIEx-2		N		N	N	C	Tb, Ek	TB348
InsectDirect pIEx-3	N	N		N	N	C	Tb, Ek	TB351
InsectDirect pIEx-4				C	C			TB350
InsectDirect pIEx-5	N			C	C			TB352
InsectDirect pIEx-6				N	C		Ek	TB398
InsectDirect pIEx-8			N	C			Ek	TB431
InsectDirect pIEx-9			N	C			3C	TB432
InsectDirect pIEx-10	N		N	C			Ek	TB433

N: N-terminal; C: C-terminal

Fusion Tags

- His•Tag fusion proteins produced in insect cells can be easily detected with the His•Tag Monoclonal Antibody and purified using Ni-NTA His•Bind® resins.
- The Strep•Tag II peptide is an 8 amino acid sequence that binds to Strep•Tactin® resin, a streptavidin engineered with an optimized binding site. Strep•Tag II fusion proteins can be purified with the Strep•Tactin resins and detected with either the Strep•Tag II Monoclonal Antibody or Strep•Tag II Antibody HRP Conjugate.
- The S•Tag peptide is a 15-amino acid sequence that allows the detection, purification, and quantification of fusion proteins based on its affinity for the 104-amino acid S-protein. S•Tag fusion proteins can be assayed using either a fluorescent homogeneous assay (FRETWorks™ S•Tag Assay) or a spectrophotometric assay (S•Tag Rapid Assay). S•Tag fusion proteins can be detected in Western blot formats using AP- or HRP-conjugated S-protein. Fusion proteins containing an N-terminal S•Tag sequence can be purified with the S•Tag Purification Kits, in which elution of the fusion protein is achieved by cleavage with Thrombin or Recombinant Enterokinase.
- The GST•Tag™ sequence is a 220-amino acid polypeptide that allows fusion proteins to be purified using Novagen GST•Bind™ Resin, detected with the GST•Tag Monoclonal Antibody, and quantified using the GST•Tag Assay Kit.
- The HSV•Tag® sequence is an 11-amino acid epitope derived from herpes simplex glycoprotein D and can be used for Western blot detection with the HSV•Tag Monoclonal Antibody Procedures

Cloning strategies to generate target proteins without fusions

Several of the InsectDirect™ pIEx™ vectors are designed to allow the expression of unfused proteins. The *Nco* I restriction site (CCATGG) in several vectors can be used for the expression of unfused protein [7] because the ATG coincides with (Met) translation start site. Similarly, vector-encoded C-terminal fusions can be avoided by including a translation stop codon in the insert.

The ATG triplet within the *Nco* I site encodes the N-terminal methionine residue. Target genes or PCR-engineered inserts that contain either an *Nco* I site or sites that generate compatible overhangs (*Bsp*H I [TCATGA], *Bsp*LU11 I [ACATGT], and subsets of *Afl* III [ACRYGT] and *Sty* I [CCWWGG]) at the beginning of the open reading frame (ORF) can be cloned into the *Nco* I site. Note that utilization of these restriction sites can be complicated if the target gene contains additional recognition sites. In addition, each of these restriction sites dictates the first nucleotide of the next triplet codon, which may prevent the generation of the authentic N-terminus.

When the insert contains these sites, it may be possible to employ an alternative strategy to allow the generation of native target protein. Several restriction enzymes that cleave "downstream" of their recognition site are commercially available (see table below).

Enzyme (isoschizomers)	Recognition and cleavage site	Overhangs generated
Bbs I (*Bpi* I, *Bpu*A I)	5′-GAAGAC(N)$_{2}$ –3′ 3′-CTTCTG(N)$_{6}$ -5′	GAAGACNN NNNNN CTTCTGNNNNNN N
Bsa I (*Eco* 3 1 I)	5′-GGTCTC(N)$_{1}$ -3′ 3′-CCAGAG(N)$_{6}$ -5′	GGTCTCN NNNNN CCAGAGNNNNN N
*Bsm*B I (*Esp* 3 I)	5′-CGTCTC(N)$_{1}$ -3′ 3′-GCAGAG(N)$_{5}$ -5′	CGTCTCN NNNNN GCAGAGNNNNN N
Bsp M I (*Bfu*A I)	5′-ACCTGC(N)$_{4}$ –3′ 3′-TGGACG(N)$_{8}$ -5′	ACCTGCNNNN NNNNN TGGACGNNNNNNNN N

Any of the restriction sites in this table can be engineered into PCR primers such that *Nco* I-compatible overhangs can be generated. Note that like any strategy employing restriction digestion, convenient utilization of this approach also can be limited if the target gene contains additional sites. However, it is relatively unlikely that a given insert will contain sites for all four of the enzymes listed above.

Target genes can also be cloned into the *Psh*A I site in InsectDirect pIEx-1, -2, -3, and -6 Vectors to create a fusion protein as outlined in the detailed plasmid maps. The entire N-terminal fusion can then be removed by cleavage with Recombinant Enterokinase to create unfused target proteins.

Another alternative for the generation of unfused protein is to utilize the HT-compatible Ek/LIC versions of InsectDirect pIEx-1, -2, –3, -7, -8, and -10 or 3C/LIC versions of InsectDirect pIEx-9 and subsequent cleavage of the fusion protein with Ek or HRV 3C, respectively (see User Protocols TB163 and TB450).

Cloning strategies to generate fusion proteins

To create N-terminal fusion proteins, utilize appropriate restriction enzyme sites downstream from the desired tag and maintain the desired reading frame. To create fusions with a C-terminal tag, the insert must lack a stop codon and maintain the desired reading frame. Restriction enzyme-mediated cloning strategies and protocols are available in many publications that describe molecular biology techniques.

Analysis of InsectDirect™ pIEx™ recombinants

Plasmid DNA from candidate recombinants should be verified for the presence of the correct insert and reading frame. This analysis should be performed prior to transfection to verify that the desired clone has been isolated. Several methods are available for analysis of transformants including colony PCR, plasmid prep, restriction analysis, and sequencing.

Colony PCR and sequencing primers

The following table designates the appropriate primer to use for PCR and sequence analysis.

Vector	Sense Primer	Antisense Primer
InsectDirect™ pIEx™-1	IE1 Promoter Primer (Cat. No. 69103-3) **or** S•Tag™ 18mer Primer (Cat. No. 70828-3)	IE1 Terminator Primer (Cat. No. 71247-3) **or** AS HSV•Tag® Primer (Cat. No. 71246-3)
InsectDirect pIEx-2 InsectDirect pIEx-3	S•Tag 18mer Primer (Cat. No. 70828-3)	IE1 Terminator Primer (Cat. No. 71247-3) **or** AS HSV•Tag Primer (Cat. No. 71246-3)
InsectDirect pIEx-4 InsectDirect pIEx-5 InsectDirect pIEx-6 InsectDirect pIEx-7	IE1 Promoter primer (Cat. No. 69103-3)	IE1 Terminator Primer (Cat. No. 71247-3) **or** AS S•Tag 18mer Primer (Cat. No. 71262-3)
InsectDirect pIEx-8 InsectDirect pIEx-9 InsectDirect pIEx-10	IE1 Promoter primer (Cat. No. 69103-3)	IE1 Terminator Primer (Cat. No. 71247-3)

Preparation of plasmid DNA for insect cell transfections

Plasmid DNA preparation intended for transfection of eukaryotic cells must not contain contaminants that interfere with transfection. Although standard plasmid miniprep DNA may work for transfection, results are often variable between different plasmids and different preparations of the same plasmid. Novagen Mobius and UltraMobius™ Plasmid Kits produce DNA of consistent quality for transfection. Please refer to scientific literature for a general protocol for isolating plasmid DNA.

Target Protein Expression and Purification

Insect cell lines and medium

The InsectDirect™ pIEx™ vectors are suitable for expression in *Spodoptera*-derived insect cells, including Sf9 and Sf21 cells. Novagen Sf9 Insect Cells (Cat. No. 71104-3) plus BacVector® Insect Cell Medium (Cat. No. 70590-3) are recommended for transfection of InsectDirect pIEx Vectors. While TriEx Sf9 Cells (Cat. No. 71023-3) can be used for transient or stable transfections, they may give lower transfection efficiencies than Sf9 Insect Cells.

Transfection with Insect GeneJuice® Transfection Reagent

Critical factors in obtaining high expression yields during transient transfection experiments are the efficiency and cytotoxicity of the transfection reagent. Insect GeneJuice Transfection Reagent (Cat. No. 71259-3; see User Protocol TB359) is a proprietary liposome formulation optimized for maximal transfection efficiency of *Spodoptera frugiperda*-derived insect cells (e.g., Sf9 and Sf21). The reagent also features extremely low toxicity to the cells and can be used for both transient and stable transfections in serum-containing or serum-free medium. Insect GeneJuice® Transfection Reagent is ideal for large-scale protein expression when using the InsectDirect pIEx Vectors.

Note: For transient transfection experiments, Novagen strongly recommends the use of Insect GeneJuice Transfection Reagent. Do NOT use Novagen Eufectin™ Transfection Reagent for this application.

Target protein extraction and purification

Insect PopCulture® Reagent

Insect PopCulture Reagent is a detergent-based lysis reagent specifically formulated for total culture extraction and affinity purification of recombinant proteins without the need for centrifugation. The improved method increases processing efficiency and target protein yields [8]. Insect PopCulture can be used for protein extraction from insect cells in suspension or adherent cells on tissue culture plates.

Note: Novagen Ni-NTA His•Bind® Resin is compatible with purification of proteins from Insect PopCulture extracts. His•Bind Resin (IDA agarose) and GST•Bind™ Resin are NOT compatible with purification of proteins from Insect PopCulture extracts.

CytoBuster™ Protein Extraction Reagent

CytoBuster Protein Extraction Reagent is a proprietary formulation of detergents optimized for efficient extraction of proteins from insect and mammalian cells. The unique composition of CytoBuster enables isolation of functionally active proteins without secondary treatment such as sonication or freeze/thaw. CytoBuster Protein Extraction Reagent is compatible with purification of proteins using Ni-NTA His•Bind and GST•Bind Resins and has been specifically formulated for use with Western blotting protocols, immunoprecipitation, and kinase/phosphatase assays.

Insect RoboPop™ Ni-NTA His•Bind Purification Kit

The Insect RoboPop Purification Kit is designed for HT purification of His•Tag® fusion proteins directly from transfected or infected Sf9 cultures without cell harvest, mechanical disruption, or extract clarification. The kits feature Insect PopCulture Reagent, Benzonase® Nuclease, Ni-NTA His•Bind Resin, and buffers for efficient protein extraction and affinity purification. The Insect RoboPop Purification Kit is designed to purify recombinant fusion protein from 10 ml cultures using a 2 ml well capacity filter plate. The 96 Well Filter Plate is compatible with standard filteration manifolds for manual and robotic processing. A Collection Plate and Sealer is provided for storage of the purified proteins. The RoboPop Ni-NTA His•Bind® Purification Kit will purify up to 38 mg of His•Tag fusion proteins per 96 well plate (up to 0.4 mg/well). Stated yields are based on 10 ml cultures and binding capacities of the resin, and can vary with the expression levels for individual fusion proteins.

Protein detection, purification, and quantification

For recommendations and protocols regarding sample preparation, purification, detection, and quantification, please see the following Technical Bulletins.

Detection/Assay Tools			
GST•Tag™ detection	**Cat. No.**	**Size**	**User Protocol No./Applications**
GST•Tag Monoclonal Antibody	71097-3 71097-4	50 µg 25 µg	TB325 Western blotting and immunofluorescence
His•Tag® detection	**Cat. No**	**Size**	**User Protocol No./Applications**
His•Tag Monoclonal Antibody	70796-4 70796-3	3 µg 100 µg	TB283 immunofluorescence, immunoprecipitation, Western blotting
His•Tag AP Western Reagents	70972-3	25 blots	TB283 colorimetric detection
His•Tag AP LumiBlot™ Reagents	70973-3	25 blots	TB283 chemiluminescent detection
His•Tag HRP LumiBlot Reagents	70974-3	25 blots	TB283 chemiluminescent detection
HSV•Tag® detection	**Cat. No**	**Size**	**User Protocol No./Applications**
HSV•Tag Monoclonal Antibody	69171-3 69171-4	40 µg 200 µg	TB067 Western blotting
S•Tag™ detection	**Cat. No**	**Size**	**User Protocol No./Applications**
S-protein AP Conjugate	69598-3	50 µl	TB097 Western blotting
S-protein HRP Conjugate	69047-3	50 µl	TB136 Western blotting
Biotinylated S-protein	69218-3	250 µl	Western blotting
S-protein FITC Conjugate	69060-3	200 µl	TB143 immunofluorescence
S•Tag AP Western Blot Kit	69213-3	25 blots	TB082 colorimetric detection
S•Tag AP LumiBlot Kit	69099-3	25 blots	TB164 chemiluminescent detection
S•Tag HRP LumiBlot Kit	69058-3	25 blots	TB145 chemiluminescent detection
Strep•Tag® II detection	**Cat. No**	**Size**	**User Protocol No./Applications**
Strep•Tag II Monoclonal Antibody	71590-3	100 µg	TB445 Western blotting
Strep•Tag II Antibody HRP Conjugate	71591-3	75 µl	TB446 Western blotting
Quantitative Assay	**Cat. No.**	**Size**	**User Protocol No./Sensitivity**
FRETWorks™ S•Tag™ Assay Kit	70724-3 70724-4	100 assays 1000 assays	TB251 fluorescent assay, Limit < 1 fmol
S•Tag Rapid Assay Kit	69212-3	100 assays	TB082 Limit 20 fmol
GST•Tag™ Assay Kit	70532-3	100 assays	TB236 Limit 8 pmol
Western Blot Protein Markers	**Cat. No**	**Size**	**User Protocol No./Size Standards**
Perfect Protein™ Western Markers	69959-3	25 lanes	TB102; 15, 25, 35, 50, 75, 100 and 150 kDa
Trail Mix™ Western Markers	70982-3	25 lanes	TB310; 15, 25, 35, 50, 75, 100 and 150 kDa, and 15, 16, 100 kDa prestained markers
Strep•Tag II Perfect Protein Markers	71614-3	100 lanes	16, 23.5, 30, 45, 60 and 100 kDa
Extraction and Purification Tools			
Extraction Reagents	**Cat. No.**	**Size**	**User Protocol No./Usage Conditions**
Insect PopCulture® Reagent	71187-3 71187-4 71187-5	15 ml 75 ml 250 ml	TB359 Use 50 µl per ml culture.
CytoBuster™ Protein Extraction Reagent	71009-3 71109-4	50 ml 250 ml	TB306 Use 150 µl per 10^6 cells
Reportasol™ Extraction Buffer	70909-3 70909-4	25 ml 125 ml	TB301 Use 150 µl per 10^6 cells
Benzonase® Nuclease, Purity > 90%	70746-3 70746-4	10,000 U 2,500 U	TB359 Use 10 U per ml insect culture volume
GST•Tag™ Purification	**Cat. No**	**Size**	**User Protocol No/Capacity and Features**
GST•Bind™ Resin	70541-3 70541-4 70541-5	10 ml 50 ml 25 ml	TB235 Capacity is 5 mg/ml settled resin

GST•Mag™ Resin	71084-3 71084-4	2 ml 10 ml	TB235 Capacity is 2 mg/ml settled resin
GST•Bind Buffer Kit	70534-3		TB235 All buffers for purification using GST•Bind or GST•Mag Resins
His•Tag® purification	**Cat. No.**	**Size**	**User Protocol No./Capacity and Features**
Ni-NTA His•Bind® Resin	70666-3 70666-4 70666-5	10 ml 25 ml 100 ml	TB273 Capacity is 5–10 mg/ml settled resin
Ni-NTA His•Bind Superflow	70691-3 70691-4 70691-5	10 ml 25 ml 100 ml	TB273 Capacity is 5–10 mg/ml settled resin, high flow rates and pressures
Ni-NTA Buffer Kit	70899-3		TB273 All buffers for native purification using Ni-NTA His•Bind and Ni-NTA Superflow Resins
Insect RoboPop™ Ni-NTA His•Bind® Purification Kit	71257-3		TB368 Purify up to 0.4 mg per 10 ml of suspension culture
S•Tag™ purification	**Cat. No.**	**Size**	**User Protocol No./Capacity and Features**
S-protein Agarose	69704-3 69704-4	2 ml 5 × 2 ml	TB087, TB160; Purify up to 1 mg per 2 ml settled resin
S•Tag Thrombin Purification Kit	69232-3		TB087 Purify and cleave up to 1 mg target protein per kit (2 ml settled resin)
S•Tag rEK Purification Kit	69065-3		TB160 Purify and cleave up to 1 mg target protein per kit (2 ml settled resin)
Strep•Tag® II purification	**Cat. No.**	**Size**	**User Protocol No./Capacity and Features**
Strep•Tactin® Superflow™ Agarose	71592-3 71592-4	2 ml 10 ml	TB449 Capacity is 50-100 nmol/ml settled resin
Strep•Tactin Superflow Column, 1 ml	71593-3	5 columns	TB449 Capacity is 50-100 nmol/column
Strep•Tactin Superflow Column, 0.2 ml	71594-3	5 columns	TB449 Capacity is 10-20 nmol/column
Strep•Tactin Superflow Cartridge, 1 ml	71595-3	5 cartridges	TB449 Capacity is 50-100 nmol/cartridge
Strep•Tactin Superflow Cartridge, 5 ml	71596-3 71596-4	1 cartridge 5 cartridges	TB449 Capacity is 250-500 nmol/cartridge
Strep•Tactin MacroPrep™ Resin	71597-3 71597-4	2 ml 10 ml	TB449 Capacity is 50-100 nmol/ml settled resin
Strep•Tactin MacroPrep Cartridge, 1 ml	71598-3	5 cartridges	TB449 Capacity is 50-100 nmol/cartridge
Strep•Tactin HT96™ Purification Kit	71605-3	1 kit	TB447 purify up to 100 µg/well or 9.6 mg/plate
Strep•Tactin SpinPrep™ Kit	71608-3	25 rxn	TB454 Purifies up to 150 µg/column

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