

## Baculovirus Titration is Essential to Maximizing Recombinant Protein Expression

Cell and Molecular Biology Group  
Clontech Laboratories, Inc.

*The baculovirus system is a highly efficient method of producing large amounts of high-quality recombinant protein in insect cells. Maximizing protein yield depends on accurate virus titration and on using an optimal multiplicity of infection (MOI). Here, we have used a virus that expresses a fluorescent protein (AcGFP1) to show that both the timing and height of peak protein expression differ with respect to increasing amounts of virus. Optimum results were obtained using an MOI of 10, while the use of excess virus led to significantly lower peak protein expression.*

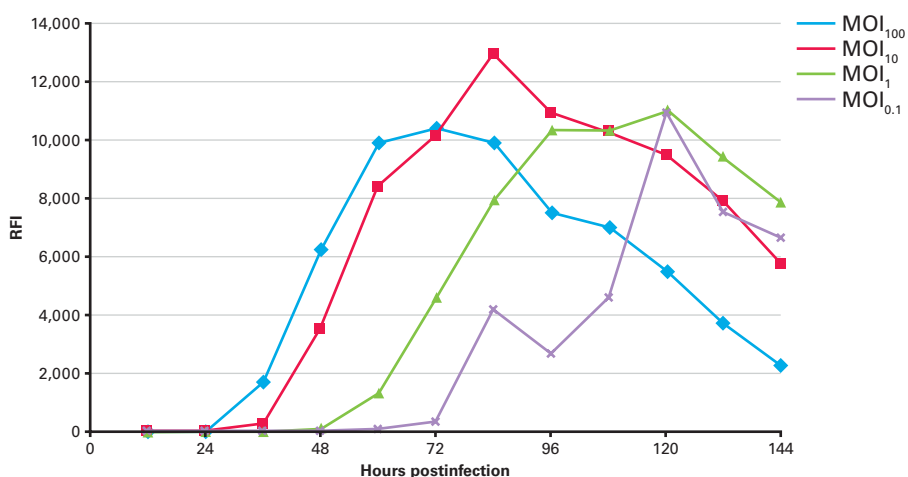
Baculovirus systems are capable of producing very high yields of recombinant protein (1, 2). Compared to bacterial systems, the qualities, activities, structures, and posttranslational features of target proteins produced in insect cells more closely resemble those of native mammalian proteins. As the virus replicates in a typical system, a protein of interest is expressed from the virus' strong, very late polyhedrin promoter, which becomes increasingly active toward the end of the infection cycle. Thus, establishing infection parameters which enable coordinated expression of the recombinant protein when the polyhedrin promoter is most active is essential to maximizing protein yield.

### MOI Matters

For the most productive infections, the ratio of virus particles per cell (commonly referred to as the multiplicity of infection or MOI) must be optimal, and that optimum value may fall within a specific range (Table I). If the MOI is too low, asynchronous replication and premature cell lysis can lead to reduced and delayed protein production. If the MOI is too high, rapid virus propagation depletes the cellular metabolic resources before the highest level of protein expression is attained. It is therefore essential to titrate your virus and use an amount that achieves a balance between the energetic demands of virus propagation and efficient protein production.

**Table I. Effects of MOI on Maximum Protein Expression in the Baculovirus System**

MOI	Effect
<1	Only a subset of cells are infected; thus not all cells will immediately express the protein of interest. Maximal expression will be delayed several days, and will not be well-synchronized.
1–20	Protein yields are usually maximized in this range. At an optimal MOI, synchronous infection leads to coordinated expression of the protein of interest.
20+	Cells are infected with excess virus particles, which deplete cellular metabolic resources before strong expression from the late-acting polyhedrin promoter can be achieved.



**Figure 1. Using the correct MOI enables high levels of recombinant protein production.** Insect cells were infected at different MOIs and fluorescent protein expression was measured every 12 hr. The highest levels of AcGFP1 expression in cells were observed at an MOI of 10 (MOI<sub>10</sub>), whereas infection with MOIs of 0.1, 1, or 100 produced lower maximum levels of recombinant protein. RFI = relative fluorescence intensity.

### Monitoring the Timing of Recombinant Protein Expression

To demonstrate how MOI can influence the timing and level of protein expression, we infected cultures of Sf21 insect cells with increasing amounts of virus (0.1–100 infectious particles per cell; MOI<sub>0.1–100</sub>), and measured cell-associated fluorescence over a period of 6 days (Figure 1).

We used the **BacPAK™ Baculovirus Expression System** (Cat. No. 631402) to construct a virus that expresses the AcGFP1 Living Colors® fluorescent protein. The virus was plaque-purified, amplified, and then titered using the **BacPAK Baculovirus Rapid Titer Kit** (Cat. No. 631406).

Infected cells were harvested every 12 hours for a period of 6 days. The cells were centrifuged and washed once with PBS to remove the medium, and frozen at –80°C. Once cells from all time points were collected, the cells were thawed, and then lysed using Clontech's **TALON® xTractor Buffer** (Cat. No. 635625). Aliquots of cell lysate were transferred to 96-well plates and relative fluorescence intensity due to AcGFP1 expression was measured using a fluorescence plate reader.

## Baculovirus Titration is Essential to Maximizing Recombinant Protein...continued

### Identifying the Optimum MOI

With MOI<sub>0.1</sub> and MOI<sub>1</sub>, protein production was delayed until 2.5–3 days after the cells were infected. Also, protein production did not peak until 5 days postinfection and then rapidly decreased as cells began to die. Infection with MOI<sub>10</sub> (i.e., within the recommended range of 1–20) resulted in protein accumulation that began at 36 hr and peaked at 84 hr; 1.5 days earlier than the lower MOIs. MOI<sub>10</sub> also produced the highest level of AcGFP1 expression. Although infection with MOI<sub>100</sub> initially produced expression levels slightly higher than MOI<sub>10</sub> and peaked more quickly, the maximum expression of MOI<sub>100</sub> was significantly lower.

The peak protein level generated by MOI<sub>10</sub> was 24% higher at 84 hr than the maximum produced by MOI<sub>100</sub> at 72 hr, and was 31% higher than MOI<sub>100</sub> at 84 hr. These data demonstrate that using the correct amount of baculovirus (e.g. MOI<sub>10</sub>) makes the most efficient use of the culture and maximizes the amount of protein available for recovery and purification.

### Rapid Virus Titration: The First Step to Higher Yields

Since production of your recombinant protein is likely just the beginning of a longer study, quickly achieving this milestone can be crucial to attaining your research goals. As we have demonstrated, the amount of virus used can significantly affect the kinetics and extent of protein expression. To obtain optimum yields, it is essential to start with accurately calibrated virus.

Clontech offers two different titration kits to quickly and easily quantify your virus so you can infect cells with confidence. Titering eliminates variability, and with it, the question of whether your infection was optimal. Virus titration helps to easily strike the most effective balance between cell culture vitality and recombinant protein production.

The BacPAK Baculovirus Rapid Titer Kit is an antibody-based assay that identifies virally infected insect cells. The **BaculoELISA Titer Kit** (Cat. No. 631412) is a simple overnight assay which calibrates virus by measuring the expression of a viral protein essential for infectivity. Neither method requires specialized cells.

These simple, time-saving kits provide advantages over traditional titration methods such as plaque assays and end-point dilution assays, which take one to two weeks to complete and require significant technical expertise to perform reliably. The time spent on traditional virus titration—and possibly having to repeat the assay—can lead to significant delays.

### Optimizing Your Infection

Baculovirus systems have the capacity to produce large amounts of recombinant protein. However, using too little virus both delays and reduces the amplitude of peak protein expression, while using too much virus also lowers maximum expression and wastes virus.

We have presented time-course data for infections performed with different amounts of a single recombinant baculovirus and found the optimum MOI to be 10. In our experience, the optimum MOI value and the infection kinetics of any particular virus will vary with different virus preparations and with different virus constructions. Titering the infectivity of your amplified virus before infecting your cells, and then monitoring protein production during the infection, will allow you to harvest your cells at the optimum time.

Titrating your virus, infecting at an optimum MOI, and harvesting cells at the peak of protein expression are the keys to producing the highest protein yields.

#### References

1. Kitts, P. A., & Possee, R. D. (1993) *BioTechniques* **14**(5):810–817.
2. Kitts, P.A. *et al.* (1990) *Nucleic Acids Res.* **18**(19):5667–5672.

Product	Size	Cat. No.
BacPAK Baculovirus Rapid Titer Kit	5 assays	631406
BaculoELISA Titer Kit	each	631412
IPLB-Sf21 Insect Cells	1 vial	631411
BacPAK Baculovirus Expression System	each	631402
BacPAK6 DNA (Bsu36 I digest)	5 transfxns	631401
TALON xTractor Buffer	500 ml	635625
TALON xTractor Buffer Kit	each	635623

#### BacPAK™ Baculovirus Rapid Titer Kit Components

- Mouse gp64 Antibody
- Goat Anti-Mouse Antibody/HRP Conjugate
- Blue Peroxidase Substrate
- Normal Goat Serum
- Methyl Cellulose Overlay
- Resealable Plastic Bags
- Control Baculovirus
- User Manual (PT3153-1)

#### BaculoELISA Titer Kit Components

- Control Virus
- Capture & Detection Antibodies
- 96-Well ELISA Plate
- Lysis Buffer
- Wash Buffer
- Avidin-HRP
- TMB Substrate
- User Manual (PT3953-1)
- Protocol-at-a-Glance (PT3953-2)

#### Related Products

- BacPAK™ Complete Medium (Cat. No. 631403)
- BacPAK™ Grace's Basic Medium (Cat. No. 631404)
- In-Fusion™ Ready BacPAK™ Vector Set (Cat. No. 631410)

#### Notice to Purchaser

Please see the TALON® Purification Products licensing statement on page 33.