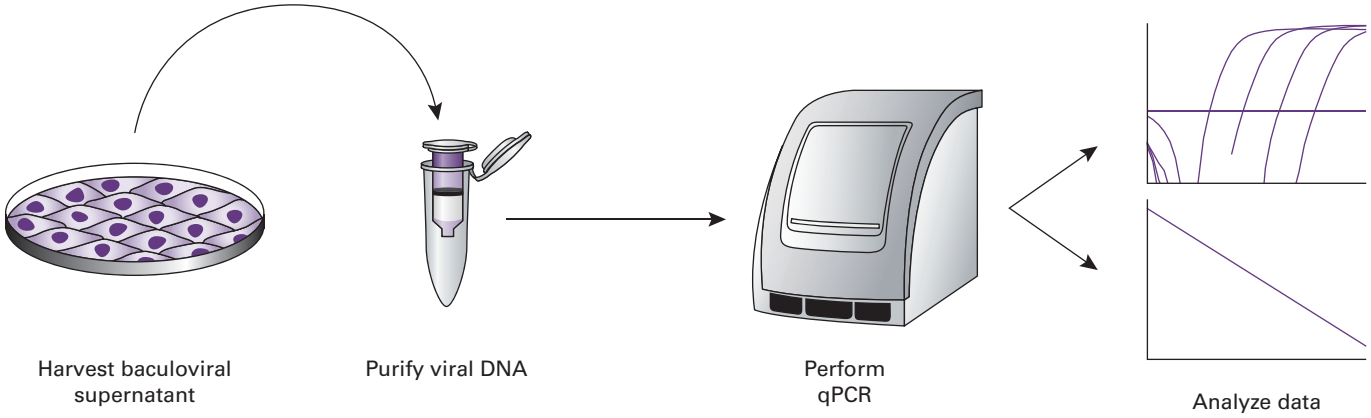
Rapid & Accurate Baculovirus Titration

- **Determine baculovirus titers in less than 4 hours**
- **Harvest, titrate, and infect in a single day**
- **Suitable for all AcMNPV-type baculoviruses**
- **Infect your target cells at known MOIs for precise and consistent results**

The **BacPAK™ qPCR Titration Kit** provides a rapid, accurate, and cost-efficient alternative to traditional methods for titrating AcMNPV-type baculoviruses (Table I). The kit employs a quick DNA purification step and allows determination of viral DNA genome content in just 4 hours, using qPCR and SYBR® Green detection technologies. Because qPCR titration is so fast, target cells can be infected with accurately titrated virus on the same day the virus is harvested. Delays and freeze-thaw cycles that reduce virus infectivity can be avoided. This method enables you to infect cells at a known multiplicity of infection (MOI), producing results that are precise, consistent, and interpretable.



The BacPAK qPCR Titration Procedure.

Table I: Comparison of BacPAK qPCR Titration to Other Titration Methods*

Titration Method	Plaque Assay	BacPAK Rapid Titer Assay	BacPAK qPCR Titration
Description	Count cleared plaques in infected cell monolayer	Immunostaining of Gp64 in infected cell monolayer	Measure viral DNA using SYBR qPCR with standard DNA as control
Time to Completion	1 week	48 hr	2–4 hr
Benefits	Traditional, visual	Simple, visual	Fast, accurate

* Clontech offers two different kits for baculovirus titration: the **BacPAK Baculovirus Rapid Titer Kit** (Cat. No. 631406) utilizes a standard immunological assay to accurately identify virus-infected cells, and the **BacPAK qPCR Titration Kit** (Cat. No. 631414) measures viral DNA copies via SYBR qPCR.

Table II: BacPAK qPCR Titer Correlation

	Titration Method			BacPAK Titration Ratios	
Baculovirus	**Plaque Assay (PFU/ml)[1]**	**BacPAK Rapid Titer Assay (FFU/ml)[2]**	**BacPAK qPCR Titration (copies/ml)**	**qPCR/Plaque Assay (copies/PFU)**	**qPCR/Rapid Titer Assay (copies/FFU)**
BacPAK 6 DNA	7.6×10^7	3.8×10^7	4×10^9	53	105

1 PFU = plaque-forming units.

2 FFU = focus-forming units.

The qPCR Titration Method

The BacPAK qPCR Titration procedure combines qPCR and SYBR Green detection technologies, allowing you to determine the viral genome copy number in baculoviral preparations from a calibrated DNA standard curve (Figure 1). The procedure is simple: viral DNA and BacPAK control DNA are serially diluted and subjected to qPCR. The DNA copy number of each viral sample is then determined by comparing its C_t value to a standard curve generated by plotting the C_t values of the diluted control samples against their respective copy numbers, as shown in Figure 1.

Delivers Consistent Results

Once the genome copy number of your viral stock has been determined, it can be correlated with viral infectivity to establish a copy number/(PFU or FFU) relationship (Table II). Determination of this ratio allows you to normalize the amount of prep used in each experiment, for consistent interassay results. Titration assays demonstrating the sensitivity and consistency of this approach are shown in Figure 2.

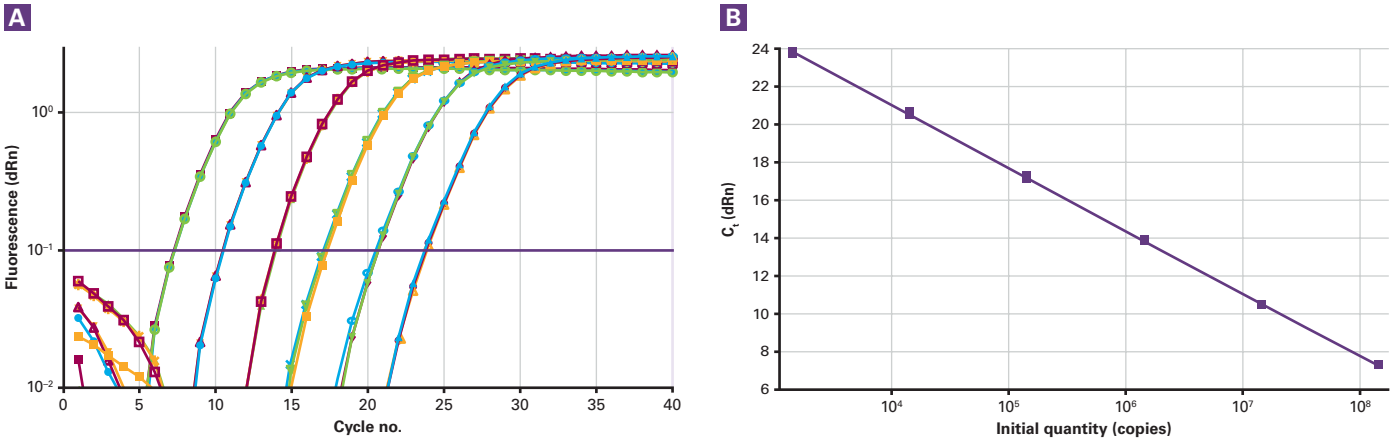


Figure 1. The BacPAK qPCR Titration Kit exhibits a wide dynamic range. The BacPAK DNA Control Template was serially diluted from 10^8–10^3 copies per sample and analyzed with the BacPAK qPCR Titration Kit. The amplification plots (**Panel A**) show a dynamic range of at least six orders of magnitude with no NTC (No-Template Control) background. The standard curve (**Panel B**) obtained by plotting the C_t values (determined from the amplification plots in Panel A) against the log of the DNA copy number in each sample, demonstrates a strong linear correlation between the C_t and the DNA copy number (log scale), with $R^2 = 1.000$ and a PCR efficiency of 100%.

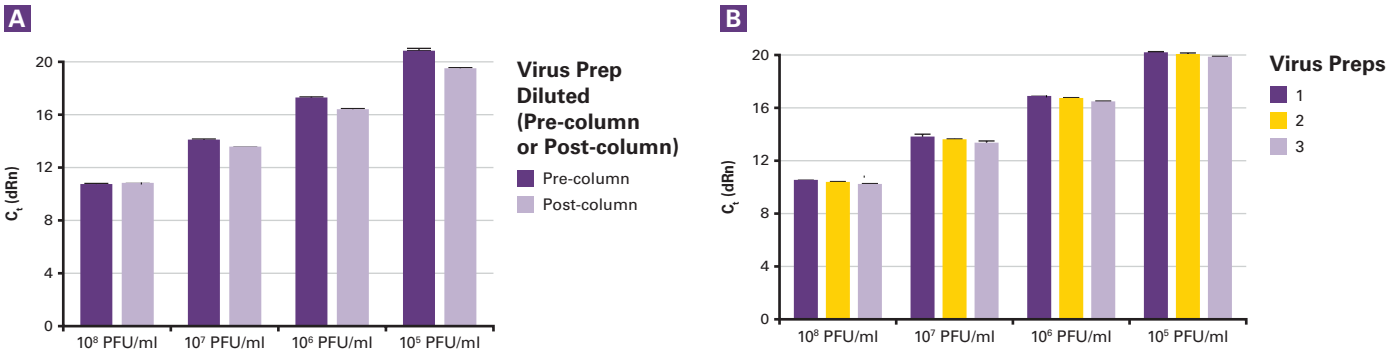


Figure 2.The BacPAK qPCR Titration method is highly sensitive and generates reproducible results. Panel A. BacPAK qPCR is sensitive enough to detect as little as 10^5 PFU/ml. One viral sample was diluted from 10^8 PFU/ml to 10^5 PFU/ml and the viral DNA in each dilution was purified (using the provided column) before qPCR was performed. A second sample of the same viral preparation was first purified (using the provided column) and then the eluted DNA was subjected to four 10-fold serial dilutions. Both treated samples showed similar C_t values. **Panel B.** BacPAK qPCR results are reproducible and consistent. One virus purified on three different days and titrated at different times showed similar C_t values at four different dilutions.

Ordering Information			
Product	**Size**	**Cat. No.**	
BacPAK qPCR Titration Kit	200 rxns	631414	**NEW!**

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