# BacPAK™ Baculovirus Rapid Titer Kit Protocol-At-A-Glance

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## I. Introduction

The **BacPAK Baculovirus Rapid Titer Kit** (Cat. No. 631406) provides a fast and simple method for accurately determining titers of baculovirus stocks within 48 hours, using a standard immunological assay. This abbreviated protocol is provided for your convenience, but is not intended for first-time users. For additional details, see the BacPAK Baculovirus Rapid Titer Kit User Manual.

## II. Protocols

### A. Assay Setup & Infection

1. Seed one row (12 wells) of a 96-well microtiter plate with early log phase Sf21 or Sf9 cells (6.5 x $10^4$ cells/well). Label wells as shown in Figure 1. The density of early log phase cells used for the viral infection in Step 3 should be 3–4 x $10^5$ cells/ml.

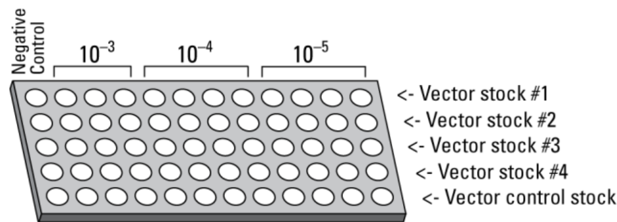


**Figure 1. Labeling a 96-well plate for the BacPAK rapid titer assay.**

2. Incubate the plate in a sealed plastic bag containing a moist towel at 27°C for 1 hr.
3. Make serial dilutions of the virus sample(s) by adding 100 µl of virus stock to 900 µl of BacPAK Complete Medium (or TNM-FH + 10% FBS) to provide final dilutions of $10^{-3}$, $10^{-4}$, and $10^{-5}$. Mix thoroughly between dilutions.

   These dilutions are appropriate for virus stocks with titers of 5 x $10^5$ to 1 x $10^8$ IFU/ml. Adjust the dilutions if the expected titer is not in this range.
4. Carefully aspirate the medium from microtiter plate using a multichannel pipettor with gel loading tips. Take care not to scrape the bottom of the wells.
5. Add 25 µl aliquots of the viral dilutions to the appropriate wells (3 wells for $10^{-3}$, 4 wells for $10^{-4}$, and $10^{-5}$). Add 25 µl of medium to the negative control well. Gently rock the plate to evenly distribute the virus.
6. Incubate for 1 hr at room temperature in a sealed plastic bag containing a moist towel.
7. Aspirate inoculum as in Step 4 above. Be careful not to scrape the cells from the bottom of the wells.
8. Add 50 µl Methyl Cellulose Overlay, wrap microtiter plate in a moist paper towel, and incubate in a sealed plastic bag at 27°C for 43–47 hr.

## B. Virus Detection

### Required Reagents:

Prepare before assay—sufficient for one assay (12 wells); increase as needed for multiple assays.

- **PBS + 0.05 % Tween 20:**
  - 25 ml PBS
  - 12.5 µl Tween 20
- **Diluted Normal Goat Serum:**
  - 80 µl concentrated serum
  - 2.3 ml PBS + 0.05% Tween 20
- **Mouse gp64 Antibody:**
  - 2.5 µl gp64 antibody
  - 497.5 µl Diluted Normal Goat Serum
- **Goat Anti-mouse Antibody/HRP Conjugate** (mix thoroughly)**:**
  - 4 µl anti-mouse conjugate
  - 996 µl Diluted Normal Goat Serum
- 4 ml of ice-cold formyl buffered acetone, **or** paraformaldehyde solution and sodium phosphate buffer (see Section III and Appendix A of the User Manual (PT3153-1)

### Protocol:

1. Carefully add 150 µl of freshly prepared ice-cold formyl buffered acetone (see User Manual) to each well containing the methyl cellulose overlay and incubate for 10 min at room temperature.
2. Shake out the reagent in a sink. Then tap the plate lightly on paper towel and wash 3X with 200 µl PBS + 0.05% Tween 20 (5 min per wash).
3. Add 50 µl diluted Normal Goat Serum. Incubate on a shaker for 5 min at room temperature.
4. Shake out the reagent in a sink and then tap the plate lightly on a paper towel. **Do not wash.**
5. Add 25 µl diluted Mouse gp64 Antibody and incubate at 37°C for 25 min.
6. Shake out the reagent in a sink. Then tap the plate lightly on a paper towel and wash 2X with 200 µl PBS + 0.05% Tween 20 (5 min per wash with shaking).
7. Add 50 µl diluted Goat Anti-mouse Antibody/HRP Conjugate and incubate for 25 min at 37°C.
8. Shake out the reagent in a sink. Then tap the plate lightly on paper towel and wash 3X with 200 µl PBS + 0.05% Tween 20 (5 min per wash with shaking).
9. Add 50 µl Blue Peroxidase Substrate and incubate for 3 hr at room temperature. You can obtain a preliminary but less accurate estimate of viral titer as early as 10 min after adding the substrate.

## C. Determining Virus Titer

1. Use light microscopy to count stained foci of infection in the highest dilution wells containing a reasonable number of foci (~5–25). Count each discrete cluster of stained cells as one focus.
2. Determine the average number of foci per well for all the wells at that dilution. You will obtain the best results when the average number of foci per well is 5–25.
3. Multiply the average number of foci by the corresponding dilution factor and an inoculum volume normalization factor of 40 to determine the virus titer in infectious units per ml (IFU/ml).

   **Virus titer (IFU/ml) =**

   **average no. of foci per well x dilution factor x 40**

   Sample calculation:

   $15 \text{ foci} \times (10^{-5})^{-1} \times 40 = 6 \times 10^{7}$ IFU/ml.

4. There may be an occasional well with no foci. This is to be expected given the variability of this assay and does not mean that you have performed the experiment incorrectly. As long as foci are counted in multiple wells at each dilution, you will get a reliable and reproducible measure of titer.


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