Baculovirus Titer Kit
Cat No#ME97101

Product Description:

Baculovirus Titer 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.

Materials Provided:

1. gp64-PE Antibody- 100 µl
2. rBV Control Virus, 1 ml ($1x10^9$ IU/ml)

Materials Provided by the End User:

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

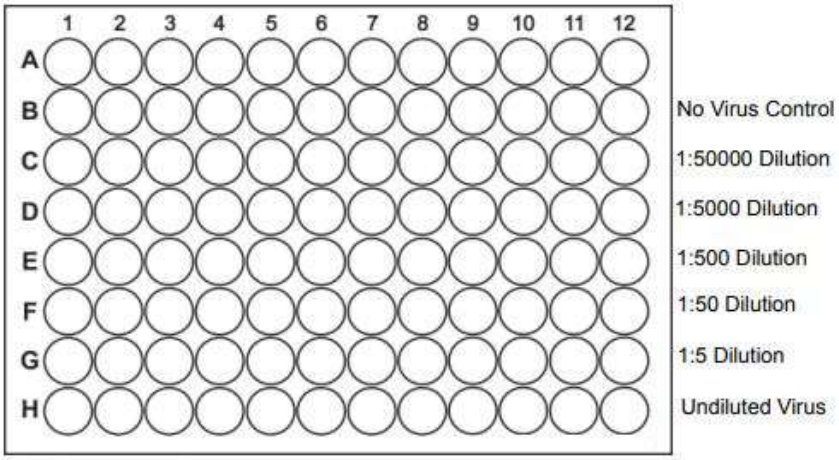
1. **Prepare virus dilutions:** Dilute control and test viruses1:5, 1:50, 1:500, 1:5000, 1:50000. When mixed 1:1 with cells in a subsequent step, these dilutions will become 1:10, 1:100, 1:1000, 1:10000, and 1:100000 respectively. It is convenient to dilute viruses using racks of 96 1.1 ml microtiter tubes.
   To make dilutions in microtiter tubes, start by pipetting fresh media into the microtiter tubes as follows:
   400 µl in Row G, and 450 µl in rows F through B. Pipette approximately 250 µl undiluted rBV control virus into well H1 and 250 µl 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 µl virus from row H to row G and mix well
   Pipette 50 µl virus from row G to row F and mix well
   Pipette 50 µl virus from row F to row E and mix well
   Pipette 50 µl virus from row E to row D and mix well
   Pipette 50 µl virus from row D to row C and mix well

2. Seed rows B through H of the 96 well ultra low adhesion plate with 100 µl insect cells at $2x10^6$ cells/ml.

3. Starting with row B and ending with row H, use a multi-channel pipette to transfer 100 µl of the solutions from the microtiter tube rack to the corresponding row of the 96 well plate containing cells

4. 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

1. Dilute gp64-PE antibody 1:200 in PBS-BSA. 50 µl diluted antibody will be required for each well of the 96 well plate.

2. 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 µl 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.

3. Pipette 200 µl 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 ul PBS. Mix gently by pipetting up and down taking care to not aspirate air and create bubbles.

4. 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.

1. Download and open the “BCT-Analysis” file from mercerexpert.com/downloads under the Data Reduction Section

2. Enter data in column C for the % PE positive control values (and column D if a replicate was prepared)

3. 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.

4. Adjust which data points are included to generate a trendline with an R2 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

5. 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.

6. 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	97.42	39.38
1:10	95.67	24.85
1:100	74.12	4.58
1:1000	31.79	2.37
1:10000	10.69	1.48
1:100000	2.89	1.95
No Virus	1.78	1.60

Procedural Notes:

1) Vortex all viruses before sampling to ensure accuracy of results.

2) Mixing 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.

3) For best results, aspirate all the liquid from the wells during the wash step. It can be challenging to aspirate all of the liquid without aspirating the cells as well. It may be useful to practice this technique several times with cells in a 96 well plate.

4) Ensure that minimal time elapses between aspiration of liquid from wells and addition of liquid. Allowing the cells to dry will lead to inaccurate results.