

## Cytokine GoStix™ Plus Protocol-At-A-Glance

GoStix Plus cytokine assays are designed to accurately quantify human cytokines extremely rapidly, using only 20 µl of cell culture supernatant or diluted serum. 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 analyte applied. The cassette is then scanned using a smartphone camera or equivalent mobile device running the GoStix Plus app that will calculate pg/ml of a given cytokine based on the intensity of the band. Control protein included with each kit allows for confirmation of GoStix function. See Section III, Table 1 for applicable assay information. To learn more about GoStix assays and the GoStix Plus app, visit [takarabio.com/gostixhelp](http://takarabio.com/gostixhelp).

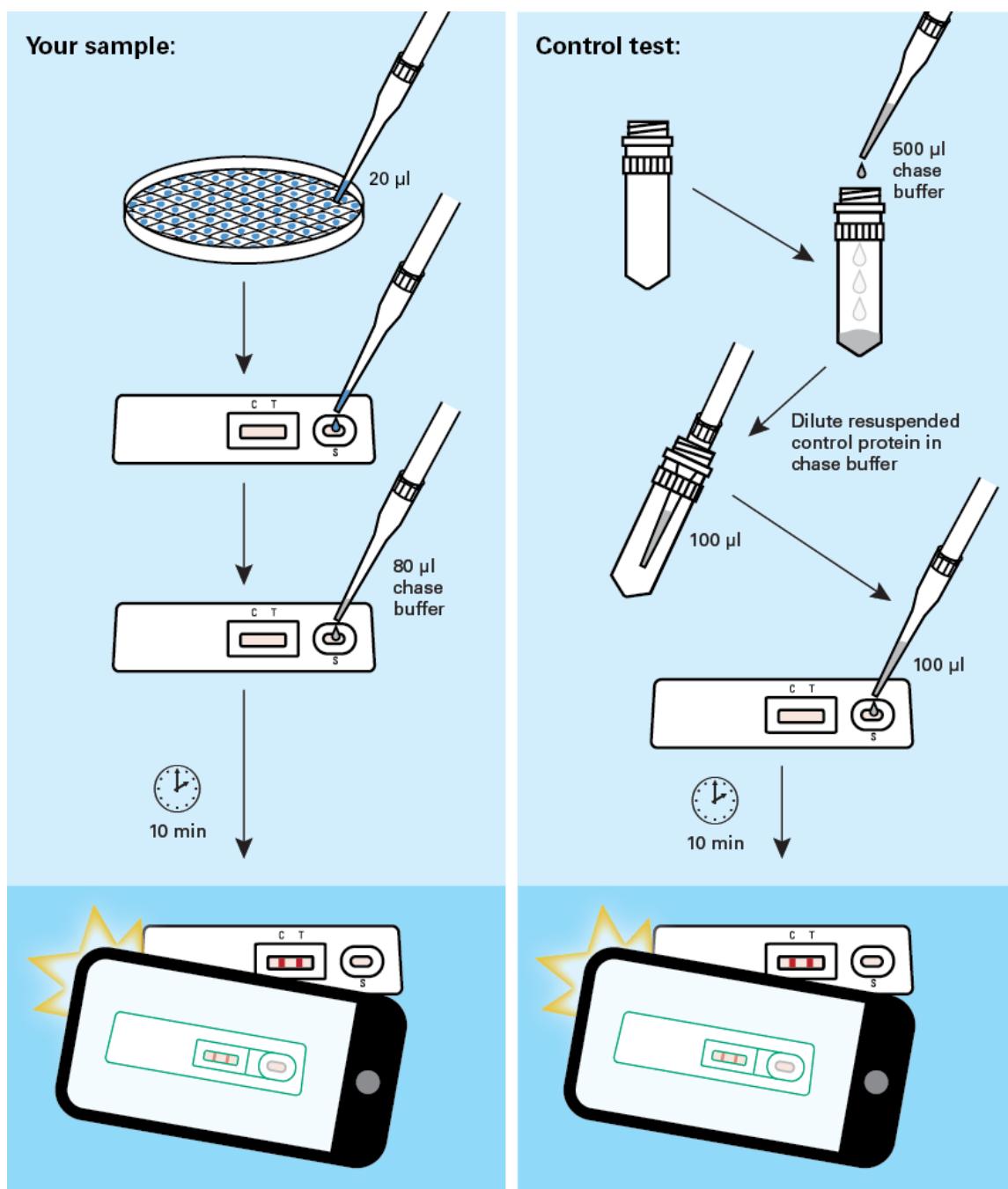


Figure 1. Cytokine GoStix Plus workflow.

When you first use the app, you will need to connect your device to the internet so that the standard curves for available lots of cytokine GoStix Plus products can be downloaded into the app. The app uses the standard curve to calculate pg/ml of cytokine. Contact your network administrator if you experience issues downloading standard curve data or if the app cannot find your lot number upon initial entry. If the problem persists, please contact Technical Support.

### I. Testing Your Sample

1. Download the GoStix Plus app from the App Store® or on 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 can be 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 foil pouch containing the GoStix cassette. (Press the QR code icon to activate the scanner.) The lot number can also be entered manually.

**NOTE:** Do not use the QR code on the outside of the box; it is not recognized by the app software.

4. Enter the number of tests to be scanned (1–8 tests). The appropriate number of sample name windows will appear below.

5. Enter in the sample names and the appropriate dilutions. Click [Start].

**NOTES:**

**Assay range:** Each cytokine GoStix Plus assay is designed to accurately quantify a given cytokine within a designated range (specified in the corresponding Certificate of Analysis for the assay). Similar to 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 assay-specific standard curve.

**Serum samples:** We recommend diluting serum samples by at least 1:10 to minimize assay variability. The GoStix Plus app includes a dropdown menu for entering dilutions as high as 1:100,000.

6. Following the prompts within the app, take 20 µl of your sample and apply it to the sample well (S) of the GoStix cassette. If you expect the sample to have high levels of analyte, you can perform dilutions prior to loading the sample.

7. Add 80 µl of chase buffer to the sample well and allow the chase buffer front to appear in the cassette window.

**NOTE:** For better reproducibility, dilute 20 µl of your sample with 80 µl of chase buffer and then load the GoStix cassette with the resulting 100 µl.

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 levels of analyte. 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 the [Skip] button 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 Gostix Value (GV) of “Off Scale” and an error message will appear recommending that the sample be diluted. If no analyte is detected in the sample, the app will return a GV of 0 pg/ml.**

## Cytokine GoStix™ Plus Protocol-At-A-Glance

10. 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. Also, if possible, avoid creating shadows when imaging the GoStix cassette.

11. Once all samples are read, the results will be displayed in the *Result detail* window.
12. If desired, add notes for each sample in the “Notes” section of the *Result detail* window, at this time.
13. Press [Done] when finished to save each data entry.
14. 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.

15. 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 all] button on the *Result history* page. Please visit [takarabio.com/gostixhelp](http://takarabio.com/gostixhelp) for further instructions on how to access results downloaded to your device.

## II. Calibrating GoStix results with other assays using a reference standard

GoStix assay results can be calibrated to international standards using conversion factors specific to each assay. These conversion factors are provided in the corresponding assay’s Certificate of Analysis.

## III. Testing the cytokine control protein

1. Control protein for each assay is supplied in a dried format. To resuspend, add 500 µl of chase buffer and vortex.
2. Dilute the control protein for your specific assay as indicated in the table at the bottom of this section.

**NOTE:** The control protein is provided to confirm the proper function of the lateral flow test. Following initial resuspension in 500 µl of chase buffer, the control protein 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 control reading falls within the standard curve.

3. Apply 100 µl of the diluted control to the sample well (S) of the GoStix cassette.
4. A control band (C) and test band (T) will appear in 10 minutes.
5. For future control tests, the reconstituted control protein can be stored at 4°C for up to 1 week. For extended storage, prepare aliquots to store at -20°C and avoid repeated freeze-thaw cycles.

## Cytokine GoStix™ Plus Protocol-At-A-Glance

**Table 1. Recommended control protein dilutions.**

Assay	Catalog Nos.	Dilution (control suspension + chase buffer)
IL-2 GoStix Plus	631283, 631284	1:40 (5 µl + 195 µl)
IFN-γ GoStix Plus	631285, 631286	1:8 (25 µl + 175 µl)
TNF-α GoStix Plus	631287, 631288	1:32 (6.25 µl + 193.75 µl)

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