

TaqPath™ COVID-19 MS2 Combo Kit 2.0

INSTRUCTIONS FOR USE

Multiplexed real-time PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 and MS2 extraction control in a single reaction well.

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Revoked

IVD

For In Vitro Diagnostic Use. For Emergency Use Authorization Only | Rx Only

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For descriptions of symbols on product labels or product documents, go to [thermofisher.com/symbols-definition](https://www.thermofisher.com/symbols-definition).

The customer is responsible for validation of assays and compliance with regulatory requirements that pertain to their procedures and uses of the instrument.

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Intended Use

The Applied Biosystems™ TaqPath™ COVID-19 MS2 Combo Kit 2.0 is a real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swabs, mid-turbinate nasal swabs, and anterior nasal swabs from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high-complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all test results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The TaqPath™ COVID-19 MS2 Combo Kit 2.0 is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.

The TaqPath™ COVID-19 MS2 Combo Kit 2.0 is only for use under the Food and Drug Administration's Emergency Use Authorization.

Product description

The TaqPath™ COVID-19 MS2 Combo Kit 2.0 is a multiplexed real-time PCR test that can detect the RNA from the SARS-CoV-2 virus and MS2 extraction control in a single reaction well.

Each kit includes the following components:

- TaqPath™ COVID-19 MS2 RT-PCR Assay 2.0—Multiplexed assay that contains primer/probe sets specific to different SARS-CoV-2 genomic regions and primers/probes for MS2 Phage Control.
- TaqPath™ COVID-19 Plus Control—*In vitro* transcribed RNA control that contains targets specific to the SARS-CoV-2 regions targeted by the assays.
- MS2 Phage Control—RNA control to verify the efficacy of the sample preparation and the absence of inhibitors in the PCR reaction. MS2 Phage Control is added to the samples before RNA extraction.
- TaqPath™ COVID-19 Control Dilution Buffer—Dilution buffer for the TaqPath™ COVID-19 Plus Control.

The TaqPath™ 1-Step Multiplex Master Mix (No ROX™) must be purchased separately (see “Required materials not supplied” on page 7).

The assay contains primers and probe sets specific to the following targets:

- SARS-CoV-2 N Gene
- SARS-CoV-2 ORF1a
- SARS-CoV-2 ORF1b
- MS2 (extraction control)

Table 1 Dyes, quenchers, and targets

Target	Dye	Quencher
SARS-CoV-2 N Gene	VIC™	None ^[1]
SARS-CoV-2 ORF1a	FAM™	
SARS-CoV-2 ORF1b	ABY™	
MS2	JUN™	

^[1] TaqPath™ COVID-19 MS2 Combo Kit 2.0 probes contain QSY™ quenchers, which do not fluoresce. Select **None** for **Quencher** in the instrument set up procedure (see page 26 and page 30).

Results are analyzed using the following software:

- Applied Biosystems™ Pathogen Interpretive Software v1.1.0
- The appropriate assay panel for your instrument:
 - C19-MS2-EUA_QS5-9602_1.0.0
 - C19-MS2-EUA_QS7-384_1.0.0
- SAE Administrator Console Dx v1.0.0 (for security and audit functions)

For more information, see Chapter 6, “Analysis and results”.

Contents and storage

Table 2 TaqPath™ COVID-19 MS2 Combo Kit 2.0, 1,000 reactions (Cat. No. [A51329](#))

Component		Amount	Storage	Shelf life
TaqPath™ COVID-19 MS2 RT-PCR Kit 2.0	TaqPath™ COVID-19 MS2 RT-PCR Assay 2.0	1 × 1,500 µL	–30°C to –10°C ^[1]	12 months ^[2]
	MS2 Phage Control	10 × 1,000 µL	–30°C to –10°C	
TaqPath™ COVID-19 Plus Control		10 × 10 µL (1×10 ⁴ copies/µL)	–30°C to –10°C	
TaqPath™ COVID-19 Control Dilution Buffer		10 × 250 µL	–30°C to –10°C	

^[1] Do not freeze-thaw more than 12 times.

^[2] From the manufacturing date.

Required materials not supplied

Unless otherwise indicated, all materials are available through [thermofisher.com](https://www.thermofisher.com). "MLS" indicates that the material is available from [fisherscientific.com](https://www.fisherscientific.com) or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
Real-time PCR instrument, one of the following:	
Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block (used with QuantStudio™ Design and Analysis Desktop Software v1.5.1)	A28569 (with laptop computer) A28574 (with desktop computer) A28139 (instrument only)
Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR Instrument, 384-well block (used with QuantStudio™ Real-Time PCR Software v1.3)	4485695 (with laptop computer) 4485701 (with desktop computer)
Equipment	
Laboratory freezers • –30°C to –10°C	MLS
Centrifuge, with a rotor that accommodates standard and deepwell microplates	MLS
Microcentrifuge	MLS
Laboratory mixer, vortex or equivalent	MLS
Single and multichannel adjustable pipettors (1.00 µL to 1,000.0 µL)	MLS
Cold block (96-well or 384-well) or ice	MLS

(continued)

Item	Source
Nucleic acid extraction system and materials	
KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head	5400630
KingFisher™ Flex 96 Deep-Well Heating Block	24075430
KingFisher™ 96 Deep-Well Plate	95040450, A48305
KingFisher™ 96 tip comb for DW magnets	97002534, A48438
96-well plate for the tip comb, one of the following:	
KingFisher™ 96 KF microplate	97002540
Tip Comb Presenting Plate for KF 96	267600
Nunc™ MicroWell™ 96-Well Microplate, Flat Bottom	167008
Nunc™ MicroWell™ 96-Well Microplate, barcoded	269787
ABgene™ 96-Well Polypropylene Storage Microplate	AB0796
ABgene™ 96-Well 1.2-mL Polypropylene Deepwell Storage Plate	AB1127
KingFisher™ 96 Deep-Well Plate	95040450, A48305
Kits and reagents	
MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit	A48383
TaqPath™ 1-Step Multiplex Master Mix (No ROX™)	A28523
Fisher BioReagents™ Ethanol, Absolute, Molecular Biology Grade ^[1] , or equivalent	BP2818100, BP2818500, BP28184
Nuclease-Free Water (not DEPC-Treated)	MLS
Calibration plates (QuantStudio™ 7 Flex Real-Time PCR Instrument)	
ABY™ Dye Spectral Calibration Plate for Multiplex qPCR, 384-well	A24736
JUN™ Dye Spectral Calibration Plate for Multiplex qPCR, 384-well	A24733
Tubes, plates, and other consumables	
MicroAmp™ Optical 96-Well Reaction Plate with Barcode, 0.2 mL	4306737, 4326659
MicroAmp™ Optical 96-Well Reaction Plate, 0.2 mL	N8010560, 4316813
MicroAmp™ Optical 384-Well Reaction Plate with Barcode	4309849, 4326270, 4343814
MicroAmp™ Optical 384-Well Reaction Plate	4343370
MicroAmp™ Clear Adhesive Film	4306311
MicroAmp™ Optical Adhesive Film	4311971, 4360954

(continued)

Item	Source
MicroAmp™ Adhesive Film Applicator	4333183
MicroAmp™ Optical Film Compression Pad ^[2]	4312639
Nonstick, RNase-free microcentrifuge tubes (1.5 mL and 2.0 mL)	thermofisher.com/plastics
Sterile aerosol barrier (filtered) pipette tips	thermofisher.com/pipettetips

^[1] Available at [fisherscientific.com](https://www.fisherscientific.com).^[2] Required for use with QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block only.

General laboratory recommendations

Follow standard operating procedures in your laboratory, such as the following:

- Follow the sample inactivation guidelines and requirements established by your laboratory and local regulatory bodies. If your laboratory inactivates samples, do so before you begin the workflow. Some inactivation methods (such as chemical inactivation) may affect the quality of primary samples and lead to deviations from the expected result.
- To prevent contamination:
 - Change gloves frequently.
 - Decontaminate surfaces, equipment, and pipettes with fresh 10% bleach (1% V/V sodium hypochlorite) or decontamination solution, followed by 70% ethanol.
 - Use ultraviolet light during biosafety cabinet decontamination (when available).
- To prevent degradation, keep eluted sample RNA, master mixes, assays, and controls on ice or in cold blocks while in use.
- Limit freeze-thaw cycles.
- Minimize exposure to light for the assay. The probes are light-sensitive.
- Aliquot reagents to prevent stock contamination and reduce the number of freeze-thaw cycles.
- After each run, review the amplification curves for signs of inadequate vortexing or centrifugation.
- To ensure reliable performance of the real-time PCR instrument, perform preventive maintenance according to the instructions provided by the manufacturer in the instrument documentation (see “Related documentation” on page 54).

Assay limitations

- The use of this assay as an *in vitro* diagnostic under the FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, that meet requirements to perform high complexity tests.
- The kit performance was established using nasopharyngeal swab, mid-turbinate nasal swabs, and anterior nasal swab samples only. Specimen types other than nasopharyngeal swab, mid-turbinate nasal swabs, and anterior nasal swab samples should not be tested with this assay.
- Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.
- Chemical inactivation and other known clinically relevant interfering substances and techniques can lead to adulteration of primary samples and may interfere with test results.
- The workflow must be performed according to the methods specified in the instructions for use.
- The quality of the biological samples is essential for the quality of the results generated with this kit.
- This kit uses purified RNA as a sample for the assay. The quality of the RNA recovered from biological samples is essential for the quality of the results generated with this kit.
- Performance was established using MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit (Cat. No. [A48383](#)). Other purification methods may lead to deviations from the expected result.
- The assay performance was established with a maximum of 12 freeze-thaw cycles. Additional freeze-thaw cycles may result in deviation from the expected results.
- False-negative results may arise from:
 - Improper sample collection
 - Degradation of the SARS-CoV-2 RNA during shipping/storage
 - Specimen collection after SARS-CoV-2 RNA can no longer be found in the specimen matrix
 - Using unauthorized extraction or assay reagents
 - The presence of RT-PCR inhibitors
 - Mutation in the SARS-CoV-2
 - Failure to follow instructions for use
 - Absence of specimen
- False-positive results may arise from:
 - Cross contamination during specimen handling or preparation
 - Cross contamination between patient samples
 - Specimen mix-up
 - RNA contamination during product handling

- Inconclusive results may arise from:
 - Sample at concentration near the limit of detection of the test
 - Assay cross-reactivity with SARS-CoV-1
 - Other factors
- Negative results do not preclude infection with SARS-CoV-2, and should not be the sole basis of a patient management decision.
- SARS-CoV-1 isolates shared $\geq 80\%$ identity with more than one assay component for the N gene and ORF1b assays. Inconclusive results may occur due to cross-reaction with SARS-CoV-1.
- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated. The TaqPath™ COVID-19 MS2 Combo Kit 2.0 cannot rule out diseases caused by other bacterial or viral pathogens.

Warnings and precautions

The TaqPath™ COVID-19 MS2 Combo Kit 2.0 workflow should be performed by qualified and trained staff to avoid the risk of erroneous results. Use separate areas for the preparation of patient samples and controls to prevent false positive results. Samples and reagents must be handled under a laminar airflow hood or biological safety cabinet.

- Samples and controls should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.
- Follow necessary precautions when handling specimens. Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples.
- Always use pipette tips with aerosol barriers. Tips that are used must be sterile and free from DNases and RNases.
- Do not eat, drink, smoke, or apply cosmetic products in the work areas.
- Do not use the kit after the expiry date.
- Dispose of waste in compliance with local, state, and federal regulations.
- Safety Data Sheets are available upon request.
- Positive results are indicative of the presence of SARS-CoV-2 RNA.
- Reagents must be stored and handled as specified in “Contents and storage” on page 7.
- The quality of the sample preparation (purified RNA) may influence the quality of the qPCR test. Laboratories shall only use the purification method described in Chapter 2, “Extract RNA”.
- The kit includes an RNA phage control to verify the efficacy of the sample preparation and the absence of inhibitors in the PCR reaction. To use the control, add MS2 Phage Control to the samples before extraction of the RNA, according to the instructions in Chapter 2, “Extract RNA”.
- Encrypt, pseudonymize, or anonymize personal data where possible following the requirements of HIPPA (Health Insurance Portability and Accountability Act).
- Modifications to assay reagents, assay protocol, or instrumentation are not permitted and are in violation of the product Emergency Use Authorization.
- For Use under Emergency Use Authorization only.
- For prescription use only.
- For *in vitro* diagnostic use.

- This product has not been FDA cleared or approved but has been authorized for emergency use by the FDA under an EUA for use by authorized laboratories.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2 RNA, not for any other viruses or pathogens.
- The emergency use of 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 declaration is terminated or authorization is revoked sooner.
- Laboratories within the United States and its territories are required to report all test results to the appropriate public health authorities.

Samples and controls

Note:

- Handle all samples and controls as if they are capable of transmitting infectious agents.
- Patient nasopharyngeal swabs, mid-turbinate swabs, or anterior nasal swabs must be collected and stored according to CDC guidelines. See the CDC website: <https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html>.
- Specimens must be packaged, shipped, and transported according to the current edition of the *International Air Transport Association (IATA) Dangerous Goods Regulations* ([iata.org/en/programs/cargo/dgr](https://www.iata.org/en/programs/cargo/dgr)).
- Refer to the *Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19)*, published by the Centers for Disease Control and Prevention, for specimen collection and storage guidelines.

Positive and negative test controls must be included to accurately interpret patient test results. Include the following controls:

Control	Description
Positive Control (TaqPath™ COVID-19 Plus Control)	Added to a single well in the RT-PCR plate; monitors RT-PCR reaction setup and reagent integrity.
MS2 Phage Control	Added to each well during RNA extraction; monitors RNA extraction and absence of inhibitors in the RT-PCR reaction.
Negative Extraction Control	A negative control on the extraction sample plate that contains water. The extracted negative control sample is run on the RT-PCR plate to monitor cross-contamination during RNA extraction and reaction setup.
No Template Control	A negative control well on the RT-PCR plate that contains Reaction Mix and water only; monitors cross-contamination during RT-PCR reaction setup.

For information on isolation and purification of viral nucleic acid from human respiratory specimens, see *MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit Instructions For Use* listed in “Related documentation” on page 54.

In-use reagent stability

Reagent	Stability information
TaqPath™ COVID-19 MS2 RT-PCR Assay 2.0	Once thawed, the TaqPath™ COVID-19 MS2 RT-PCR Assay 2.0 is stable for up to 24 hours at 2°C–8°C. Do not exceed 12 freeze/thaw cycles.
MS2 Phage Control	Single-use only.
TaqPath™ COVID-19 Plus Control	Single-use only.
TaqPath™ COVID-19 Plus Control working stock (1/2,675 dilution)	The working stock of the TaqPath™ COVID-19 Plus Control is stable for up to 24 hours at 2°C–8°C.
TaqPath™ COVID-19 Control Dilution Buffer	Single-use only.

Workflow

TaqPath™ COVID-19 MS2 Combo Kit 2.0 workflow

Extract RNA from patient samples (see Chapter 2, Extract RNA)

Perform automated RNA extraction using the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head and the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit with a 200-µL sample input volume.

Perform RT-PCR

1. Prepare RT-PCR reactions (see page 20).
2. Perform RT-PCR using one of the following real-time PCR instruments:
 - QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block (see page 25)
 - QuantStudio™ 7 Flex Real-Time PCR System, 384-well block (see page 29)

Analyze data and interpret the results using the Pathogen Interpretive Software v1.1.0 (see Chapter 6, Analysis and results)

1. Install the appropriate assay panel for your instrument (see page 35).
2. Analyze the data (see page 36).
3. Review run control results and interpret patient samples (see page 36).

The workflow begins with nucleic acid extraction from nasopharyngeal swab, mid-turbinate nasal swab, or anterior nasal swab specimens that were collected in transport media according to appropriate laboratory procedures.

Nucleic acids are isolated and purified from the specimens using the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit. Nucleic acid isolation is performed via an automated process using the KingFisher™ Flex Purification System (KingFisher™). For more information about using the kit, see “Related documentation” on page 54.

The nucleic acid is reverse transcribed into cDNA and amplified using the TaqPath™ COVID-19 MS2 Combo Kit 2.0 with one of the following real-time PCR instruments:

- QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block
- QuantStudio™ 7 Flex Real-Time PCR Instrument, 384-well block

In the process, the probes anneal to specific SARS-CoV-2 target sequences located between unique forward and reverse primers for the following targets:

- SARS-CoV-2 N Gene
- SARS-CoV-2 ORF1a
- SARS-CoV-2 ORF1b
- MS2 (extraction control)

During the extension phase of the PCR cycle, the 5' exonuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by the real-time PCR instrument.

The data are analyzed, then interpreted by the Pathogen Interpretive Software v1.1.0 and the appropriate assay panel for your instrument.

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■ Prepare a Proteinase K and MS2 Phage Control Mix	17
■ Prepare sample plate	18
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IMPORTANT! The RNA extraction protocols in this chapter are compatible with nasopharyngeal swabs, mid-turbinate nasal swabs, or anterior nasal swabs.

Automated RNA extraction is performed using the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head, KingFisher™ Flex 96 Deep-Well Heating Block, and the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit with a sample input volume of 200 µL.

Before you begin

Note: During the wash steps, the Wash Solution may develop inert white or brown particulates that float in solution. This is not a cause for concern and does not negatively affect performance.

- Determine the number of required reactions based on the number of samples, plus one Negative Extraction Control per plate.

Note: Each plate can accommodate up to 93 samples *plus* one Negative Extraction Control.

- Prepare fresh 80% ethanol solution in accordance with standard laboratory procedures using Ethanol, Absolute, Molecular Biology Grade and Nuclease-Free Water (not DEPC-Treated) for the required number of reactions, sufficient for 1 mL per reaction, plus 10% overage.
- Mark the Negative Extraction Control well on the plate.
- Label the short side of each KingFisher™ 96 Deep-Well Plate (4):

Label	Number of plates
Sample plate	1
Wash 1	1
Wash 2	1
Elution plate	1

- Label the short side of the KingFisher™ 96 KF microplate (1):

Label	Number of plates
Tip comb	1

Note: The following plates can be used in place of the KingFisher™ 96 KF microplate to hold the tip comb:

- Tip Comb Presenting Plate for KF 96
- Nunc™ MicroWell™ 96-Well Microplate, Flat Bottom
- Nunc™ MicroWell™ 96-Well Microplate, barcoded
- ABgene™ 96-Well Polypropylene Storage Microplate
- ABgene™ 96-Well 1.2-mL Polypropylene Deepwell Storage Plate
- KingFisher™ 96 Deep-Well Plate

Set up the instrument

1. Ensure that the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head is set up with the KingFisher™ Flex 96 Deep-Well Heating Block.

IMPORTANT! Failure to use the proper magnetic head and heat block results in lower yields and potential harm to the instrument.

2. Ensure that the **MVP_2Wash_200_Flex** program has been downloaded from the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit product page at www.thermofisher.com and loaded onto the instrument.

Prepare the processing plates

Prepare the processing plates according to the following table. Cover the plates with a temporary seal (such as MicroAmp™ Clear Adhesive Film), then store at room temperature for up to 1 hour while you set up the sample plate.

Plate ID	Plate position	Plate type	Reagent	Volume per well
Wash 1 Plate	2	KingFisher™ 96 Deep-Well Plate	Wash Solution	500 µL
Wash 2 Plate	3		80% Ethanol	1,000 µL
Elution Plate	4		Elution Solution	50 µL
Tip Comb Plate	5	Place a KingFisher™ 96 tip comb for DW magnets in a KingFisher™ 96 KF microplate		

Prepare Binding Bead Mix



WARNING! Do not use bleach or bleached pipette tips with the Binding Bead Mix. The Binding Solution contains guanidium thiocyanate, which produces cyanide gas when combined with bleach.

Prepare the required amount of Binding Bead Mix on each day of use.

1. Vortex the Total Nucleic Acid Magnetic Beads to ensure that the bead mixture is homogeneous.
2. For the number of required reactions, prepare the Binding Bead Mix according to the following table:

Component	Volume per well ^[1]	Volume per 96-well plate
Binding Solution	265 µL	28.0 mL
Total Nucleic Acid Magnetic Beads	10 µL	1.1 mL
Total volume	275 µL	29.1 mL

^[1] Include 10% overage when preparing the Binding Bead Mix for use with multiple reactions.

3. Invert at least five times to mix, then store at room temperature.

Prepare a Proteinase K and MS2 Phage Control Mix

Prepare the required amount of the Proteinase K and MS2 Phage Control Mix on each day of use. Keep on ice.

1. Thaw the vial of MS2 Phage Control.
2. For the number of required samples plus one Negative Extraction Control, prepare the Proteinase K and MS2 Phage Control Mix according to the following table:

Component	Volume per well ^[1]	Volume for 94 ^[2] reactions plus 10% overage
Proteinase K	5 µL	517 µL
MS2 Phage Control	5 µL	517 µL
Total volume per well	10 µL	1,034 µL

^[1] Include 10% overage when preparing the Proteinase K and MS2 Phage Control Mix for use with multiple reactions.

^[2] 93 samples plus one Negative Extraction Control

3. Mix well by inversion, then store on ice.

Prepare sample plate

Each 96-well RT-PCR plate requires three controls:

- Positive Control (PC)
- No Template Control (NTC)
- Negative Extraction Control (NC)

Therefore, each 96-well plate can accommodate 93 samples plus 1 Negative Extraction Control.

Note: NTC is a control on the RT-PCR plate only. It is not derived from the extraction plate.

1. Invert the prepared Binding Bead Mix 5 times gently to mix, then add 275 μ L to each sample well and to the Negative Extraction Control well in the Sample Plate.

Note:

- Remix the Binding Bead Mix by inversion frequently during pipetting to ensure even distribution of beads to all samples or wells. The Binding Bead Mix is viscous, so pipet slowly to ensure that the correct amount is added. Do NOT reuse pipette tips to add Binding Bead Mix to the samples, as the high viscosity will cause variations in the volumes added.
 - Do NOT vortex the Binding Bead Mix.
-

2. Add 200 μ L of sample to each sample well.

Note: Change tips between samples to eliminate cross-contamination.

3. Add 200 μ L of Nuclease-Free Water (not DEPC-Treated) to the Negative Extraction Control well.
4. Add 10 μ L of the prepared Proteinase K and MS2 Phage Control Mix to each well, including the Negative Extraction Control well, in the KingFisher™ 96 Deep-Well Plate labeled **Sample Plate**.

Process the samples

1. Select the **MVP_2Wash_200_Flex** on the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head.
2. Start the run, then load the prepared plates into position when prompted by the instrument.
3. After the run is complete (~24 minutes after start), immediately remove the Elution Plate from the instrument, then cover the plate with MicroAmp™ Clear Adhesive Film.

IMPORTANT! To prevent evaporation, seal the plate containing the eluate immediately.

The samples are eluted in 50 μ L of Elution Solution (see “Prepare the processing plates” on page 16).

4. Place the Elution Plate on ice for immediate use in real-time RT-PCR.

Note:

- Significant bead carry over may adversely impact RT-PCR performance. If bead carry over is observed, re-extract a new aliquot of the sample.
 - To ensure reliable performance of the KingFisher™ Flex Magnetic Particle Processor, perform preventive maintenance as instructed by the manufacturer.
-

Revoked

Prepare RT-PCR reactions

■ Guidelines for RT-PCR	20
■ Prepare the RT-PCR reactions (96-well reaction plate)	20
■ Prepare the RT-PCR reactions (384-well reaction plate)	22

Guidelines for RT-PCR

IMPORTANT!

- Prepare and keep the run plate on ice (or cold block) until it is loaded into the real-time PCR instrument.
- Run the plate immediately after preparation. Failure to do so could result in degraded RNA samples.
- To prevent contamination, prepare reagents in a PCR workstation or equivalent amplicon-free area. Do not use the same pipette for controls and RNA samples, and always use aerosol barrier pipette tips.
- Maintain a RNase-free environment. Periodically decontaminate surfaces.
- Protect assays from light.
- Keep RNA samples and components on ice during use.
- Add samples (templates) to reactions in an area that is designated for handling templates.
- For each RT-PCR plate, include the following controls:
 - One Positive Control (PC)
 - One No Template Control (NTC)
 - One Negative Extraction Control (NC) from each extraction run.

For example, if RNA samples from 4 extraction runs are combined on one 384-well real-time RT-PCR plate, then 4 Negative Extraction Control wells must be run on that 384-well RT-PCR plate.

Prepare the RT-PCR reactions (96-well reaction plate)

If frozen, thaw the reagents on ice.

1. Gently vortex the reagents, then centrifuge briefly to collect liquid at the bottom of the tube.
2. Dilute TaqPath™ COVID-19 Plus Control to a working stock (1/2,675 dilution):
 - a. Pipet 98 µL of TaqPath™ COVID-19 Control Dilution Buffer into a microcentrifuge tube, then add 2.0 µL of TaqPath™ COVID-19 Plus Control. Mix well, then centrifuge briefly.

- b. Pipet 105 µL of TaqPath™ COVID-19 Control Dilution Buffer into a second microcentrifuge tube, then add 2.0 µL of the dilution created in substep 2a. Mix well, then centrifuge briefly.
3. Prepare the Reaction Mix.
 - a. For each run, combine the following components sufficient for the number of RNA samples plus one Positive Control, one Negative Extraction Control, and one No Template Control.

IMPORTANT! The volumes in the following table assume that you extracted sample RNA using an original sample input volume of up to 200 µL.

Note: All volumes include 10% overage.

Component	Volume per RNA Sample or Control	Volume for n RNA Samples plus 3 Controls ^[1]	Volume for 93 RNA Samples plus 3 Controls ^[1]
TaqPath™ 1-Step Multiplex Master Mix (No ROX™) (4X)	6.25 µL	$6.875 \times (n + 3)$ µL	660 µL
TaqPath™ COVID-19 MS2 RT-PCR Assay 2.0	1.25 µL	$1.375 \times (n + 3)$ µL	132 µL
Total Reaction Mix volume	7.5 µL	—	792 µL

^[1] 1 NC, 1 NTC, and 1 PC

4. Set up the reaction plate.
 - a. Pipet 7.5 µL of the Reaction Mix prepared in step 3 into each well of a MicroAmp™ Optical 96-Well Reaction Plate with Barcode, 0.2 mL.
- Note:** For other reaction plates that can be used, see “Required materials not supplied” on page 7.
- b. Gently vortex the sealed plate containing the purified sample RNA and Negative Extraction Control from the RNA extraction procedure, then centrifuge briefly to collect liquid at the bottom of the plate.
 - c. Unseal the plate containing the purified sample RNA and Negative Extraction Control from the RNA extraction procedure. Add either sample RNA, Negative Extraction Control, No Template Control, or Positive Control to each well of the reaction plate according to Table 3.
 - d. Seal the plate thoroughly with MicroAmp™ Optical Adhesive Film.

IMPORTANT!

- Use ONLY MicroAmp™ Optical Adhesive Film (Cat. No. [4311971](#), [4360954](#)).
- Do NOT use optical caps, MicroAmp™ Clear Adhesive Film (Cat. No. [4306311](#)), or any other film or sealing methods.
- Do NOT heat seal the plates.
- When applying the MicroAmp™ Optical Adhesive Film, ensure that pressure is applied across the entire plate and that there is a tight seal across every individual well. Failure to do so runs the risk of an improperly sealed well, leading to potential well-to-well contamination during vortexing and evaporation during PCR.

- e. Vortex the plate at the highest setting speed for 10–30 seconds with medium pressure. Move the plate around to ensure equal contact on the vortex mixer platform.

IMPORTANT! Vortex for 10–30 seconds to ensure proper mixing. Failure to do so might result in inaccurate sample results.

- f. Centrifuge the reaction plate for 1–2 minutes at $\geq 650 \times g$ (≥ 650 RCF) to remove bubbles and to collect the liquid at the bottom of the reaction plate.

IMPORTANT! Centrifuge the plate for 1–2 minutes to ensure bubbles are removed. Failure to do so can produce inaccurate test results.

Table 3 Reaction plate volumes

Component	Volume per reaction			
	RNA Sample	Positive Control	Negative Extraction Control	No Template Control
Reaction Mix (from step 3)	7.5 μ L	7.5 μ L	7.5 μ L	7.5 μ L
Purified sample RNA (from RNA extraction)	17.5 μ L	—	—	—
Positive Control (diluted TaqPath™ COVID-19 Plus Control from step 2)	—	17.5 μ L	—	—
Negative Extraction Control (from RNA extraction)	—	—	17.5 μ L	—
Nuclease-free water	—	—	—	17.5 μ L
Total volume	25.0 μL	25.0 μL	25.0 μL	25.0 μL

Proceed immediately to set up and run the real-time PCR instrument.

Prepare the RT-PCR reactions (384-well reaction plate)

If frozen, thaw the reagents on ice.

1. Gently vortex the reagents, then centrifuge briefly to collect liquid at the bottom of the tube.
2. Dilute TaqPath™ COVID-19 Plus Control to a working stock (1/2,675 dilution):
 - a. Pipet 98 μ L of TaqPath™ COVID-19 Control Dilution Buffer into a microcentrifuge tube, then add 2.0 μ L of TaqPath™ COVID-19 Plus Control. Mix well, then centrifuge briefly.
 - b. Pipet 105 μ L of TaqPath™ COVID-19 Control Dilution Buffer into a second microcentrifuge tube, then add 2.0 μ L of the dilution created in substep 2a. Mix well, then centrifuge briefly.

3. Prepare the Reaction Mix.

- a. For each run, combine the following components sufficient for the number of RNA samples, plus one Positive Control per 384-well RT-PCR reaction plate, one No Template Control per 384-well RT-PCR reaction plate, and one Negative Extraction Control from each extraction run.

For example, if RNA samples from 4 extraction runs are combined on one 384-well RT-PCR reaction plate, then 4 Negative Extraction Control wells (one from each extraction plate) need to be run on that 384-well RT-PCR reaction plate.

IMPORTANT! The volumes in the following table assume that you extracted sample RNA using an original sample input volume of up to 200 µL.

Note: All volumes include 10% overage.

Component	Volume per RNA sample or control	Volume for n RNA samples plus 3 controls ^[1]	Volume for 378 RNA Samples + 6 Controls ^[2]
TaqPath™ 1-Step Multiplex Master Mix (No ROX™) (4X)	5 µL	$5.5 \times (n + 3)$ µL	2,112.0 µL
TaqPath™ COVID-19 MS2 RT-PCR Assay 2.0	1 µL	$1.1 \times (n + 3)$ µL	422.4 µL
Total Reaction Mix volume	6 µL	—	2,534.4 µL

^[1] 1 NC, 1 NTC, and 1 PC

^[2] 4 NCs (from 4 extraction plates), 1 NTC, and 1 PC. If more than 4 NCs are used, adjust the number of samples accordingly.

4. Set up the reaction plate.

- a. Pipet 6 µL of the Reaction Mix prepared in step 3 into each well of a MicroAmp™ Optical 384-Well Reaction Plate with Barcode.

Note: For other reaction plates that can be used, see “Required materials not supplied” on page 7.

- b. Gently vortex the sealed plate containing the purified sample RNA and Negative Extraction Control from the RNA extraction procedure, then centrifuge briefly to collect liquid at the bottom of the plate.
- c. Unseal the plate containing the purified sample RNA and Negative Extraction Control from the RNA extraction procedure. Add either sample RNA, Negative Extraction Control, No Template Control, or Positive Control to each well of the reaction plate according to Table 4.

IMPORTANT! To prevent sample contamination, unseal one extraction plate at a time, then reseal it after adding the samples to the RT-PCR reaction plate.

- d. Seal the plate thoroughly with MicroAmp™ Optical Adhesive Film.

IMPORTANT!

- Use ONLY MicroAmp™ Optical Adhesive Film (Cat. No. [4311971](#), [4360954](#)).
- Do NOT use MicroAmp™ Clear Adhesive Film (Cat. No. [4306311](#)) or any other film or sealing methods.
- Do NOT heat seal the plates.
- When applying the MicroAmp™ Optical Adhesive Film, ensure that pressure is applied across the entire plate and that there is a tight seal across every individual well. Failure to do so runs the risk of an improperly sealed well, leading to potential well-to-well contamination during vortexing and evaporation during PCR.

- e. Vortex the plate at the highest setting speed for 10–30 seconds with medium pressure. Move the plate around to ensure equal contact on the vortex mixer platform.

IMPORTANT! Vortex for 10–30 seconds to ensure proper mixing. Failure to do so might result in inaccurate sample results.

- f. Centrifuge the reaction plate for 1–2 minutes at $\geq 650 \times g$ (≥ 650 RCF) to remove bubbles and to collect the liquid at the bottom of the reaction plate.

IMPORTANT! Centrifuge the plate for 1–2 minutes to ensure bubbles are removed. Failure to do so can produce inaccurate test results.

Table 4 Reaction plate volumes

Component	Volume per reaction			
	RNA Sample	Positive Control	Negative Extraction Control	No Template Control
Reaction Mix (from step 3)	6.0 μ L	6.0 μ L	6.0 μ L	6.0 μ L
Purified sample RNA (from RNA extraction)	14.0 μ L	—	—	—
Positive Control (diluted TaqPath™ COVID-19 Plus Control from step 2)	—	14.0 μ L	—	—
Negative Extraction Control (from RNA extraction)	—	—	14.0 μ L	—
Nuclease-free water	—	—	—	14.0 μ L
Total volume	20.0 μL	20.0 μL	20.0 μL	20.0 μL

Proceed immediately to set up and run the real-time PCR instrument.



Perform RT-PCR using the QuantStudio™ 5 Real-Time PCR Instrument (96-well, 0.2-mL block)

- Dye calibration for the QuantStudio™ 5 Real-Time PCR Instrument 25
- Transfer the template (EDT) file for the QuantStudio™ 5 Real-Time PCR Instrument (96-well, 0.2-mL block) 25
- Set up and run the QuantStudio™ 5 Real-Time PCR Instrument 26

Dye calibration for the QuantStudio™ 5 Real-Time PCR Instrument

Ensure the system calibrations are current. In addition, ABY™ dye and JUN™ dye must be calibrated for use with this kit. Refer to the standard calibration process in the instrument user guide.

Transfer the template (EDT) file for the QuantStudio™ 5 Real-Time PCR Instrument (96-well, 0.2-mL block)

The template (EDT) file contains the settings for the instrument run.

The file is in the same compressed folder as the Pathogen Interpretive Software v1.1.0: MS2_2.0_EUA_QS5_96_0.2mL_AssayPanel_Template.

The template must be transferred via a USB drive or other method to the computer on which QuantStudio™ Design and Analysis Desktop Software v1.5.1 is installed.

IMPORTANT! Be careful to select the appropriate template file for your instrument and block type. Failure to do so can cause errors in the analysis.

1. After extracting the files from the compressed folder, select the following EDT file:
TaqPath COVID-19 MS2_2_0_Template_QS5_0_2mL_da1_5_1_v1_0.edt
2. Transfer the EDT file to the computer with QuantStudio™ Design and Analysis Desktop Software v1.5.1, using a USB drive or other method.

Set up and run the QuantStudio™ 5 Real-Time PCR Instrument

For more information about the instrument, see “Related documentation” on page 54.

Note: For the QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block, use the system defaults for the PCR filters.

1. In the QuantStudio™ Design and Analysis Desktop Software v1.5.1 home screen, in the **New Experiment** box, select **Create New Experiment ▶ Template**.
2. Browse to, then open the EDT file that you transferred in “Transfer the template (EDT) file for the QuantStudio™ 5 Real-Time PCR Instrument (96-well, 0.2-mL block)” on page 25.

IMPORTANT! Be careful to select the appropriate template file for your instrument and block type. Failure to do so can cause errors in the analysis.

3. In the **Properties** screen, enter or confirm the following information.
 - **Name:** Enter a unique name
 - **Instrument type:** QuantStudio™ 5 System
 - **Block type:** 96-Well 0.2-mL Block
 - **Experiment type:** Standard Curve
 - **Chemistry:** TaqMan™ Reagents
 - **Run Mode:** Standard
4. In the **Method** screen, confirm that the **Volume** is 25 µL, then confirm the thermal protocol.

Step	Temperature ^[1]	Time	Number of cycles
UNG incubation	25°C	2 minutes	1
Reverse transcription	53°C	10 minutes	1
Preincubation	85°C	10 minutes	1
Activation	95°C	2 minutes	1
Denaturation	95°C	3 seconds	40
Annealing / extension	60°C	30 seconds	

^[1] Confirm that the ramp rate for each step is 1.6°C per second.

5. In the **Plate** screen, click **Quick Setup**.
6. In the **Plate Attributes** pane, confirm that the **Passive Reference** is set to **None**.
7. Click **Advanced Setup**.

8. In the **Targets** table, confirm that the targets, reporter dyes, and quencher are listed correctly.

Target name	Reporter dye	Quencher
N Gene	VIC™	None
ORF1a	FAM™	
ORF1b	ABY™	
MS2	JUN™	

IMPORTANT! Target names are case-sensitive and must be named as described.

9. In the plate layout pane, confirm that the targets from step 8 are assigned to each well in the plate. To assign a target to a well, select the well in the plate layout, then select the targets from the **Targets** table.

10. In the plate layout pane, confirm the labeling of the control wells.

- The template has one Positive Control, one Negative Extraction Control, and one No Template Control assigned to wells for reference.
- The Positive Control must be named **PC**. If additional characters are included, it must be named **PC<>**, where <> is defined by the user, for example **PC1**.
- The Negative Extraction Control must be named **NC**. If additional characters are included, it must be named **NC<>**, where <> is defined by the user, for example **NC1**.
- The No Template Control must be named **NTC**. If additional characters are included, it must be named **NTC<>**, where <> is defined by the user, for example **NTC1**.
- Move the control well assignments by copying the existing control wells and pasting them according to their location on the physical plate.

IMPORTANT! The Positive Control, Negative Extraction Control, and No Template Control must be named as described.

11. In the plate layout pane, confirm the **Task** assignments.

- For wells with a Positive Control (**PC**), confirm that the **Task** is set to **S (Standard)** for all of the targets.
- For wells with a Negative Extraction Control (**NC**) and No Template Control (**NTC**), confirm that the **Task** is set to **N (Negative Control)** for all of the targets.
- For the wells with a patient sample, confirm that the **Task** is set to **U (Unknown)** for all of the targets.

12. In the **Samples** table, click **Add** to define a sample name for each sample. Create a unique sample name for each well in the physical plate.

IMPORTANT! Encrypt, pseudonymize, or anonymize personal data where possible following the requirements of HIPPA (Health Insurance Portability and Accountability Act).

13. Assign a sample name to each well to match the physical plate.

To assign a sample to a well, select the well in the plate layout, then select the sample from the **Samples** table.

Note: Wells that do not have a sample name will not be analyzed by the software.

14. Load the prepared and sealed RT-PCR reaction plate into the real-time PCR instrument.

15. Place a MicroAmp™ Optical Film Compression Pad gray side down on the surface of the RT-PCR reaction plate, to ensure a proper seal between the thermal cycler and the adhesive film.

IMPORTANT!

- Be careful to place the compression pad with the brown side up and the gray side down, centered on top of the plate.
 - Ensure that the compression pad is free from wrinkles and signs of deterioration prior to use.
-

16. In the **Run** screen, click **Start Run**, then select your instrument from the drop-down list.

17. Enter a file name in the dialog box that prompts you to save the run file, then save the file.

18. At the end of the run, remove the MicroAmp™ Optical Film Compression Pad from the plate and store the compression pad inside the pack.

IMPORTANT!

- If the compression pad becomes stuck inside the thermal cycler, call service to clean the heated cover.
 - Between each use, place the pad back in the pouch so that it does not dry out.
 - Each compression pad may be used up to 20 times before discarding. Do not use more than 20 times.
 - Do NOT use the pad with other instruments, unless expressly instructed to do so in the user documentation.
-



Perform RT-PCR using the QuantStudio™ 7 Flex Real-Time PCR System (384-well block)

- Dye calibration for the QuantStudio™ 7 Flex Real-Time PCR Instrument 29
- Transfer the template (EDT) file for the QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block) 29
- Set up and run the QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block) 30

Dye calibration for the QuantStudio™ 7 Flex Real-Time PCR Instrument

Ensure the system calibrations are current. In addition, ABY™ dye and JUN™ dye must be calibrated for use with this kit. Refer to the standard calibration process in the instrument user guide.

Transfer the template (EDT) file for the QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block)

The template (EDT) file contains the settings for the instrument run.

The file is in the same compressed folder as the Pathogen Interpretive Software v1.1.0:
MS2_2.0_EUA_QS7_384_AssayPanel_Template.

The template must be transferred via a USB drive or other method to the computer on which QuantStudio™ Real-Time PCR Software v1.3 is installed.

IMPORTANT! Be careful to select the appropriate template file for your instrument and block type. Failure to do so can cause errors in the analysis.

1. After extracting the files from the compressed folder, select the following EDT file:
TaqPath COVID-19 MS2_2_0_Template_QS7_384_1_3_v1_0.edt
2. Transfer the EDT file to the computer with QuantStudio™ Real-Time PCR Software v1.3, using a USB drive or other method.

Set up and run the QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block)

For more information about the instrument, see “Related documentation” on page 54.

1. In the QuantStudio™ Real-Time PCR Software v1.3 home screen, click **Template**.
2. Browse to, then open the EDT file that you transferred in “Transfer the template (EDT) file for the QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block)” on page 29.

IMPORTANT! Be careful to select the appropriate template file for your instrument and block type. Failure to do so can cause errors in the analysis.

3. In the **Experiment Properties** screen, enter or confirm the following.
 - **Experiment Name:** Enter a unique name
 - **Instrument type:** QuantStudio™ 7 Flex System
 - **Block:** 384-Well
 - **Type of Experiment:** Standard Curve
 - **Reagents:** TaqMan™ Reagents
 - **Properties:** Standard
4. In the **Define** screen, in the **Targets** table, confirm that the targets, reporter dyes, and quencher are listed correctly.

Target name	Reporter dye	Quencher
N Gene	VIC™	None
ORF1a	FAM™	
ORF1b	ABY™	
MS2	JUN™	

IMPORTANT! Target names are case-sensitive and must be named as described.

5. In the **Define** screen, in the **Samples** table, define a sample name for each sample. Create a unique sample name for each well in the physical plate.

IMPORTANT! Encrypt, pseudonymize, or anonymize personal data where possible following the requirements of HIPPA (Health Insurance Portability and Accountability Act).

6. In the **Define** screen, confirm that the **Passive Reference** is set to **None**.
7. In the **Assign** screen, confirm that targets from step 4 are assigned to each well in the **Plate Layout** tab.
To assign a target to a well, select the well in the plate layout, then select the targets from the **Targets** table.

8. In the **Assign** screen, in the **Plate Layout** tab, confirm the labeling of the control wells.

- The template has one Positive Control, one Negative Extraction Control, and one No Template Control assigned to wells for reference.
- The Positive Control must be named **PC**. If additional characters are included, it must be named **PC<>**, where <> is defined by the user, for example **PC1**.
- The Negative Extraction Control must be named **NC**. If additional characters are included, it must be named **NC<>**, where <> is defined by the user, for example **NC1**.
- The No Template Control must be named **NTC**. If additional characters are included, it must be named **NTC<>**, where <> is defined by the user, for example **NTC1**.
- Move the control well assignments by copying the existing control wells and pasting them according to their location on the physical plate.

IMPORTANT! The Positive Control, Negative Extraction Control, and No Template Control must be named as described.

9. In the **Assign** screen, confirm the **Task** assignments.

- For wells with a Positive Control (**PC**), confirm that the **Task** is set to **S (Standard)** for all of the targets.
- For wells with a Negative Extraction Control (**NC**) and No Template Control (**NTC**), confirm that the **Task** is set to **N (Negative Control)** for all of the targets.
- For the wells with a patient sample, confirm that the **Task** is set to **U (Unknown)** for all of the targets.

10. In the **Assign** screen, assign a sample name to each well to match the physical plate.

To assign a sample to a well, select the well in the plate layout, then select the sample from the **Samples** table.

Note: Wells that do not have a sample name will not be analyzed by the software.

11. In the **Run Method** screen, confirm that the **Reaction Volume Per Well** is 20 µL, then confirm the thermal protocol.

Step	Temperature ^[1]	Time	Number of cycles
UNG incubation	25°C	2 minutes	1
Reverse transcription	53°C	10 minutes	1
Preincubation	85°C	10 minutes	1
Activation	95°C	2 minutes	1
Denaturation	95°C	3 seconds	40
Annealing / extension	60°C	30 seconds	

^[1] Confirm that the ramp rate for each step is 1.6°C per second.

12. In the **Run Method** screen, select **Optical Filters**, then select the 8 PCR filters that are listed in Table 5.

To enable **Optical Filters**, navigate to **Tools ▶ Preferences**, then in the **Defaults** tab, select **Show optical filters for run method**.

Note: Five filters are pre-selected by default.

Table 5 PCR Filters

		Emission Filter					
		m1(520±15)	m2(558±11)	m3(586±10)	m4(623±14)	m5(682±14)	m6(711±12)
Excitation Filter	x1(470±15)	✓	✓				
	x2(520±10)		✓	✓			
	x3(550±11)			✓	✓		
	x4(580±10)				✓		
	x5(640±10)					✓	
	x6(662±10)						

13. Load the prepared and sealed RT-PCR reaction plate into the real-time PCR instrument.
14. In the **Run** screen, click **Start Run**, then select your instrument from the drop-down list.
15. Enter a file name in the dialog box that prompts you to save the run file, then save the file.

Revoked

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Software and instrument compatibility

Software	Description
Pathogen Interpretive Software v1.1.0	Data analysis and results interpretation software
SAE Administrator Console Dx v1.0.0	Security and audit tool
C19-MS2-EUA_QS5-9602_1.0.0	Assay panel for QuantStudio™ 5 Real-Time PCR Instrument (96-well, 0.2-mL block)
C19-MS2-EUA_QS7-384_1.0.0	Assay panel for QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block)

For information on how to obtain the software, see “Obtain the software package” on page 34.

The following table lists the assay panel that is compatible with the Pathogen Interpretive Software v1.1.0, your instrument, and its associated analysis software.

Instrument	Analysis software associated with the instrument	Pathogen Interpretive Software version	Assay panel code and version ^[1]
QuantStudio™ 5 Real-Time PCR Instrument with instrument firmware v1.3.3 <ul style="list-style-type: none"> 96-well, 0.2-mL block 	QuantStudio™ Design and Analysis Desktop Software v1.5.1	v1.1.0	C19-MS2-EUA_QS5-9602_1.0.0
QuantStudio™ 7 Flex Real-Time PCR Instrument with instrument firmware v1.0.4 <ul style="list-style-type: none"> 384-well block 	QuantStudio™ Real-Time PCR Software v1.3	v1.1.0	C19-MS2-EUA_QS7-384_1.0.0

^[1] For information on how to install the assay panel, see “Install the assay panel” on page 35.

To obtain the analysis software or firmware for your real-time PCR instrument, go to [thermofisher.com/qpcrsoftware](https://www.thermofisher.com/qpcrsoftware), then select your instrument in the **Real-Time PCR** section.

Obtain the software package

To perform data analysis and results interpretation, you must use the following software:

- Pathogen Interpretive Software v1.1.0
- SAE Administrator Console Dx v1.0.0
- Assay panel appropriate for your instrument (see “Software and instrument compatibility” on page 33):
 - C19-MS2-EUA_QS5-9602_1.0.0
 - C19-MS2-EUA_QS7-384_1.0.0

The software and assay panel can be installed on a customer-provided computer with the following minimum computer system specifications:

- Operating system—Windows™ 10 (64-bit), with language set to English
- Processor—Pentium® 4 or higher
- Memory—8 GB RAM minimum
- Hard drive—10 GB minimum free space
- Monitor—1280 × 1024 resolution or higher

Note: Do not install the Pathogen Interpretive Software v1.1.0 and SAE Administrator Console Dx v1.0.0 if it is already installed on your computer. The software can be used with several assay panels.

To obtain the software, contact your local technical support team. Go to <https://www.thermofisher.com/contactus>.

For more information about the software, see “Related documentation” on page 54.

Install the assay panel

An assay panel contains the analysis settings that are used for data analysis in the Pathogen Interpretive Software v1.1.0.

The assay panel is located in the same compressed folder as the software and is instrument-specific. Select the folder for your instrument.

Note: The software must be installed before installing the assay panel.

Only one matching assay panel is required to analyze a data file.

1. Extract the files from the compressed folder for your instrument.

Instrument	Folder name
QuantStudio™ 5 Real-Time PCR Instrument	MS2_2.0_EUA_QS5_96_0.2mL_AssayPanel_Template
QuantStudio™ 7 Flex Real-Time PCR Instrument	MS2_2.0_EUA_QS7_384_AssayPanel_Template

2. In the software, click  **System** ▶ **Assay Panels** ▶ **Actions** ▶ **Install**.

3. Navigate to, then open the assay panel for your instrument.

Instrument	Assay panel file name
QuantStudio™ 5 Real-Time PCR Instrument	C19-MS2-EUA_QS5-9602_1.0.0.zip
QuantStudio™ 7 Flex Real-Time PCR Instrument	C19-MS2-EUA_QS7-384_1.0.0.zip

4. Click **Install**.

Note: If the assay panel is already installed, you will be prompted to confirm the assay panel upgrade.

The assay panel is added to the **Assay Panels** library.

For more information about assay panels, including uninstalling assay panels, see the documentation for the Pathogen Interpretive Software v1.1.0 (Appendix F, “Documentation and support”).

Analyze the data

For detailed instructions about using the software, click the **Help** menu in the Pathogen Interpretive Software v1.1.0.

1. Using a USB drive or other method, transfer the EDS files from the computer with the data collection software to the computer with the Pathogen Interpretive Software v1.1.0.
2. In the software screen, select one of the following options:

Option	Description
In the Data Gallery , click Actions ▶ Open File .	The data file will open in the current window.
In the Data Gallery , click Actions ▶ Open File in New Window .	The data file will open in a new window.

3. Navigate to, then open the data file.
 - The data file opens and the results are displayed in the **Presence Absence** tab.
 - The data file is added to the **Data Gallery**.

Note: If the data file has already been added to the **Data Gallery**, click the file to open the file in the current window or hover over the file, then click ... **(Actions)** ▶ **Open in new window** to open the file in a new window.

Interpretation of the results

Interpretation of the results is performed by the Pathogen Interpretive Software v1.1.0 using one of the following assay panels:

Instrument	Assay panel code	Assay panel version
QuantStudio™ 5 Real-Time PCR Instrument	C19-MS2-EUA_QS5-9602	1.0.0
QuantStudio™ 7 Flex Real-Time PCR Instrument	C19-MS2-EUA_QS7-384	1.0.0

For information about the C_t values that are used by the software to interpret results, see Appendix C, “ C_t cutoff values for assay targets”.

Quality control and validity of results

A minimum of one Negative Extraction Control, one No Template Control, and one Positive Control must be present for each run.

Negative Extraction Control wells must be run for each extraction that is represented on a real-time RT-PCR plate. All control wells must pass for the real-time RT-PCR plate to be considered valid.

Validation of results is performed automatically by the software based on performance of the positive and negative controls.

Table 6 Results interpretation for control wells

Sample	Target				Call	Status	Assessment
	N Gene	ORF1a	ORF1b	MS2			
Positive Control (PC)	POS	POS	POS	POS or NEG ^[1]	Presence	Pass	VALID
Negative Extraction Control (NC)	NEG	NEG	NEG	POS	Absence	Pass	VALID
No Template Control (NTC)	NEG	NEG	NEG	NEG	Absence	Pass	VALID
PC, NC, or NTC	All other scenarios				Invalid	Fail	RETEST ^[2]

^[1] Spectral cross-talk can result in JUN™/MS2 signal in the PC sample. This signal is not applicable to the validity of the PC call.

^[2] Retesting must be performed by re-extracting the RNA from the original sample and repeating the RT-PCR.

Table 7 Results interpretation for viral targets in patient samples

Sample ^[1]	Target				Call	Assessment
	N Gene	ORF1a	ORF1b	MS2		
Patient Sample	NEG	NEG	NEG	NEG	Invalid	RETEST ^[2]
Patient Sample	NEG	NEG	NEG	POS	Absence	REPORT – SARS-CoV-2 Not Detected
Patient Sample	Only one SARS-CoV-2 target = POS			POS or NEG	Inconclusive	RETEST ^[2] /REPORT – SARS-CoV-2 Inconclusive
Patient Sample	Two or more SARS-CoV-2 targets = POS			POS or NEG	Presence	REPORT – SARS-CoV-2 Detected

^[1] Controls must pass for viral targets to be interpreted (Table 6).

^[2] Retesting must be performed by re-extracting the RNA from the original sample and repeating the RT-PCR. If the repeat result remains invalid, consider collecting a new specimen. If the repeat result remains inconclusive, the healthcare provider should conduct additional confirmation testing with a new specimen, if clinically indicated.



Conditions of authorization for labs

The TaqPath™ COVID-19 MS2 Combo Kit 2.0 Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: [fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas](https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas).

However, to assist clinical laboratories using the TaqPath™ COVID-19 MS2 Combo Kit 2.0, the relevant Conditions of Authorization are listed below.

- Authorized laboratories^[1] using the TaqPath™ COVID-19 MS2 Combo Kit 2.0 must include with test result reports all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories using the TaqPath™ COVID-19 MS2 Combo Kit 2.0 must perform the TaqPath™ COVID-19 MS2 Combo Kit 2.0 as outlined in the *TaqPath™ COVID-19 MS2 Combo Kit 2.0 Instructions for Use*. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents, and authorized materials required to perform the TaqPath™ COVID-19 MS2 Combo Kit 2.0 are not permitted.
- Authorized laboratories that receive the TaqPath™ COVID-19 MS2 Combo Kit 2.0 must notify the relevant public health authorities of their intent to run the test prior to initiating testing.
- Authorized laboratories using the TaqPath™ COVID-19 MS2 Combo Kit 2.0 must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories must collect information on the performance of the test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Thermo Fisher Scientific (techservices@thermofisher.com; 1 800 955 6288) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.
- All laboratory personnel using the test must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the test in accordance with the authorized labeling.
- Thermo Fisher Scientific, its authorized distributor(s), and authorized laboratories using the TaqPath™ COVID-19 MS2 Combo Kit 2.0 must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

^[1] For ease of reference, this letter will refer to “Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests” as “authorized laboratories.”



Performance characteristics

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Analytical performance of the TaqPath™ COVID-19 MS2 Combo Kit 2.0 was evaluated by determining limit of detection (LoD) and characterizing the impact of interfering substances and cross-reactivity, as described in the following sections.

Limit of Detection (LOD)

The LoD study established the lowest SARS-CoV-2 viral concentration (Genomic Copy Equivalents or GCE) that can be detected by the TaqPath™ COVID-19 MS2 Combo Kit 2.0 at least 95% of the time. Negative nasopharyngeal swab (NP) specimens were pooled and spiked with gamma-irradiated SARS-CoV-2 isolate USA-WA1/2020 at several concentrations and processed through the TaqPath™ COVID-19 MS2 Combo Kit 2.0 workflow. A two-phase approach was used to determine the LoD. A preliminary LoD was established in phase I and LoD was confirmed in phase II by testing 20 replicates at 75 GCE/mL.

Table 8 LoD determination (75 GCE/mL) in NP specimens spiked with gamma-irradiated SARS-CoV-2 isolate USA-WA1/2020 (QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block, 17.5 µL of purified sample RNA)

Concentration (per mL)		Replicate	C _t value ^[1]				Result ^[2]	% Positive
GCE ^[3]	TCID ₅₀ ^[4]		N Gene	ORF1a	ORF1b	MS2		
75	1.24×10 ⁻²	1	32.921	32.324	34.536	21.243	Positive	100%
		2	33.083	32.207	Undet.	21.441	Positive	
		3	32.293	31.878	39.607	21.359	Positive	
		4	32.389	30.991	Undet.	21.430	Positive	
		5	33.001	32.378	Undet.	21.544	Positive	
		6	32.266	31.272	36.156	21.557	Positive	
		7	32.986	31.344	37.421	21.683	Positive	

Table 8 LoD determination (75 GCE/mL) in NP specimens spiked with gamma-irradiated SARS-CoV-2 isolate USA-WA1/2020 (QuantStudio 5 Real-Time PCR Instrument, 96-well, 0.2-mL block, 17.5 µL of purified sample RNA) (continued)

Concentration (per mL)		Replicate	C _t value ^[1]				Result ^[2]	% Positive	
GCE ^[3]	TCID ₅₀ ^[4]		N Gene	ORF1a	ORF1b	MS2			
75	1.24×10 ⁻²	8	32.888	34.888	Undet.	21.707	Positive	100%	
		9	32.077	31.339	36.252	21.398	Positive		
		10	32.581	31.968	Undet.	21.442	Positive		
		11	32.516	32.009	36.815	21.656	Positive		
		12	32.954	31.079	36.459	21.510	Positive		
		13	32.931	31.781	38.386	21.524	Positive		
		14	32.770	31.558	35.981	21.411	Positive		
		15	32.553	31.596	Undet.	21.446	Positive		
		16	32.937	32.331	36.415	21.408	Positive		
		17	31.718	30.507	34.213	21.397	Positive		
		18	34.046	35.201	Undet.	21.479	Positive		
		19	32.179	31.200	36.810	21.258	Positive		
		20	33.111	32.534	Undet.	21.519	Positive		
		Mean ^[5]	32.710	32.019	35.961	21.471	N/A		
		Std. Dev.	0.498	1.166	0.994	0.123			
		CV	1.52%	3.64%	2.62%	0.57%			

^[1] Undet.: Undetermined

^[2] N/A: Not Applicable

^[3] GCE: Genomic Copy Equivalents

^[4] TCID₅₀: Tissue Culture Infective Dose-50%

^[5] Only those replicates with positive results (C_t ≤ 37 for SARS-CoV-2 targets; C_t ≤ 32 for MS2) were factored into the mean C_t value.

Table 9 LoD determination (75 GCE/mL) in NP specimens spiked with gamma-irradiated SARS-CoV-2 isolate USA-WA1/2020 (QuantStudio™ 7 Flex Real-Time PCR Instrument, 384-well block, 14 µL of purified sample RNA)

Concentration (per mL)		Replicate	C _t value ^[1]				Result ^[2]	% Positive	
GCE ^[3]	TCID ₅₀ ^[4]		N Gene	ORF1a	ORF1b	MS2			
75	1.24×10 ⁻²	1	32.337	31.674	34.702	21.465	Positive	100%	
		2	32.660	34.828	Undet.	21.292	Positive		
		3	32.780	34.497	Undet.	21.317	Positive		
		4	31.967	30.484	35.784	21.072	Positive		
		5	32.295	31.106	Undet.	21.399	Positive		
		6	32.262	30.738	35.157	21.517	Positive		
		7	33.383	31.109	Undet.	21.561	Positive		
		8	32.151	30.481	Undet.	21.433	Positive		
		9	31.938	27.704	35.357	21.329	Positive		
		10	32.444	34.523	Undet.	21.346	Positive		
		11	31.590	30.719	Undet.	21.512	Positive		
		12	32.303	30.537	34.830	21.346	Positive		
		13	32.885	30.674	Undet.	21.334	Positive		
		14	31.761	30.588	Undet.	21.460	Positive		
		15	32.513	31.188	Undet.	21.319	Positive		
		16	31.903	30.998	Undet.	21.437	Positive		
		17	31.282	29.977	34.422	21.358	Positive		
		18	31.834	35.578	Undet.	21.232	Positive		
		19	32.485	30.827	Undet.	21.334	Positive		
		20	33.942	31.370	36.579	21.287	Positive		
		Mean ^[5]		32.436	31.479	35.262	21.373	N/A	
		Std. Dev.		0.685	1.912	0.734	0.110		
		CV		2.11%	6.07%	2.08%	0.51%		

^[1] Undet.: Undetermined

^[2] N/A: Not Applicable

^[3] GCE: Genomic Copy Equivalents

^[4] TCID₅₀: Tissue Culture Infective Dose-50%

^[5] Only those replicates with positive results (C_t ≤ 37 for SARS-CoV-2 targets; C_t ≤ 32 for MS2) were factored into the mean C_t value.

Table 10 LoD results for NP specimens

Real-Time PCR Instrument	Limit of Detection of SARS-CoV-2 isolate USA-WA1/2020	
QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block	75 GCE/mL ^[1]	1.24 × 10 ⁻² TCID ₅₀ /mL
QuantStudio™ 7 Flex Real-Time PCR Instrument, 384-well block		

^[1] LoD according to the reporting algorithm. At 75 GCE/mL, there were only 9/20 and 7/20 three-target positive replicates on the QuantStudio™ 5 Real-Time PCR Instrument and QuantStudio™ 7 Flex Real-Time PCR Instrument, respectively. Testing at 100 GCE/mL showed 100% detection (20/20 replicates) for all 3 targets.

Reactivity (Inclusivity)

In silico analysis was executed using 1,802,689 complete SARS-CoV-2 genomes from the GISAID database, as of June 09, 2021, which includes the following variants of concern.

- 819,801 genomes of the Alpha variant
- 20,057 genomes of the Beta variant
- 25,068 genomes of the Gamma variant
- 29,149 genomes of the Delta variant

All (100%) SARS-CoV-2 sequences of human origin exhibited 100% homology to one or more primer and probe sets within each of the three targets amplified by the TaqPath™ COVID-19 MS2 Combo Kit 2.0 (ORF1a, ORF1b, and N Gene), and are thus predicted to be detected.

Interfering substances

Pooled SARS-CoV-2 negative nasopharyngeal swab (NP) specimens were spiked with inactivated SARS-CoV-2 (USA-WA1/2020) at 4X LOD (75 GCE/mL) and potential interfering substances at the concentrations listed in Table 11. In addition, pooled negative swab specimens without spiked SARS-CoV-2 were spiked with potential interfering substances at the same concentrations. Each substance was tested with triplicate extractions. The results are presented in Table 11.

No false-positive or negative results were observed for any of the substances at the concentrations tested.

Table 11 Interfering substances results

Interfering substance	Final concentration in sample	Agreement with expected results			
		Positive NP samples		Negative NP samples	
		QuantStudio™ 5 Real-Time PCR Instrument	QuantStudio™ 7 Flex Real-Time PCR Instrument	QuantStudio™ 5 Real-Time PCR Instrument	QuantStudio™ 7 Flex Real-Time PCR Instrument
None	N/A	100%	100%	100%	100%
Mucin: bovine submaxillary gland, type I-S	0.1 mg/mL	100%	100%	100%	100%
Blood (human)	1% v/v	100%	100%	100%	100%
Nasal spray (Afrin™)	10% v/v	100%	100%	100%	100%
Nasal corticosteroids — Fluticasone Propionate	5 µg/mL	100%	100%	100%	100%
Nasal gel — NeilMed™ Nasogel™	1% w/v	100%	100%	100%	100%
Homeopathic allergy relief medicine — bioAllers®	10% v/v	100%	100%	100%	100%
Throat lozenges, oral anesthetic and analgesic — Dorithricin®	1% w/v	100%	100%	100%	100%
Oseltamivir phosphate	33 µg/mL	100%	100%	100%	100%
Nasal ointment, antibiotic — Pseudomonic Acid	5 µg/mL	100%	100%	100%	100%
Antibacterial, systemic — Tobramycin	0.6 mg/mL	100%	100%	100%	100%

Cross-reactivity

Functional testing was performed using the following 32 microorganisms and pooled human nasal wash. No calls of Presence were generated using the TaqPath™ COVID-19 MS2 Combo Kit 2.0 and one call of Inconclusive was obtained with the SARS-CoV-1 due to positive amplification of one of the three SARS-CoV-2 targets.

Table 12 Organisms used for *in vitro* cross-reactivity analysis

Human CoV 229E	Rhinovirus
Human CoV OC43	Epstein-Barr virus (human herpesvirus 4)
Human CoV HKU1	<i>Chlamydomyphila pneumoniae</i>
Human CoV NL63	<i>Haemophilus influenzae</i>
SARS-CoV-1 ^[1]	<i>Legionella pneumophila</i>
MERS CoV	<i>Mycobacterium tuberculosis</i>
Adenovirus	<i>Streptococcus pneumoniae</i>
Human metapneumovirus	<i>Streptococcus pyogenes</i>
Parainfluenza virus 1	<i>Bordetella pertussis</i>
Parainfluenza virus 2	<i>Mycoplasma pneumoniae</i>
Parainfluenza virus 3	<i>Pseudomonas aeruginosa</i>
Parainfluenza virus 4	<i>Staphylococcus epidermidis</i>
Influenza A virus	<i>Streptococcus salivarius</i>
Influenza B virus	<i>Pneumocystis jirovecii</i> (PJP)
Enterovirus	<i>Candida albicans</i>
Respiratory syncytial virus	Pooled human nasal wash
<i>Pneumocystis carinii</i>	

^[1] SARS-CoV-1 generated a call of Inconclusive (amplification was observed for the N Gene, while no amplification was observed for the ORF1a and ORF1b targets).

In addition to functional testing, an *in silico* study was performed to assess cross-reactivity between the TaqPath™ COVID-19 MS2 Combo Kit 2.0 primer/probe sequences and the organisms in Table 13, using BLAST analysis. With the exception of SARS-CoV-1, no bacterial, viral, or fungal microbe sequence aligned with ≥80% identity to more than one TaqPath™ COVID-19 MS2 Combo Kit 2.0 assay component. The majority of the 285 SARS coronavirus isolates tested in this study shared ≥80% identity with more than one assay component for N gene and ORF1b assays.

Table 13 Organisms used for *in silico* cross-reactivity analysis

<i>Bacillus anthracis</i>	Adenovirus
<i>Bacteroides oralis</i>	Cytomegalovirus (human herpesvirus 5)
<i>Bordetella pertussis</i>	Enterovirus
<i>Chlamydia psittaci</i>	Epstein-Barr virus (human herpesvirus 4)
<i>Chlamydia pneumoniae</i>	Hepatitis B Virus
<i>Corynebacterium diphtheriae</i>	Hepatitis C Virus
<i>Coxiella burnetii</i>	Herpes Simplex Virus type 1
<i>Eikenella</i> spp.	Human coronavirus 229E
<i>Haemophilus influenzae</i>	Human coronavirus HKU1
<i>Legionella pneumophila</i>	Human coronavirus NL63
<i>Legionella pneumophila</i>	Human coronavirus OC43
<i>Leptospira</i> spp.	Human Immunodeficiency Virus
<i>Moraxella catarrhalis</i>	Human Metapneumovirus (hMPV)
<i>Mycobacterium tuberculosis</i>	Influenza A virus
<i>Mycoplasma pneumoniae</i>	Influenza B virus
<i>Neisseria elongata</i>	Influenza C virus
<i>Neisseria meningitidis</i>	MERS Coronavirus
<i>Nocardia</i> spp.	Parainfluenza 1 virus
<i>Porphyromonas gingivalis</i>	Parainfluenza 2 virus
<i>Pseudomonas aeruginosa</i>	Parainfluenza 3 virus
<i>Staphylococcus aureus</i>	Parainfluenza 4 virus
<i>Staphylococcus epidermidis</i>	Parechovirus
<i>Streptococcus mutans</i>	Respiratory Syncytial Virus A
<i>Streptococcus pneumoniae</i>	Respiratory Syncytial Virus B
<i>Streptococcus pyogenes</i>	Rhinovirus
<i>Streptococcus salivarius</i>	SARS-coronavirus (SARS-CoV-1)
<i>Candida albicans</i>	Varicella-zoster virus (human herpesvirus 3)
<i>Pneumocystis jirovecii</i>	

Clinical evaluation

A clinical evaluation study was carried out to evaluate the performance of the TaqPath™ COVID-19 MS2 Combo Kit 2.0 using archived nasopharyngeal swab and anterior nasal swab specimens.

The following specimens were tested:

- 60 specimens positive for SARS-CoV-2 (54 nasopharyngeal swabs and 6 anterior nasal swabs)
- 60 specimens negative for SARS-CoV-2 (45 nasopharyngeal swabs and 15 anterior nasal swabs)

Samples were tested using the TaqPath™ COVID-19 MS2 Combo Kit 2.0 and an FDA EUA-Authorized comparator assay.

Samples were extracted using the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit for testing with TaqPath™ COVID-19 MS2 Combo Kit 2.0 and RT-PCR was performed on QuantStudio™ 5 Real-Time PCR System (96-well, 0.2-mL block) and QuantStudio™ 7 Flex Real-Time PCR System (384-well block) real-time PCR instruments. The comparator method was conducted according to the Instructions for Use for the comparator assays.

Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were calculated relative to the comparator test. Results are shown below.

Table 14 Clinical evaluation study—nasopharyngeal swabs and anterior nasal swabs (QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block)

TaqPath™ COVID-19 MS2 Combo Kit 2.0	Comparator assay		
	SARS-CoV-2 Positive	SARS-CoV-2 Negative	Total
SARS-CoV-2 Positive	58	0	58
SARS-CoV-2 Negative	2	60	62
Total	60	60	120

PPA: 96.7% (95% CI: 88.6% LCL - 99.1% UCL)

NPA: 100% (95% CI: 94% LCL - 100% UCL)

Table 15 Clinical evaluation study—nasopharyngeal swabs and anterior nasal swabs (QuantStudio™ 7 Flex Real-Time PCR System, 384-well block)

TaqPath™ COVID-19 MS2 Combo Kit 2.0	Comparator assay		
	SARS-CoV-2 Positive	SARS-CoV-2 Negative	Total
SARS-CoV-2 Positive	58	0	58
SARS-CoV-2 Negative	2	60	62
Total	60	60	120

PPA: 96.7% (95% CI: 88.6% LCL - 99.1% UCL)

NPA: 100% (95% CI: 94% LCL - 100% UCL)

Table 16 Clinical evaluation study—nasopharyngeal swabs only (QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block)

TaqPath™ COVID-19 MS2 Combo Kit 2.0	Comparator assay		
	SARS-CoV-2 Positive	SARS-CoV-2 Negative	Total
SARS-CoV-2 Positive	52	0	52
SARS-CoV-2 Negative	2	45	47
Total	54	45	99

PPA: 96.4% (95% CI: 87.9% LCL - 99.0% UCL)

NPA: 100% (95% CI: 92.1% LCL - 100% UCL)

Table 17 Clinical evaluation study—nasopharyngeal swabs only (QuantStudio™ 7 Flex Real-Time PCR System, 384-well block)

TaqPath™ COVID-19 MS2 Combo Kit 2.0	Comparator assay		
	SARS-CoV-2 Positive	SARS-CoV-2 Negative	Total
SARS-CoV-2 Positive	52	0	52
SARS-CoV-2 Negative	2	45	47
Total	54	45	99

PPA: 96.4% (95% CI: 87.9% LCL - 99.0% UCL)

NPA: 100% (95% CI: 92.1% LCL - 100% UCL)

Table 18 Clinical evaluation study—anterior nasal swabs only (QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block)

TaqPath™ COVID-19 MS2 Combo Kit 2.0	Comparator assay		
	SARS-CoV-2 Positive	SARS-CoV-2 Negative	Total
SARS-CoV-2 Positive	6	0	6
SARS-CoV-2 Negative	0	15	15
Total	6	15	21

PPA: 100% (95% CI: 61.0% LCL - 100% UCL)

NPA: 100% (95% CI: 79.6% LCL - 100% UCL)

Table 19 Clinical evaluation study—anterior nasal swabs only (QuantStudio™ 7 Flex Real-Time PCR System, 384-well block)

TaqPath™ COVID-19 MS2 Combo Kit 2.0	Comparator assay		
	SARS-CoV-2 Positive	SARS-CoV-2 Negative	Total
SARS-CoV-2 Positive	6	0	6
SARS-CoV-2 Negative	0	15	15
Total	6	15	21

PPA: 100% (95% CI: 61.0% LCL - 100% UCL)

NPA: 100% (95% CI: 79.6% LCL - 100% UCL)

Revoked



C_t cutoff values for assay targets

The Pathogen Interpretive Software v1.1.0 uses the following C_t cutoff values for assay targets during interpretation of the results.

Table 20 COVID-19 Assay C_t cutoff values

Sample or Control	Target	C _t cutoff
Positive Control	MS2	Not applicable
	Viral targets	Valid C _t values are ≤37
Negative Extraction Control	MS2	Valid C _t values are ≤32
	Viral targets	Valid C _t values are >37
No Template Control	MS2	Valid C _t values are >37
	Viral targets	Valid C _t values are >37
Clinical samples	MS2	Valid C _t values are ≤32 ^[1]
	Viral targets	Positive C _t values are ≤37

^[1] If any of the viral targets is positive, a C_t value >32 is acceptable.

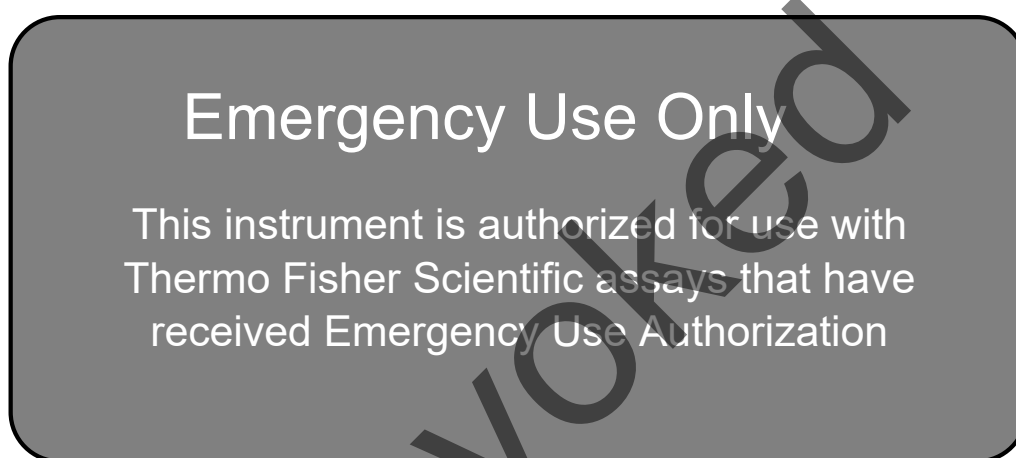


EUO label for RUO instruments

Affix the Emergency Use Only (EUO) label on each of the following instruments. Retain this labeling throughout the Emergency Use Authorization (EUA) use of the following instruments.

- QuantStudio™ 5 Real-Time PCR Instrument
- QuantStudio™ 7 Flex Real-Time PCR Instrument

1. Download or print the following EUO label:



2. Visibly affix the EUO instrument verification label on your instrument. If the instrument includes labeling indicating “For Research Use Only”, cover with the EUO instrument verification label.



Safety

■ Chemical safety	52
■ Biological hazard safety	53



WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the “Documentation and Support” section in this document.

Revoked

Chemical safety



WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety



WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.



WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020; found at:
<https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2020-P.pdf>
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs); found at:
www.who.int/publications/i/item/9789240011311



Documentation and support

Related documentation

Document	Publication Number
<i>TaqPath™ COVID-19 MS2 Combo Kit 2.0 Product Information Sheet</i>	100102583
<i>MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit Instructions For Use</i>	MAN0019746
<i>Thermo Scientific™ KingFisher™ Flex User Manual</i>	MAN0019870
<i>QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide</i>	MAN0010407
<i>QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Maintenance and Administration Guide</i>	4489821
<i>QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Quick Reference</i>	4489826
<i>QuantStudio™ Real-Time PCR Software Getting Started Guide</i>	4489822
<i>Pathogen Interpretive Software Help</i>	MAN0019534 (available in the software)
<i>Pathogen Interpretive Software Installation Quick Reference</i>	MAN0019535
<i>SAE Administrator Console Dx User Guide</i>	MAN0019574

Customer and technical support

For additional documentation and information about this kit, visit:

[thermofisher.com/TaqPathC19MS2v2EUA](https://www.thermofisher.com/TaqPathC19MS2v2EUA)

For download instructions for the software package, see “Obtain the software package” on page 34.

Visit: [thermofisher.com/contactus](https://www.thermofisher.com/contactus) for service and support information for this kit, including the following:

- Worldwide contact telephone numbers
- Product support information
- Order and web support
- Product documentation such as:
 - Certificates of Analysis
 - Safety Data Sheet (SDS; also known as Material Safety Data Sheet or MSDS)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have any questions, please contact Life Technologies at www.thermofisher.com/support.

Revoked

TaqPath™ COVID-19 MS2 Combo Kit 2.0

Multiplexed real-time PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 and MS2 extraction control in a single reaction well.

Catalog Number A51329

Pub. No. 100102583 Rev. A

For Emergency Use Authorization Only

Intended Use

The Applied Biosystems™ TaqPath™ COVID-19 MS2 Combo Kit 2.0 is a real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swabs, mid-turbinate nasal swabs, and anterior nasal swabs from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high-complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all test results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The TaqPath™ COVID-19 MS2 Combo Kit 2.0 is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.

The TaqPath™ COVID-19 MS2 Combo Kit 2.0 is only for use under the Food and Drug Administration's Emergency Use Authorization.

Instructions for Use/Interpretation/Limitations

The complete *TaqPath™ COVID-19 MS2 Combo Kit 2.0 Instructions for Use* (Pub. No. MAN0024938) can be downloaded from the following link:

thermofisher.com/findyourifu

If you require a printed copy (no charge) or cannot access the document at this location, visit thermofisher.com/askaquestion or call 1 800 955 6288 (U.S. only).

This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.

This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.

The emergency use of 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 declaration is terminated or authorization is revoked sooner.

Obtain the software package

To perform data analysis and results interpretation, you must use the following software:

- Applied Biosystems™ Pathogen Interpretive Software v1.1.0
- SAE Administrator Console Dx v1.0.0
- Assay panel for your instrument

To obtain the software, contact your local instrument service team. Go to <https://www.thermofisher.com/contactus>.

Contents and storage

Table 1 TaqPath™ COVID-19 MS2 Combo Kit 2.0, 1,000 reactions (Cat. No. [A51329](#))

Component		Amount	Storage	Shelf life
TaqPath™ COVID-19 MS2 RT-PCR Kit 2.0	TaqPath™ COVID-19 MS2 RT-PCR Assay 2.0	1 × 1,500 µL	–30°C to –10°C ^[1]	12 months ^[2]
	MS2 Phage Control	10 × 1,000 µL	–30°C to –10°C	
TaqPath™ COVID-19 Plus Control		10 × 10 µL (1 × 10 ⁴ copies/µL)	–30°C to –10°C	
TaqPath™ COVID-19 Control Dilution Buffer		10 × 250 µL	–30°C to –10°C	

^[1] Do not freeze-thaw more than 12 times.

^[2] From the manufacturing date.

Customer and technical support

For additional documentation and information about this kit, visit: [thermofisher.com/TaqPathC19MS2v2EUA](https://www.thermofisher.com/TaqPathC19MS2v2EUA). For download instructions for the software, see “Obtain the software package” on page 1. Refer to the Software Release Notes provided with the software before contacting support for the software.

Visit: <https://www.thermofisher.com/contactus> for service and support information for this kit, including worldwide contact telephone numbers, product support information, order and web support, Certificates of Analysis, and Safety Data Sheets (SDSs; also known as MSDSs).

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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For descriptions of symbols on product labels or product documents, go to [thermofisher.com/symbols-definition](https://www.thermofisher.com/symbols-definition).

The customer is responsible for validation of assays and 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, THERMO FISHER SCIENTIFIC 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.

Revision history: Pub. No. 100102583

Revision	Date	Description
A	02 August 2021	New document.

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