

Lenti-X™ GoStix™ Plus Protocol-At-A-Glance

Lenti-X GoStix Plus are designed to accurately quantify the amount of lentivirus in your viral prep extremely rapidly, using only 20 μl of packaging 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 lentivirus applied. The cassette is then scanned using a smartphone camera or equivalent mobile device running the GoStix Plus app that will then calculate a titer (i.e., a GoStix Value [GV] equivalent to ng/ml p24) based on the intensity of the band. The included p24 Control provides confirmation of GoStix function. If you do not have access to a mobile device, you can still use Lenti-X GoStix Plus as a qualitative test to confirm the successful production of lentivirus in your supernatant. To learn more about the app and GoStix Plus assays, visit takarabio.com/gostixhelp.

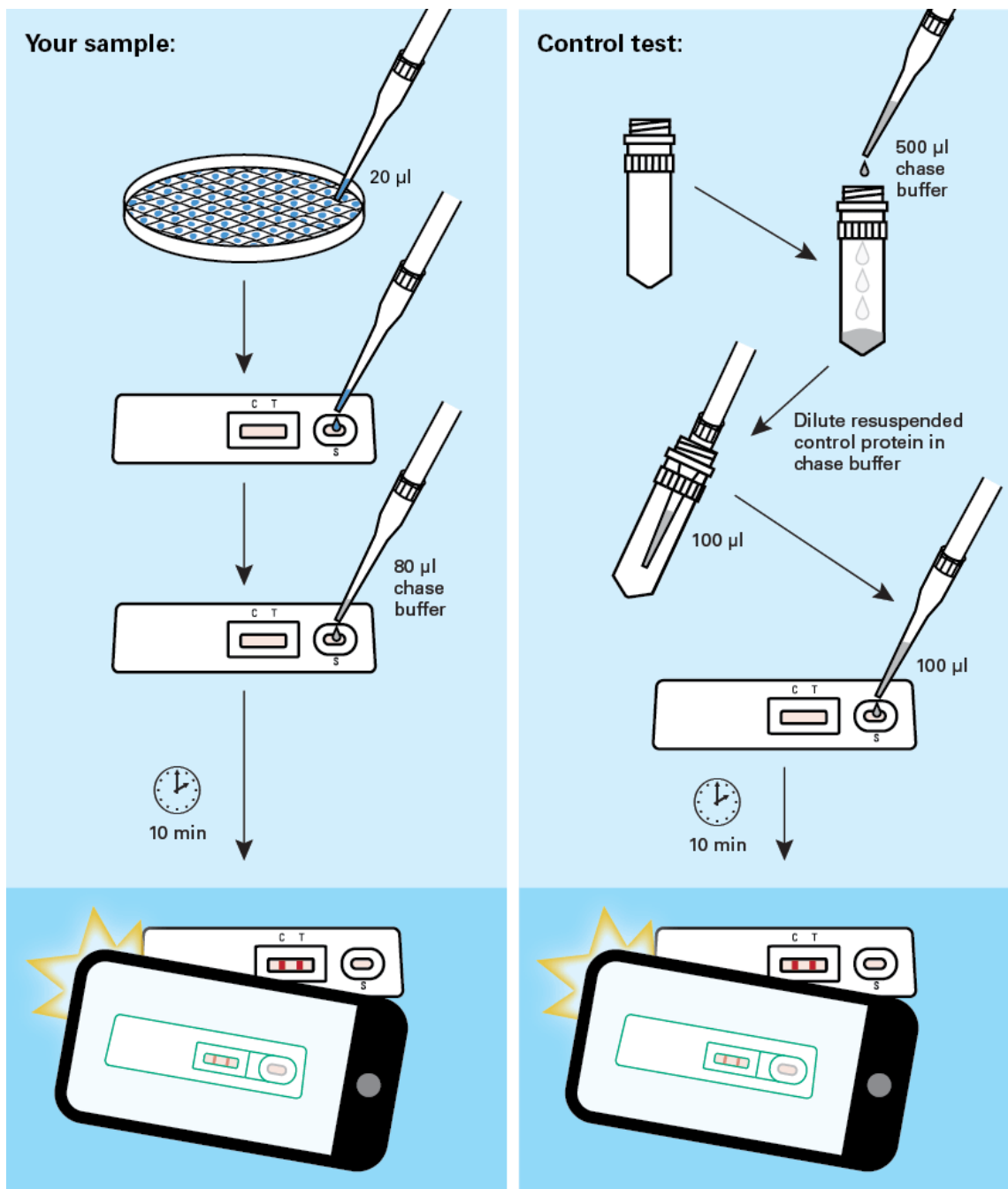


Figure 1. Lenti-X GoStix Plus workflow.

NOTE: As with all other titration methods (e.g., p24 ELISA, or qRT-PCR) used to produce comparable titer quantitation between experiments, the following variables must be consistent: lentiviral packaging systems, lentiviral vectors, transfection methods, harvest times, and scanning devices. We recommend first testing a sample of known titer to determine the corresponding GV titer provided by the kit. For example, when Lenti-X GoStix Plus were used to test viral supernatants produced using Lenti-X Packaging Single Shots, Lenti-X 293T Cells, and pLVX vector expressing our ZsGreen1 fluorescent protein, a clear band was generated by a supernatant containing 1×10^7 IFU/ml (as measured by flow cytometry of transduced HT-1080 cells), which correlated to a GV of 610 ng/ml p24 at a 1:2 dilution.

When you first use the GoStix Plus app, you will need to connect your device to the internet so that the standard curves for available lots of Lenti-X GoStix Plus can be downloaded into the app. The app uses the standard curve to calculate the GV in ng/ml p24. Contact your network administrator if you experience issues downloading standard curve data or if the app cannot find your lot number upon initial entry.

I. Testing Your Sample

1. Download the GoStix Plus app from the App Store or Google Play.

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

2. Open the app on your mobile device and enter a valid email address to begin. Click [Start].

NOTE: This email is used for sending results to the user from the mobile device (see Step 15).

3. Enter the lot number by scanning the QR code on the Lenti-X GoStix Plus foil pouch. (Press the QR code icon to activate the scanner.) The lot number can also be entered manually.
4. Enter the number of tests to be scanned (1–8 tests). The appropriate number of sample name windows will appear below.
5. Enter the sample names and the appropriate dilutions. Click [Start].

NOTE: FOR UNCONCENTRATED SAMPLES, we recommend using a 1:2 or 1:5 dilution in addition to testing an undiluted sample to ensure a proper reading within the range of the standard curve. **FOR CONCENTRATED SAMPLES**, we recommend running additional, and higher-fold, dilutions. The app includes a dropdown menu for entering dilutions as high as 1:100,000.

6. Following the prompts within the app, take 20 μ l of your lentiviral supernatant and apply it to the sample well (S) of the Lenti-X GoStix Plus cassette. If you expect the supernatant to have a high titer, you can perform dilutions prior to loading the sample.
7. Add 80 μ l of Chase Buffer to the sample well and allow the Chase Buffer front to appear in the cassette window.
8. Press [Start] to activate the timer on the app.
9. Allow the lateral flow test to run for the full 10 minutes. A test band (T) will start to appear within 5 minutes and reach maximum intensity at 10 minutes if your sample contains sufficient lentivirus. The control band (C) will always appear when the test is functioning properly.

NOTE: The test will not give consistent results if the full development time is not observed. A warning within the app will appear if [Skip] is pressed before the 10 minutes has expired. For accurate results, it is important that the intensity of the test band is less than that of the control band. The closer this ratio is to 1, the closer the sample is to exceeding the standard curve. **If a reading exceeds the standard curve, the app will return a GV of “Off Scale” and an error message will appear recommending that the sample be diluted. If no lentivirus is detected in the sample, the app will return a GV of 0 ng/ml p24.**

- After 10 minutes, the app will alert you to take a picture of the cassette. Proper alignment and focal length for imaging is 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.

NOTE: Depending on the device used, it may be necessary to tap the device screen in order to focus prior to scanning.

- Once all samples are read, the results will be displayed in the *Result detail* window.
- Add notes for each sample, if desired, at this time.
- Press [Done] when finished to save each data entry.
- If you wish to rescan a sample, repeat steps 3, 4, and 5, skip the protocol page(s) and timer (press [Start], [Skip], and then [Yes]) to proceed. Do not exceed 20 minutes of total development time (i.e., rescanning must take place within 10 minutes of initial timer expiration).

NOTE: 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 <15%, respectively.

- If desired, individual results can be emailed to the address previously entered into the app (Step 2) by pressing the [Share] button at the bottom of each *Result detail* page. Additionally, all saved results can be downloaded as a .csv file to your mobile device by pressing the [Download all] button in the upper-right section of the *Result history* page. Please visit takarabio.com/gostixhelp for additional guidance on retrieving downloaded results.

NOTE: Because the assay quantifies ng/ml p24, similar to an ELISA, it can be used as a titer value to maintain consistency from prep to prep with the same lentiviral system, so long as the preparation methods are consistent.

II. Calculating IFU/ml from the GoStix Value (GV, ng/ml p24)

- 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.
- Calculate the IFU/GV ratio for the reference virus.
- Analyze the unknown sample using Lenti-X GoStix Plus to obtain the GV (ng/ml p24).
- Perform calculations to determine your IFU/ml (see Table 1, below).

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.

Table 1. Calculating IFU/ml from the GV.

Viral prep	Infectious titer (IFU/ml)	GV (ng/ml p24)	(IFU/ml)/GV
LVXZsGreen1 lentivirus (reference)	1 x 10 ⁷	1,220	8,197
LVXZsGreen1 lentivirus (unknown)	?	850	8,197 (from reference)

Formula: GV (unknown) x (IFU/ml)/GV (reference) = IFU/ml (unknown)

Example from Table I, above: 850 ng/ml x 8,197 IFU/ng = 7.0 x 10⁶ IFU/ml

III. Testing the p24 Control

1. The p24 Control is supplied in a dried format. To resuspend, add 500 µl of Chase Buffer and vortex.
2. Dilute 5 µl of the resuspended p24 Control in 195 µl of Chase Buffer.
3. Apply 100 µl of the diluted p24 Control to the sample well (S) of the Lenti-X GoStix Plus cassette. No additional Chase Buffer should be added to the cassette.
4. A control band (C) and test band (T) will appear in 10 min.
5. For future control tests, add 0.1% sodium azide to the vial containing resuspended p24 Control and store at 4°C for up to 12 months.

NOTE: The p24 Control is provided to confirm the proper function of the lateral flow test. Following initial resuspension in 500 µl of Chase Buffer, the p24 Control is too highly concentrated to yield a reading within the range of the standard curve referenced in the app, and will cause the app to return a GV of “Off Scale”. Further dilution is required to ensure that the p24 Control reading falls within the standard curve.

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