I. Introduction

The **BacPAK™ p35 ELISA Kit (Single Wash)** (Cat. No. 631477) enables you to determine baculovirus titers based on the expression of p35, an early-expressed viral protein that prevents cellular apoptosis and is essential for infectivity. The kit accurately measures the ability of the virus to infect cells and express virally encoded p35, reliably quantifying titers greater than 10^4 PFU/ml (IFU/ml).

The following information is provided as a high-level overview of the protocol to perform the workflow. For more detailed information, please see the BacPAK p35 ELISA Kit (Single Wash) User Manual.

II. Required Materials

This protocol applies to the following Takara Bio products:

- BacPAK p35 ELISA Kit (Single Wash) (Cat. No. 631477)

Additional materials required:

- Latex gloves, safety glasses, and other appropriate protective garments
- Biohazard infectious waste containers
- A reference baculovirus with known titer (recommended)
- Sf21, Sf9, or HighFive insect cells
- Insect cell complete medium
- Distilled or deionized water
- Micropipettes for delivering volumes of 2 µl, 20 µl, 100 µl, 200 µl, and 1,000 µl
- Filtered pipetting devices for 1 ml, 5 ml, 10 ml, and 25 ml pipettes
- 25 ml reagent reservoirs
- Two 96-well tissue culture plates
- Cell culture incubator (27°C; humidified by water)
- Hemacytometer and Trypan Blue, or equivalent cell counting instrument
- Automatic microtiter plate washer or a vacuum line fitted with a vacuum pump trap to collect liquid
- Microtiter plate reader with 450 nm filter
- Absorbent paper towels
- Standard laboratory equipment

III. Protocol Overview

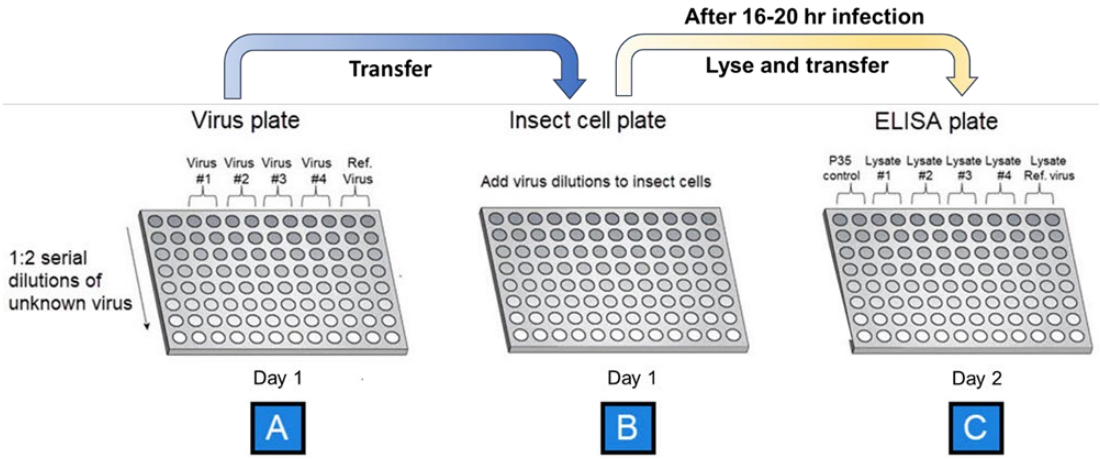

Figure 1. The BacPAK p35 ELISA Kit (Single Wash) workflow. **(Not shown)** Initial sample dilutions are made off plate. **Panel A.** Serial dilutions are made of baculovirus samples of an unknown titer. **Panel B.** Insect cells are infected with the prepared baculovirus dilutions. After incubation (16-20 hr), the cells are lysed. **Panel C.** The lysate is transferred to the ELISA plate included in the kit. ELISA is performed, and the absorbance measured at 450 nm. The standard curve generated from the p35 control (provided) is used to determine the titers of the sample baculoviruses.

IV. Protocol

IMPORTANT:

- Please read the entire protocol before starting.
- We strongly recommend using a reference sample of known titer to determine the correlation between titer and p35 values.
- Reference the User Manual for general requirements and sample recommendations for the protocol.

A. Day 1

1. Seed Insect Cells

1. Collect cultured insect cells with ≥90% viability from culture (spinner flask or plate).
2. Count viable cells and dilute in insect medium to a final concentration of 1 x 10^6 cells/ml.
3. Seed 100 µl of insect cell suspension (10^5 cells/well) into each well of the required number of columns of a sterile 96-well culture plate (2X columns as the total number of virus samples being tested).
4. Let the cells attach for at least 15 min at room temperature. They should be ~90% confluent.

2. Prepare Serial Dilutions of Virus Samples

- Initial Dilution
  - Early Passage/Low Titer: Use the virus sample either as-is or dilute 1:10 in a total volume of 250 µl per single replicate.
  - Late Passage/High Titer: Dilute the virus sample 1:100 or 1:1,000 in a total volume of 250 µl per single replicate.
- Serial Dilution
  - Perform seven 1:2 serial dilutions using 125 µl of media for each dilution for each replicate.

3. Infect Insect Cells

1. Gently transfer 100 µl of the diluted virus sample from Section 2 to the corresponding wells containing the insect cells (seeded in Section 1).
2. Incubate in a cell culture incubator (27°C; humidified by water) for 16–20 hr.

B. Day 2

4. Prepare p35 Standard Dilutions

1. Allow all reagents to reach room temperature (18–25°C).
2. Prepare a p35 stock solution by adding 1,250 µl of p35 Buffer to the p35 Control vial (500 ng lyophilized powder). Vortex for 5 sec.

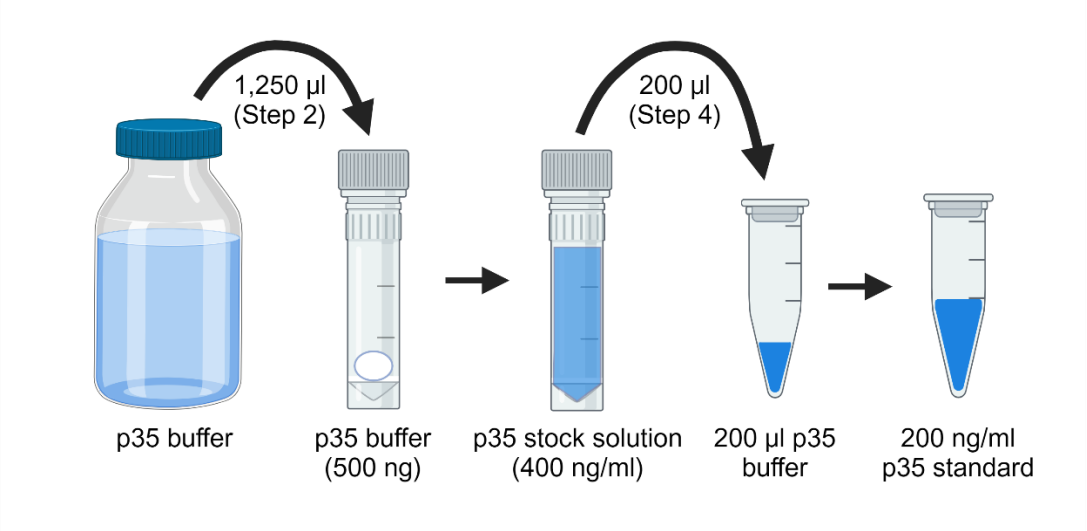

Figure 2. Preparing the p35 stock solution and 200 ng/ml p35 standard solution.

3. Label eight 2 ml microcentrifuge tubes for the 200 ng/ml, 100 ng/ml, 50 ng/ml, 25 ng/ml, 12.5 ng/ml, 6.25 ng/ml, and 3.125 ng/ml dilution standards and the negative control (0 ng/ml).
4. Dispense 200 µl of p35 Buffer into each of the eight tubes. Set aside the negative control tube.
5. Pipette 200 µl of the p35 stock solution (400 ng/ml) into the tube labeled for the 200 ng/ml dilution. Vortex for 5 sec.
6. Repeat Step 5 six times, pipetting 200 µl from the 200, 100, 50, 25, 12.5, and 6.25 ng/ml dilutions respectively into the tube for the next step in the series, for each subsequent dilution (Figure 3).

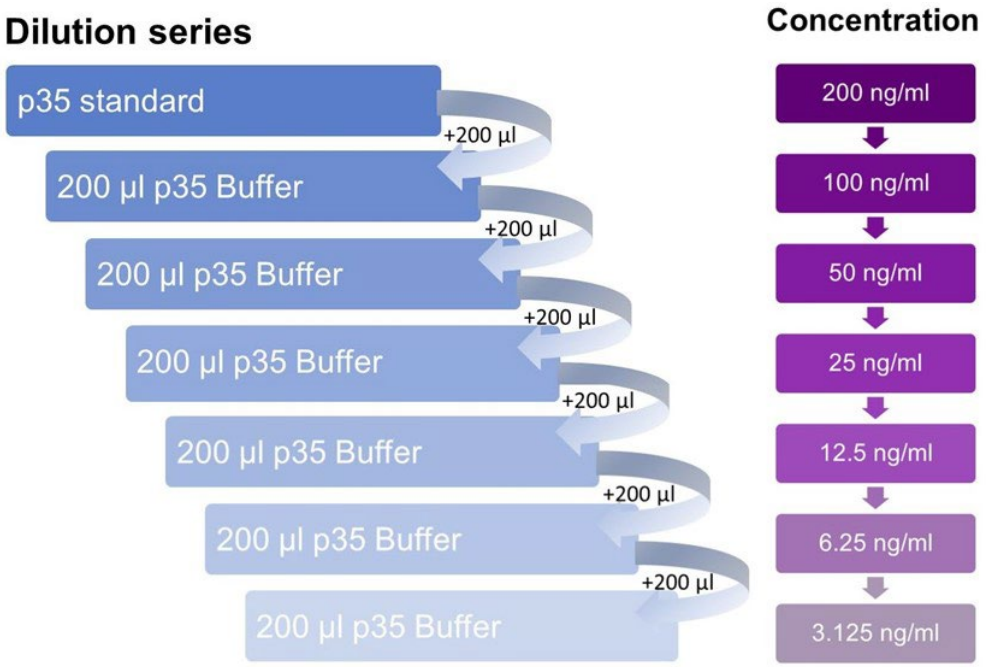

Figure 3. Preparing the p35 curve serial dilutions.

5. Lyse Infected Cells

1. Take the infected insect cells from Day 1 and aspirate the medium from the plate.
2. Add 60 µl of ice-cold lysis buffer to each well containing cells.
3. Incubate the plate at room temperature for 20 min to lyse the cells.

6. Perform ELISA

1. Place the Anti-p35 Coated Plate microtiter strips into the holding frame.
2. Transfer 50 µl of p35 standard dilutions to the corresponding p35 standard wells of the ELISA plate.
3. Transfer 50 µl of the insect cell lysates to the corresponding sample wells of the ELISA plate.
4. Add 100 µl of Anti-p35 (HRP conjugate) to each well then gently agitate the plate.
5. Incubate the plate at room temperature (18–25°C) for 60 min ± 5 min.
6. Aspirate the contents of the wells and wash the microtiter plate either via automatic or manual plate washing. A single six-rinse/wash cycle is required.
7. After washing, immediately, dispense 100 µl of TMB Substrate into each well.
8. Incubate the plate in the dark at room temperature (18–25°C) for 10 ± 2 min.
9. Add 100 µl of Stop Solution to each well to stop the reaction and mix gently. The blue solution should change to a homogeneous yellow color in each well.
10. Ensure that the undersides of the wells are dry and that there are no air bubbles in the wells.
11. Immediately read the absorbance values at 450 nm using a microtiter plate reader.
12. Calculate the standard curve based on the standard dilution series, then use the curve to determine the p35 concentration values of both your known and unknown samples.

Contact Us	
Customer Service/Ordering	**Technical Support**
tel: 800.662.2566 (toll-free)	tel: 800.662.2566 (toll-free)
fax: 800.424.1350 (toll-free)	fax: 800.424.1350 (toll-free)
web: takarabio.com/service	web: takarabio.com/support
e-mail: ordersUS@takarabio.com	e-mail: technical_support@takarabio.com

Notice to Purchaser

Our products are to be used for **Research Use Only**. They may not be used for any other purpose, including, but not limited to, use in humans, therapeutic or diagnostic use, or commercial use of any kind. Our products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without our prior written approval.

Your use of this product is also subject to compliance with any applicable licensing requirements described on the product's web page at takarabio.com. It is your responsibility to review, understand and adhere to any restrictions imposed by such statements.

This document has been reviewed and approved by the Quality Department.