

Product Description

Expression Systems Baculovirus Titering Kit facilitates accurate determination of infectious baculovirus titers in less than 24 hours. The assay is based on detection of the baculovirus gp64 fusion protein which is expressed on the surface of infected insect cells within six hours of infection. Staining of cells with a fluorescently labeled anti-gp64 antibody allows for identification of infected insect cells. By inoculating cultures with a series of log dilutions of virus, and staining of the cultures 18-20 hours post inoculation, the ratio of infected to un-infected insect cells can be determined by flow cytometry. Statistical analysis of the percentage of infected cells in the virus dilution series enables accurate infectious titer determination. Alternative assays that also claim results in under 24 hours such as particle counting instruments and qPCR, are indeed fast, but do not deliver true infectious titer values. In contrast to the plaque assay, the culturing conditions employed for the flow cytometric assay closely reflect actual culturing conditions utilized for expression cultures, resulting in a more relevant titer value in a fraction of the time. Provided access to a flow cytometer equipped to handle 96 well plates, the flow cytometric assay is easily adapted to high throughput processes.

Product	Catalog Number	Components	Storage	Shelf Life
Baculovirus Titering Kit	97-101	gp64-PE Antibody, 200 μ l rBV Control Virus, 1 ml (1×10^9 IU/ml)	2°C to 8°C, protected from light	rBV Control: 6 months from date of manufacture gp64-PE Antibody: 2 year recommended use by date

Important Information

This protocol is designed for use with a flow cytometer equipped to handle 96 well plates. If 96 well plate handling is not available the protocol may be adapted for manual sampling.

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

The cell cycle stage at the time of virus addition has a significant impact on the cells' permissivity to baculovirus infection, therefore the state of the cells used for this assay is of great importance for generating reliable and consistent titer data. To ensure that cultures used for this assay are in the exponential growth phase and maximally susceptible to infection, Expression Systems recommends passing cultures to a low density and incubating them 24-48 hours before using them for the assay

It is critical that the cells and virus are not incubated longer than 20 hours before staining. The objective is to detect the percentage of cells infected by the initial virus inoculation. After 20 hours, the infected cells will start producing progeny virus that will infect the remaining un-infected cells and skew the titer data.

References

Mulvania T, Hayes B, Hedin D. 2004. A flow cytometric assay for rapid, accurate determination of baculovirus titers. *BioProcess J* 3: 47-53.

Materials Required But Not Provided

1. Biosafety cabinet
2. Centrifuge with plate holders
3. Micropipettes, multi-channel pipette
4. Vortex mixer
5. 27°C shaker incubator
6. Flow cytometer equipped for Phycoerythrin detection
7. Ultra low attachment 96 well plate (Corning #3474)
8. Dilution tubes

9. Insect cells
10. Cell culture medium
11. PBS-BSA (PBS supplemented with 1% Bovine Serum Albumin)
12. PBS

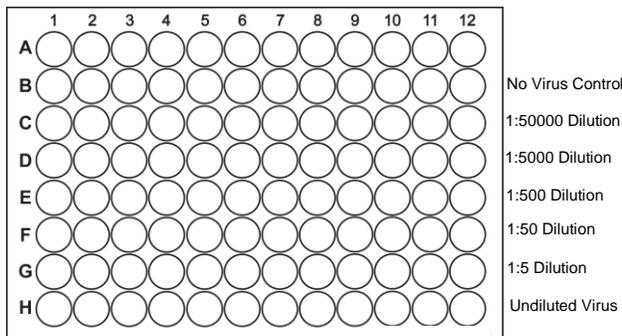
Virus Titration Protocol

DAY 1

1. **Prepare virus dilutions:** Dilute control and test viruses 1:5, 1:50, 1:500, 1:5000, 1:50000. When mixed 1:1 with cells in a subsequent step, these dilutions will become 1:10, 1:100, 1:1000, 1:10000, and 1:100000 respectively. It is convenient to dilute viruses using racks of 96 1.1 ml microtiter tubes (E&K Scientific #604508-RCS). To make dilutions in microtiter tubes, start by pipetting fresh media into the microtiter tubes as follows: 400 μ l in Row G, and 450 μ l in rows F through B. Pipette approximately 250 μ l undiluted rBV control virus into well H1 and 250 μ l undiluted test virus into the remaining tubes of row H. Serially dilute the viruses in row H following the dilution scheme below. There will be one column of dilutions in the 96 well plate for every virus to be titered.

Pipette 100 μ l virus from row H to row G and mix well
 Pipette 50 μ l virus from row G to row F and mix well
 Pipette 50 μ l virus from row F to row E and mix well
 Pipette 50 μ l virus from row E to row D and mix well
 Pipette 50 μ l virus from row D to row C and mix well

- Plate Cells:** Seed rows B through H of the 96 well ultra low adhesion plate with 100 μ l insect cells at 2×10^6 cells/ml.



- Inoculate wells:** Starting with row B and ending with row H, use a multi-channel pipette to transfer 100 μ l of the solutions from the microtiter tube rack to the corresponding row of the 96 well plate containing cells
- Incubate:** Incubate the plate at 27°C shaking at 155 rpm in a sealed plastic bag to prevent evaporation. Incubate for 18-20 hours.

DAY 2

- Dilute Antibody:** dilute gp64-PE antibody 1:200 in PBS-BSA. 50 μ l diluted antibody will be required for each well of the 96 well plate.
- Stain Cells:** Centrifuge the plate at 500g for 5 minutes to pellet cells. Aspirate the supernatant taking care to not aspirate cells on the bottom surface of the wells. Pipette 50 μ l diluted antibody into wells. Mix cells and antibody gently by pipetting up and down, taking care to not aspirate air and create bubbles. Incubate the cells with the antibody for 20 minutes at 4°C.
- Wash:** pipette 200 μ l PBS into wells containing cells and antibody, centrifuge for 5 minutes at 500g, aspirate. Repeat PBS wash two more times. After aspirating PBS from the final wash, re-suspend cells in 200 μ l PBS. Mix gently by pipetting up and down taking care to not aspirate air and create bubbles.
- Flow Cytometric Analysis:** Analyze the samples using a flow cytometer equipped for Phycoerythrin detection. Set the gates on the no virus control wells (% positive for PE should be less than 2). Record the Percentage of PE positive cells for each virus dilution

Data Analysis

The standard curve provides a direct comparison of data generated for unknown viruses to a virus of known titer. Data generated from the control virus are plotted on a logarithmic scale. A trendline is generated using an exponential curve and an equation determined using the data analysis features of Microsoft

excel. The equation from the standard curve is applied to data from the unknown samples and the average of the results from each dilution is used to determine the virus titer

- Download and open the “BTK Analysis” file from expressionsystems.com
- Enter data in column C for the % PE positive control values (and column D if a replicate was prepared)
- The titer of the standard control virus (reported on the rBV control technical data sheet) should be entered in N10 and ten fold dilutions entered in cells N11 to N15. A scatter graph with an exponential trendline and equation will be generated.
- Adjust which data points are included to generate a trendline with an R^2 value greater than 90 and preferably greater than 95. For example, the stock and 1:10 dilution of the control virus are typically saturating and should be excluded from the trendline if this is the case
- Enter % PE positive values for the test viruses into cells in column Q (and R if samples were tested in duplicate) and replace the old equation with the new equation in cells under the heading “equation” in column T. This will generate a titer value calculated for each dilution of the virus. The titers calculated for each dilution are then averaged to generate the final value in the yellow cell.
- Refine the final titer value by ensuring that only relevant data are averaged. Double click the yellow cell to display the range of values that are included in the average. Do not include titer values generated by % positive PE values less than 2%, as this is background and will tend to overestimate the titer. For high titer viruses, the titer value generated from the stock virus dilution is usually not included because the virus is saturating and the titer value will be under-estimated. The example below highlights the % positive PE data that should be included for a high titer virus and low titer virus.

	High Titer	Low Titer
Stock	98.68	48.4
1:10	97.96	23.5
1:100	76.4	3.4
1:1000	32.4	1.68
1:10000	12.62	1.42
1:100000	2.84	1.88
No Virus	1.84	1.8

Tips For Success

- Vortex all viruses before sampling
- When preparing serial dilutions of the virus, take care to minimize carryover of sample on the outside of pipette tips and ensure that all tips of a multi-channel pipettor fill to an equal level.

- All mixing steps should be done very gently with large bore pipette tips. Infected insect cells are more sensitive to shear stress than un-infected cells, so cell lysis caused by vigorous pipetting will artificially lower the percentage of PE positive cells and result in underestimation of virus titer.
- For best results, nearly all of the liquid should be aspirated from the wells during the wash steps. It can be challenging to aspirate all of the liquid without aspirating the cells as well. It may be helpful to practice this technique several times with cells in a 96 well plate
- Make an effort to minimize the amount of time that elapses between aspiration of liquid from wells and addition of liquid. Allowing the cells to dry will negatively impact results

Related Products

Product	Catalog Number
ESF 921	96-001
Adapted Sf9 Cells	94-001 or 94-006
BestBac™ Linearized DNA	91-001 or 91-002
Transfection Medium	95-020
gp64-PE antibody	97-201
Titering Service	94-901

Legend of Labeling Symbols

Symbol	Interpretation
	Catalog Number
	Lot Number
	<i>Research Use Only</i>
	Manufacturer
	Temperature Limitation
	Date of Manufacture
	Instruction for Use

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

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Doc ID: 36431
Rev. No.: 1