I. Introduction

dsRNA GoStix Plus (Cat. No. 635860) assay is designed to accurately and rapidly quantify the amount of dsRNA in an mRNA IVT reaction. Ten minutes after applying your sample, a band will appear in the window of the GoStix cassette at an intensity that can be correlated with the amount of dsRNA in the sample. The cassette is then scanned using a smartphone camera or equivalent mobile device* running the GoStix Plus app that will then calculate a concentration (i.e., a GoStix Value [GV] equivalent to ng/ml Poly I:C) based on the intensity of the band. The included lyophilized Poly I:C Control provides confirmation of the GoStix function. 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.

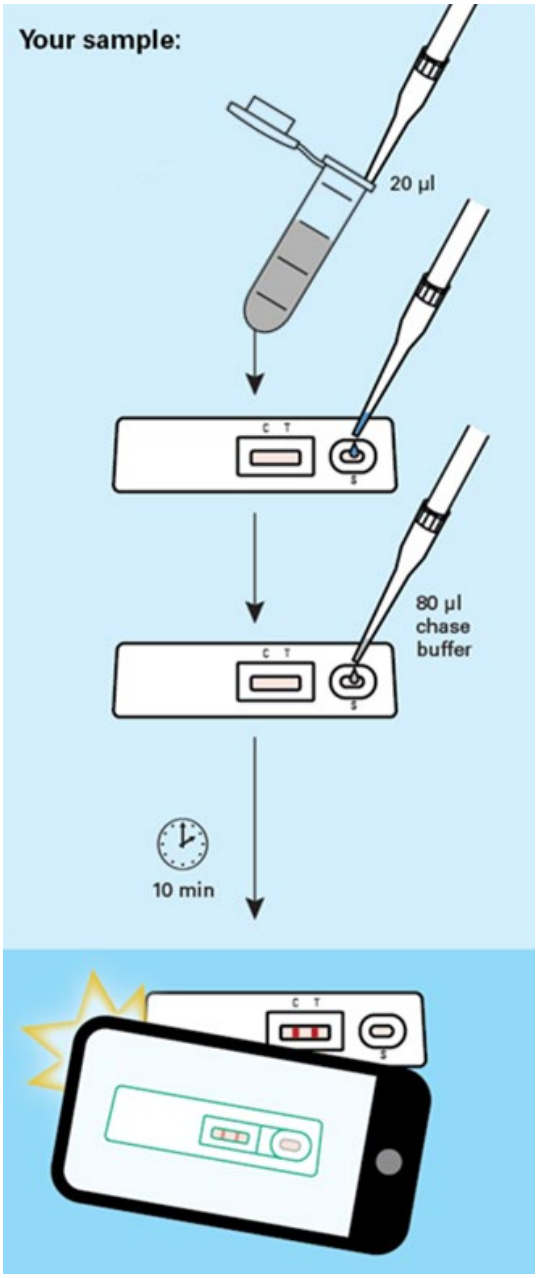

Figure 1. dsRNA GoStix Plus workflow.

II. Before You Begin

A. Experimental Considerations

- Use clean, RNase-free micro-centrifuge tubes with caps.
- Similar to antibody-based detection methods such as ELISA or dot-blot, the GoStix signal intensity from dsRNA samples can vary based on length, modification and G/C content of the dsRNA produced as a side product in your mRNA IVT reaction.
- A lot-specific standard curve was generated from a series of Poly I:C standards (0–125 ng/ml). This curve is preloaded into the app and does not need to be recreated by the user. With Poly I:C used as the reference, the app automatically quantifies dsRNA levels in unknown mRNA samples using a linearized equation derived from this curve. Results are reported in ng/ml of Poly I:C equivalents.

NOTE: We recommend first testing a reference dsRNA sample with a known concentration to determine the corresponding GV using the kit, with assistance from the online calculator.

Example: When tested with the dsRNA GoStix Plus assay, a 300 ng/ml dsRNA reference standard gave a GV of 17 ng/ml Poly I:C. For an unknown sample, test it using the assay, then input its GV (automatically provided by the app) and the calculator will convert it to a dsRNA concentration in ng/ml. See Section IV for more information.

B. Use of the App (Open App Before Adding Sample to GoStix)

1. Download the GoStix Plus app 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. Read and accept the End User License Agreement (EULA).
4. When prompted, enter a valid email address and press [Start]. This email address is used to log into your account and can be used to export data from your mobile device to an external location from the mobile device (Section III, Step 14).

When the app is first opened, the standard curves for all available lots of dsRNA GoStix Plus products will automatically be downloaded for later use. The app uses the standard curve to calculate ng/ml of Poly I:C equivalents.

NOTE: Granting data-storage (retention) and camera (test imaging) permissions is necessary to ensure stable app operation.

If you have trouble downloading standard curve data or using the app, contact your network administrator. If issues continue, reach out to 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. Once the lot number is entered correctly, the test type will appear in the window.

NOTES:

The app will return the message "**The lot number entered may be incorrect or a product is expired**" if any of the following conditions occur:

- The QR code on the outside of the box was scanned for lot-number information, but it is not recognized by the app.
- The device is not connected to a network and cannot download the most up-to-date control-curve information.
- The lot number in use has exceeded the established expiration date for that specific GoStix lot. In this case, we recommend using the GoStix only as a qualitative assay.

If the app cannot connect to a network, contact your network administrator. If the issue 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 into the windows and the related dilutions. Click [Continue].

NOTE: As with ELISA-based methods, first-time users are encouraged to run the assay with multiple sample dilutions, alongside an undiluted sample, to ensure the reading falls within the designated range of the standard curve (0–125 ng/ml). We recommend diluting samples in STE Buffer provided in the kit. The GoStix Plus app includes a dropdown menu for entering dilutions as high as 1:100,000. The calculated GV will automatically use the entered dilution factor to calculate the actual, undiluted concentration of Poly I:C equivalent in your sample.

If you anticipate a high dsRNA concentration in your sample, dilute it before proceeding to the next step. Conversely, if you expect a low dsRNA concentration, either test the sample as-is or concentrate it prior to the next step.

6. Following the prompts within the app, take 20 µl of your dsRNA preparation and apply it to the sample well (S) of the dsRNA GoStix Plus cassette (see Figure 2).
7. Add 80 µl of Chase Buffer to the same sample well and allow the Chase Buffer front to appear in the cassette window.

NOTE: For ease of use when handling multiple samples, and to improve % CV, you may combine 20 µl of sample with 80 µl of chase buffer and dispense the full 100 µl mixture into the sample well (S) of the GoStix cassette. Allow the chase-buffer front to appear in the cassette window before proceeding.

Ng/ml values are calculated based on the 20 µl sample applied. The additional 80 µl of chase buffer functions solely to drive flow and does not dilute or otherwise alter the effective sample concentration.

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 dsRNA. The control band (C) will always appear when the test is functioning properly (see Figure 2).

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 min has expired.

10. After 10 min, the app will alert you to scan 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. If the outline appears yellow, the alignment has been lost, and the scanning is paused until alignment is reestablished.

NOTES:
- Avoid creating shadows when imaging the GoStix cassette. If bright light causes shadows, place cassettes on a white background before scanning.
- Depending on the device used, it may be necessary to tap the device screen to focus before scanning.
- On newer smartphones, the scanning process can be quite rapid once proper alignment is achieved.

The GoStix Plus app can return three types of reads, depending on how the sample signal compares to the standard curve (see Figure 2):

1) Off Scale: When the test-line signal exceeds the upper limit of the standard curve (i.e., the T-line is stronger than the C-line), the app will display a GoStix Value (GV) of "Off Scale" and provide a message recommending that the sample be diluted and retested.

2) Valid: When the signal falls within the calibrated range of the standard curve, the app will return a valid GV result corresponding to the ng/ml of dsRNA in the sample.

3) Invalid: When no dsRNA is detected or the control signal is too low to confirm proper cassette function, the app will return an invalid result and display 0 ng/ml dsRNA.

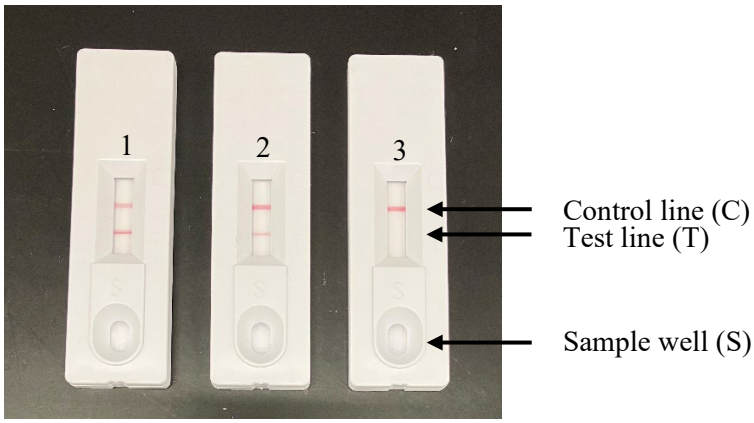

Figure 2. Expected GoStix Assay results.

11. Once all samples are read, the results will be displayed in the *Result detail* window. If desired, you may 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).

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 on different GoStix 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 icon at the upper right 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.

Understanding GoStix Value and dsRNA quantification:
The GoStix Value measured in ng/ml Poly I:C equivalents reflects the affinity of dsRNA-specific antibodies to Poly I:C. Variations in dsRNA length or chemical modifications may alter the binding affinity of the dsRNA-specific antibody to the respective dsRNA sample. For a comparison of GoStix Values across different dsRNA species at the same concentration, refer to Figure 3.

The Poly I:C included in this kit is intended only as a positive control to confirm that the lateral flow assay cassette is functioning (see Section V). For accurate dsRNA quantification, a purified version of the dsRNA of interest or reference dsRNA must be used as a standard. Detailed instructions for converting the GoStix Value into actual dsRNA concentration using the correct standard are provided in Section IV.

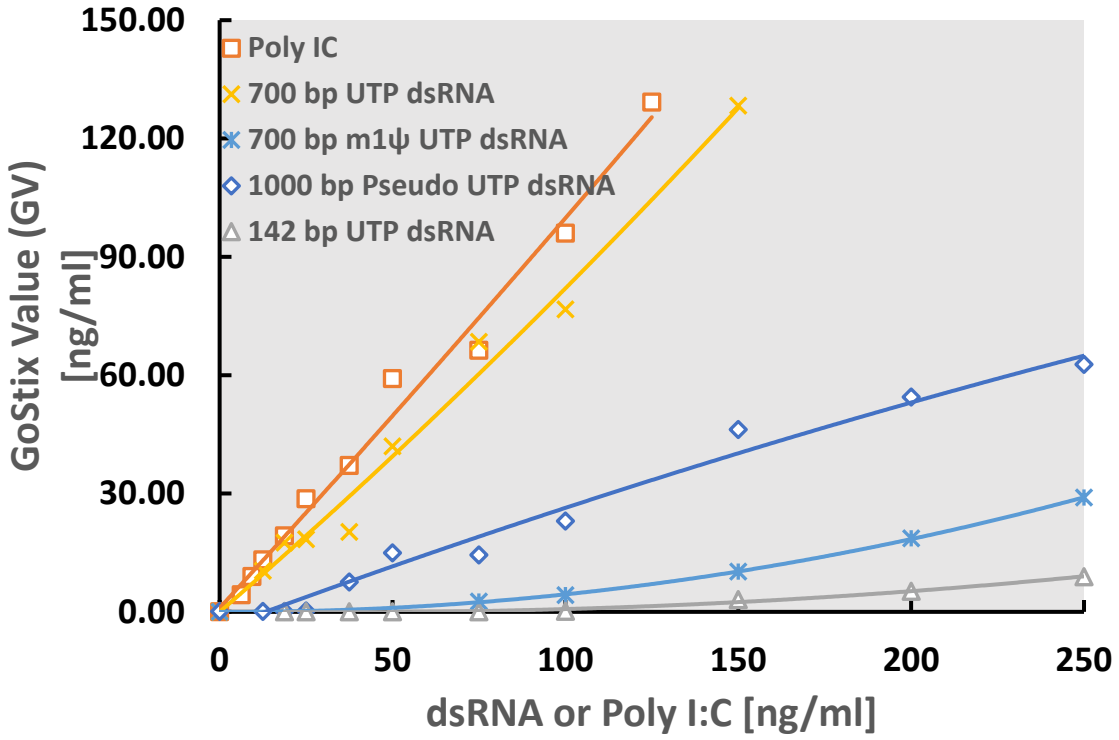

Figure 3. Comparison of the impact of dsRNA length and modifications on GoStix Value (equivalent to ng/ml Poly I:C) for dsRNA quantitation using dsRNA GoStix Plus. Data was generated using the 100 µl combined protocol.

To determine the actual concentration of an unknown dsRNA stock, a reference dsRNA material (the dsRNA for which the concentration is known) must first be tested to obtain both concentration as well as a GV.

1. Prepare up to three serial dilutions of the reference dsRNA.
2. Process reference dilutions and unknowns using the protocol detailed in Section III.
3. Open the online calculator tool. In the designated area, enter the known concentration for each reference dilution alongside its corresponding GV. In the designated area, enter the known concentration for each reference dilution alongside its corresponding GV.In the designated area, enter the known concentration for

each reference dilution alongside its corresponding GV.In the designated area, enter the known concentration for each reference dilution alongside its corresponding GV.

4. Find the section for unknown samples and enter their measured GVs. The tool is designed to automatically calculate and display the final dsRNA concentration for your samples based on the reference curve.

IV. Using the Poly I:C Control

Resuspend the Poly I:C Control

The Poly I:C Control is supplied in a lyophilized format and is used to confirm GoStix function. Prior to use, add 1,000 µl of STE Buffer to the vial and vortex to resuspend. The Poly I:C Control should be used immediately after reconstitution.

Testing protocol

1. Dilute 10 µl of the resuspended Poly I:C Control with 30 µl of STE Buffer.
2. Take 20 µl of diluted Poly I:C Control from Step 1 above and apply it to the sample well (S) of the dsRNA GoStix cassette.
3. Add 80 µl of Chase Buffer to the sample well and allow the Chase Buffer front to appear in the cassette window.
4. Press [Start timer] to activate the timer on the app.
5. A control band (C) and test band (T) will appear in 10 min.

V. Appendix

Problem	Possible explanation	Solution
The App states “The lot number entered may be incorrect or a product is expired. Please verify the expiration date on the box. For further assistance, contact technical support.”	Lot number was not entered correctly.	Enter in the correct lot number or scan the QR code from the GoStix pouch (not the kit box).
	The GoStix lot being used has expired (>2 years).	Use GoStix in qualitative manner.
		Discontinue use and repurchase.
The App crashes after I attempt to read the test with my device.	GoStix App has not been given permission to access either the camera or file system of the device.	Proceed to phone settings and allow access to camera and files. Photos of tests cannot be analyzed by the App.
How does my GoStix Value translate into a value I can use?	See Section IV, “Calculating the actual concentration from the GoStix Value”.	Test a sample of known concentration (qPCR, ELISA, etc.) to establish a correlation factor and multiply it by the GoStix Value for unknown samples.
My signal is consistently “Off Scale”.	Too much analyte is being placed onto the test. The Test-line signal must always be less than the Control-line signal to produce a valid read. (See Figure 2.)	Dilute samples and retest.
My signal is “Invalid”.	Low dsRNA concentration.	Concentrate sample and retest.
	The sample buffer may negatively impact assay performance, suppressing both Test-line and Control-line signal development.	Dilute sample in Chase Buffer and retest.

Problem	Possible explanation	Solution
My device crashed and I no longer have access to my data.	Device issues.	Contact Technical Support (Technical_Support@takarabio.com).
I am observing some variability in my readings.	Improper dilutions.	Repeat dilutions and retest. Prepare 100 µl of sample by mixing sample and chase buffer first before adding to the sample well (S).
	Improper reads.	Collect reads consistently after 10 min of incubation.
	Device is moving too much during read.	Steady device.
	Different devices were utilized.	Use same device for all reads.

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