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TaqPath™ COVID-19 Combo Kit

INSTRUCTIONS FOR USE

Multiplex real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2

Catalog Number A47814

Publication Number MAN0019181

Revision C.0

IVD

For *in vitro* diagnostic use.
For Emergency Use Authorization Only | Rx Only

ThermoFisher SCIENTIFIC

Life Technologies Corporation | 6055 Sunol Blvd | Pleasanton, CA 94566
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

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

Revision history: Pub. No. MAN0019181

Revision	Date	Description
C.0	19 April 2020	• Removed 100-reaction kit. • Added a catalog number for the KingFisher™ Deepwell 96 Plate. • Updated the catalog number for the Compact Digital Microplate Shaker. • Added catalog number for the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit and removed catalog numbers for individual components of the kit. • Added the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit. • Added an option to extract RNA with 200 µL of sample. • Added Applied Biosystems™ 7500 Real-Time PCR Instrument and Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instruments. • Added Applied Biosystems™ COVID-19 Interpretive Software v1.2 and Applied Biosystems™ COVID-19 Interpretive Software v2.0. • Removed Applied Biosystems™ COVID-19 Interpretive Software v1.0 and Applied Biosystems™ and COVID-19 Interpretive Software v1.1. • Added specific instructions to vortex and centrifuge the reaction plate for RT-PCR ("Prepare the RT-PCR reactions (400-µL sample input volume)" on page 29 and "Prepare the RT-PCR reactions (200-µL sample input volume)" on page 27). • Specified that retesting must be done with the original sample ("Interpretation of the results" on page 48). • Reorganized the content to perform RT-PCR based on the real-time PCR instrument. • Added "" on page 0 . • Added information to customer and technical support (page 58).
B.0	24 March 2020	• Added MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit components for 1,000 preparations to Required Materials. • Added manual RNA extraction protocol and required materials for the manual RNA extraction protocol. • Added Applied Biosystems™ 7500 Fast Real-Time PCR Instrument. • Removed storage options for RNA after extraction. • Updated guidelines for RT-PCR to run the plate immediately after preparation and to keep the plate on ice until it is loaded into the real-time PCR instrument. • When setting up the RT-PCR reaction, added instructions to mix by pipetting up and down 10 times and seal and centrifuge the reaction plate. • Added COVID-19 Interpretive Software v1.1 (compatible with Applied Biosystems™ 7500 Fast Real-Time PCR Instrument and Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument). • Added that the run file must be opened, analyzed, and saved in the instrument software before it is opened in COVID-19 Interpretive Software. • For TaqPath™ RT-PCR COVID-19 Kit, 1,000 reactions (Cat. No. A47817), changed MS2 Phage Control from 20 tubes × 500 µL to 10 tubes × 1,000 µL. • Updated instructions to obtain the COVID-19 Interpretive Software. • Changed Limit of Detection and Clinical Evaluation data to 1 decimal place.
A.0	15 March 2020	New document.

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1 TaqPath™ COVID-19 Combo Kit product information

Intended Use

TaqPath™ COVID-19 Combo Kit contains the assays and controls for a real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens (such as nasopharyngeal, oropharyngeal, nasal, and mid-turbinate swabs, and nasopharyngeal aspirate) and bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19 by their healthcare provider. TaqPath™ COVID-19 Combo Kit is for use only under Emergency Use Authorization (EUA). Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, 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 and bronchoalveolar lavage (BAL) 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 positive 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.

Testing with the TaqPath™ COVID-19 Combo Kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The TaqPath™ COVID-19 Combo Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

Product description

The TaqPath™ RT-PCR COVID-19 Kit, packaged as part of the TaqPath™ COVID-19 Combo Kit, includes the assays and controls for a multiplex real-time RT-PCR test for the qualitative detection of RNA from SARS-CoV-2 in upper respiratory specimens (such as nasopharyngeal, oropharyngeal, nasal, and mid-turbinate swabs, and nasopharyngeal aspirate) and bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19 by their healthcare provider. TaqPath™ COVID-19 Combo Kit includes the following components:

- TaqPath™ RT-PCR COVID-19 Kit
  - COVID-19 Real Time PCR Assay Multiplex—Multiplexed assays that contain three primer/probe sets specific to different SARS-CoV-2 genomic regions and primers/probes for bacteriophage MS2
  - MS2 Phage Control—Internal process control for nucleic acid extraction
- TaqPath™ COVID-19 Control—RNA control that contains targets specific to the SARS-CoV-2 genomic regions targeted by the assays

Contents and storage

Table 1 TaqPath™ COVID-19 Combo Kit, 1,000 reactions (Cat. No. A47814)

Component	Contents	Amount	Storage
TaqPath™ RT-PCR COVID-19 Kit, 1,000 reactions (Cat. No. A47817)[1]	COVID-19 Real Time PCR Assay Multiplex (ORF1ab, N gene, S gene, MS2)	1,500 µL	–30°C to –10°C
	MS2 Phage Control	10 × 1,000 µL	–30°C to –10°C
TaqPath™ COVID-19 Control Kit (Cat. No. A47816)[1]	TaqPath™ COVID-19 Control (1×10^4 copies/µL)	2 × 10 µL per kit; 5 kits included	≤ –70°C
	TaqPath™ COVID-19 Control Dilution Buffer	2 × 250 µL per kit; 5 kits included	–30°C to –10°C

[1] This kit can be ordered as a stand-alone kit.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Source
Real-time PCR instrument	
Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument (used with SDS Software v1.4.1)	4406984 (with laptop computer) 4406985 (with tower computer)
Applied Biosystems™ 7500 Fast Real-Time PCR Instrument (used with SDS Software v1.5.1 or 7500 Software v2.3)	4351106 (with laptop computer) 4351107 (with desktop computer)
Applied Biosystems™ 7500 Real-Time PCR Instrument (used with 7500 Software v2.3)	4351104 (with laptop computer) 4351105 (with desktop computer)
Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument, 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™ 5 Real-Time PCR Instrument, 0.1-mL block (used with QuantStudio™ Design and Analysis Desktop Software v1.5.1)	A28568 (with laptop computer) A28573 (with desktop computer) A28138 (instrument only)
Equipment	
Laboratory freezers • –30°C to –10°C • ≤ –70°C	MLS
Centrifuge, with a rotor for 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 or ice	MLS
Automated 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

(continued)

Item	Source
KingFisher™ Deepwell 96 Plate	95040450 A48305
KingFisher™ 96 KF microplate	97002540
KingFisher™ 96 tip comb for DW magnets	97002534
Manual nucleic acid extraction system and materials	
Magnetic Stand-96	AM10027 AM10050
Compact Digital Microplate Shaker	88882005
Incubator capable of reaching 65°C with slatted shelves	MLS
KingFisher™ Deepwell 96 Plate	95040450 A48305
KingFisher™ 96 KF microplate	97002540
MicroAmp™ Clear Adhesive Film	4306311
Kits and reagents	
MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (up to 200 preparations, when 200 µL of sample is used)	A42352
MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (up to 2,000 preparations, when 200 µL of sample is used)	A48310
MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit (up to 2,000 preparations, when 200 µL of sample is used)	A48383
TaqPath™ 1-Step Multiplex Master Mix (No ROX™)	A28521, A28522, A28523
100% ethanol, ACS reagent grade or equivalent	MLS
Nuclease-free Water (not DEPC-Treated)	MLS
Calibration plates (7500 real-time PCR instrument series)	
ABY™ Dye Spectral Calibration Plate for Multiplex qPCR, Fast 96-well (0.1-mL)	A24734
JUN™ Dye Spectral Calibration Plate for Mutliplex qPCR, Fast 96-well (0.1-mL)	A24735
ABY™ Dye Spectral Calibration Plate for Multiplex qPCR, 96-well (0.2-mL)	A24738
JUN™ Dye Spectral Calibration Plate for Mutliplex qPCR, 96-well (0.2-mL)	A24737

(continued)

Item	Source
Calibration plates (QuantStudio™ 5 Real-Time PCR Instrument)	
QuantStudio™ 3/5 Spectral Calibration Plate 2 (ABY™, JUN™, MUSTANG PURPLE™ dyes), 96-well Fast (0.1-mL) Plate	A26337
QuantStudio™ 3/5 Spectral Calibration Plate 2, 96-Well 0.2-mL (ABY™, JUN™, and MUSTANG PURPLE™ dyes)	A26332
Tubes, plates, and other consumables	
MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL	4346906, 4366932
MicroAmp™ Optical 96-Well Reaction Plate with Barcode, 0.2 mL	4306737, 4326659
MicroAmp™ Clear Adhesive Film	4306311
MicroAmp™ Optical Adhesive Film	4311971, 4360954
MicroAmp™ Adhesive Film Applicator	4333183
Nonstick, RNase-free microcentrifuge tubes (1.5 mL and 2.0 mL)	**thermofisher.com/plastics**
Sterile aerosol barrier (filtered) pipette tips	**thermofisher.com/pipettetips**

Instrument and software compatibility

The following table lists the version of the Applied Biosystems™ COVID-19 Interpretive Software that is compatible with your instrument and its associated analysis software.

For information on how to obtain the Applied Biosystems™ COVID-19 Interpretive Software, see “Obtain the Applied Biosystems™ COVID-19 Interpretive Software” on page 47.

Instrument	Analysis software used with the instrument	Compatible COVID-19 Interpretive Software version
7500 Fast Dx Real-Time PCR Instrument	SDS Software v1.4.1	v1.2
7500 Fast Real-Time PCR Instrument	SDS Software v1.5.1 *or* 7500 Software v2.3	v1.2
7500 Real-Time PCR Instrument	7500 Software v2.3	v1.2
QuantStudio™ 5 Real-Time PCR Instrument	QuantStudio™ Design and Analysis Desktop Software v1.5.1	v2.0

Warnings and precautions

The TaqPath™ RT-PCR COVID-19 Kit 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.

- The assay is for *in vitro* diagnostic use under the FDA Emergency Use Authorization Only.
- 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.
- Modifications to assay reagents, assay protocol, or instrumentation are not permitted, and are in violation of the product Emergency Use Authorization.
- Reagents must be stored and handled as specified in Table 1 on page 8.
- Do not use the kit after the indicated expiry date.
- Dispose of waste in compliance with local, state, and federal regulations.
- Safety Data Sheets are available upon request.
- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- Positive results are indicative of the presence of SARS-CoV-2 RNA.

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, to perform high complexity tests.
- The TaqPath™ RT-PCR COVID-19 Kit performance was established using nasopharyngeal and oropharyngeal swab, nasopharyngeal aspirate, and bronchoalveolar lavage samples only. Other specimen types have not been evaluated and should not be tested with this assay.
- Nasal swabs and mid-turbinate swabs are considered acceptable specimen types for use with the TaqPath™ RT-PCR COVID-19 Kit, but performance with these specimen types has not been established. Testing of nasal and mid-turbinate nasal swabs (self-collected under supervision of or collected by a healthcare provider) is limited to patients with symptoms of COVID-19. Refer to FDA's *FAQs on Diagnostic Testing for SARS-CoV-2* for additional information.
- 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.
- Extraction and amplification of nucleic acid from clinical samples must be performed according the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.
- Manual extraction of 400-µL sample input volumes using the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit has not been evaluated.
- 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 virus
  - Failure to follow instructions for use
- 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
- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated. The TaqPath™ RT-PCR COVID-19 Kit cannot rule out diseases caused by other bacterial or viral pathogens.

- Negative results do not preclude infection with SARS-CoV-2 virus, and should not be the sole basis of a patient management decision.
- Laboratories are required to report all positive results to the appropriate public health authorities.

Samples and controls

Patient samples must be collected according to appropriate laboratory guidelines. Positive and negative test controls must be included to accurately interpret patient test results.

Include the following controls:

Control	Used to monitor	Assays
Positive Control (TaqPath™ COVID-19 Control Kit)	RT-PCR reaction setup and reagent integrity	All three SARS-CoV-2 assays
MS2 Phage Control	RNA extraction	MS2 assay
Negative Control	Cross-contamination during RNA extraction and reaction setup	All three SARS-CoV-2 assays
		MS2 assay

Sample collection, transport, and storage

Note: Handle all samples and controls as if they are capable of transmitting infectious agents.

Workflow

Extract RNA from patient sample

▼

Perform RT-PCR

▼

Analyze data using the Applied Biosystems™ COVID-19 Interpretive Software

▼

Review run control results

▼

Review results interpretation for patient samples

The workflow begins with nucleic acid extraction from upper respiratory specimens (such as nasopharyngeal, oropharyngeal, nasal, and mid-turbinate swabs, and nasopharyngeal aspirate) and bronchoalveolar lavage (BAL) specimens that arrive in the testing site in transport media. Nucleic acids are isolated and purified from the specimens using the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit or the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit. Nucleic acid isolation can be performed manually or via an automated process using the KingFisher™ Flex Purification System (KingFisher). For more information about using the kit, see “Related documentation” on page 58.

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

- Applied Biosystems™ 7500 Fast Dx Real-Time PCR instrument
- Applied Biosystems™ 7500 Fast Real-Time PCR Instrument
- Applied Biosystems™ 7500 Real-Time PCR Instrument
- Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument, 0.2-mL block
- Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument, 0.1-mL block

In the process, the probes anneal to three (3) specific SARS-CoV-2 target sequences located between three (3) unique forward and reverse primers for the following genes:

- ORF1ab
- N Protein
- S Protein

During the extension phase of the PCR cycle, the 5’ nuclease 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 Applied Biosystems™ COVID-19 Interpretive Software.

2 Extract RNA (automated method)

Automated RNA extraction is performed using the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head and the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit or MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit with a sample input volume of 200 µL or 400 µL.

Before you begin

- Determine the number of required reactions based on the number of patient samples to be processed, plus one Negative Control per plate.
- Prepare fresh 80% Ethanol using 100% absolute Ethanol and Nuclease-free Water (not DEPC-Treated), sufficient for 1.5 mL per reaction, plus 10% overage.
- Label each KingFisher™ Deepwell 96 Plate (5):

Label	Number of plates
Sample plate	1
Wash 1	1
Wash 2	1
Wash 3	1
Elution plate	1

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

Label	Number of plates
Tip comb	1

- Mark the Negative Control well on the plate.

Extract RNA—Automated method (200-µL sample input volume)

The following procedure uses components from the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit or the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit.

Set up the instrument (200-µL sample input volume)

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_Flex_200ul** program has been downloaded from the product page and loaded onto the instrument.

Prepare the processing plates (200-µL sample input volume)

Prepare the processing plates according to the following table. Cover the plates with a temporary seal, 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™ Deepwell 96 Plate	Wash Buffer	500 µL
Wash 2 Plate	3		80% Ethanol	500 µL
Wash 3 Plate	4		80% Ethanol	250 µL
Elution Plate	5		Elution Solution	50 µL
Tip Comb	6	Place a KingFisher™ 96 tip comb for DW magnets in a KingFisher™ 96 KF microplate		

Prepare Binding Bead Mix (200-µL sample input volume)

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]
Binding Solution	265 µL
Total Nucleic Acid Magnetic Beads	10 µL
Total volume per well	275 µL

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

3. Mix well by inversion, then store at room temperature.

Prepare sample plate (200-µL sample input volume)

1. Add 5 µL of Proteinase K to each well in the KingFisher™ Deepwell 96 Plate labeled "Sample Plate".
2. Add 200 µL of sample to each sample well.
3. Add 200 µL of Nuclease-free Water (not DEPC-Treated) to the Negative Control well.
4. Invert the Binding Bead Mix 5 times gently to mix, then add 275 µL to each sample well and the Negative Control well in the Sample Plate.

   Note: Remix Binding Bead Mix by inversion frequently during pipetting to ensure even distribution of beads to all samples or wells. 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.
5. Add 5 µL of MS2 Phage Control to each sample well and to the Negative Control well.

Process the samples (200-µL sample input volume)

1. Select the **MVP_Flex_200ul** 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 (~25 minutes after start), immediately remove the Elution Plate from the instrument, then cover the plate with MicroAmp™ Clear Adhesive Film.
   The samples are eluted in 50 µL of Elution Solution (see “Prepare the processing plates (200-µL sample input volume)” on page 17).

Note: Significant bead carry over may adversely impact RT-PCR performance.

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

Extract RNA—Automated method (400-µL sample input volume)

The following procedure uses components from the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit or the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit.

Set up the instrument (400-µL sample input volume)

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_Flex** program has been downloaded from the product page and loaded onto the instrument.

Prepare the processing plates (400-µL sample input volume)

Prepare the processing plates according to the following table. Cover the plates with a temporary seal, 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™ Deepwell 96 Plate	Wash Buffer	1,000 µL
Wash 2 Plate	3		80% Ethanol	1,000 µL
Wash 3 Plate	4		80% Ethanol	500 µL
Elution Plate	5		Elution Solution	50 µL
Tip Comb	6	Place a KingFisher™ 96 tip comb for DW magnets in a KingFisher™ 96 KF microplate		

Prepare Binding Bead Mix (400-µL sample input volume)

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]
Binding Solution	530 µL
Total Nucleic Acid Magnetic Beads	20 µL
Total volume per well	550 µL

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

3. Mix well by inversion, then store at room temperature.

Prepare sample plate (400-µL sample input volume)

1. Add 10 µL of Proteinase K to each well in the KingFisher™ Deepwell 96 Plate labeled "Sample Plate".
2. Add 400 µL of sample to each sample well.
3. Add 400 µL of Nuclease-free Water (not DEPC-Treated) to the Negative Control well.
4. Invert the Binding Bead Mix 5 times gently to mix, then add 550 µL to each sample well and the Negative 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.
5. Add 10 µL of MS2 Phage Control to each sample well and to the Negative Control well.

Process the samples (400-µL sample input volume)

1. Select the **MVP_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 (~25 minutes after start), immediately remove the Elution Plate from the instrument, then cover the plate with MicroAmp™ Clear Adhesive Film.
   The samples are eluted in 50 µL Elution Solution (see “Prepare the processing plates (400-µL sample input volume)” on page 19).

Note: Significant bead carry over may adversely impact RT-PCR performance.

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

3 Extract RNA (manual method)

Manual RNA extraction can be performed from a sample input volume of 200 µL using either the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit or the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit.

Manual RNA extraction can be performed from a sample input volume of 400 µL using only the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit.

Before you begin

- Determine the number of required reactions based on the number of patient samples to be processed, plus one Negative Control per plate.
- Prepare fresh 80% Ethanol using 100% absolute Ethanol and Nuclease-free Water (not DEPC-Treated), sufficient for 1.5 mL per reaction, plus 10% overage.
- Mark the Negative Control well on the plate.

Extract RNA—Manual method (200-µL sample input volume)

The following procedure uses components from the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit or the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit.

Prepare Binding Bead Mix (200-µL sample input volume)

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]
Binding Solution	265 µL
Total Nucleic Acid Magnetic Beads	10 µL
Total volume per well	275 µL

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

3. Mix well by inversion, then store at room temperature.

Digest with Proteinase K (200-µL sample input volume)

1. Add 5 µL of Proteinase K to each well of a KingFisher™ Deepwell 96 Plate.
2. Add 200 µL of sample to each sample well.
3. Add 200 µL of Nuclease-free Water (not DEPC-Treated) to the Negative Control well.
4. Invert the Binding Bead Mix 5 times gently to mix, then add 275 µL to each sample well and Negative Control well.

   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.
5. Add 5 µL of MS2 Phage Control to each sample well and to the Negative Control well.
6. Seal the plate with MicroAmp™ Clear Adhesive Film, then shake the sealed plate at 1,050 rpm for 2 minutes.
7. Incubate the sealed plate at 65°C for 5 minutes (ensure the bottom of the plate is uncovered), then shake the plate at 1,050 rpm for 5 minutes.
8. Place the sealed plate on the magnetic stand for 10 minutes or until all of the beads have collected.

Wash the beads (200-µL sample input volume)

1. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well.

   IMPORTANT! Avoid disturbing the beads.

2. Remove the plate from the magnetic stand, then add 500 µL of Wash Buffer to each sample.
3. Reseal the plate, then shake at 1,050 rpm for 1 minute.
4. Place the plate back on the magnetic stand for 2 minutes, or until all the beads have collected.
5. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well.

   IMPORTANT! Avoid disturbing the beads.

6. Repeat step 2 to step 5 using 500 µL of 80% Ethanol.
7. Repeat step 2 to step 5 using 250 µL of 80% Ethanol.
8. Dry the beads by shaking the plate (uncovered) at 1,050 rpm for 2 minutes.

Elute the nucleic acid (200-µL sample input volume)

1. Add 50 µL of Elution Solution to each sample, then seal the plate with MicroAmp™ Clear Adhesive Film.
2. Shake the sealed plate at 1,050 rpm for 5 minutes.
3. Place the plate in an incubator at 65°C for 10 minutes.
4. Remove the plate from the incubator, then shake the plate at 1,050 rpm for 5 minutes.
5. Place the sealed plate on the magnetic stand for 3 minutes or until clear to collect the beads against the magnets.
6. Keeping the plate on the magnet, carefully remove the seal, transfer the eluates to a fresh standard (not deep-well) plate, then seal the plate with MicroAmp™ Clear Adhesive Film.

   IMPORTANT! To prevent evaporation, seal the plate containing the eluate immediately after the transfers are complete.

Note: Significant bead carry over may adversely impact RT-PCR performance.

Place the plate on ice for immediate use in real-time RT-PCR.

Extract RNA—Manual method (400-µL sample inputvolume)

The following procedure uses components from only the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit.

Note: The MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit was not evaluated with this procedure.

Prepare Binding Bead Mix (400-µL sample input volume)

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]
Binding Solution	530 µL
Total Nucleic Acid Magnetic Beads	20 µL
Total volume per well	550 µL

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

3. Mix well by inversion, then store at room temperature.

Digest with Proteinase K (400-µL sample input volume)

1. Add 10 µL of Proteinase K to each well of a KingFisher™ Deepwell 96 Plate.
2. Add 400 µL of sample to each sample well.
3. Add 400 µL of Nuclease-free Water (not DEPC-Treated) to the Negative Control well.
4. Invert the Binding Bead Mix 5 times gently to mix, then add 550 µL to each sample well and Negative Control well.

   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.
5. Add 10 µL of MS2 Phage Control to each sample well and to the Negative Control well.
6. Seal the plate with MicroAmp™ Clear Adhesive Film, then shake the sealed plate at 1,050 rpm for 2 minutes.

7. Incubate the sealed plate at 65°C for 5 minutes (ensure the bottom of the plate is uncovered), then shake the plate at 1,050 rpm for 5 minutes.

8. Place the sealed plate on the magnetic stand for 10 minutes or until all of the beads have collected.

Wash the beads (400-µL sample input volume)

1. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well.

   IMPORTANT! Avoid disturbing the beads.

2. Remove the plate from the magnetic stand, then add 1 mL of Wash Buffer to each sample.

3. Reseal the plate, then shake at 1,050 rpm for 1 minute.

4. Place the plate back on the magnetic stand for 2 minutes, or until all the beads have collected.

5. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well.

   IMPORTANT! Avoid disturbing the beads.

6. Repeat step 2 to step 5 using 1 mL of 80% Ethanol.

7. Repeat step 2 to step 5 using 500 µL of 80% Ethanol.

8. Dry the beads by shaking the plate (uncovered) at 1,050 rpm for 2 minutes.

Elute the nucleic acid (400-µL sample input volume)

1. Add 50 µL of Elution Solution to each sample, then seal the plate with MicroAmp™ Clear Adhesive Film.

2. Shake the sealed plate at 1,050 rpm for 5 minutes.

3. Place the plate in an incubator at 65°C for 10 minutes.

4. Remove the plate from the incubator, then shake the plate at 1,050 rpm for 5 minutes.

5. Place the sealed plate on the magnetic stand for 3 minutes or until clear to collect the beads against the magnets.

6. Keeping the plate on the magnet, carefully remove the seal, transfer the eluates to a fresh standard (not deep-well) plate, then seal the plate with MicroAmp™ Clear Adhesive Film.

   IMPORTANT! To prevent evaporation, seal the plate containing the eluate immediately after the transfers are complete.

Note: Significant bead carry over may adversely impact RT-PCR performance.

Place the plate on ice for immediate use in real-time RT-PCR.

4 Prepare RT-PCR reactions

Note: The procedure used to prepare the RT-PCR reactions will depend on the original sample input volume that was used during RNA extraction (200 µL or 400 µL).

Guidelines for RT-PCR

IMPORTANT!

- Prepare the run plate on ice and keep it on ice 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 samples, and always use aerosol barrier pipette tips.
- Maintain an RNase-free environment.
- Protect assays from light.
- Keep samples and components on ice during use.
- Include one Positive Control and one Negative Control on each plate.

Prepare the RT-PCR reactions (200-µL sample input volume)

Use this procedure if you extracted sample RNA using an original sample input volume of 200 µL.

1. If frozen, thaw the reagents on ice.
2. Gently vortex the samples and reagents, then centrifuge briefly to collect liquid at the bottom of the 96-well plate.
3. Dilute TaqPath™ COVID-19 Control (1×10^4 copies/µL) to a working stock of 25 copies/µL:
   a. Pipet 98 µL of TaqPath™ COVID-19 Control Dilution Buffer into a microcentrifuge tube, then add 2 µL of diluted TaqPath™ COVID-19 Control. Mix well, then centrifuge briefly.
   b. Pipet 87.5 µL of TaqPath™ COVID-19 Control Dilution Buffer into a second microcentrifuge tube, then add 12.5 µL of the dilution created in substep 3a. Mix well, then centrifuge briefly.

Note: The TaqPath™ COVID-19 Control does not contain the MS2 template.

4. Prepare the Reaction Mix:
   a. For each run, combine the following components sufficient for the number of tests plus one Positive Control and one Negative Control.

All volumes include 10% overage for pipette error.

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

Component	Volume per sample or control	Volume for *n* samples plus 2 controls	Volume for 94 Samples plus 2 Controls
TaqPath™ 1-Step Multiplex Master Mix (No ROX™) (4X)	6.25 µL	6.875 x (*n* + 2) µL	660 µL
COVID-19 Real Time PCR Assay Multiplex	1.25 µL	1.375 x (*n* + 2) µL	132 µL
Nuclease-free Water	7.50 µL	8.25 x (*n* + 2) µL	792 µL
Total Reaction Mix volume	15.0 µL	—	1584 µL

5. Set up the reaction plate:
   a. Pipette 15.0 µL of the Reaction Mix prepared in step 4 into each well of a MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL or a MicroAmp™ Optical 96-Well Reaction Plate with Barcode, 0.2 mL.

b. Unseal the plate containing the purified sample RNA and Negative Control from the RNA extraction procedure. Add either sample RNA, Negative Control, or Positive Control to each well of the reaction plate according to the following table. After each addition, mix by pipetting up and down 10 times.

Component	Volume per reaction		
	Sample reaction	Positive Control reaction	Negative Control reaction
Reaction Mix	15.0 µL	15.0 µL	15.0 µL
Purified sample RNA (from RNA extraction)	10.0 µL	—	—
Positive Control (diluted TaqPath™ COVID-19 Control, from step 3)	—	2.0 µL	—
Nuclease-free Water	—	8.0 µL	—
Purified Negative Control (from RNA extraction)	—	—	10.0 µL
Total volume	25.0 µL	25.0 µL	25.0 µL

6. Seal the plate with MicroAmp™ Optical Adhesive Film, vortex the plate for 10 seconds to ensure proper mixing, then centrifuge for 1 minute at 2000 rpm to collect the liquid at the bottom of the reaction plate.

Prepare the RT-PCR reactions (400-µL sample input volume)

Use this procedure if you extracted sample RNA using an original sample input volume of 400 µL.

1. If frozen, thaw the reagents on ice.

2. Gently vortex the samples and reagents, then centrifuge briefly to collect liquid at the bottom of the 96-well plate.

3. Dilute TaqPath™ COVID-19 Control (1×10^4 copies/µL) to a working stock of 25 copies/µL:

   a. Pipet 98 µL of TaqPath™ COVID-19 Control Dilution Buffer into a microcentrifuge tube, then add 2 µL of diluted TaqPath™ COVID-19 Control. Mix well, then centrifuge briefly.

   b. Pipet 87.5 µL of TaqPath™ COVID-19 Control Dilution Buffer into a second microcentrifuge tube, then add 12.5 µL of the dilution created in substep 3a. Mix well, then centrifuge briefly.

   Note: The TaqPath™ COVID-19 Control does not contain the MS2 template.

4. Prepare the Reaction Mix:
   a. For each run, combine the following components sufficient for the number of tests plus one Positive Control and one Negative Control.

   All volumes include 10% overage for pipette error.

   IMPORTANT! The volumes in this table assume that you extracted sample RNA using an original sample input volume of 400 µL.

Component	Volume per Sample or Control	Volume for *n* Samples plus 2 Controls	Volume for 94 Samples plus 2 Controls
TaqPath™ 1-Step Multiplex Master Mix (No ROX™) (4X)	6.25 µL	6.875 x (*n* + 2) µL	660 µL
COVID-19 Real Time PCR Assay Multiplex	1.25 µL	1.375 x (*n* + 2) µL	132 µL
Nuclease-free Water	12.50 µL	13.75 x (*n* + 2) µL	1320 µL
Total Reaction Mix volume	20.0 µL	—	2112 µL

5. Set up the reaction plate:
   a. Pipette 20.0 µL of the Reaction Mix prepared in step 4 into each well of a MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL or a MicroAmp™ Optical 96-Well Reaction Plate with Barcode, 0.2 mL.

b. Unseal the plate containing the purified sample RNA and Negative Control from the RNA extraction procedure. Add either sample RNA, Negative Control, or Positive Control to each well of the reaction plate according to the following table. After each addition, mix by pipetting up and down 10 times.

Component	Volume per reaction		
	Sample reaction	Positive Control reaction	Negative Control reaction
Reaction Mix	20.0 µL	20.0 µL	20.0 µL
Purified sample RNA (from RNA extraction)	5.0 µL	—	—
Positive Control (diluted TaqPath™ COVID-19 Control from step 3)	—	2.0 µL	—
Nuclease-free Water	—	3.0 µL	—
Purified Negative Control (from RNA extraction)	—	—	5.0 µL
Total volume	25.0 µL	25.0 µL	25.0 µL

6. Seal the plate with MicroAmp™ Optical Adhesive Film, vortex the plate for 10 seconds to ensure proper mixing, then centrifuge for 1 minute at 2000 rpm to collect the liquid at the bottom of the reaction plate.

5 Perform RT-PCR using the Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument

Dye calibration for the 7500 Real-Time PCR Instrument series

A maintained instrument will be calibrated for many dyes. In addition to those dyes, the instrument operator must calibrate the instrument for ABY™ dye and JUN™ dye that are used with this kit. For all other assays, refer to the standard calibration process.

Transfer the template (SDT) file for the 7500 Fast Dx Real-Time PCR Instrument

The template (SDT) file contains the settings for the instrument run. It is installed on the computer with Applied Biosystems™ COVID-19 Interpretive Software, and must be transferred via a USB drive or other method to the computer on which SDS Software v1.4.1 is installed.

1. On the computer with Applied Biosystems™ COVID-19 Interpretive Software, navigate to the following directory and locate the SDT file:

   ```
   <installation directory>\Applied Biosystems\COVID-19
   Interpretive Software\Client\docs\User Documents\TaqPath
   COVID-19 Kit Template 7500fastDx sds1_4_1 v1-2.sdt
   ```

2. Transfer the SDT file to the computer with SDS Software v1.4.1, using a USB drive or other method.

   IMPORTANT! Be careful to select the appropriate SDT file for the instrument and software version that you are using. Failure to do so can cause errors in the analysis.

Set up and run the 7500 Fast Dx Real-Time PCR Instrument

For more information about the 7500 Fast Dx Real-Time PCR Instrument, see the documents listed in “Related documentation” on page 58.

1. Using SDS Software v1.4.1, open the SDT file that you transferred in “Transfer the template (SDT) file for the 7500 Fast Dx Real-Time PCR Instrument” on page 32.

   IMPORTANT! Be careful to select the appropriate template file for the instrument and software version that you are using. Failure to do so can cause errors in the analysis.

2. Confirm the run settings in the template and adjust as necessary.
   - **Assay**: **Standard Curve (Absolute Quantitation)**
   - **Run mode**: **Standard 7500**
   - **Passive reference**: **None**
   - **Sample volume**: **25 µL**

   IMPORTANT! The passive reference must be set to **None**.

3. Confirm that the reporter dye and the detector pairs are correct in the **Detector Manager** in the **Tools** menu.

Reporter dye	Detector
FAM	ORF1ab
VIC	N gene
ABY	S gene
JUN	MS2

4. Confirm that the targets above are assigned to each well in the plate layout.

5. Set up the controls.
   - The template has one positive control and one negative control assigned to wells for reference.
   - Move the control well assignments by copying the existing control wells and pasting them according to their location on the physical plate.

6. For the positive control, confirm that **Task** is set to **Standard**.

7. For the negative control, confirm that **Task** is set to **NTC**.

8. Edit the plate layout to assign a unique sample name to each well with a patient sample in the physical plate.

   For wells with a patient sample, confirm that **Task** is set to **Unknown** for all detectors.

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

9. Confirm the thermal protocol.

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

10. Click **Save As**, enter a file name, then click **Save**.

11. Reopen the file, load the plate, then start the run on the real-time PCR instrument.

12. After the instrument run is complete, open the SDS file in SDS Software v1.4.1. Analyze, then save the file.

6 Perform RT-PCR using the Applied Biosystems™ 7500 Fast Real-Time PCR Instrument

Dye calibration for the 7500 Real-Time PCR Instrument series

A maintained instrument will be calibrated for many dyes. In addition to those dyes, the instrument operator must calibrate the instrument for ABY™ dye and JUN™ dye that are used with this kit. For all other assays, refer to the standard calibration process.

Transfer the template (SDT or EDT) file for the 7500 Fast Real-Time PCR Instrument

The template (SDT or EDT) file contains the settings for the instrument run. It is installed on the computer with Applied Biosystems™ COVID-19 Interpretive Software,

and must be transferred via a USB drive or other method to the computer on which instrument data collection software is installed.

1. On the computer with Applied Biosystems™ COVID-19 Interpretive Software, navigate to the directory that contains the SDT or EDT file.

   Note: The file name depends on the version of the instrument software you are using, as shown in the following table.

Data collection software version	Template file
SDS Software v1.5.1	`<installation directory>\Applied Biosystems\COVID-19 Interpretive Software\Client\docs\User Documents\TaqPath COVID-19 Kit Template 7500fast sds1_5_1 v1-2.sdt`
7500 Software v2.3	`<installation directory>\Applied Biosystems\COVID-19 Interpretive Software\Client\docs\User Documents\TaqPath COVID-19 Kit Template 7500fast sds2_3 v1-2.edt`

2. Transfer the appropriate SDT or EDT file to the computer with your data collection software, using a USB drive or other method.

IMPORTANT! Be careful to select the appropriate SDT or EDT file for the instrument and software version that you are using. Failure to do so can cause errors in the analysis.

Set up and run the 7500 Fast Real-Time PCR Instrument (SDS Software v1.5.1)

This procedure is specific for the 7500 Fast Real-Time PCR Instrument using SDS Software v1.5.1. For more information, see the documents listed in “Related documentation” on page 58.

1. Using SDS Software v1.5.1, access the appropriate template file.
   a. Create a new experiment.
   b. In the **Template** field, browse to, then open the SDT file that you transferred in “Transfer the template (SDT or EDT) file for the 7500 Fast Real-Time PCR Instrument” on page 35.

IMPORTANT! Be careful to select the appropriate template file for the instrument and software version that you are using. Failure to do so can cause errors in the analysis.

2. Confirm the run settings in the template and adjust as necessary.
   - **Assay**: **Standard Curve (Absolute Quantitation)**
   - **Run mode**: **Standard 7500**
   - **Passive reference**: **None**
   - **Sample volume**: **25 µL**

   IMPORTANT! The passive reference must be set to **None**.

3. Confirm that the reporter dye and the detector pairs are correct in the **Detector Manager** in the **Tools** menu.

Reporter dye	Detector
FAM	ORF1ab
VIC	N gene
ABY	S gene
JUN	MS2

4. Confirm that the targets above are assigned to each well in the plate layout.

5. Set up the controls.

   The template has one positive control and one negative control assigned to wells for reference.

   Move the control well assignments by copying the existing control wells and pasting them according to their location on the physical plate.

6. For the positive control, confirm that **Task** is set to **Standard**.

7. For the negative control, confirm that **Task** is set to **NTC**.

8. Edit the plate layout to assign a unique sample name to each well with a patient sample in the physical plate.

   For wells with a patient sample, ensure that **Task** is set to **Unknown** for all detectors.

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

9. Confirm the thermal protocol.

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

10. Click **Save As**, enter a file name, then click **Save**.

11. Reopen the file, load the plate, then start the run on the instrument.

12. After the instrument run is complete, open the SDS file in SDS Software v1.5.1. Analyze, then save the file.

Set up and run the 7500 Fast Real-Time PCR Instrument (7500 Software v2.3)

This procedure is specific for the 7500 Fast Real-Time PCR Instrument using 7500 Software v2.3. For more information, see the documents listed in “Related documentation” on page 58.

1. In the 7500 Software v2.3 home page, click **Template**.

2. Browse to, then open the SDT or EDT file that you transferred in “Transfer the template (SDT or EDT) file for the 7500 Fast Real-Time PCR Instrument” on page 35.

   IMPORTANT! Be careful to select the appropriate template file for the instrument and software version that you are using. Failure to do so can cause errors in the analysis.

3. In the **Experiment Properties** window, enter or confirm the following information:

   - **Experiment name**: Enter a name
   - **Instrument type**: **7500 Fast (96 wells)**
   - **Type of experiment**: **Quantitation - Standard Curve**
   - **Reagents**: **TaqMan™**
   - **Ramp Speed**: **Standard**

4. In the **Plate Setup** window, in the **Define Targets and Samples** tab and the **Define Targets** pane, confirm that the targets, reporter dyes, and quenchers are listed correctly.

Target	Reporter dye	Quencher
MS2	JUN	None
N gene	VIC	None
ORF1ab	FAM	None
S gene	ABY	None

5. In the **Plate Setup** window, in the **Define Targets and Samples** tab and the **Define Samples** pane, confirm the labeling of the controls.

   The template has one positive control (PC) and one negative control (NC) assigned to the wells for reference. To include additional controls, select **Add New Sample**.

6. Select **Add New Sample** to assign a unique sample name to each well that has a patient sample in the physical plate.

7. In the **Plate Setup** window, in the **Assign Targets and Samples** tab, confirm that four targets are assigned to each well in the plate layout. To designate a target or sample to a well, select the well, then check the **Assign** checkbox.

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

8. For wells with a positive control, confirm that **Task** is set to **S** for Standard.

9. For wells with a negative control, confirm that **Task** is set to **N** for Negative.

10. For wells with a patient sample, confirm that **Task** is set to **U** for Unknown.

11. Confirm that **Passive Reference** is set to **None**.

12. In the **Run Method** window, confirm that **Reaction Volume Per Well** is 25 µL, then confirm the thermal protocol.

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

13. Select **Start Run**, enter a file name, then click **Save**.

14. After the instrument run is complete, click **Analyze**, then save the file.

7 Perform RT-PCR using the Applied Biosystems™ 7500 Real-Time PCR Instrument

Dye calibration for the 7500 Real-Time PCR Instrument series

A maintained instrument will be calibrated for many dyes. In addition to those dyes, the instrument operator must calibrate the instrument for ABY™ dye and JUN™ dye that are used with this kit. For all other assays, refer to the standard calibration process.

Transfer the template (EDT) file for the 7500 Real-Time PCR Instrument

The template (EDT) file contains the settings for the instrument run. It is installed on the computer with Applied Biosystems™ COVID-19 Interpretive Software, and must be transferred via a USB drive or other method to the computer on which 7500 Software v2.3 is installed.

1. On the computer with Applied Biosystems™ COVID-19 Interpretive Software, navigate to the following directory and locate the EDT file:

   ```
   <installation directory>\Applied Biosystems\COVID-19 Interpretive Software\Client\docs\User Documents\TaqPath COVID-19 Kit Template 7500std sds2_3 v1-2.edt
   ```

2. Transfer the EDT file to the computer with your data collection software, using a USB drive or other method.

   IMPORTANT! Be careful to select the appropriate template file for the instrument and software version that you are using. Failure to do so can cause errors in the analysis.

Set up and run the 7500 Real-Time PCR Instrument (7500 Software v2.3)

This procedure is specific for the 7500 Real-Time PCR Instrument using 7500 Software v2.3. For more information, see the documents listed in “Related documentation” on page 58.

1. In the 7500 Software v2.3 home page, click **Template**.
2. Browse to, then open the template file that you transferred in “Transfer the template (EDT) file for the 7500 Real-Time PCR Instrument” on page 41.

   IMPORTANT! Be careful to select the appropriate template file for the instrument and software version that you are using. Failure to do so can cause errors in the analysis.

3. In the **Experiment Properties** window, enter or confirm the following information:
   - **Experiment name**: Enter a name
   - **Instrument type**: **7500 (96 wells)**
   - **Type of experiment**: **Quantitation - Standard Curve**
   - **Reagents**: **TaqMan™**
   - **Ramp Speed**: **Standard**
4. In the **Plate Setup** window, in the **Define Targets and Samples** tab and the **Define Targets** pane, confirm that the targets, reporter dyes, and quenchers are listed correctly.

Target	Reporter dye	Quencher
MS2	JUN	None
N gene	VIC	None
ORF1ab	FAM	None
S gene	ABY	None

5. In the **Plate Setup** window, in the **Define Targets and Samples** tab and the **Define Samples** pane, confirm the labeling of the controls.

   The template has one positive control (**PC**) and one negative control (**NC**) assigned to the wells for reference. To include additional controls, select **Add New Sample**.

6. Select **Add New Sample** to assign a unique sample name to each well that has a patient sample in the physical plate.

7. In the **Plate Setup** window, in the **Assign Targets and Samples** tab, confirm that four targets are assigned to each well in the plate layout. To designate a target or sample to a well, select the well, then check the **Assign** checkbox.

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

8. For wells with a positive control, confirm that **Task** is set to **S** for Standard.

9. For wells with a negative control, confirm that **Task** is set to **N** for Negative.

10. For wells with a patient sample, confirm that **Task** is set to **U** for Unknown.

11. Confirm that **Passive Reference** is set to **None**.

12. In the **Run Method** window, confirm that **Reaction Volume Per Well** is 25 µL, then confirm the thermal protocol.

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

13. Select **Start Run**, enter a file name, then click **Save**.

14. After the instrument run is complete, click **Analyze**, then save the file.

8 Perform RT-PCR using the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument

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

A maintained instrument will be calibrated for all dyes that are used with this kit. Ensure that the calibrations for FAM™ dye, VIC™ dye, ABY™ dye, and JUN™ dye are current. For all other assays, refer to the standard calibration process.

Transfer the template (EDT) file for the QuantStudio™ 5 Real-Time PCR Instrument

The template (EDT) file contains the settings for the instrument run. It is installed on the computer with Applied Biosystems™ COVID-19 Interpretive Software, and 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.

1. On the computer with Applied Biosystems™ COVID-19 Interpretive Software, navigate to the following directory and locate the EDT file:

   `<installation directory>\Applied Biosystems\COVID-19 Interpretive Software\Client\docs\User Documents`

2. Select the correct EDT file for your instrument:

Instrument	Template file
QuantStudio™ 5 Real-Time PCR Instrument (0.1-mL block)	`TaqPath COVID-19 Kit Template QS5 0_1ml_da1_5_1 v2-0.edt`
QuantStudio™ 5 Real-Time PCR Instrument (0.2-mL block)	`TaqPath COVID-19 Kit Template QS5 0_2ml_da1_5_1 v2-0.edt`

3. Transfer the EDT file to the computer with QuantStudio™ Design and Analysis Desktop Software v1.5.1, using a USB drive or other method.

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

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

For more information about the QuantStudio™ 5 Real-Time PCR Instrument, see the documents listed in “Related documentation” on page 58.

1. In the QuantStudio™ Design and Analysis Desktop Software v1.5.1, 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” on page 44.

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

3. In the **Properties** tab, enter or confirm the following.
   - **Name**: Enter a name
   - **Instrument type**: **QuantStudio™ 5 System**
   - **Block type**: **96-Well 0.2-mL Block** *or* **96-Well 0.1-mL Block**
   - **Experiment type**: **Standard Curve**
   - **Chemistry**: **TaqMan™ Reagents**
   - **Run Mode**: **Standard**

4. In the **Method** tab, confirm that the **Volume** is 25 µL, then confirm the thermal protocol.

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

5. In the **Plate** tab, click **Quick Setup**.

6. In the **Plate Attributes** pane, confirm that **Passive Reference** is set to **None**.

IMPORTANT! The passive reference must be set to **None**.

7. In the **Plate** tab, click **Advanced Setup**.

8. In the **Targets** table, confirm that the reporter dye and the target pairs are correct.

Reporter dye	Detector
FAM	ORF1ab
VIC	N gene
ABY	S gene
JUN	MS2

9. Confirm that the targets above are assigned to each well in the plate layout.

10. In the plate layout pane, set up the control wells.

    The template has one positive control and one negative control assigned to wells for reference.

    Default control wells are provided in the template. If your control wells are in a different location, move the control well assignments by copying the existing control wells and pasting them according to their location on the physical plate.

11. For all targets in the positive control well, confirm that **Task** is set to **S (Standard)**.

12. For all targets in the negative control well, confirm that **Task** is set to **N (NTC)**.

13. In the **Samples** table, click **Add** to define the sample names. Create a unique sample name for each well in the physical plate that has a patient sample.

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

    For all targets in the patient sample wells, confirm that **Task** is set to **U (Unknown)**.

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

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

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

9 Analysis and results

Obtain the Applied Biosystems™ COVID-19 Interpretive Software

To perform data analysis and results interpretation, you must use the Applied Biosystems™ COVID-19 Interpretive Software.

To obtain the software, contact your local support team or look up the local service phone number on the website (see below).

1. Go to **https://www.thermofisher.com/contactus**.
2. In the **Step One** pane, click **Instrument Service**.
3. In the **Step Two** pane, enter the name of your real-time PCR instrument, then select your location from the dropdown list.
4. To obtain the software, call the local service phone number that is displayed on the screen.

For software installation instructions, see the document listed in “Related documentation” on page 58.

Analyze the data

For detailed instructions about using the software, click the **Help** menu in the COVID-19 Interpretive Software.

1. Using a USB drive or other method, transfer the SDS or EDS files from the computer with the data collection software to the computer with the COVID-19 Interpretive Software.
2. In the COVID-19 Interpretive Software **Home** screen, click the **Import Samples** button.
3. Select the SDS files or the EDS files to import, then click **Open**.
   After import, the software analyzes the run data, performs Quality Check (QC) analysis, and calculates the interpretive results for each sample and control.
4. In the **Batches** pane of the **Home** screen, select a batch to view the status and result for each sample in the **Samples** list.

5. To generate a batch export file (CSV or XLSX), select the checkbox for the batch, then click the **Export Batch** button at the top of the **Home** screen. Click **Open folder location** in the dialog box, then navigate to the exported file.

6. To generate a batch report file (PDF), select the checkbox for the batch, then click the **Report Batch** button at the top of the **Home** screen. Click **Open folder location** in the dialog box, then navigate to the report.

Interpretation of the results

Interpretation of the results is performed by the Applied Biosystems™ COVID-19 Interpretive Software.

Quality control and validity of results

One Negative Control and one Positive Control are processed with each run.

Validation of results is performed automatically by the Applied Biosystems™ COVID-19 Interpretive Software based on performance of the Positive and Negative Controls.

Table 2 Result interpretation for patient samples

ORF1ab	N gene	S gene	MS2	Status	Result	Action
NEG	NEG	NEG	NEG	INVALID	NA	Repeat test. If the repeat result remains invalid, consider collecting a new specimen.
NEG	NEG	NEG	POS	VALID	SARS-CoV-2 Not Detected	Report results to healthcare provider. Consider testing for other viruses.
Only one SARS-CoV-2 target = POS			POS or NEG	VALID	SARS-CoV-2 Inconclusive[1]	Repeat test. If the repeat result remains inconclusive, additional confirmation testing should be conducted if clinically indicated.
Two or more SARS-CoV-2 targets = POS			POS or NEG	VALID	Positive SARS-CoV-2	Report results to healthcare provider and appropriate public health authorities.

[1] Samples with a result of SARS-CoV-2 Inconclusive shall be retested one time. Retesting must happen from the original sample.

10 Conditions of authorization for labs

The TaqPath™ RT-PCR COVID-19 Kit 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: **https:// www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm**.

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

- Authorized laboratories[1] using the TaqPath™ COVID-19 Combo Kit will include with result reports of the TaqPath™ COVID-19 Combo Kit 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 Combo Kit will perform the TaqPath™ COVID-19 Combo Kit as outlined in the *TaqPath™ COVID-19 Combo Kit 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 Combo Kit are not permitted.
- Authorized laboratories that receive the TaqPath™ COVID-19 Combo Kit 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 Combo Kit will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories will 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.

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

- Thermo Fisher Scientific, its authorized distributor(s), and authorized laboratories using the TaqPath™ COVID-19 Combo Kit will 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.

11 Performance characteristics

Analytical performance of the TaqPath™ RT-PCR COVID-19 Kit was evaluated by determining limit of detection (LoD), 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 Combo Kit in a particular specimen type at least 95% of the time. Banked Nasopharyngeal swab (NP) and Bronchoalveolar lavage (BAL) samples, obtained from U.S. patients in the years 2015-2019, were pooled, respectively, and spiked with purified SARS- CoV-2 viral genomic RNA at several concentrations and processed through the TaqPath™ COVID-19 Combo Kit workflow. A three-phase approach was used to determine the LoD for each specimen type. In phases I and II, the preliminary LoD was established and confirmed in phase III by testing 20 replicates.

Table 3 LoD determination in BAL

Effective Concentration	Replicate	Mean C_t				Interpretation	% Positive
		ORF1ab	N	S	MS2		
10 GCE/reaction	1	29.9	29.1	28.5	23.1	Positive	100%
	2	30.1	29.3	29.7	24.0	Positive	
	3	30.0	29.7	29.3	24.0	Positive	
	4	30.3	29.7	29.1	23.8	Positive	
	5	30.2	29.6	29.6	23.7	Positive	
	6	30.3	29.3	29.7	23.5	Positive	
	7	29.9	29.6	32.8	23.4	Positive	
	8	30.2	29.8	29.2	23.8	Positive	
	9	30.1	29.4	28.6	23.8	Positive	
	10	30.1	29.4	29.1	24.0	Positive	
	11	29.8	29.5	29.4	24.3	Positive	
	12	30.1	29.7	29.1	24.6	Positive	

Table 3 LoD determination in BAL *(continued)*

Effective Concentration	Replicate	Mean C_t				Interpretation	% Positive
		ORF1ab	N	S	MS2		
10 GCE/reaction	13	30.7	30.1	28.4	25.1	Positive	100%
	14	30.4	29.8	29.1	24.8	Positive	
	15	30.2	29.8	29.7	24.9	Positive	
	16	30.3	29.8	29.4	24.6	Positive	
	17	30.4	30.0	31.5	24.7	Positive	
	18	30.4	30.1	29.3	24.9	Positive	
	19	30.9	29.7	29.2	25.4	Positive	
	20	30.3	29.9	29.4	25.7	Positive	

Table 4 LoD determination in Nasopharyngeal specimens

Effective Concentration	Replicate	Mean C_t				Interpretation	% Positive
		ORF1ab	N	S	MS2		
10 GCE/reaction	1	30.0	28.9	35.7	25.7	Positive	100%
	2	30.6	28.9	33.6	25.8	Positive	
	3	30.2	28.8	32.0	25.8	Positive	
	4	30.4	28.7	34.2	25.7	Positive	
	5	30.5	29.0	31.4	25.8	Positive	
	6	31.0	29.3	36.6	26.0	Positive	
	7	30.3	29.2	31.1	25.8	Positive	
	8	31.1	29.2	31.8	26.5	Positive	
	9	30.5	28.9	33.0	26.2	Positive	
	10	30.3	28.8	34.7	26.8	Positive	
	11	30.5	29.8	38.7	27.4	Positive	
	12	31.6	29.7	35.0	27.6	Positive	
	13	30.7	29.3	36.4	27.4	Positive	
	14	31.6	28.8	31.3	27.2	Positive	
	15	31.0	29.3	36.0	27.0	Positive	
	16	30.5	29.1	35.7	27.0	Positive	

Table 4 LoD determination in Nasopharyngeal specimens *(continued)*

Effective Concentration	Replicate	Mean C_t				Interpretation	% Positive
		ORF1ab	N	S	MS2		
10 GCE/reaction	17	30.7	29.4	34.8	27.4	Positive	100%
	18	30.7	29.3	34.6	27.5	Positive	
	19	31.0	29.3	35.9	28.7	Positive	
	20	30.4	29.2	32.7	28.4	Positive	

Table 5 LoD results

Specimen type	Limit of Detection (GCE/reaction)
Bronchoalveolar lavage	10 GCE/reaction
Nasopharyngeal swab	10 GCE/reaction

Reactivity (Inclusivity)

The assays were mapped to 185 complete SARS-CoV-2 genomes of human host in GenBank and GISAID databases as of March 5, 2020. Primer and probes sequences for SARS-CoV-2 ORF1ab, S gene, and N gene assays had 100% homology to all SARS-CoV-2 isolates analyzed, with one exception. EPI_ISL_407084 (Beta Coronavirus/Japan/AI/I-004/2020) showed a mismatch at position 7 from the 5’ end of the reverse primer (23 nt length) corresponding to 95.6% homology. The mismatch is located at the 5’end of the primer and does not affect the test performance.

Interfering substances

Pooled SARS-CoV-2-negative nasopharyngeal swab and bronchoalveolar lavage specimens were spiked with purified SARS-CoV-2 viral RNA at 3X the Limit of Detection (30 GCE/reaction) and potential interfering substances at the concentrations above. Each substance was tested with triplicate extractions. The results are presented in the table below.

Pooled SARS-CoV-2-negative nasopharyngeal swab and bronchoalveolar lavage specimens were spiked with potential interfering substances at the concentrations above. Each substance was tested with triplicate extractions. No false positive results were observed for any of the substances at the concentrations tested.

Interfering substance	Final concentration in sample	Agreement with expected results			
		Positive BAL samples	Positive NP samples	Negative BAL samples	Negative NP samples
None	N/A	100%[1]	100%	100%	100%
Mucin: bovine submaxillary gland, type I-S	0.1 mg/mL	100%[2]	100%	100%	100%
Blood (human)	1% v/v	100%[3]	100%	100%	100%
Nasal sprays or drops—Nasacort™	10% v/v	100%[4]	100%[4]	100%	100%
Nasal corticosteroids—Dymista™	5 µg/mL	100%[2]	100%	100%	100%
NeilMed™ Nasogel™	1% w/v	100%[2]	100%	100%	100%
Influenza A H1N1 Brisbane/59/07	1×10^5 $TCID_{50}$/mL	100%[2]	100%	100%	100%
Throat lozenges, oral anesthetic and analgesic—Chloraseptic™	1% w/v	100%[3]	100%	100%	100%
Oseltamivir phosphate	33 µg/mL	100%[2]	100%	100%	100%
Antibiotic, nasal ointment—Bactroban™	5 µg/mL	100%[2]	100%	100%	100%
Antibacterial, systemic—Tobramycin	0.6 mg/mL	100%[2]	100%	100%	100%
Homeopathic allergy relief medicine—Similasan™ Nasal	10% v/v	100%	100%	100%	100%

[1] Two of six replicates produced a C_t >37 or Undetermined for S Gene, but all replicates were called Positive based on the interpretation algorithm.
[2] Two of three replicates produced a C_t >37 or Undetermined for S Gene, but all replicates were called Positive based on the interpretation algorithm.
[3] All three replicates produced a C_t >37 or Undetermined for S Gene but were called Positive based on the interpretation algorithm.
[4] One of three replicates produced a C_t >37 or Undetermined for S Gene, but all replicates were called Positive based on the interpretation algorithm

Cross-reactivity

In silico analysis of the following forty-three (43) organisms:

Table 6 Organisms used for *in silico* cross-reactivity analysis

Human coronavirus 229E	Rhinovirus/Enterovirus
Human coronavirus OC43	Parechovirus
Human coronavirus HKU1	*Candida albicans*
Human coronavirus NL63	*Corynebacterium diphtheriae*
SARS-coronavirus	*Legionella* (non-pneumophila)
MERS-coronavirus	*Bacillus anthracis* (Anthrax)
Adenovirus	*Moraxella catarrhalis*
Human Metapneumovirus (hMPV)	*Neisseria elongata* and *Neisseria meningitidis*
Parainfluenza 1	*Pseudomonas aeruginosa*
Parainfluenza 2	*Staphylococcus epidermidis*
Parainfluenza 3	*Streptococcus salivarius*
Parainfluenza 4	*Leptospira* sp.
Influenza A	*Chlamydophila pneumoniae*
Influenza B	*Chlamydophila psittaci*
Influenza C	*Coxiella burnetii* (Q-Fever)
Enterovirus	*Staphylococcus aureus*
Respiratory Syncytial Virus A	*Haemophilus influenzae*
Respiratory Syncytial Virus B	*Legionella pneumophila*
Bordetella pertussis	*Mycobacterium tuberculosis*
Mycoplasma pneumoniae	*Streptococcus pneumoniae*
Pneumocystis jirovecii (PJP)	*Streptococcus pyogenes*

Among the tested organisms, *Neisseria elongata* showed homology for the forward and reverse primers and probe for the N gene. The forward primer showed ≥80% homology while the reverse primer and probe showed 36% homology. The N gene reverse primer and probe show low homology, therefore the risk of the non-specific amplification is low.

Blast analysis showed ≥80% homology for one assay component (forward primer, reverse primer, or probe) for select isolates. Despite ≥80% homology of one assay component for select isolates, there is no anticipated amplification because hybridization of all three assay components are necessary to generate a signal. We also found multiple instances where different assay components had ≥80% homology to different isolates of the same species. For example, *Bacillus anthracis* strain AFS029987 had ≥80% homology to the ORF1ab forward primer while strain MCCC 1A01412 had ≥80% homology to the ORF1ab reverse primer. Since these are two different organisms, amplification is not likely to occur. The *in silico* analysis indicates that significant amplification of non-target sequences that result in cross- reactivity or potentially interfere with detection of SARS-CoV-2 is not likely to occur.

Clinical evaluation

A clinical evaluation study was performed to evaluate the performance of the TaqPath™ RT-PCR COVID-19 Kit using nasopharyngeal swab (NP) and bronchoalveolar lavage (BAL) specimens.

A total of sixty (60) contrived positive specimens were tested:

- 30 contrived positive nasopharyngeal swab (NP) specimens
- 30 contrived positive bronchoalveolar lavage (BAL) specimens

Samples were contrived by spiking known concentrations of extracted SARS-CoV-2 viral genomic RNA, relative to the product LoD, into matrices which were determined to be negative by the TaqPath™ RT-PCR COVID-19 Kit prior to spiking in the RNA.

In addition to the contrived positive specimens, sixty (60) negative specimens were tested:

- 30 negative nasopharyngeal swab (NP) specimens
- 30 negative samples bronchoalveolar lavage (BAL) specimens
- All negative samples yielded negative results

Results for positive samples are shown in the tables below:

Table 7 BAL Clinical Evaluation Study

Final RNA Concentration in Sample	Number of Positives	Mean C_t		
		S gene	ORF1ab	N gene
2X LoD	20/20[1]	28.9	29.5	28.7
3X LoD	5/5[1]	28.8	29.2	28.5
5X LoD	5/5	27.4	28.2	27.4

[1] Two samples initially gave inconclusive results and were retested. The results were positive after the retest. Mean C_t values are calculated from the retest results.

Table 8 NP Clinical Evaluation Study

Final RNA Concentration in Sample	Number of Positives	Mean C_t		
		S gene	ORF1ab	N gene
2X LoD	20/20[1]	30.9	30.6	29.3
3X LoD	5/5	30.0	30.1	28.8
5X LoD	5/5	28.7	29.0	27.9

[1] One sample initially gave an inconclusive result and was retested. The result was positive after the retest. Mean C_t values are calculated from the retest results.

A Documentation and support

Related documentation

Document	Publication Number
Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument Reference Guide	4406991
Applied Biosystems™ 7500/7500 Fast Real-Time PCR System: Maintenance Guide	4387777
QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide	MAN0010407
MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (automated extraction) User Guide	MAN0018073
MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (manual extraction) User Guide	MAN0018072
Thermo Scientific™ KingFisher™ Flex User Manual	N07669
COVID-19 Interpretive Software Installation Quick Reference	MAN0019257

Customer and technical support

For additional documentation and information about this kit, visit:
https://www.thermofisher.com/covid19

For download instructions for the COVID-19 Interpretive Software, see “Obtain the Applied Biosystems™ COVID-19 Interpretive Software” on page 47.

Refer to the Read Me file provided with the COVID-19 Interpretive Software before contacting support for the software.

Visit: **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 Sheets (SDSs; also known as MSDSs)

    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**.