The BacPAK™ System—Simplicity Meets Great Results

- **Provides high yields of biologically active proteins**
- **Specially engineered BacPAK6 DNA produces a recombination efficiency close to 100%**
- **Easy-to-work-with insect cells**
- **Quickly determine baculovirus titers without time-consuming plaque assays**
- **Polyhistidine-tagged recombinant proteins facilitate purification via TALON® resins**
- **From gene to purified protein in as little as two weeks**

Baculoviral expression systems are widely used to produce large quantities of recombinant proteins in insect host cells. These systems are particularly well-suited for proteins that are difficult to express in bacteria due to size, complexity, or posttranslational processing requirements. Proteins expressed in insect cells have biological activities and immunological reactivities comparable to proteins that naturally exist in mammalian cells, as a result of similar posttranslational processing. Another advantage of the baculovirus system is its ability to express multiple proteins simultaneously, including large and/or secreted proteins, all without the need to generate cell lines. Furthermore, baculoviruses are inherently safe to use, since baculoviral genes are inactive in mammalian cells, and thus the viruses are unable to replicate and are quickly inactivated by the complement system.

Clontech's **BacPAK Baculovirus Expression System** is used to routinely express target proteins at levels from 1–500 mg/liter. The system provides everything necessary for efficiently producing recombinant proteins. Clontech offers a variety of tools for your BacPAK system that, when used together, make the process of expression, analysis, and purification as easy as 1-2-3. You can either purchase the all-in-one BacPAK system, or you can combine separate components for your specific needs: In-Fusion™ technology enables rapid and accurate cloning; the BacPAK Baculovirus system yields robust protein expression; and 6xHN tags greatly facilitate protein purification via Clontech's TALON resins. A protocol for the concerted use of Clontech's complete line of baculovirus and protein expression products is outlined below.

STEP 1: Clone your gene of interest into a transfer vector (1–3 days)

The first step in generation of recombinant baculovirus is to clone your gene of interest into the multiple cloning site (MCS) of a "transfer" vector. The Clontech BacPAK expression system includes transfer vectors that can be used to clone your gene of interest. Alternatively, you can speed up your initial cloning steps by cloning into our **In-Fusion Ready BacPAK Vectors** (1; Figure 1). The In-Fusion Ready Vectors come prelinearized and are ready for use (no restriction digestion, phosphatase treatment, or gel purification required). The same PCR product can be cloned in parallel into both prelinearized vectors, to generate clones encoding your gene of interest with a 6xHN tag at either the N- or C-terminus. The histidine-rich 6xHN tag allows for convenient protein purification using immobilized metal affinity chromatography (IMAC), for which our TALON resins are recommended.

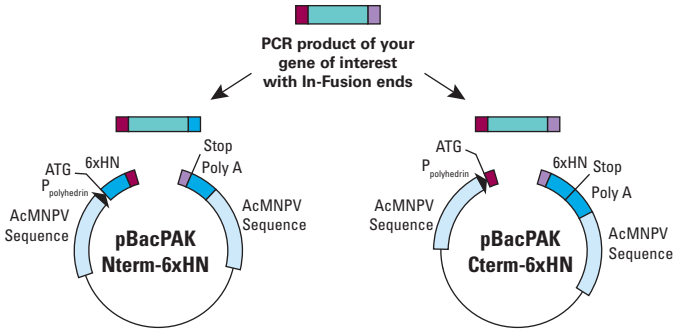

Figure 1. Simplify recombinant baculovirus production with the In-Fusion Ready BacPAK Vector Set. Insert your gene of interest into the transfer vector, then transform into *E. coli.*

STEP 2: Create a recombinant baculovirus expressing your gene of interest (3–4 days)

Baculoviruses expressing your gene of interest are generated by cotransfection of **Sf21 insect cells** with the transfer vector and linearized **BacPAK6 baculoviral DNA.** The cells are robust and easy to work with: they do not require CO_2 and can be freely converted from monolayer to suspension cultures without the use of trypsin. At the optimum growth temperature of 27°C, the cell doubling time is a mere 20–24 hours. Once your insect cells are ready and your gene of interest resides in the BacPAK transfer vector (Step 1), cotransfect the plasmid and linear BacPAK6 Viral DNA into the insect cells to make recombinant baculovirus (Figure 2).

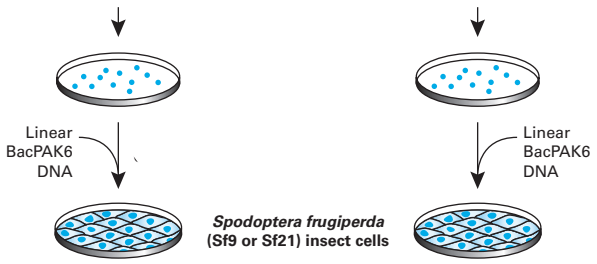

Figure 2. Cotransfect pBacPAK plasmid (from Step 1) and BacPAK6 linear DNA into Sf21 Insect Cells.

Then wait 3–4 days for the recombinant virus to accumulate in the medium. Recombinant virus can be collected from the medium by a quick low-speed centrifugation to remove any floating cells, and stored at 4°C for many months.

The BacPAK™ System...continued

STEP 3: Collect and amplify the virus (3–4 days)

Typical recombination efficiencies are 96–99%, meaning that virus can be amplified for protein production without the need for plaque purification. Simply collect the medium from the transfected cells (Step 2) and add a small aliquot of supernatant to fresh cells in order to amplify the recombinant virus for 3–4 days. The resulting high-titer stock can be used to infect cell cultures for analysis of protein expression (Step 5) or production of the recombinant protein (Step 6).

STEP 4: Determine viral titer (1–2 days)

Knowing the titer of your virus stock will enable you to maximize recombinant protein yield. Clontech has created new techniques for determining titer in a fraction of the time required for traditional titering methods. With Clontech's **BaculoELISA** and **BacPAK Baculovirus Rapid Titer Kits,** you can measure the ability of the virus to infect and express virally-encoded proteins within 24 and 48 hours, respectively (2).

STEP 5: Characterize gene expression (2–3 days)

Before producing target protein on a large scale, we recommend performing a small-scale infection to analyze gene expression from the recombinant virus and determine the time course of protein production. Most proteins will reach maximal expression in 2–3 days. Protein expression can be analyzed by Western blotting or with polyhistidine-tag antibodies. Clontech's **Universal His Western Blot Kit 2.0** (Figure 3) utilizes a TALON-based detection reagent that binds to a variety of polyhistidine tags and allows you to detect as little as 0.5 ng of purified protein (Step 7).

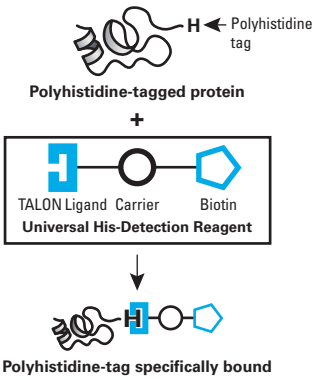

Figure 3. Analyze expression of your protein of interest with the Universal His Western Blot Kit 2.0.

STEP 6: Large-scale target protein production

Use the optimized conditions determined in Step 5 to scale-up protein expression. Using an optimal multiplicity of infection, determined in Step 4, will synchronize the infection in all cells, without over-burdening the cells with too much virus. Harvest the cells at the appropriate time, spin down, and freeze the cell pellet. Proceed to purification at your convenience.

STEP 7: Purify your target protein (1 hour)

TALON® Single Step Columns simplify purification of polyhistidine-tagged protein (Figure 4). These columns combine our exclusive **TALON xTractor Buffer** with the patented TALON resin to allow consolidation of the preliminary purification steps: cell lysis, centrifugation, and resin binding. Your entire purification can be completed in less than 1 hour.

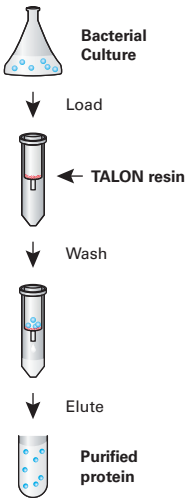

Figure 4. Extract and purify your target proteins in one step with TALON Single-Step Columns.

Product	Size	Cat. No.
BacPAK Baculovirus Expression System	each	631402
In-Fusion Ready BacPAK Vector Set	3 vectors	631410
In-Fusion 2.0 Dry-Down PCR Cloning Kit	8 rxns	639609
BacPAK6 DNA (Bsu36 I digest)	5 trnsfxns	631401
IPLB-Sf21 Insect Cells	1 vial	631411
BacPAK Complete Medium	1 L	631403
BacPAK Grace's Basic Medium	500 ml	631404
BacPAK Baculovirus Rapid Titer Kit	each	631406
BaculoELISA Titer Kit	each	631412
Universal His Western Blot Kit 2.0	each	635642
TALON Single Step Columns (5 ml)	25 columns	635628
TALON Single Step Columns (20 ml)	10 columns	635632

BacPAK™ Baculovirus Expression System Components

- pBacPAK8 Transfer Vector
- pBacPAK9 Transfer Vector
- BacPAK6 Viral DNA (Bsu36 I digest)
- Bacfectin Transfection Reagent
- IPLB-Sf21 Insect Cells
- BacPAK6 Virus Stock (positive control)
- Bac1 Sequencing/PCR Primer
- Bac2 Sequencing/PCR Primer
- pBacPAK8-GUS Positive Control Transfer Vector

Notice to Purchaser

Please see the In-Fusion™ Cloning Products and TALON® Purification Products licensing statements on page 42.

References

1. In-Fusion™ Ready BacPAK Vector Set (July 2006) *Clontechniques* **XXI**(2):18–19.
2. BaculoELISA Titer Kit (April 2007) *Clontechniques* **XXII**(2):8–9.