I. Introduction

BacPAK™ Baculovirus GoStix™ Plus are designed to accurately and rapidly quantify the amount of baculovirus in your viral prep, using only 20 µl of cell supernatant. Ten minutes after applying your sample, a band will appear in the window of the GoStix cassette at an intensity that correlates with the amount of baculovirus applied. The cassette is scanned using a smartphone camera or equivalent mobile device* running the GoStix Plus app, which will then calculate a titer (i.e., a GoStix Value [GV] equivalent to ng/ml gp64) based on the intensity of the band. The included gp64 Control confirms the GoStix function.

If you do not have access to a mobile device, you can still use BacPAK Baculovirus GoStix Plus as a qualitative test to confirm the successful production of baculovirus in your supernatant.

To learn more about the GoStix assays and the GoStix Plus app, visit takarabio.com/gostixhelp.

*The GoStix Plus app has not been validated for use with tablets.

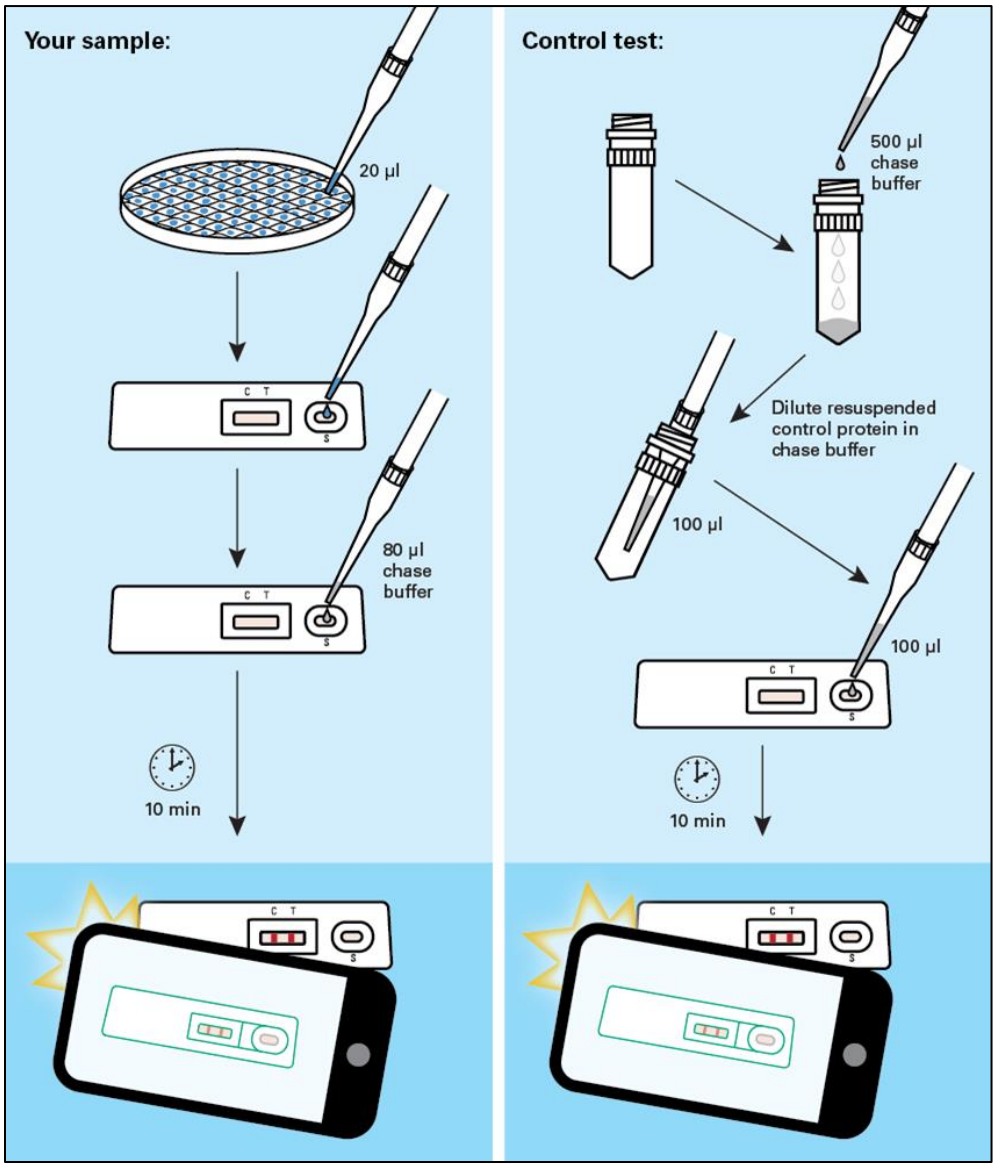

Figure 1. BacPAK Baculovirus GoStix Plus workflow.

II. Before You Begin

A. Experimental Considerations

As with all other titration methods (e.g., ELISA, or qPCR) used to produce comparable titer quantitation between experiments, the following variables must be consistent:

- Baculoviral production systems
- Baculoviral vectors
- Transfection methods
- Harvest times
- Scanning devices

NOTE: We recommend first testing a sample of known titer to determine the corresponding GV titer provided by the kit.

Example:

When BacPAK Baculovirus GoStix Plus were used to test viral supernatants produced using the BacPAK Baculovirus Expression System (Cat. No. 631402) or IPLB-Sf21 Insect Cells (Cat. No. 631411) and expressing ZsGreen1 fluorescent protein, a clear band was generated by a supernatant containing 1.5 x 10^6 IFU/ml, as measured by the BacPAK Baculovirus Rapid Titer Kit (Cat. No. 631406) on infected IPLB-Sf21 cells. This result correlated to a GV of 75 ng/ml gp64. See section IV for more information.

B. First Use of the App

1. Download the GoStix Plus app (if needed) from the Apple App Store (iOS) or Google Play (Android) onto your smartphone or equivalent mobile device.

   NOTE: The GoStix Plus app has not been validated for use with tablets.

2. While still connected to the internet, open the app on your mobile device.
3. When prompted, enter a valid email address, then press [Start]. This email address is your account and can be used to have results sent to you from your mobile device (Section III, Step 14).

The app uses a standard curve to calculate ng/ml of gp64, the baculoviral envelope glycoprotein. When the app is first opened, the standard curves for all available lots of BacPAK Baculovirus GoStix Plus will automatically be downloaded for later use.

Contact your network administrator if you experience issues downloading standard curve data. If the problem persists, please contact technical support.

III. Testing Your Sample

1. Open the app on your mobile device. If prompted, enter a valid email address and press [Start].
2. Enter the lot number by scanning the QR code **on the foil pouch** containing the GoStix cassette. (Press the QR code icon to activate the scanner.) The lot number can also be entered manually.

   NOTES:
   - **Do not use** the QR code on the outside of the box; it is not recognized by the app software.
   - Contact your network administrator if the app cannot find your lot number upon initial entry. If the problem persists, please contact technical support.
3. Enter the number of tests to be scanned (1–8 tests).
4. Press [Start test]. The equivalent number of sample name windows will appear.
5. Enter the sample names and their related dilutions into the appropriate windows. Click [Continue].

   NOTES:
   - Like ELISA methods, first-time users are advised to consider running the assay on several sample dilutions in addition to testing an undiluted sample to ensure that they obtain a valid reading within the designated range of the standard curve.
   - We recommend diluting samples by at least 1:2 with PBS or media. The GoStix Plus app includes a dropdown menu for entering dilutions as high as 1:100,000.
   - If you expect the sample to have high titer, perform dilutions before the next step.
6. Add 20 µl of your baculoviral supernatant to 80 µl of chase buffer.
7. Add this entire 100 µl of supernatant and chase buffer to the sample well (S) of the GoStix cassette. Allow the chase buffer front to appear in the cassette window.
8. Press [Start timer**]** to activate the timer on the app.
9. Allow the lateral flow test to run for the full 10 min. A test band (T) will start to appear within 5 min and reach maximum intensity at 10 min if your sample contains sufficient levels of baculovirus. The control band (C) will always appear when the test is functioning properly.

   NOTES:
   - The test will not give consistent results if the full development time is not observed. A warning within the app will appear if the [Skip] button is pressed before the 10 min has expired.
   - For accurate results, the intensity of the test band must be less than that of the control band. The closer this ratio is to one, the closer the sample is to exceeding the standard curve.
   - **If the reading exceeds the standard curve, the app will return a Gostix Value (GV) of "Off Scale" and an error message will appear recommending that the sample be diluted. If no baculovirus is detected in the sample, the app will return a GV of 0 ng/ml gp64 and show the result as invalid.**
10. After 10 min, the app will alert you to take a picture of the cassette. Proper alignment and focal length for imaging are achieved by using the outline of the cassette in the scanning window. Your sample name will appear below the outline of the cassette.

    Once proper alignment is achieved, the outline will turn green, and the cassette will automatically be scanned.

NOTES:
- Depending on the device used, it may be necessary to tap the device screen to focus before scanning.
- On newer smartphones, the scanning progress can be quite rapid once proper alignment is achieved
- Avoid creating shadows when imaging the GoStix cassette.

11. Once all samples are read, the results will be displayed in the *Result detail* window. If desired, add notes for each sample in the "Notes" section of the *Result detail* window.
12. Press [Upload result**]** when finished to save each data entry.
13. If you wish to rescan a sample, repeat steps 2–5, skip the protocol page(s) and timer (press [Start], [Skip], and then [Yes]) to proceed. Do not exceed 20 min of total development time (i.e., rescanning must take place within 10 min of initial timer expiration).

NOTES:
- Lateral flow tests can continue to develop after the initial 10-min development time, such that variation in development time can contribute to read variability. If replicate reads from the same test are desired, we recommend timing the acquisition of the images as close to each other as possible.
- Typical coefficients of variation (%CV) for replicate reads of the same test vs. replicate tests of the same sample are <10% and <20%, respectively.

14. If desired, individual results from the *Result history* page (accessed from the main menu) can be emailed or sent via SMS by pressing the [Share] button at the bottom of each overview page. Accumulated results can also be downloaded as a single batch to your device using the [Download] button at the top of the *Result history* page.

    Please visit takarabio.com/gostixhelp for further instructions on how to access results downloaded to your device.

IV. Calculating IFU/ml from the GoStix Value

1. To calculate the actual IFU/ml for an unknown stock, a reference virus (a virus stock for which the IFU/ml is known) must first be tested to obtain both an infectious unit value as well as a GV.

NOTE: To be able to accurately calculate IFU/ml for an unknown virus using a reference virus, the unknown virus, and reference virus must be produced using similar protocols.

2. Calculate the IFU/GV ratio for the reference virus.
3. Analyze the unknown sample using BacPAK Baculovirus GoStix Plus to obtain the GV (ng/ml gp64).
4. Perform calculations to determine your IFU/ml (see Table I, below).

Table 1. Calculating IFU/ml from the GV.

Viral prep	Infectious Titer (IFU/ml)	GV (ng/ml gp64)	(IFU/ml)/GV
ZsGreen1 baculovirus (reference)	1.5×10^6	75	20,000
New baculovirus (unknown)	*N*	1,500	20,000*

*Value from the reference virus stock.

Formula

$$\text{IFU/ml of the unknown sample} = \frac{\textbf{<GV of the unknown sample>} \times \textbf{<Infectious titer of the reference>}}{\textbf{<GV of the reference>}}$$

Example

Using the values listed in Table 1, solving for *N*:

$$\text{IFU/ml of the unknown sample } (N) = \frac{\textbf{1,500 ng/ml} \times \textbf{1.5 x } \mathbf{10^6} \text{ ng/ml gp64}}{\textbf{75 ng/ml gp64}} = \boxed{\mathbf{3.0 \times 10^7 \text{ IFU/ml}}}$$

V. Testing the gp64 Control

NOTE: The gp64 Control is supplied in a dried-down format. Before testing, add 100 µl of Chase Buffer to the vial and vortex to resuspend. The gp64 Control should be used immediately after reconstitution.

1. Dilute 10 µl of the resuspended gp64 Control in 90 µl of chase buffer.

   NOTE: Do not skip this step. If this dilution is not performed, the app will return a GV of "Off Scale".

2. Apply 100 µl of the diluted gp64 Control to the sample well (S) of the BacPAK Baculovirus GoStix Plus cassette.
3. A control band (C) and test band (T) will appear in 10 min.

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