pBiEx™ Vectors

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About the Kits

pBiEx™-1 DNA	20 µg	71234-3
pBiEx-2 DNA	20 µg	71233-3
pBiEx-3 DNA	20 µg	71232-3

Description

pBiEx vectors are dual-host expression vectors that facilitate rapid expression screening in both *E. coli* and *Spodoptera*-derived insect cells. Expression in appropriate *E. coli* strains is controlled by the T7*lac* promoter. For expression in insect cells, the vectors employ an optimal combination of AcNPV baculovirus derived transcription elements, 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 may also be used for the generation of drug-resistant stable cell lines by performing cotransfection with the plasmid pIE1-Neo (See Technical Bulletin 176).

The BiEx Vectors are designed with the option of producing native, unfused proteins or fusions with a variety of tags to aid in the detection, purification and quantification of the fusion protein. The *lacI* gene is included on the plasmid backbone to ensure that an adequate amount of *lac* repressor is expressed for regulation of the T7*lac* promoter in appropriate *E. coli* strains. The vectors also carry a high-copy pUC origin of replication facilitate the preparation of high-quality plasmid DNA for transfection.

Components

- 20 µg   pBiEx DNA

Storage

Store DNA at –20°C.

Multiple Cloning Sites and Fusion Tags

Vector features

The pBiEx vectors contain a wide array of restriction sites including an *Nco* I site that provides the option for production of native 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. pBiEx-1 and pBiEx-2 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 antibody epitope tag, the HSV•Tag® sequence. pBiEx-3 encodes optional C-terminal His•Tag® and S•Tag™ sequences.

pBiEx Vector Characteristics Table

Vector	Fusion tags				Protease Cleavage Sites
	GST•Tag	**His•Tag**	**S•Tag**	**HSV•Tag**	
pBiEx-1		N	N	C	Tb, Ek
pBiEx-2	N	I	I	C	Tb, Ek
pBiEx-3		C	C		

N = N-terminal, I = Internal, 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 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 performed with cleavage by 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 native target proteins

Several of the pBiEx vectors are designed to allow the expression of native proteins. The *Nco* I site (CCATGG) in several vectors can be used for the expression of native unfused protein (5) because the ATG coincides with (Met) translation initiation 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 restriction 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 their 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.

In these cases 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 CTTCTGNNNNNNN N
Bsa I (*Eco*31 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 these restriction sites 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 will also 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.

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 open reading frame (ORF). To create fusions with a C-terminal tag, the insert must lack a stop codon and maintain the desired ORF. Restriction enzyme-based cloning strategies and protocols are available in many molecular biology techniques publications.

Analysis of pBiEx 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 isolate verify that the desired cloned 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
pBiEx-1	T7 Promoter Primer (Cat. No. 69348-3) **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)
pBiEx-2	S•Tag 18mer Primer (Cat. No. 70828-3)	IE1 Terminator Primer (Cat. No. 71247-3) **or** AS HSV•Tag Primer (Cat. No. 71246-3)
pBiEx-3	T7 Promoter primer (Cat. No. 69348-3)	IE1 Terminator Primer (Cat. No. 71247-3) **or** AS S•Tag 18mer Primer (Cat. No. 71262-3)

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. Please use a general protocol from scientific literature to purify larger amounts of plasmid DNA. Alternatively, transfection quality plasmid DNA may be prepared by using a CsCl/EtBr protocol.

Target Protein Expression and Purification in *E. coli*

Target protein expression can be induced from pBiEx recombinants by induction of the T7*lac* promoter. Induction can be achieved in the indicated host strains in the following ways.

Induction in NovaBlue by λCE6 infection

Expression can be induced from the NovaBlue host used for cloning by infection with the bacteriophage λCE6. The λp_L and λp_I promoters control the expression of T7 RNA polymerase in this phage. Therefore, T7 RNA polymerase is produced in host cells upon λCE6 infection. This in turn results in transcription of the target gene from the T7*lac* promoter present in the pBiEx vectors. T7 RNA polymerase transcribes target DNA so actively that normal λCE6 phage development cannot proceed. No T7 RNA polymerase is present in the cell before infection, so it should be possible to express any target DNA that can be cloned under control of a T7 promoter in this way. Bacteriophage CE6 is available separately and is described in Technical Bulletin 007.

Induction in (DE3) expression hosts

pBiEx recombinants can be transformed into an *E. coli* expression host containing a chromosomal copy of the gene for T7 RNA polymerase (λDE3 lysogens). These hosts carry a chromosomal copy of T7 RNA polymerase under the control of the *lacUV5* promoter (6, 7). The pBiEx plasmids supply sufficient *lac* repressor from the plasmid *lacI* gene to ensure stringent repression in the uninduced state. Induction is intiated by the addition of IPTG to the culture.

A variety of host strains are available and differ in several characteristics:

- BL21(DE3) is one of the most widely use host strains and has the advantage of being deficient in both *lon* (8) and *ompT* proteases.
- Tuner™(DE3) strains allow for uniform dose-dependent IPTG induction of the target protein throughout the culture because of a lac permease (*lac Y1*) mutation.
- Origami™(DE3) and Origami B(DE3) strains enhance disulfide-bond formation for proper folding and activity.
- The Rosetta™(DE3) and RosettaBlue™(DE3) strains are designed to enhance the expression of eukaryotic proteins that contain codons rarely used in *E. coli*.
- The Rosetta-gami™(DE3) and Rosetta-gami B(DE3) strains combine the attributes of the Rosetta and Origami strains.

Complete descriptions of the host strain characteristics and protocols for transformation can be found in our Competent Cell Technical Bulletin (Technical Bulletin 009).

After the plasmids are established in a λDE3 lysogenic host strain, expression of the target DNA is induced by the addition of IPTG to a growing culture. A final concentration of 1 mM IPTG is recommended for full induction at an OD_{600} of 0.6. The typical time of induction is 2–3 h. A detailed induction protocol can be found in the pET System Manual (Technical Bulletin 055).

Induction analysis, protein detection, purification and quantification

For recommendations and protocols regarding induction analysis and optimization, sample preparation, purification, detection and quantification please refer to the pET System Manual (Technical Bulletin 055) and the Technical Bulletins listed in the table on page 8.

Target Protein Expression and Purification from Sf9 cells

Insect cell lines and medium

The pBiEx vectors are suitable for expression in *Spodoptera*-derived insect cells, including Sf9 and Sf21 cells. Novagen® Sf9 Insect Cells (Cat. No. 71023-3) plus BacVector® Insect Cell Medium (Cat. No. 70590-3) are recommended for transfection of pBiEx 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. The Novagen Insect GeneJuice Transfection Reagent (Cat. No. 71259-3, See Technical Bulletin 359) is a proprietary liposome formulation optimized for maximal transfection efficiency of *Spodoptera*-derived insect cells (e.g Sf9 or 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 is ideal for large-scale protein expression when using the pBiEx Vectors.

Note: It is highly recommended to use Insect GeneJuice Transfection Reagent for transient transfection experiments. The Novagen Eufectin™ Transfection Reagent is sold for the purpose of creating baculovirus recombinants and is not recommended for transient transfections.

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 (9). 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 Reagents 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 high throughput (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 filter 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 will 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	**Cat. No.**	**Size**	**Technical Bulletin No./Applications**
GST•Tag™ detection			
GST•Tag Monoclonal Antibody	71097-3 71097-4	50 µg 25 µg	TB325 Western blotting and immunofluorescence
His•Tag® detection			
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			
HSV•Tag MonoclonalAntibody	69171-3 69171-4	40 µg 200 µg	TB067 Western blotting
S•Tag™ detection	**Cat. No**	**Size**	**Technical Bulletin/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
Quantitative Assay	**Cat. No.**	**Size**	**Technical Bulletin 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**	**Technical Bulletin 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
***E. coli* Extraction Reagents**	**Cat. No.**	**Size**	**Technical Bulletin No./Capacity and Features**
BugBuster® Protein Extraction Reagent	70584-3 70584-4	100 ml 500 ml	TB245 Use 5 ml/g wet cell paste, Tris-buffered
BugBuster HT Protein Extraction Reagent	70922-3 70922-4	100 ml 500 ml	TB245 Use 5 ml/g wet cell paste, Tris-buffered and pre-mixed with Benzonase Nuclease
BugBuster 10X Protein Extraction Reagent	70921-3 70921-4 70921-5	10 ml 50 ml 100 ml	TB245 Dilute to 1X with choice of buffer and use 5 ml/g wet cell paste
BugBuster (primary amine-free) Extraction Reagent	70923-3 70923-4	100 ml 500 ml	TB245 Use 5ml/g wet cell paste, PIPPS-buffered
PopCulture™ Reagent	71092-3 71092-4 71092-5	15 ml 75 ml 250 ml	TB323 Use 0.1 volume of culture
rLysozyme™ Solution	71110-3 71110-4 71110-5	300 KU 1200 KU 6000 KU	TB334 and TB323 Use 40 U per 1 ml of culture volume with PopCulture 1 KU per 1 ml BugBuster Reagent
Benzonase® Nuclease, Purity > 90%	70746-3 70746-4	10,000 U 2,500 U	TB245, 323, 261 Use 25 U per ml original culture volume with PopCulture and BugBuster Reagent

Insect Extraction Reagents	Cat. No.	Size	Technical Bulletin No./Capacity and Features
Insect PopCulture™ Reagent	71187-3 71187-4 71187-5	15 ml 75 ml 250 ml	TB359 Use 50 µl per ml insect cell culture
CytoBuster™ Protein Extraction Reagent	71009-3 71109-4	50 ml 250 ml	TB306 Use 150 µl per 10^6 insect cells
Reportasol™ Extraction Buffer	70909-3 70909-4	25 ml 125 ml	TB301 Use 150 µl per 10^6 insect cells
Benzonase® Nuclease	70746-3 70746-4	10,000 U 2,500 U	TB359 Use 10 U per ml original culture volume

GST•Tag™ Purification	Cat. No	Size	Technical Bulletin 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
RoboPop™ GST•Bind™ Purification Kit	71189-3		TB346 Purify 0.8 mg/well from 5 ml *E. coli* cultures

His•Tag® Purification	Cat. No.	Size	Technical Bulletin 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
His•Bind Resin	69670-3 69670-4 69670-5	10ml 50 ml 100ml	TB054 Capacity is 8 mg/ml settled resin
His•Bind Buffer Kit	69755-3		TB054 All buffers for native purification using His•Bind Resin
His•Bind Columns	70971-3 70971-4	pkg/5 pkg/25	TB054 pre-packed, pre-charged; Capacity is 10 mg per column.
His•Mag™ Agarose Beads	71002-3 71002-4	2 ml 10 ml	TB054 magnetic agarose beads, pre-charged, Capacity is 5 mg per ml settled beads
His•Bind Quick Buffer Kit	70665-3		TB054 all buffers for native purification using His•Bind Columns, Quick Columns, Cartridges and His•Mag Agarose Beads, No charge buffer included
His•Bind Purification Kit	70239-3		TB054 10 ml His•Bind Resin, Buffers and Chromatography Columns
BugBuster® Ni-NTA His•Bind Purification Kit	70751-3		TB273 10 ml Ni-NTA His•Bind Resin, BugBuster, Benzonase and Chromatography Columns
BugBuster His•Bind Purification Kit	70793-3		TB054 10 ml His•Bind Resin and Buffer, BugBuster, Benzonase and Chromatography Columns
PopCulture His•Mag Purification Kit	71114-3		TB054 Process 40 × 3 ml cultures purifying up to 375 µg per 3 ml culture

His•Tag® purification	Cat. No	Size	Technical Bulletin No./Capacity and Features
RoboPop™ His•Mag™ Purification Kit	71103-3		TB327 Purify up to 12 mg per 96 wells
RoboPop Ni-NTA His•Bind® Kit	71188-3		TB346 Purify up to 96 mg per 96 wells
Insect RoboPop™ Ni-NTA His•Bind Purification Kit	71257-3		TB368 Purify up to 0.4mg per 10 ml suspension culture

S•Tag™ purification	Cat. No.	Size	Technical Bulletin 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)

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