PRODUCT SHEET

Biomeme

Biomeme SARS-CoV-2 Bulk Vials

Last Updated: 01/26/2021
Version 1.5

Brief Overview

Biomeme **SARS-CoV-2 Bulk Vial** detects the RNA of severe acute respiratory syndrome coronavirus 2 that causes coronavirus disease 2019 (COVID-19), also known as “2019-nCoV” or “Wuhan coronavirus.”

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). The SARS-CoV-2 Bulk Vial 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)

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 Bulk Vial

CONTENTS	DESCRIPTION
Buk Vial	Each vial contains enough lyophilized assay for up to 260 reactions depending on your chosen reaction volume: • 260x 5 µL reactions (2.5 µL template + 2.5 µL assay resuspended at a 2x concentration) • 130x 10 µL reactions (5 µL template + 5 µL assay resuspended at a 2x concentration) • 65x 20 µL reactions (10 µL template + 10 µL assay resuspended at a 2x concentration)
1x small clear bag	The small clear bag includes: • 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) • 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. • 1x transfer pipette

Technical Characteristics

SPECIFICATIONS	VALUE
DNA-dependent DNA-polymerase	Hotstart Taq polymerase (1 min. activation @ 95°C)
Reverse transcriptase	Thermostable RNase H+ recombinant MMuLV (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

Dissolution time	~60s

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

Multiplex Assay Characteristics

Target	COLOR CHANNEL
SARS-CoV-2-Orf1ab gene	Green (FAM)
SARS-CoV-2-S gene	Red (ATTO647N)
RNA Process Control (Exogenous RNA Extraction and RT-PCR Process Control (MS2 bacteriophage))	Amber (TexasRedX)

Reconstitution Volume

ASSAY CONCENTRATION SOUGHT	DILUENT VOLUME TO ADD
2x	675 µL

Prepare RNA Process Control (RPC)

Each Biomeme SARS-CoV-2 Test includes pouches containing your RNA Process Control (RPC), RPC Pellets (MS2 bacteriophage), and 1mL transfer pipettes.

1. Open a foil pouch and remove the screw cap tube containing your RPC pellet. Open tube.
2. Open the 5mL screw cap tube containing RPC buffer.
3. Using the 1mL transfer pipette, pull 0.5 - 0.75 mL of RPC buffer and add to the RPC pellet tube to resuspend.
4. Pipette up and down with the transfer pipette to mix.
5. Transfer the entire volume back into the 5 mL RPC buffer tube, again pipetting up and down to mix.
6. Your RPC is now ready to add to your upcoming sample extractions:
    a. Each lyophilized pellet of MS2 bacteriophage is at a 5e6 pfu concentration.
    b. Resuspending with 5 mL of buffer yields a concentration of 1000 pfu/µL.
    c. Adding 5 µL of RPC (5000 pfu) per sample extraction to an extraction protocol that yields an elution volume of 50 µL would have an end RPC concentration of approximately 100 pfu/µL.
    d. Assuming a 5 µL final PCR reaction volume (2.5 µL assay at 2x concentration + 2.5 µL template), the RPC would have a concentration of 250 pfu per PCR reaction.

Once resuspended, the RPC Pellet can be refrigerated at 4 ℃ for up to one week. It can also be frozen at -20 ℃ but we caution against frequent freezing and re-thawing as it will degrade the control.

Assay Setup

1. Add 675 µL of DNase/RNase free molecular grade water to the SARS-CoV-2 Bulk Vial to resuspend the assay at a 2x concentration.
2. Pipette the solution up and down several times to mix.
3. Add 10 µL of assay into each of your PCR reaction wells. *Remember to change pipette tips each time you add your assay to a new well.* Each Bulk Vial contains enough assay for 65 reactions (without pipetting waste).
4. Transfer 10 µL of eluate (containing purified nucleic acids) to each PCR reaction Biomeme's SARS-CoV-2 assay. 1 sample extraction per PCR reaction (not including any wells you may reserve for additional controls or replicates). *Remember to change pipette tips each time you add your purified sample to a new PCR reaction.*
5. Transfer your PCR reactions to your thermocycler and begin your run!

Note: *For additional tips, How-To videos, and best practices for our Sample Prep system, please visit our Biomeme Sample Prep Guide, available at:* https://help.biomeme.com/sample-prep-guide

Thermocycling Parameters

		DURATION	
	TEMPERATURE (℃)	Biomeme Franklin™	Lab Thermocycler
RT Step	55	120 secs	120 secs
Initial Denature	95	60 secs	60 secs
Cycling Denature	95	3 secs	1 sec
Annealing	60	30 secs	20 secs
Extension	N/A	N/A	N/A
Melt Curve	N/A	N/A	N/A
Number of Cycles: 45		**Total Reaction Volume:** 20 µL	

Note: *The above thermocycling parameters have been confirmed on the Bio-Rad CFX96, Applied Biosystems (ABI) 7500 Fast and/or QuantStudio5 using the "fast" block.*

In Silico Analysis

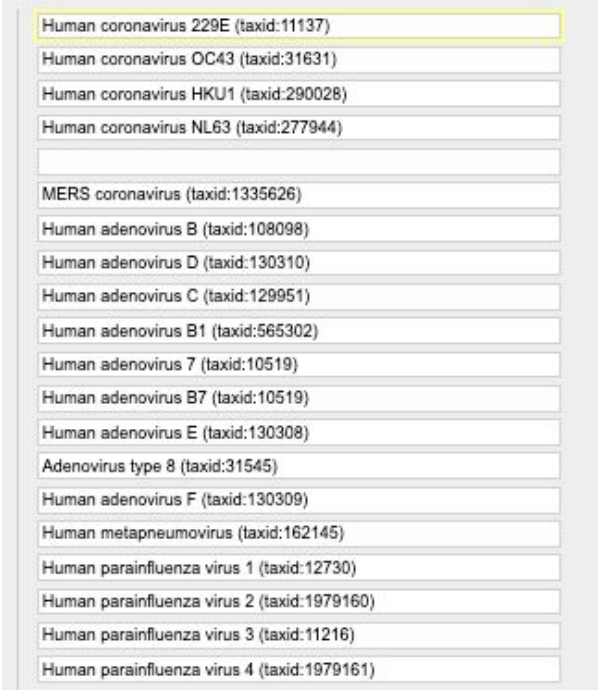
Inclusivity:

The SARS-CoV-2 primer and probe sets for both Orf1ab and S gene targets have 100% homology to the published sequence from NCBI.

Cross-reactivity (Exclusivity):

The Orf1ab R primer returned 90% homology to SARS-CoV, however when primer BLAST was performed (which takes into account the Forward primer and the ability for PCR amplicon production) no target templates were found to SARS-CoV.

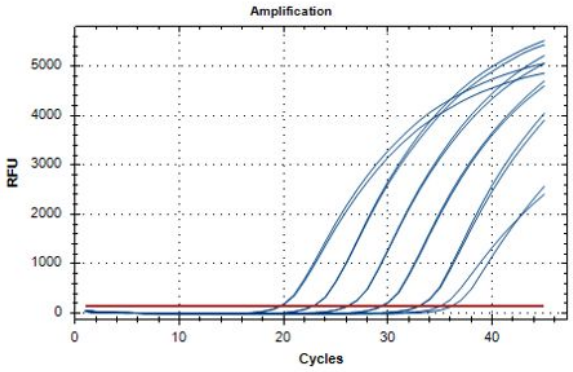
All other primers and probes had <80% homology to exclusive organisms listed below.

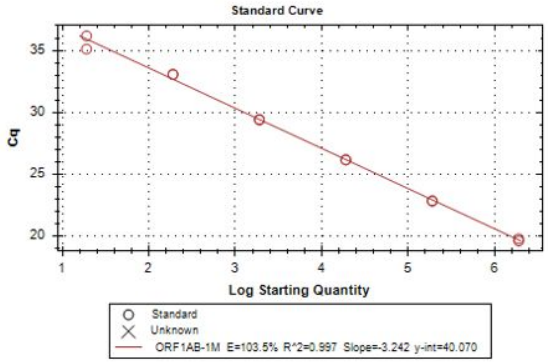


Performance Characteristics

SARS-CoV-2 Orf1ab

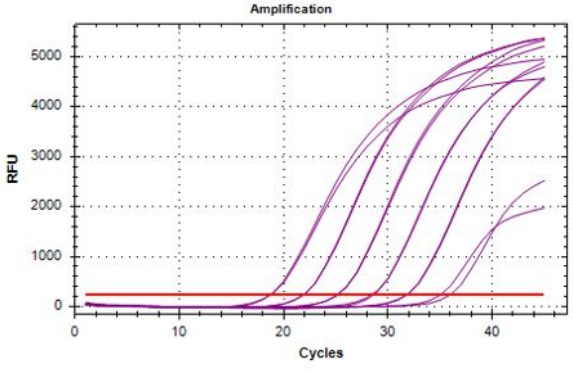
Amplification plots of multiplexed Orf1ab ivt-RNA in serial dilution from 2e6 — 20 copies/reaction in lyophilized LyoRNA 2.0 Master Mix. Efficiency of 103.5%. Limit of detection not determined.

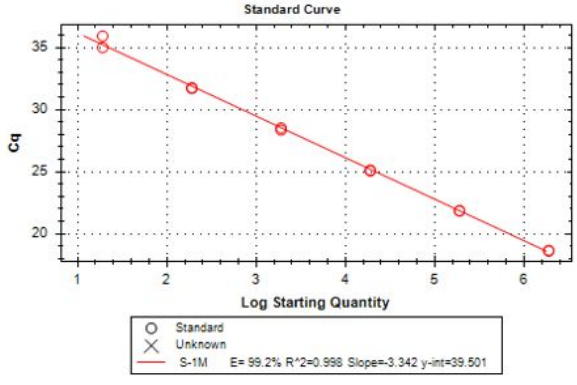




SARS-CoV-2 S

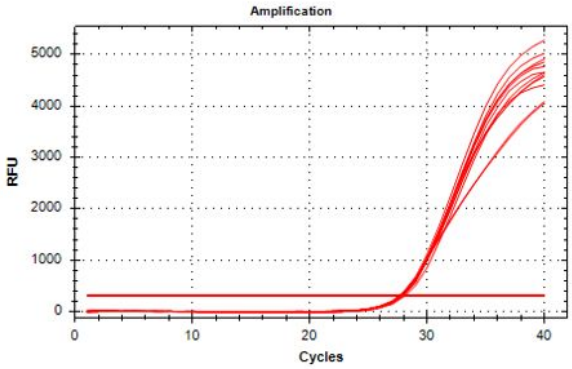
Amplification plots of multiplexed S ivt-RNA in serial dilution from 2e6 —20 copies/reaction in lyophilized LyoRNA 2.0 Master Mix. Efficiency of 99.2%. Limit of detection not determined.





RNA Process Control (Exogenous RNA Extraction and RT-PCR Control - MS2)

Amplification of multiplexed MS2 RNA extraction and RT-PCR process control in lyophilized LyoRNA 2.0 Master Mix.



Storage

Bulk Vials should be stored in a dry place, at room temperature (15-30°C). See the vial label for expiration date. After resuspension, use as quickly as possible and store at 4°C for up to eight hours

Disclaimer

For Research Use Only. Not for use in human or veterinary diagnostics. The performance characteristics of this product have not been Established.

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.

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