

# Biomeme SARS-CoV-2 Go-Plates

For IVD Use. In the U.S. only, for use under an Emergency Use Authorization (EUA) only.

This product has not been FDA cleared or approved; the product has been authorized by FDA for use with the Biomeme SARS-CoV-2 Real-Time RT-PCR Test under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

This product has been authorized only for use with the Biomeme SARS-CoV-2 Real-Time RT-PCR Test for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.

This product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

Last Updated: October 18<sup>th</sup>, 2022

# Biomeme SARS-CoV-2 Go-Plates

Biomeme **SARS-CoV-2 Go-Plates** detect the RNA of severe acute respiratory syndrome coronavirus 2 that causes coronavirus disease 2019 (COVID-19).

The Biomeme test detects two different SARS-CoV-2 genes and is multiplexed together with Biomeme's RNA Process Control (RPC) for RNA extraction and RT-PCR (MS2 bacteriophage). Each of reaction well of the 96-well Go-Plate contains lyophilized master mix, enzymes, and multiplexed primer/probes for the following triplex reaction:

- **1ab** Open reading frame 1ab gene
- **S** Spike gene
- **RPC** RNA Process Control (MS2 bacteriophage)

Each order contains one 96-well Go-Plate, which is shelf-stable if unopened. Biomeme Go-Plates are compatible for use on standard lab-bound real-time PCR thermocyclers (e.g., ABI 7500 Fast Dx, QuantStudio5 Fast, Bio-Rad CFX96). Please contact <a href="mailto:support@biomeme.com">support@biomeme.com</a> for further instruction on cutting Go-Plates for use on a Franklin three9.

**Safety Warning**: When working with our products, always wear appropriate personal protective equipment (PPE) (e.g. lab coat, disposable gloves with adequate chemical resistance, mouth/face protection, goggles, etc.) For more information, please review the product's safety data sheet(s) (SDS).

## **Contents of Go-Plate Pouch**

CONTENTS	DESCRIPTION
1x large foil pouch	The test pouch contains a 96-well PCR Go-Plate with your lyophilized assay reactions and void filling caps.
RNA Process Control (RPC) Kit	<ul> <li>RPC Kit includes:</li> <li>1x small foil pouch with a screw cap tube containing a lyophilized pellet of quantified MS2 to be used as your RNA Process Control (RPC)</li> <li>1x screw cap tube containing 5 mL of pre-aliquoted RPC Buffer used to resuspend the lyophilized RPC pellet. 1x resuspended RPC is enough positive control for 250 sample extractions when adding 20 uL to each extraction.</li> <li>1x transfer pipette</li> </ul>

## **Technical Characteristics**

SPECIFICATIONS	VALUE
PCR Tube Capacity	0.1 mL
PCR Reaction Volume	20 µL
Caps	Seal Go-Plates with adhesive thermal PCR seal ( <b>not</b> included)
DNA-dependent DNA-polymerase	Hotstart Taq polymerase (1 min. activation @ 95°C)
Reverse transcriptase	RT enzyme (2 min. RT step @ 55°C)
Nucleotides	Proprietary mix of dNTPs
Buffer	Tris pH 8.8 Salts and enhancers for 5' nuclease assays
Mg++	6 mM
Storage	15-30°C
<b>Dissolution time</b>	~60s

*Note:* Contains Bovine Serum Albumin of USA origin. Certified BSE free.

## **Multiplex Assay Characteristics**

Target	WELL LOCATION	COLOR CHANNEL
SARS-CoV-2-Orf1ab gene	All	Green (FAM)

SARS-CoV-2-S gene	All	Red (ATTO647N)
<b>RNA Process Control</b> (Exogenous RNA Extraction and RT-PCR Process Control (MS2))	All	Amber (TexasRedX)

## Thermocycling Protocol Parameters for Biomeme

## SARS-CoV-2 Assay

	Temperature (°C)	Duration
Reverse Transcriptase Step (Cycle 0)	55	120 secs
Initial Denature	95	60 secs
Cycling Denature	95	3 secs
Annealing	60	30 secs
Extension	N/A	N/A
Melt Curve	N/A	N/A
Number of Cyc	: <b>les:</b> 45	Total Reaction Volume: 20 uL

## Protocol

#### Preparing SARS-CoV-2 Go-Plates for Use on the Bio-Rad CFX

#### Maestro 96-well plate instrument

- 1. Open the contents of a Biomeme SARS-CoV-2 Go-Plate (REF# <u>3000562</u>).
  - If not loading the entire 96-well Go-Plate, cut the desired number of wells away from the Go-Plate using sterile scissors.
  - Insert the unused portion of the Go-Plate back into the foil pouch.
  - Remove any excess air from the pouch and reseal the ziplock.
  - Ensure the ziplock is fully sealed to maximize shelf-life of the unused reactions.
- 2. Place the wells you into to use into a 96-well plate tray for setup of your PCR run.
- 3. Working on one row at a time, carefully peel away the foil from the wells.
- 4. Attach a pipette tip to a 20μL fixed volume pipette (REF# <u>3000011</u>) or use your own 20uL pipette.
- 5. Unscrew the cap of your first purified sample and transfer  $20\mu$ L of the extracted RNA into the first reaction well.

**Note**: The strip connections between the tubes of your Go-Plate will face the back of the thermocycler once inserted. When transferring your extracted RNA into the different reaction wells, replicate this orientation to ensure accurate result interpretation (e.g. transfer sample 1 into the far-left reaction well of your first Go-Plate Well, moving from a left to right orientation.

6. Pipette up and down 3-5 times to mix your PCR reaction. When mixing your samples try to avoid introducing bubbles.

**Note**: If bubbles have been introduced, remove them from the lower portion of the PCR tubes by gently tapping them against your work surface before sealing. Bubbles may remain at the top of the tube, but bubbles at the bottom are not acceptable.

7. Discard your pipette tip and repeat this process for the remaining reaction wells. Once all reaction wells are filled, apply an optical adhesive sealer. Firmly press down with a plastic sealer while moving around the outer edges of the top of the to ensure a good seal. Cut away any excess adhesive when less than 96 wells are used.

*Note*: You can use a plate spinner to make sure your PCR reactions are at the bottom of the plate.

#### Setup and Loading the Bio-Rad CFX-96:

- 1. Turn on Bio-Rad CFX-96 instrument
- 2. Open the Bio-Rad CFX Maestro 1.1 version 4.1.2433.1219 (REF 1855195)
- 3. Select "Start Up Wizard" shown below:

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4. Select "User-defined" tab. This will take you to Run set up page. Then click on "Create New" as shown below:

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5. On the protocol editor page, click on "Insert Step" to add the RT step to the protocol

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- 6. Set up your PCR thermocycling parameters as follows:
  - a. Step 1 (RT): 55 C for 2 minutes
  - b. Step 2 (Initial Denature): 95°C for 1 minute
  - c. Step 3 (Denature): 95°C for 3 seconds
  - d. Step 4 (Anneal): 60°C for 30 seconds
- 7. Change the cycles from a default of 39X to 45X.
- 8. Change the reaction volume shown below from default 25 uL to 20 uL.

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9. Press "Ok" once you are finished setting up the thermocycling parameters. The program will now ask whether you want to save your protocol file. Click on "Yes" and save your file where appropriate.

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10. Once finished press "Next". This will take you plate set up screen. Click on "Create New". Now you will set up your plate layout.



11. To start click on "Select Fluorophores". From the list you want to select "FAM, Texas Red, and Cy5" as your dyes.



12. Depending on how you have loaded your plate, you can select "Sample Type" and give either "Unknown", "Positive control", or "NTC" tags to each well. For example, in the image below, 24 unknown samples were selected with one positive control and one NTC.

- 13. Name each target by typing the target name into each of the appropriate dyes:
  - a. FAM: "Orf1ab"
  - b. Texas Red: "MS2" or "RPC"
  - c. Cy5: "S"



- 14. The software will ask you whether you want to save the plate setup. Click on "Yes" and save your plate file where appropriate.
- 15. Press on next to go to the "Start Run" page. If multiple Bio-Rad CFX-96 instruments are connected to the computer, select the appropriate one. Click on "Start Run". The software will ask you again to save your data file. Click on "Yes" and save your data file where appropriate with a unique name.



16. Once the run is finished, the result will pop up automatically on the screen. If it does not, then you can click on the "Open a Data or Gene Study File" icon shown below and open up your data file with the unique ID to view the results.



17. From the results screen, pick "Target" from the tab on the right-hand corner of the screen shown below. Select only one target, for example Orf1ab as shown. Right click on the screen and select "Baseline Threshold".



- 18. On the baseline threshold page, click on "User Defined" and change the value to a specific number for each target:
  - a. Orf1ab = 150
  - b. S= 200
  - c. RPC or MS2 = 200



19. Once the thresholds are set for each target, you can begin analyzing your sample by clicking on each well. On the right side of the page (shown below), you can see the Cq values for each given sample. Refer to the interpretation result table to analyze your data.



#### Preparing SARS-CoV-2 Go-Plates for Use on the Applied Biosystems Quant Studio 5 96-well plate instruments

- 1. Open the contents of a Biomeme SARS-CoV-2 Go-Plate (REF#<u>3000562</u>).
  - If not loading the entire 96-well Go-Plate, cut the desired number of wells away from the Go-Plate using sterile scissors.
  - Insert the unused portion of the Go-Plate back into the foil pouch.
  - Remove any excess air from the pouch and reseal the ziplock.
  - Ensure the ziplock is fully sealed to maximize shelf-life of the unused reactions.
- 2. Place the wells you into to use into a 96-well plate tray for setup of your PCR run.
- 3. Working on one row at a time, carefully peel away the foil from the wells.
- 4. Attach a pipette tip to a 20µL fixed volume pipette (REF# 3000011) or use your own 20 uL pipette
- 5. Unscrew the cap of your first purified sample and transfer  $20\mu$ L of the extracted RNA into the first reaction well.

**Note**: The strip connections between the tubes of your Go-Plate will face the back of the thermocycler once inserted. When transferring your extracted RNA into the different reaction wells, replicate this orientation to ensure accurate result interpretation (e.g. transfer sample 1 into the far-left reaction well of your first Go-Plate Well, moving from a left to right orientation.

6. Pipette up and down 3-5 times to mix your PCR reaction. When mixing your samples try to avoid introducing bubbles.

**Note**: If bubbles have been introduced, remove them from the lower portion of the PCR tubes by gently tapping them against your work surface before sealing. Bubbles may remain at the top of the tube, but bubbles at the bottom are not acceptable.

- 7. Discard your pipette tip and repeat this process for the remaining reaction wells. Once all reaction wells are filled, apply an optical adhesive sealer. Firmly press down with a plastic sealer while moving around the outer edges of the top of the to ensure a good seal. Cut away any excess adhesive when less than 96 wells are used.
- 8. Turn on the QuantStudio5. Once the instrument is booted up, press the "open drawer" button on the touch screen of the instrument
- 9. Carefully load your reaction wells into the blue-plate adapter provided with the QuantStudio5. Close the adapter.
- 10. Load the adapter containing your reaction wells into the 96 well block of the QuantStudio5.
- 11. Close the drawer by pressing on the "open drawer" button. *Note*: You can use a plate spinner to make sure your PCR reactions are at the bottom of the plate.

#### Setup and Loading the QuantStudio5:

- 1. Open the QuantStudio<sup>™</sup> Design and Analysis Software
- 2. Select "Create New Experiment" shown below:



3. Change the run name if needed as shown in the image below. Once you have entered a unique name, click on "Next".

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- 4. On the "method" tab make sure that PCR thermocycling parameters are set as follows:
  - a. Step 1 (RT): 55 C for 2 minutes
  - b. Step 2 (Initial Denature): 95 C for 1 minute
  - c. Step 3 (Denature): 95 C for 3 seconds
  - d. Step 4 (Anneal): 60 C for 30 seconds
- 5. Change the cycles to 45X cycles

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- 6. Change the reaction volume shown below from default 50 uL to 20 uL.

7. Press next once you are finished setting up the thermocycling parameters. In the "Plate" tab, click on the drop down on "Passive reference" and change the default from "ROX" to "None".

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- 8. In the plate tab, click on "Advanced setup". In that page, click on the "Add" button as shown below and add 2 other targets (total of 3 targets). Change the name of the target and pick appropriate reporter dye for each target:
  - a. FAM:Orflab
  - b. TexasRedX: RPC
  - c. ATTO647N: S

*Note*: Make sure that your instrument is calibrated with specified dyes (FAM, ATTO647N and TexasRedX) before use.

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9. Click on the "Add button" shown below to add number of samples you are running based on your plate layout. You can change the sample name as preferred. Click on each well on the right side and make sure all three targets are selected for each well.

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10. Click next, now you want to save your run where you prefer and then click on "Start Run".



11. Once your run is finished and on "Results" tab, click on the "eye" icon and from the dropdown show in the image, change the graph type from "Log" to "Linear".



- 12. Next, from the "Target" drop down, pick each target one by one and change the threshold from auto to the following:
  - a. Orf1ab: 36000
  - b. S: 38000
  - c. RPC: 56000

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13. Once you have set the threshold for all the targets individually, click on "All" from the target drop down. Then click on "Save" followed by "Analyze" as shown below.

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14. As shown in the image below, click on the tab and you can see the CT values given for each sample and target. Refer to interpretation result table to analyze your data.



## Storage

Unopened Go-Plate pouches and RPC Kit should be stored in a dry place, at room temperature (15-30°C). The individual foil pouch containing a single Go-Plate is stable under these conditions if unsealed. See the pouch label for the expiration date. Individual Go-Plates should be used promptly after removal from individual foil pouch.

RPC Buffer, once resuspended, has a maximum shelf life of 2 days when stored at room temperature. For longer-term storage, aliquot out and freeze at -20°C for up to three months.

## Disclaimer

Biomeme products may not be transferred to third parties, resold, modified for resale or used to manufacture commercial products or to provide a service to third parties without written approval of Biomeme, Inc.

Biomeme warrants every thermocycler to be free of defects in material and workmanship for one year from the date of shipment to buyer. All warranties are subject to our <u>Terms and Conditions and Privacy Policy</u> (https://biomeme.com/privacy-policy-and-terms-of-use/).

Biomeme, Inc. 401 North Broad Street, Suite 222 Philadelphia, PA, USA 19108 <u>support@biomeme.com</u> Patent Protected
(https://biomeme.com/patents/)

## **Technical Support**

Biomeme, Inc. 401 North Broad Street, Suite 222 Philadelphia, PA 19108

Phone: 267-930-7707 FAX: 855-940-0157 Email: <u>support@biomeme.com</u>

The customer is responsible for compliance with regulatory requirements that pertain to their procedures and uses of the instrument. The information in this guide is subject to change without notice.

**DISCLAIMER**: TO THE EXTENT ALLOWED BY LAW, BIOMEME INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

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v2.0

## **Revision History**

Revision	Date	Description
1.0		New document
2.0	October 18, 2022	Removed instructions for RNA Process Control Added instructions for Preparing and Loading Bio-Rad CFX Maestro 96- well plate instruments Added instructions for Preparing and Loading Applied Biosystems Quant Studio 5 96-well plate instruments Removed <i>in silico</i> Analysis

v2.0