Expression Systems
An Advancion Company

BestBac™ Linearized Baculovirus DNA INSTRUCTIONS FOR USE

Product Description

BestBac Linearized Baculovirus DNA is derived from the E2 strain of *Autographa californica Multiple Nuclear Polyhedrosis Virus* (AcMNPV). Recombinant virus generation is achieved by co-transfection of BestBac DNA with a transfer vector containing an expression cassette flanked by regions of homology to the AcMNPV polyhedrin locus. BestBac DNA contains a deletion in the essential ORF1629 gene, preventing replication of the non-recombinant parental DNA. Upon homologous recombination between the transfer vector and BestBac DNA inside the Insect cell, the gene of interest is introduced into the baculovirus genome and ORF1629 is restored allowing for replication of only recombinant baculovirus. BestBac is available in two varieties: BestBac 1.0 and BestBac 2.0. BestBac 1.0 is minimally modified from wild-type AcMNPV, while the viral proteases Chitinase and Cathepsin have been deleted from the viral genome in BestBac 2.0. Deletion of Chitinase and Cathepsin has been demonstrated to enhance yields of some recombinant proteins. Both BestBac products are compatible with transfer plasmids from any system that is based on polyhedrin locus based recombination.

Product	Catalog Number	Contents	Storage and Stability
BestBac 1.0, Linearized Baculovirus DNA	91-001	2.5 µg Linearized Baculovirus DNA	All Components are guaranteed stable for one year after the date of manufacture when stored at 2-8°C. Do NOT freeze the viral DNA.
BestBac 2.0, Linearized Baculovirus DNA	91-002		
BestBac 1.0, Baculovirus Cotransfection Kit	91-100	2.5 µg Linearized Baculovirus DNA 75 µl Expres^{2}TR Transfection Reagent 20 ml Transfection Medium	
BestBac 2.0, Baculovirus Cotransfection Kit	91-200		

Important Information

This protocol is optimized for Sf9 cells, but other Insect cell lines may be used successfully. All cell cultures used for transfection should be greater than 95% viability and be in the mid-logarithmic growth phase at the start of the procedure.

All steps of this procedure should be performed using sterile technique in a biosafety cabinet.

Antibiotics inhibit transfection and should not be used during the incubation step with the transfection mixture. Antibiotics may be used once the transfection mixture is replaced with fresh medium.

Expression Systems transfection medium is formulated to maximize transfection efficiency of Insect cells for generation of high titer P0 supernatants. However, standard insect cell culture medium may still be used successfully during transfection.

Safety Information

Read the Safety Data Sheets (SDS) and follow the handling instructions. Wear appropriate protective eyewear, clothing and gloves.

Additional Materials Required

Biosafety Cabinet
Centrifuge
Micropipettes
Sterile Polypropylene Tubes
Tissue Culture Treated 6-well Plate
27°C Static Incubator
Sterile Centrifuge Tubes
Transfer Vector containing the Gene of Interest

Compatible Transfer Vectors

BestBac DNA is compatible with these common families of baculovirus transfer vectors: pOET, pTriEx, pBAC, pBacPAK, and BD pVL and pAc.

Contact technical support if you have questions regarding compatibility of other transfer vectors.

Co-transfection Protocol

1. **Plate Cells:** Plate 0.9x10^6 Sf9 cells in a well of a 6 well plate for each co-transfection and control reaction. Incubate the plate undisturbed at room temperature for 30 minutes to allow the cells to adhere. Co-transfection mixtures may be prepared during this incubation.
2. **Prepare Co-transfection Mixtures:** For each co-transfection, pipette 100 µl transfection medium into each of two sterile polypropylene tubes, A and B. To tube A, add 5 µl (0.5 µg) BestBac DNA and 2 µg plasmid DNA. To Tube B, add 6 µl Expres^{2}TR transfection reagent. Incubate solutions A and B at room temperature for 5 minutes before combining the two solutions in one tube. Mix gently by slowly pipetting up and down two times using a large bore 1000 µl pipette tip. Incubate this mixture at room temperature for 20 minutes.
3. **Addition of Transfection Mixtures:** Add 800 µl transfection medium to each transfection mixture to increase the volume to 1000 µl. Aspirate the cell culture medium from each well and add the transfection solution to the appropriate well. To prevent drying of the monolayer, only aspirate and add transfection mixture to one well at a time.
4. **Incubation:** Incubate the plate at 27°C for 4-5 hours in a plastic bag or other sealed container with a damp paper towel or reservoir of water to maintain a humid environment.
5. **Replace with Fresh Medium:** Aspirate the transfection solution and immediately replace with 3 mL fresh cell culture medium. Antibiotics may be added at this point if desired. Incubate the plate at 27°C for 4-5 days in a plastic bag or other sealed container with a damp paper towel or reservoir of water to maintain a humid environment.
6. **Harvest P0 virus:** Transfer supernatant to sterile centrifuge tubes, centrifuge supernatant at 2000 g for 5 minutes, and transfer to a sterile tube. Store the P0 virus at 4°C and determine the viral titer for further use.

Expression Systems
An Advancion Company

P0 Virus Operations

Subsequent steps taken with the P0 virus depend on the intended use. Below are typical actions taken with the P0 virus:

- Cells or supernatant from the P0 co-transfection may be screened for expression of recombinant protein. However, expression levels may be low due to the sub-optimal expression conditions of the co-transfection.
- Although the recombination efficiency of BestBac is 100%, some intrinsically unstable sequences introduced by the gene of interest may result in excision of the gene during replication of progeny virus. If this is a concern, recombinant virus may be purified from the P0 supernatant by plaque or end point dilution purification.
- The P0 supernatant may be amplified to produce a larger volume high titer stock. Refer to the protocol below:

Amplification to High Titer P1 Virus Stock

1. Seed a culture of Sf9 cells at a density of $1x10^6$ cells/ml and incubate overnight at 27°C.
2. The following day, infect culture with P0 virus at a Multiplicity of Infection (MOI) of 0.1.
3. Incubate the culture for approximately 72 hours at 27°C.
4. Harvest P1 virus: pellet cells by centrifugation at 2000 g, pour off supernatant, and sterile filter through 0.2µm filter.
5. Store the P1 virus at 4°C and determine the viral titer for further use. For long term storage (years), aliquots of virus may be stored frozen at -80°C. Freezing and thawing of baculovirus supernatants will result in a drop in virus titer of approximately one log, but the titer will remain stable apart from this drop in titer.

Important Licensing Information

This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Limited Product Warranty

Expression Systems LLC warrants that this product meets its specifications, as stated in our product brochures and certificates. This warranty lasts from the time we deliver the consumable until the completion of the consumable's shelf life, when the product has been handled and stored in accordance with this IFU.

Related Products

Product	Catalog Number
ESF 921	96-001
pVL1392, 1393 Transfer Vector Set	91-030
Expres^{2}TR Transfection Reagent	95-055
Baculovirus Titering Kit	97-101
gp64-PE Antibody	97-201
Adapted Sf9 Cells Adapted Sf9-AF Cells	94-001 94-006
Transfection Medium	95-020

Legend of Labeling Symbols

Symbol	Interpretation
	Catalog Number
	Lot Number
	*Research Use Only*
	Manufacturer
	Temperature Limitation
	Date of Manufacture
	Instruction for Use

For technical assistance or documentation, such as Certificates of Analysis or Safety Data Sheets, email support@expressionsystems.com

Expression Systems LLC
2537 2nd Street, Davis, CA 95618
P: (530) 747-2035
expressionsystems.com

Doc ID: 34524
Rev. No.: 6