Baculovirus Titration with the BD Accuri™ C6 Flow Cytometer

Technical Bulletin

Contents

Introduction

The baculovirus expression vector system (BEVS) is increasingly popular among researchers as a tool for generating large amounts of recombinant protein in insect cells, which post-translationally process proteins in a manner similar to mammalian cells. Titration of the viral stock is an important step in optimizing recombinant protein expression. Established methods include the plaque assay (the gold standard of virus titration methods), endpoint dilution, immunostaining, use of fluorescent tags in fusion expression products, and real-time PCR. Recently, flow cytometry has emerged as a fast, accurate, and often less expensive, more efficient method to determine baculovirus titers.[1-4]

The BD Accuri™ flow cytometer, equipped with a BD CSampler™ accessory (CSampler), is an ideal analysis tool to assess the BEVS. The affordable BD Accuri C6 has a small footprint, and allows hands-free analysis of a 96-well plate.

Several features make the BD Accuri C6 uniquely suited for BEVS analysis. First, the BD Accuri C6 fluorescence and light scatter detectors have a broad dynamic range and are pre-optimized, greatly reducing instrument setup time and the probability of data loss due to improper instrument settings. Second, the use of microprocessor-controlled peristaltic pumps to drive the system fluidics allows the events per microliter of sample to be directly determined and used as a statistical parameter for any gated cell population. Thus, direct determination of viable, infected cell numbers per well is easily accomplished. Third, a user-designed statistics table for all 96 wells of data is easily built-out and can be transferred quickly to a spreadsheet for viral-titer calculations.

Materials and Methods

Sf9 insect cells in Sf9-00 II media plus antibiotics (Invitrogen) were plated in 96-well plates (100 µL of 2 x 10^6/mL stock) and infected with AcNPV virus stocks (final dilutions of 1:2, 1:10, 1:100, 1:1000, 1:10000, and 0) under the following conditions: 18 to 22 hours, 27°C, 180 rpm. Cells were washed and stained in the same plates using standard methods with PE-labeled anti-gp64 antibody (Expression Systems, LLC), in order to monitor expression of viral gp64 on the surface of the host insect cells.

After washing, cells were resuspended in 200 µL of phosphate buffered saline (PBS) and analyzed directly from the 96-well plate using the BD Accuri C6 and CSampler. Only cells with high forward and side light scatter values were gated for fluorescence analysis.

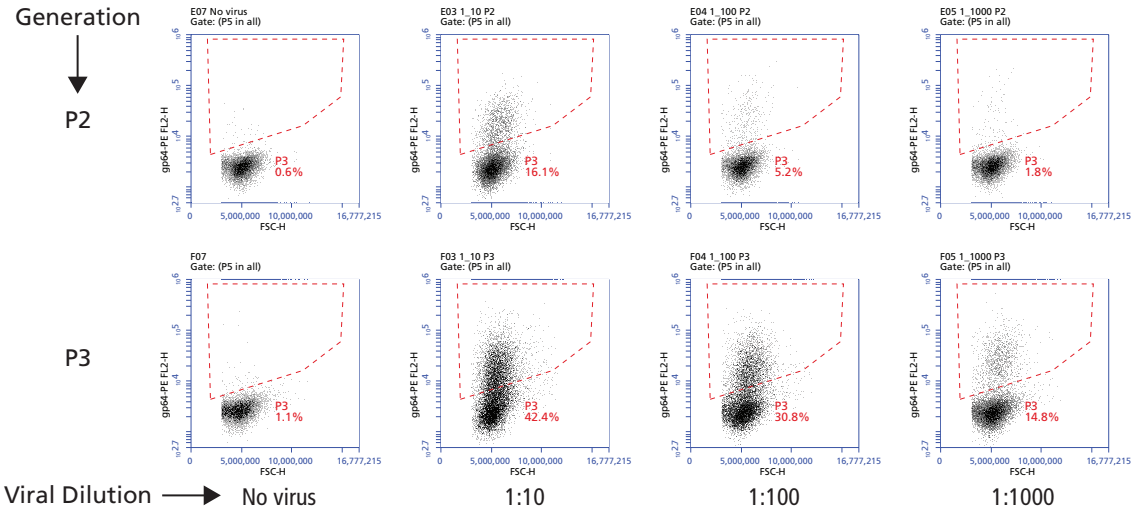

Figure 1. Examples of FSC vs gp64 expression in Sf9 cells infected with either generation 2 (row P2) or generation 3 (row P3) of AcNPV baculovirus cultures. The third generation of virus was more infective than generation 2 (compare rows P2 and P3).

Plate Row	Virus Description	Titer (IU mL^{-1})	Titer P3/P2
B	Pos. Control	7.589E+07	
C	V1:P2	2.586E+07	
D	V1:P3	3.263E+07	1.26
E	V2:P2	1.960E+07	
F	V2:P3	8.063E+07	4.11

Table 1. Viral titers were determined for the positive control and generations 2 and 3 (P2, P3) for two different viruses (V1, V2). V2:P3 is ~4 times more infective than V2:P2, while V1:P3 is only slightly (1.26 times) more infective than V1:P2.

Results

The percentage of anti-gp64-PE positive cells for each sample was determined by a gate (P3) set on FSC vs FL2 (gp64-PE) (Figure 1). The Master Statistics Tab in BD Accuri™ C6 software was used to generate a data table for all samples, which displayed the event count, percentage of anti-gp64-PE positive, and number of anti-gp64-PE positive cells per microliter of sample (Figure 2). The data from this table was transferred to a spreadsheet and viral titers were determined using the "normalization" method[1] (Table 1). As the viral titer determinations show, the third generation of virus preparation 2 (V2:P3) showed the highest infectivity. These results were comparable to those obtained with the same virus preparations analyzed with a BD FACSCalibur™ flow cytometer (data not shown).

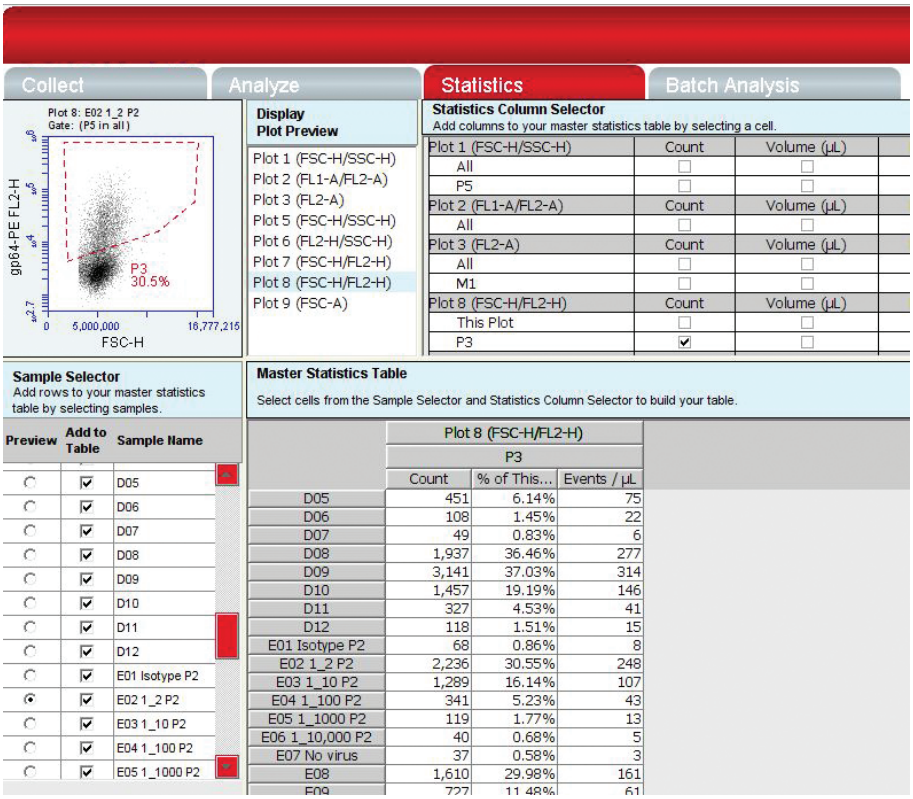

Figure 2. Both the percentage and absolute number per microliter of PE-positive events in gate P3 (Figure 1) were selected for display in the BD Accuri C6 software Master Statistics Table. This data was transferred to Microsoft® Excel and calculations of the viral titers were made using the "normalization" method described in Mulvania et al.[1]

Data courtesy of Brad Bennett and Mark Yeager, Department of Molecular Physiology and Biological Physics, University of Virginia, Charlottesville.

References

1. Mulvania T, Hayes B, Hedin D. A flow cytometric assay for rapid, accurate determination of baculovirus titers. *Bioprocessing.* 2004;May/June.
2. Roldao A, Oliveira R, Carrando MJ, Alves PM. Error assessment in recombinant baculovirus titration: evaluation of different methods. *J Virol Methods.* 2009;159:69-80.
3. Chan Z, Lai C, Lee H, Chen H, Hu Y. Determination of the baculovirus transducing titer in mammalian cells. *Biotechnol and Bioeng.* 2006;93:564-571.
4. Janakiraman V, Forrest WF, Seshagiri S. Estimation of baculovirus titer based on viable cell size. *Nature Protocols.* 2006;1:2271-2276.