

# TaqPath™ COVID-19 Pooling Kit

## INSTRUCTIONS FOR USE

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

Catalog Number A49918

Publication Number MAN0019598

Revision B.0

**IVD**

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

**ThermoFisher**  
SCIENTIFIC



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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).

**Revision history: MAN0019598 B.0 (English)**

Revision	Date	Description
B.0	31 May 2023	"Warnings and precautions" on page 11, "Assay limitations" on page 12, and "Reactivity (Inclusivity)" on page 41 were updated.
A.0	26 May 2021	New Instructions for Use for the TaqPath™ COVID-19 Pooling Kit.

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.

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# TaqPath™ COVID-19 Pooling Kit product information

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## Intended Use

The TaqPath™ COVID-19 Pooling Kit contains the assays and controls for a real-time reverse transcription polymerase chain reaction (RT-PCR) test that is intended for the qualitative detection of nucleic acid from SARS-CoV-2 in pooled samples containing up to 5 individual nasopharyngeal, oropharyngeal, anterior nasal, or mid-turbinate nasal swabs where each specimen is collected by a healthcare provider using individual vials containing transport media from individuals suspected of COVID-19. 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.

Negative results from pooled testing should not be treated as definitive. If a patient's clinical signs and symptoms are inconsistent with a negative result or results are necessary for patient management, then the patient should be considered for individual testing. Specimens included in pools with positive, inconclusive, or invalid results must be tested individually using the TaqPath™ COVID-19 Pooling Kit prior to reporting a result. Specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing.

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 for individual samples 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 Pooling Kit is intended to be used with 14.0 µL of nucleic acid template extracted from an individual sample or sample pool and run on the Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block) 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 Pooling Kit is only for use under the Food and Drug Administration’s Emergency Use Authorization.

## Product description

The TaqPath™ COVID-19 Pooling 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 pooled upper respiratory specimens (such as nasopharyngeal, oropharyngeal, anterior nasal, and mid-turbinate swabs) specimens from individuals suspected of COVID-19 by their healthcare provider.

The kit includes the following components:

- TaqPath™ COVID-19 Pooling Assay Kit
  - TaqPath™ COVID-19 Pooling Multiplex Assay—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
- TaqPath™ COVID-19 Control Dilution Buffer—Buffer formulation for diluting the TaqPath™ COVID-19 Control

## Contents and storage

Table 1 TaqPath™ COVID-19 Pooling Kit, 200 reactions (Cat. No. A49918)

Box	Components	Amount	Storage
TaqPath™ COVID-19 Pooling Assay Kit	TaqPath™ COVID-19 Pooling Multiplex Assay (ORF1ab, N gene, S gene, MS2)	300 µL	–30°C to –10°C
	MS2 Phage Control	2 × 1,000 µL	–30°C to –10°C
TaqPath™ COVID-19 Control (1 × 10 <sup>4</sup> copies/µL)		2 × 10 µL	≤ –70°C
TaqPath™ COVID-19 Control Dilution Buffer		2 × 250 µL	–30°C to –10°C

## 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
<b>Real-time PCR instrument</b>	
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)
<b>Equipment</b>	
Laboratory freezers <ul style="list-style-type: none"> <li>-30°C to -10°C</li> <li>≤ -70°C</li> </ul>	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
<b>Automated nucleic acid extraction system and materials</b>	
KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head	<a href="#">5400630</a>
KingFisher™ Flex 96 Deep-Well Heating Block	<a href="#">24075430</a>
KingFisher™ 96 Deep-Well Plate	<a href="#">95040450</a> , <a href="#">A48305</a> , <a href="#">A48424</a> , <a href="#">95040455</a>
96-well plate for the tip comb, one of the following: <ul style="list-style-type: none"> <li>KingFisher™ 96 KF microplate</li> <li>Tip Comb Presenting Plate for KF 96</li> <li>Nunc™ MicroWell™ 96-Well Microplate, Flat Bottom</li> <li>Nunc™ MicroWell™ 96-Well Microplate, barcoded</li> <li>Abgene™ 96-Well Polypropylene Storage Microplate</li> <li>Abgene™ 96-Well 1.2-mL Polypropylene Deepwell Storage Plate</li> <li>Nunc™ F96 MicroWell™ Black Polystyrene Plate</li> <li>Nunc™ F96 MicroWell™ White Polystyrene Plate</li> <li>KingFisher™ 96 Deep-Well Plate</li> </ul>	<ul style="list-style-type: none"> <li><a href="#">97092540</a></li> <li><a href="#">267600</a></li> <li><a href="#">167008</a></li> <li><a href="#">269787</a></li> <li><a href="#">AB0796</a></li> <li><a href="#">AB1127</a></li> <li><a href="#">137101</a></li> <li><a href="#">136101</a></li> <li><a href="#">95040450</a>, <a href="#">A48305</a>, <a href="#">A48424</a>, <a href="#">95040455</a></li> </ul>

(continued)

Item	Source
KingFisher™ 96 tip comb for deep-well magnets	<a href="#">97002534</a> , <a href="#">A48438</a> , <a href="#">A48414</a>
<b>Manual nucleic acid extraction system and materials</b>	
Magnetic Stand-96	<a href="#">AM10027</a> <a href="#">AM10050</a>
Compact Digital Microplate Shaker	<a href="#">88882005</a>
Incubator capable of reaching 65°C with slatted shelves	MLS
KingFisher™ 96 Deep-Well Plate	<a href="#">95040450</a> , <a href="#">A48305</a> , <a href="#">A48424</a> , <a href="#">95040455</a>
Standard 96-well plate for the eluate, one of the following: <ul style="list-style-type: none"> <li>• KingFisher™ 96 KF microplate</li> <li>• MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL</li> <li>• MicroAmp™ Fast Optical 96-Well Reaction Plate, 0.1 mL</li> <li>• MicroAmp™ Optical 96-Well Reaction Plate with Barcode, 0.2 mL</li> <li>• MicroAmp™ Optical 96-Well Reaction Plate, 0.2 mL</li> </ul>	<ul style="list-style-type: none"> <li>• <a href="#">97002540</a></li> <li>• <a href="#">4346906</a>, <a href="#">4366932</a></li> <li>• <a href="#">4346907</a></li> <li>• <a href="#">4306737</a>, <a href="#">4326659</a></li> <li>• <a href="#">N8010560</a>, <a href="#">4316813</a></li> </ul>
MicroAmp™ Clear Adhesive Film	<a href="#">4306311</a>
<b>Kits and reagents</b>	
MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit	<a href="#">A42352</a> <a href="#">A48310</a>
MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit	<a href="#">A48383</a>
TaqPath™ 1-Step Multiplex Master Mix (No ROX™)	<a href="#">A28521</a> , <a href="#">A28522</a> , <a href="#">A28523</a>
Fisher BioReagents™ Ethanol, Absolute, Molecular Biology Grade <sup>[1]</sup> , or equivalent	<a href="#">BP2818100</a> , <a href="#">BP2818500</a> , <a href="#">BP28184</a>
Nuclease-Free Water (not DEPC-Treated)	MLS
<b>Calibration plates (QuantStudio™ 7 Flex Real-Time PCR Instrument)</b>	
ABY™ Dye Spectral Calibration Plate for Multiplex qPCR, 384-well	<a href="#">A24736</a>
JUN™ Dye Spectral Calibration Plate for Multiplex qPCR, 384-well	<a href="#">A24733</a>
<b>Tubes, plates, and other consumables</b>	
MicroAmp™ Optical 384-Well Reaction Plate with Barcode	<a href="#">4309849</a> , <a href="#">4326270</a> , <a href="#">4343814</a>
MicroAmp™ Optical 384-Well Reaction Plate	<a href="#">4343370</a>
MicroAmp™ Clear Adhesive Film	<a href="#">4306311</a>
MicroAmp™ Optical Adhesive Film	<a href="#">4311971</a> , <a href="#">4360954</a>

(continued)

Item	Source
MicroAmp™ Adhesive Film Applicator	4333183
Nonstick, RNase-free microcentrifuge tubes (1.5 mL and 2.0 mL)	<a href="https://www.thermofisher.com/plastics">thermofisher.com/plastics</a>
Sterile aerosol barrier (filtered) pipette tips	<a href="https://www.thermofisher.com/pipettetips">thermofisher.com/pipettetips</a>

[1] Available at [fisherscientific.com](https://www.fisherscientific.com).

## Instrument and software compatibility

The following table lists the version of the COVID-19 Interpretive Software that is compatible with your instrument and pooling of samples.

**Note:** The TaqPath™ COVID-19 Pooling Kit is not compatible with samples collected using the Everlywell™ COVID-19 Test Home Collection Kit, and therefore does not use the RNase P test as part of the workflow. Select the **COVID-19 Test** button when you sign in to the COVID-19 Interpretive Software.

For information on how to obtain the COVID-19 Interpretive Software, see “Obtain the Applied Biosystems™ COVID-19 Interpretive Software” on page 33.

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.

Instrument	Analysis software used with the instrument	Compatible COVID-19 Interpretive Software version
QuantStudio™ 7 Flex Real-Time PCR Instrument with instrument firmware v1.0.4 <ul style="list-style-type: none"> <li>• 384-well block</li> </ul>	QuantStudio™ Real-Time PCR Software v1.3	v2.5

## Warnings and precautions

The TaqPath™ COVID-19 Pooling 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 in a biological safety cabinet.

- For *in vitro* diagnostic use under the FDA Emergency Use Authorization Only. Rx only.
- This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- This 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 Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or the authorization is revoked sooner.
- 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 7.
- 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 results to the appropriate public health authorities.
- A positive result for a sample pool indicates the presence of SARS-CoV-2 RNA in at least one of the samples that were pooled. Deconvolution by testing the individual samples is required.
- An invalid or inconclusive result for a sample pool requires deconvolution by testing the individual samples.

## 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.
- Analytical performance of the TaqPath™ COVID-19 Pooling Kit was determined by leveraging limit of detection (LoD), interfering substances, and cross-reactivity data for individual samples using the TaqPath™ COVID-19 Combo Kit and TaqPath™ COVID-19 Combo Kit Advanced.
- TaqPath™ COVID-19 Pooling Kit performance was established using nasopharyngeal swab samples only. Anterior nasal swabs, oropharyngeal swabs, and mid-turbinate nasal swabs are considered acceptable specimen types for use with the TaqPath™ COVID-19 Pooling Kit, but performance with these specimen types has not been established. Refer to FDA's *FAQs on Diagnostic Testing for SARS-CoV-2* for additional information. Specimen types other than nasopharyngeal, anterior nasal, oropharyngeal, and mid-turbinate nasal swabs should not be tested with this assay.
- Samples should only be pooled when testing exceeds laboratory capacity and/or when testing reagents are in short supply.
- TaqPath™ COVID-19 Pooling Kit performance was established using only the TaqPath™ COVID-19 Combo Kit Advanced workflow performed on the Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR Instrument, 384-well block (with QuantStudio™ Real-Time PCR Software v1.3). The TaqPath™ COVID-19 Combo Kit and the TaqPath™ COVID-19 Combo Kit Advanced workflows on the following instruments have not been validated for pooling:
  - 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 (96-well plates, 0.1-mL block)
  - Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument (96-well plates, 0.2-mL block)
  - Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument (384-well plates)
- 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 or sample pools must be performed according the specified methods listed in this procedure. Other extraction approaches and processing systems have 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
  - Improper vortexing and centrifuging when preparing RT-PCR reactions.
- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated. The TaqPath™ COVID-19 Pooling 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.
- Positive, inconclusive, and invalid results for pooled samples require deconvolution by testing the individual samples in the pool.
- Specimens with low SARS-CoV-2 RNA concentrations may not be detected in sample pools due to the decreased sensitivity of pooled testing.
- Laboratories are required to report all SARS-CoV-2 results for individual samples to the appropriate public health authorities.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. 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.
- Certain mutations may affect detection of individual targets with the TaqPath™ COVID-19 Pooling Kit, including the B.1.1.7 variant of SARS-CoV-2, which may exhibit positive results for the N and ORF1ab targets and negative results for the S-gene target. If such a pattern of detection is observed, further characterization of the specimen by sequence analysis should be considered. If such services are not readily available, local or state clinical laboratories should consider contacting the Centers for Disease Control and Prevention at [EQCenter177@cdc.gov](mailto:EQCenter177@cdc.gov) for additional information.

## General laboratory recommendations

- Implement standard operating procedures in your laboratory to prevent contamination, such as the following:
  - Frequent glove changes
  - Frequent decontamination of surfaces, equipment, and pipettes with 10% bleach or decontamination solution, followed by 70% ethanol
  - Use of 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.

- Aliquot reagents to prevent stock contamination and reduce the number of freeze-thaw cycles.
- After each run, review the amplification curves in the interpretive software for signs of inadequate vortexing or centrifugation. Contact your Applications Support team for additional information or training on data QC in your instrument software.

## Samples and controls

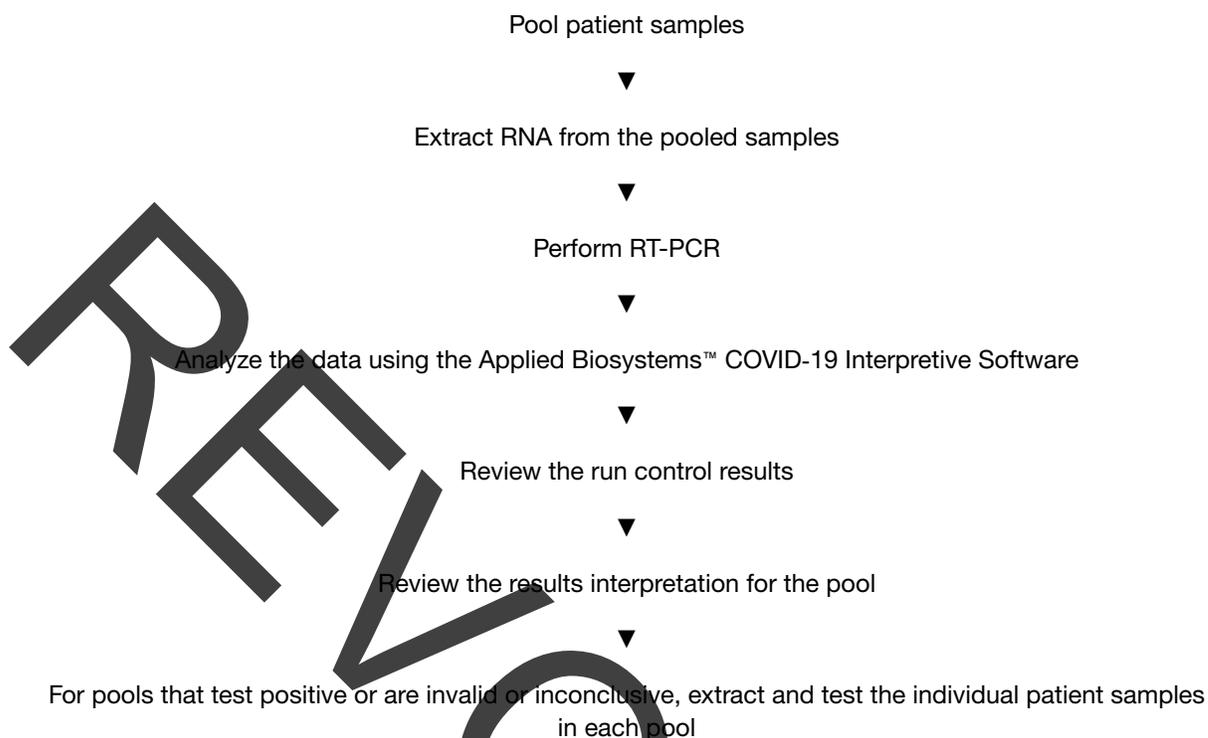
Patient samples must be collected according to appropriate laboratory guidelines. Positive and negative test controls must be included to accurately interpret patient test results.

Store patient samples according to CDC guidelines. See the CDC website: <https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html>.

Include the following controls:

Control	Used to monitor	Assays
Positive Control (TaqPath™ COVID-19 Control)	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

## Workflow



The workflow begins by pooling up to 5 patient samples into a single tube at a total volume of  $\geq 400$   $\mu\text{L}$ , using an equal volume from each sample to create the pool. Samples may include upper respiratory specimens (nasopharyngeal, oropharyngeal, anterior nasal, and mid-turbinate swabs) that arrive in the testing site in transport media. Nucleic acids are isolated and purified from the pooled samples 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 Appendix D, “Documentation and support”.

The nucleic acid is reverse transcribed into cDNA and amplified using the TaqPath™ COVID-19 Pooling Kit and the Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR Instrument, 384-well 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 Gene
- S Gene

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.

For pools that test negative, then each sample is reported as negative with the following statement added to the report:

*"Negative results are not definitive and, if inconsistent with clinical signs and symptoms or necessary for patient management, pooled samples should be tested individually. Negative results do not preclude SARS-CoV-2 infection and must not be used as the sole basis for patient management decisions. Negative results must be considered in the context of a patient's recent exposures, history, and presence of clinical signs and symptoms consistent with COVID-19."*

For pools that test positive, invalid, or inconclusive, the patient samples in each pool must be extracted and tested individually using only the TaqPath™ COVID-19 Pooling Kit and the individual result reported.

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## Sample collection, transport, and storage

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

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## Defining a sample pooling strategy

Sample pooling is generally recommended only if laboratories have backlogs in testing patient samples, resulting in delayed test result reporting. Pooling can lead to a reduction in the sensitivity for detecting SARS-CoV-2 RNA in a patient sample, because patient samples are combined, leading to overall sample dilution. In general, the larger the sample pool, the greater the risk of generating a false-negative result from testing pooled samples.<sup>[1,2]</sup> The TaqPath™ COVID-19 Pooling Kit has been validated for pool sizes ranging from 2 to 5 samples per pool.

### **Before Implementation of Pooling: Determine Appropriate Pool Size**

Before a pooling strategy is implemented, a laboratory should determine the appropriate pool size based on percent positivity rate and desired testing efficiency.

#### **If historical laboratory data for individual specimens are available:**

- If historical data for individual specimens from the previous 7-10 days are available, estimate the percent positivity rate ( $P_{\text{individual}}$ ) based on individual results.  
$$(P_{\text{individual}}) = (\text{Number of positive specimens over chosen date range} \div \text{Total number of specimens tested over chosen date range}) \times 100$$
- Using the calculated  $P_{\text{individual}}$  and Table 2, identify the appropriate  $n$  number of samples to pool.
- If  $P_{\text{individual}}$  is less than 7%, the maximum pool size validated, ( $n=5$ ), should be selected to maximize the efficiency of specimen pooling. Pooling with greater than 5 samples has not been validated and should not be performed.
- If  $P_{\text{individual}}$  is greater than 25%, pooling of patient specimens is not efficient and should not be implemented.

#### **If historical laboratory data for individual specimens are unavailable:**

- If historical data from the previous 7-10 days are unavailable, 5-, 4-, or 3-specimen pooling may still be implemented.
- **Note:** without calculating  $P_{\text{individual}}$ , the pooling size implemented may not maximize pooling efficiency.

[1] <https://www.who.int/bulletin/volumes/98/9/20-257188/en/>

[2] <https://www.cap.org/covid-19/pooled-testing-guidance-from-cap-microbiology-committee>

**Table 2** Number of samples to pool based on the percent of positive individuals in the population in order to achieve the maximum efficiency

(P <sub>individual</sub> ) Percent of positive individuals in the tested population	(n <sub>maxefficiency</sub> ) Number of samples to pool for maximum efficiency	(F) Efficiency of pooling n samples
0.1%–6%	5	4.99–2.15
7%–12%	4	1.99–1.54
13%–25%	3	1.48–1.10

## Monitor the pooling strategy

### If historical laboratory data for individual specimens are available:

- After implementing a pooling strategy, evaluate the performance of pooled testing by comparing the percent positivity rate of pooled testing to that of individual testing.
- Calculate the percent positivity rate among patient specimens during specimen pooling ( $P_{\text{pools}}$ ) on a daily basis using a moving average of the data from the previous 7–10 days of testing.  

$$(P_{\text{pools}}) = (\text{Number of patient specimens with a positive result as determined by individual specimen reflex testing of positive pools over chosen date range} \div \text{Total number of patient specimens tested in pools over chosen date range}) \times 100$$
- Compare  $P_{\text{pools}}$  to  $P_{\text{individual}}$ . If  $P_{\text{pools}}$  is less than 85% of  $P_{\text{individual}}$ , ( $P_{\text{pools}} < 0.85 \times P_{\text{individual}}$ ), it is recommended that the pool size be reassessed and adjusted to maximize pooling efficiency (if necessary), according to the criteria in Table 2 on page 18.
- To ensure maximum pooling efficiency, it is recommended that  $n_{\text{maxefficiency}}$  be reassessed periodically while sample pooling is implemented by the laboratory.

### If historical laboratory data for individual specimens are unavailable:

- After initiating a pooling strategy, evaluate the performance of pooled testing by calculating the initial percent positivity rate for pooled specimens ( $P_{\text{pools-initial}}$ ).  
 $(P_{\text{pools-initial}})$  is the percent positivity rate for pooled specimens for the first 7–10 days of pooled testing.
- Calculate the initial percent positivity rate for individual specimens from pool testing ( $P_{\text{pools-initial}}$ ) from the first 7–10 days of testing.  

$$P_{\text{pools-initial}} = (\text{Number of patient specimens with a positive result as determined by individual specimen reflex testing of positive pools in first 7–10}^{[3]} \text{ days} \div \text{Total number of patient specimens tested in pools in the first 7–10 days}) \times 100$$
- If  $P_{\text{pools-initial}}$  is greater than 25%, pooling of patient specimens is not efficient and should be discontinued until the percent positivity rate decreases.
- If  $P_{\text{pools-initial}}$  is less than or equal to 25%, pooling of patient specimens can be continued.
- Continue to monitor pooling strategy by calculating the percent positivity rate among patient specimens during specimen pooling ( $P_{\text{pools-x}}$ ) for subsequent 7–10 day periods. ( $P_{\text{pools-x}}$ ) should be updated daily using a moving average.

<sup>[3]</sup> Authorized laboratories must keep records of their sample pooling strategies, including type of strategy, date implemented, quantities tested, and test result data generated as part of monitoring the pooling strategy.

- Compare  $P_{\text{pools-x}}$  to  $P_{\text{pools-initial}}$ . If  $P_{\text{pools-x}}$  is less than 90% of  $P_{\text{pools-initial}}$  ( $P_{\text{pools-x}} < 0.90 \times P_{\text{pools-initial}}$ ), it is recommended that the pool size be reassessed and potentially adjusted to maximize pooling efficiency.
- To ensure maximum pooling efficiency, it is recommended that  $n_{\text{maxefficiency}}$  be reassessed periodically while sample pooling is implemented by the laboratory.

**Note:** When resource availability is sufficient to meet testing demand, the FDA recommends that laboratories consider whether the risks of reduced test sensitivity with pooling outweigh the benefits of resource conservation.

## Pool patient samples

Up to 5 patient samples can be combined into a single pool before RNA extraction. Each RT-PCR reaction plate can contain pools of different numbers of samples (2–5) as well as individual samples. The volume of each individual patient sample or sample pool used in RNA extraction is 400  $\mu\text{L}$ .

**Note:** The pooling of samples has only been validated using the automated and manual extraction methods with 400  $\mu\text{L}$  of pooled clinical samples.

Use your laboratory protocols and systems to name and track the samples in pools throughout the workflow.

**IMPORTANT!** Pools with test results that are positive, invalid, or inconclusive require extraction and testing of the individual patient samples.

1. Combine equal volumes of up to 5 patient samples into a single tube at a total volume of  $\geq 400 \mu\text{L}$ . Example volumes are shown in the following table.

**Table 3** Example volumes of pooled samples

Sample	Volume		
	N = 5	N = 4	N = 3
1	100 $\mu\text{L}$	120 $\mu\text{L}$	150 $\mu\text{L}$
2	100 $\mu\text{L}$	120 $\mu\text{L}$	150 $\mu\text{L}$
3	100 $\mu\text{L}$	120 $\mu\text{L}$	150 $\mu\text{L}$
4	100 $\mu\text{L}$	120 $\mu\text{L}$	—
5	100 $\mu\text{L}$	—	—
<b>Total volume</b>	<b>500 <math>\mu\text{L}</math></b>	<b>480 <math>\mu\text{L}</math></b>	<b>450 <math>\mu\text{L}</math></b>

2. Store the remaining unpooled volumes of patient samples according to CDC guidelines.

# 3

## Extract RNA (automated method)

- Before you begin ..... 20
- Extract RNA—Automated method (400-µL sample input volume) ..... 21

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 an individual or pooled sample input volume of 400 µL.

### Before you begin

**IMPORTANT!** The Binding Bead Mix is not compatible with bleach. For more information, see the SDS.

**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 individual patient samples or sample pools to be processed, plus one Negative Control per plate.
- Prepare 1 mL of fresh 80% Ethanol per reaction using Ethanol, Absolute, Molecular Biology Grade and Nuclease-Free Water (not DEPC-Treated) for the required number of reactions, plus 10% overage.
- 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 items can be used to hold the tip comb instead of the KingFisher™ 96 KF microplate:

- 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
- Nunc™ F96 MicroWell™ Black Polystyrene Plate
- Nunc™ F96 MicroWell™ White Polystyrene Plate
- KingFisher™ 96 Deep-Well Plate

- 
- Mark the Negative Control well on the plate.

## Extract RNA—Automated method (400- $\mu$ L 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- $\mu$ L 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\_2Wash\_400\_Flex** program has been downloaded from the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit product page at [www.thermofisher.com](http://www.thermofisher.com) and loaded onto the instrument.

## Prepare the processing plates (400- $\mu$ L sample input volume)

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	1,000 $\mu$ L
Wash 2 Plate	3		80% Ethanol	1,000 $\mu$ L
Elution Plate	4		Elution Solution	50 $\mu$ L
Tip Comb Plate	5	Place a KingFisher™ 96 tip comb for deep-well magnets in a KingFisher™ 96 KF microplate		

**Note:** The following items can be used to hold the tip comb instead of the KingFisher™ 96 KF microplate:

- 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
- Nunc™ F96 MicroWell™ Black Polystyrene Plate
- Nunc™ F96 MicroWell™ White Polystyrene Plate
- KingFisher™ 96 Deep-Well Plate

## Prepare Binding Bead Mix (400- $\mu$ L 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 <sup>[1]</sup>
Binding Solution	530 $\mu$ L
Total Nucleic Acid Magnetic Beads	20 $\mu$ L
<b>Total volume per well</b>	<b>550 <math>\mu</math>L</b>

<sup>[1]</sup> 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- $\mu$ L sample input volume)

This section provides volumes for the sample plate. Your plate layout will depend on the number of samples you run.

1. Add 10  $\mu$ L of Proteinase K to each well in the KingFisher™ 96 Deep-Well Plate labeled "Sample Plate".
2. Add 400  $\mu$ L of individual patient sample or sample pool to each sample well.
3. Add 400  $\mu$ L 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  $\mu$ L 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  $\mu$ L of MS2 Phage Control to each sample well and to the Negative Control well.

## Process the samples (400- $\mu$ L sample input volume)

1. Select the **MVP\_2Wash\_400\_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  $\mu$ L of Elution Solution (see "Prepare the processing plates (400- $\mu$ L sample input volume)" on page 22).

---

**Note:**

- Significant bead carry over may adversely impact RT-PCR performance. If bead carry over is observed, repeat the test by re-extracting 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.
- 

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

# 4

## Extract RNA (manual method)

- Before you begin ..... 24
- Extract RNA—Manual method (400- $\mu$ L sample input volume) ..... 24

Manual RNA extraction can be performed from an individual or pooled sample input volume of 400  $\mu$ L using either the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit or the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit.

### Before you begin

---

**IMPORTANT!** The Binding Bead Mix is not compatible with bleach. For more information, see the SDS.

---

**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 individual patient samples or sample pools to be processed, plus one Negative Control per plate.
- Prepare 1.5 mL of fresh 80% Ethanol per reaction using Ethanol, Absolute, Molecular Biology Grade and Nuclease-Free Water (not DEPC-Treated) for the required number of reactions, plus 10% overage.
- Mark the Negative Control well on the plate.

### Extract RNA—Manual method (400- $\mu$ L 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 (400- $\mu$ L 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 <sup>[1]</sup>
Binding Solution	530 $\mu$ L
Total Nucleic Acid Magnetic Beads	20 $\mu$ L
<b>Total volume per well</b>	<b>550 <math>\mu</math>L</b>

<sup>[1]</sup> 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- $\mu$ L sample input volume)

This section provides volumes for the sample plate. Your plate layout will depend on the number of samples you run.

1. Add 10  $\mu$ L of Proteinase K to each well of a KingFisher™ 96 Deep-Well Plate.
2. Add 400  $\mu$ L of individual patient sample or sample pool to each sample well.
3. Add 400  $\mu$ L 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  $\mu$ L 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  $\mu$ L 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- $\mu$ L 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 Solution 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  $\mu$ L of 80% Ethanol.
8. Dry the beads by shaking the plate (uncovered) at 1,050 rpm for 2 minutes.

## Elute the nucleic acid (400- $\mu$ L sample input volume)

1. Add 50  $\mu$ L 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) 96-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. If bead carry over is observed, repeat the test by re-extracting a new aliquot of the sample.

---

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



# Prepare RT-PCR reactions

## Guidelines for RT-PCR

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### IMPORTANT!

- For each RT-PCR reaction plate, include the following controls:
    - One Positive Control
    - One Negative 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 Control wells must be run on that 384-well reaction plate.
  - Prepare the RT-PCR reaction 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 RNA samples, and always use aerosol barrier pipette tips.
  - Maintain an RNase-free environment.
  - Protect assays from light.
  - Keep RNA samples and components on ice during use.
- 

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

Use this procedure to prepare RT-PCR reactions for the Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block).

1. If frozen, thaw the reagents on ice.
2. Gently vortex the reagents, then centrifuge briefly to collect liquid at the bottom of the tube.
3. Dilute TaqPath™ COVID-19 Control ( $1 \times 10^4$  copies/ $\mu\text{L}$ ) to a working stock of 25 copies/ $\mu\text{L}$ :
  - a. Pipet 98  $\mu\text{L}$  of TaqPath™ COVID-19 Control Dilution Buffer into a microcentrifuge tube, then add 2  $\mu\text{L}$  of TaqPath™ COVID-19 Control. Mix well, then centrifuge briefly.
  - b. Pipet 87.5  $\mu\text{L}$  of TaqPath™ COVID-19 Control Dilution Buffer into a second microcentrifuge tube, then add 12.5  $\mu\text{L}$  of the previous dilution. 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 RNA samples, plus one Positive Control per 384-well real-time RT-PCR plate, and one Negative Control from each extraction run.

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

All volumes include 10% overage for pipette error.

Component	Volume per RNA Sample or control	Volume for $n$ RNA Samples plus $y$ Negative Controls plus 1 Positive Control	Volume for 379 RNA Samples plus 4 Negative Controls plus 1 Positive Control
TaqPath™ 1-Step Multiplex Master Mix (No ROX™) (4X)	5.00 $\mu$ L	$5.50 \times (n + y + 1)$ $\mu$ L	2112.0 $\mu$ L
TaqPath™ COVID-19 Pooling Multiplex Assay	1.00 $\mu$ L	$1.10 \times (n + y + 1)$ $\mu$ L	422.4 $\mu$ L
<b>Total Reaction Mix volume</b>	<b>6.0 <math>\mu</math>L</b>	—	<b>2534.4 <math>\mu</math>L</b>

## 5. Set up the reaction plate:

- a. Pipet 6.0  $\mu$ L of the Reaction Mix prepared in step 4 into each well of a MicroAmp™ Optical 384-Well Reaction Plate with Barcode.  
Plates without a barcode can be used (see “Required materials not supplied” on page 8).
- b. Gently vortex the sealed plate containing the purified sample RNA and Negative 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 Control from the RNA extraction procedure. Add either sample RNA, Negative Control, or Positive Control to each well of the reaction plate according to Table 4 on page 29.

**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!** 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 false classification of samples.

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

**Table 4 Reaction plate volumes**

Component	Volume per reaction		
	RNA Sample reaction	Positive Control reaction	Negative Control reaction
Reaction Mix	6.0 $\mu$ L	6.0 $\mu$ L	6.0 $\mu$ L
Purified sample RNA (from RNA extraction)	14.0 $\mu$ L	—	—
Positive Control (diluted TaqPath™ COVID-19 Control from step 3)	—	2.0 $\mu$ L	—
Nuclease-free water	—	12.0 $\mu$ L	—
Purified Negative Control (from RNA extraction)	—	—	14.0 $\mu$ L
<b>Total volume</b>	<b>20.0 <math>\mu</math>L</b>	<b>20.0 <math>\mu</math>L</b>	<b>20.0 <math>\mu</math>L</b>



# Perform RT-PCR using the Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block)

- Dye calibration for the QuantStudio™ 7 Flex Real-Time PCR Instrument ..... 30
- Transfer the template (EDT) file for the QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block) ..... 30
- Set up and run the QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block) ..... 31

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

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 QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block)

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 the QuantStudio™ Real-Time PCR Software v1.3 is installed.

**IMPORTANT!** Be careful to select the correct template file. Failure to do so can cause errors in the analysis.

1. On the computer with Applied Biosystems™ COVID-19 Interpretive Software, navigate to the following directory (where <...> is the installation directory):  

```
<...>\Applied Biosystems\COVID-19 Interpretive Software\Client\docs\User Documents\COVID-19
```
2. Select the EDT file: TaqPath COVID-19 Kit Template QS7 384 1\_3 v2-2.edt
3. 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 the documents listed in Appendix D, “Documentation and support”.

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 30.

**IMPORTANT!** Be careful to select the correct template file. Failure to do so can cause errors in the analysis.

3. In the **Experiment Properties** tab, 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™
  - **Properties:** Standard
4. In the **Define** tab, in the **Targets** pane, confirm that the targets, reporter dyes, and quenchers are listed correctly.

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

5. In the **Define** tab, in the **Samples** pane, define a unique sample name for each well in the physical plate.

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

**IMPORTANT!** Do not use a patient name or associated identifying information in the sample name.

6. In the **Define** tab, confirm that the **Passive Reference** is set to **None**.
7. In the **Assign** tab, confirm that four targets are assigned to each well in the plate layout. To assign a target to a well, select the well, then check the **Assign** checkbox.

8. In the **Assign** tab, in the **Samples** pane, confirm the labeling of the control wells.
  - The template has one positive control (PC) and one negative control (NC) 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.

---

**Note:** If the 384-well plate includes additional negative controls (from combined extraction runs), define each additional negative control and assign it to a well to match the physical plate. Give each negative control a unique name (for example, **NC1**, **NC2**, **NC3**, and **NC4**).

---

9. In the **Assign** tab, confirm the **Task** assignments.
  - For wells with a Positive Control (PC), confirm that the **Task** is set to **S** for Standard for all of the targets.
  - For wells with a Negative Control (NC), confirm that the **Task** is set to **N** for Negative for all of the targets.
  - For the wells with a patient sample, confirm that the **Task** is set to **U** for Unknown for all of the targets.
10. In the **Assign** tab, assign a sample name to each well to match the physical plate. To assign a sample to a well, select the well, then check the **Assign** checkbox.
11. In the **Run Method** tab, confirm that the **Reaction Volume Per Well** is 20 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	

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



# 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 instrument service team. Go to <https://www.thermofisher.com/contactus>.

## 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. Sign in to the COVID-19 Interpretive Software, then click **COVID-19 Test**.

---

**Note:** The TaqPath™ COVID-19 Pooling Kit is not compatible with samples collected using the Everlywell™ COVID-19 Test Home Collection Kit, and therefore does not use the **COVID-19 + RNase P Test** option in the software.

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3. In the **Home** screen, click the **Import Samples** button.
4. 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.
5. In the **Home** screen, under **Batches**, click the <Batch ID> link for the batch of interest to display the **Batch Details** screen.
  - In the **Batch Details** screen, view the status and result for each sample in the **Sample Information** tab.
  - **Optional:** To identify signs of inadequate vortexing or centrifugation, select the **Amplification Plot** tab to view the amplification curve for each sample.
  - **Optional:** To enter a comment for the sample, select the **Amplification Plot** tab, select the **Comment** tab, enter a comment, then click **Add**.  
The comment will appear in all exports (CSV files) and reports (PDF files). It will include the user name and date.

6. To generate a Batch Export (CSV file), return to the **Home** screen, select the checkbox for the batch, then click **Export Batch** at the top of the **Home** screen. Click **Open folder location** in the dialog box to locate and open the CSV file.
7. To generate a Batch Report (PDF file), select the checkbox for the batch, then click **Report Batch** at the top of the **Home** screen. Click **Open folder location** in the dialog box to locate and open the PDF file.

REVOKED

## Interpretation of the results

Interpretation of the results is performed by the COVID-19 Interpretive Software. For information about the  $C_t$  values that are used by the software to interpret results, see Appendix A, “Ct cutoff values for assay targets”.

### Quality control and validity of results

A minimum of one Negative Control and one Positive Control must be present for each run.

If multiple extraction plates are combined on an RT-PCR plate, one Negative Control well must be included for each extraction plate. All control wells must pass for the RT-PCR plate to be considered valid.

Validation of results is performed automatically by the COVID-19 Interpretive Software based on performance of the Positive and Negative Controls. If the controls do not pass, the run is considered invalid and must be repeated.

**Table 5 Result interpretation for pooled patient samples and individual samples if applicable**

ORF1ab	N gene	S gene	MS2	Status	Result	Action	
						Pooled samples	Individual sample
NEG	NEG	NEG	NEG	INVALID	NA	Deconvolute by re-extracting and testing the individual samples in each pool. <sup>[1]</sup> (Proceed to the "Individual sample" Action column.)	Repeat the test by re-extracting the original sample and repeating the RT-PCR. If the repeat result remains invalid, consider collecting a new specimen.

Table 5 Result interpretation for pooled patient samples and individual samples if applicable (continued)

ORF1ab	N gene	S gene	MS2	Status	Result	Action	
						Pooled samples	Individual sample
NEG	NEG	NEG	POS	VALID	SARS-CoV-2 Not Detected	<p>Report the results to the healthcare provider and appropriate public health authorities. Consider testing for other viruses.</p> <p>Negative results are not definitive and, if inconsistent with clinical signs and symptoms or necessary for patient management, pooled samples should be tested individually.</p> <p>Negative results do not preclude SARS-CoV-2 infection and must not be used as the sole basis for patient management decisions. Negative results must be considered in the context of a patient's recent exposures, history, presence of clinical signs, and symptoms consistent with COVID-19.</p>	<p>N/A<sup>[2]</sup></p> <p>Testing of individual specimens that make up the negative pool is not necessary.</p>

**Table 5** Result interpretation for pooled patient samples and individual samples if applicable (continued)

ORF1ab	N gene	S gene	MS2	Status	Result	Action	
						Pooled samples	Individual sample
Only one SARS-CoV-2 target = POS			POS or NEG	VALID	SARS-CoV-2 Inconclusive	Deconvolute by extracting and testing the individual samples in each pool. <sup>[1]</sup> (Proceed to the "Individual sample" Action column.)	<ol style="list-style-type: none"> <li>Repeat the test by re-extracting the original sample and repeating the RT-PCR.</li> <li>After retesting one time, report the results to the healthcare provider and appropriate public health authorities.</li> </ol> <p>If the repeat result remains inconclusive, the healthcare provider should conduct additional confirmation testing with a new specimen, if clinically indicated.</p>
Two or more SARS-CoV-2 targets = POS			POS or NEG	VALID	Positive SARS-CoV-2	Deconvolute by extracting and testing the individual samples in each pool. <sup>[1]</sup> (Proceed to the "Individual sample" Action column.)	Repeat the test by re-extracting the original samples that make up the pool and repeating the RT-PCR. If the repeat result is invalid for any individual specimen, consider collecting a new specimen.

<sup>[1]</sup> Individual samples must only be tested using the workflow validated for the TaqPath™ COVID-19 Pooling Kit (400 µL of clinical sample for extraction, 50 µL eluate volume, 14 µL of purified sample RNA, 20-µL final PCR volume, run on the QuantStudio™ 7 Flex Real-Time PCR Instrument, 384-well block)

<sup>[2]</sup> N/A: Testing scenario is not applicable.

## Conditions of authorization for labs

The TaqPath™ COVID-19 Pooling 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/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas>.

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

- Authorized laboratories<sup>[4]</sup> using the TaqPath™ COVID-19 Pooling Kit must include with 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 specimen pooling strategies when testing patient specimens with the TaqPath™ COVID-19 Pooling Kit must include with negative test result reports for specific patients whose specimen(s) were the subject of pooling, a notice that pooling was used during testing and that "Patient specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing."
- Authorized laboratories must keep records of specimen pooling strategies implemented, including type of strategy, date implemented, quantities tested, and test result data generated as part of "Monitor the pooling strategy" on page 18. For the first 12 months from the date of their creation, such records must be made available to FDA within 48 business hours for inspection upon request. After 12 months from the date of their creation, upon FDA's request, such records must be made available for inspection within a reasonable time.
- Authorized laboratories using the TaqPath™ COVID-19 Pooling Kit must perform the procedures as outlined in the *TaqPath™ COVID-19 Pooling 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 Pooling Kit are not permitted.
- Authorized laboratories implementing pooling strategies for testing patient specimens must use the information provided in Chapter 2, "Pool samples" in the *TaqPath™ COVID-19 Pooling Kit Instructions for Use* to evaluate the appropriateness of continuing to use such strategies based on the recommendations in the protocol.
- Authorized laboratories that receive the TaqPath™ COVID-19 Pooling 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 Pooling Kit must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

<sup>[4]</sup> 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."

- 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](mailto:CDRH-EUA-Reporting@fda.hhs.gov)) and Thermo Fisher Scientific ([techservices@thermofisher.com](mailto: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 TaqPath™ COVID-19 Pooling Kit 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 Pooling Kit 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.

Analytical performance of the TaqPath™ COVID-19 Pooling Kit was determined by leveraging limit of detection (LoD), interfering substances, and cross-reactivity data for individual samples using the TaqPath™ COVID-19 Combo Kit and TaqPath™ COVID-19 Combo Kit Advanced, as described in the following sections.

## Limit of detection (LoD) for RT-PCR with 14.0 µL of purified sample RNA

The study results in this section were generated using the TaqPath™ COVID-19 Combo Kit Advanced workflow described in the *TaqPath™ COVID-19 Combo Kit Instructions for Use*. Note that the LoD results in Table 6 are for the unpooled TaqPath™ COVID-19 Combo Kit Advanced workflow. The TaqPath™ COVID-19 Pooling Kit utilizes the same RNA sample input and PCR workflow.

The LoD study with 14.0 µL of purified sample RNA established the lowest SARS-CoV-2 viral concentration (Genomic Copy Equivalents or GCE) that can be detected by the TaqPath™ COVID-19 Combo Kit Advanced at least 95% of the time. Negative nasopharyngeal swab (NP) matrix was spiked with gamma-irradiated SARS-CoV-2 virus at several concentrations and processed through the TaqPath™ COVID-19 Combo Kit Advanced 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 6** QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well), 14.0 µL of purified sample RNA

Concentration	Replicate	ORF1ab	N gene	S gene	MS2	Interpretation	% Positive
50 GCE/mL (≤5.6 GCE/reaction)	1	33.064	33.380	34.410	22.847	Positive	100%
	2	34.727	33.488	33.894	22.465	Positive	
	3	33.364	33.584	33.596	22.569	Positive	
	4	32.872	33.518	34.151	22.291	Positive	
	5	33.359	34.923	33.267	22.099	Positive	
	6	33.379	Not detected	35.909	22.514	Positive	
	7	33.738	33.190	36.431	21.908	Positive	
	8	33.430	34.375	33.968	21.933	Positive	
	9	34.121	35.457	34.250	21.829	Positive	
	10	32.608	34.032	34.510	21.825	Positive	

**Table 6** QuantStudio 7 Flex Real-Time PCR Instrument (384-well), 14.0 µL of purified sample RNA (continued)

Concentration	Replicate	ORF1ab	N gene	S gene	MS2	Interpretation	% Positive
50 GCE/mL (≤5.6 GCE/reaction)	11	32.958	Not detected	33.301	22.152	Positive	100%
	12	33.760	33.723	33.910	22.473	Positive	
	13	33.657	33.837	35.406	22.137	Positive	
	14	Not detected	34.200	33.961	21.745	Positive	
	15	33.560	35.388	33.713	22.517	Positive	
	16	34.338	34.091	34.007	21.838	Positive	
	17	33.350	34.315	34.410	21.657	Positive	
	18	34.025	33.251	34.292	21.584	Positive	
	19	33.277	34.955	34.468	21.541	Positive	
	20	33.562	33.137	34.488	21.551	Positive	

## Reactivity (Inclusivity)

*In silico* analysis was updated using 82,596 sequenced SARS-CoV-2 genomes in the GISAID database from January 18, 2023 to March 18, 2023, which include instances of emerging SARS-CoV-2 variants that are under global surveillance. Mismatch melting temperature analysis was performed, in which a positive is called when at least two of the three target assays (ORF1ab, S-gene, and N-gene) show a melting temperature higher than the annealing temperature. Based on the melting temperature analysis, the TaqPath™ COVID-19 Pooling Kit correctly identified 82,350 (99.7%) of known SARS-CoV-2 strains/isolates in GISAID. Evaluation of assay components that did not match 100% to target sequences indicated that most of the primer or probe mismatches are unlikely to impact assay function, thus the COVID-19 test is expected to be highly inclusive for SARS-CoV-2 strain detection.

## Interfering substances

The study results in this section were generated using the TaqPath™ COVID-19 Combo Kit workflow described in the *TaqPath™ COVID-19 Combo Kit Instructions for Use*. The TaqPath™ COVID-19 Pooling Kit also utilizes the ORF1ab, S gene, and N gene, therefore the results of the study are applicable.

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 listed in Table 7 on page 42. Each substance was tested with triplicate extractions. The results are presented in Table 7 on page 42.

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.

Table 7 Interfering substances

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% <sup>[1]</sup>	100%	100%	100%
Mucin: bovine submaxillary gland, type I-S	0.1 mg/mL	100% <sup>[2]</sup>	100%	100%	100%
Blood (human)	1% v/v	100% <sup>[3]</sup>	100%	100%	100%
Nasal sprays or drops—Nasacort™	10% v/v	100% <sup>[4]</sup>	100% <sup>[4]</sup>	100%	100%
Nasal corticosteroids—Dymista™	5 µg/mL	100% <sup>[2]</sup>	100%	100%	100%
NeilMed™ Nasogel™	1% w/v	100% <sup>[2]</sup>	100%	100%	100%
Influenza A H1N1 Brisbane/59/07	1 × 10 <sup>5</sup> TCID <sub>50</sub> /mL	100% <sup>[2]</sup>	100%	100%	100%
Throat lozenges, oral anesthetic and analgesic—Chloraseptic™	1% w/v	100% <sup>[3]</sup>	100%	100%	100%
Oseltamivir phosphate	33 µg/mL	100% <sup>[2]</sup>	100%	100%	100%
Antibiotic, nasal ointment—Bactroban™	5 µg/mL	100% <sup>[2]</sup>	100%	100%	100%
Antibacterial, systemic—Tobramycin	0.6 mg/mL	100% <sup>[2]</sup>	100%	100%	100%
Homeopathic allergy relief medicine—Similasan™ Nasal	10% v/v	100%	100%	100%	100%

<sup>[1]</sup> Two of six replicates produced a C<sub>t</sub> >37 or Undetermined for S Gene, but all replicates were called Positive based on the interpretation algorithm.

<sup>[2]</sup> Two of three replicates produced a C<sub>t</sub> >37 or Undetermined for S Gene, but all replicates were called Positive based on the interpretation algorithm.

<sup>[3]</sup> All three replicates produced a C<sub>t</sub> >37 or Undetermined for S Gene but were called Positive based on the interpretation algorithm.

<sup>[4]</sup> One of three replicates produced a C<sub>t</sub> >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 was performed.

**Table 8** Organisms used for *in silico* cross-reactivity analysis

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

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

Blast analysis showed  $\geq 80\%$  homology for one assay component (forward primer, reverse primer, or probe) for select isolates. Despite  $\geq 80\%$  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  $\geq 80\%$

homology to different isolates of the same species. For example, *Bacillus anthracis* strain AFS029987 had  $\geq 80\%$  homology to the ORF1ab forward primer while strain MCCC 1A01412 had  $\geq 80\%$  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 study evaluated the performance of the TaqPath™ COVID-19 Pooling Kit by testing pools of 3 samples and 5 samples containing a mix of positive and negative nasopharyngeal swab specimens, and comparing the results to tests of the individual positive specimens in those pools.

The following samples and sample pools were used:

- 42 individual SARS-CoV-2-positive clinical specimens
- 42 3-sample pools, formulated using material from two negative specimens and one positive specimen
- 42 5-sample pools, formulated using material from four negative specimens and one positive specimen

The tests were performed using the TaqPath™ COVID-19 Pooling Kit on the QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block). The positive percent agreement (PPA) was calculated by comparing the results for each sample pool to the corresponding results for the individual positive specimens used to formulate each pool. The negative percent agreement (NPA) was calculated by comparing the results for each sample pool to the corresponding results for the individual negative specimens used to formulate each pool.

The concordance between all positive pool results and individual positive specimen results was 100%.

**Table 9 Clinical evaluation study**

Pool type	Number of pools	Mean C <sub>t</sub> (individual)	Mean C <sub>t</sub> (pooled)	PPA	NPA
3-sample positive pool	42	ORF1ab = 19.9	ORF1ab = 21.8	100% (95% CI [91.6%–100%]) <sup>[1]</sup>	N/A <sup>[2]</sup>
		N gene = 20.7	N gene = 22.3		
		S gene = 20.6	S gene = 22.3		
5-sample positive pool	41 <sup>[3]</sup>	ORF1ab = 19.9	ORF1ab = 22.3	100% (95% CI [91.4%–100%])	N/A
		N gene = 20.7	N gene = 22.9		
		S gene = 20.6	S gene = 23.0		
5-sample negative pool <sup>[4]</sup>	29	ORF1ab = ND <sup>[5]</sup>	ORF1ab = ND	N/A	100% (95% CI [88.8%–100%])

**Table 9 Clinical evaluation study (continued)**

Pool type	Number of pools	Mean C <sub>t</sub> (individual)	Mean C <sub>t</sub> (pooled)	PPA	NPA
5-sample negative pool <sup>[4]</sup>	29	N gene = ND	N gene = ND	N/A	100% (95% CI [88.8%–100%])
		S gene = ND	S gene = ND		

<sup>[1]</sup> 95% CI = Two-sided 95% score confidence interval.

<sup>[2]</sup> N/A: Not applicable.

<sup>[3]</sup> One pool was called invalid on the QuantStudio™ 7 Flex Real-Time PCR Instrument and removed from performance calculations.

<sup>[4]</sup> Only 5-sample negative pools were evaluated (worst case), since validation for 5-sample pooling is considered validated for 2-, 3-, or 4-sample pools.

<sup>[5]</sup> ND: Not Detected (Negative).

A linear relationship was observed between individual and pooled specimens with an expected Ct shift for each target. By using a Passing-Bablok linear regression model, the slope and y-intercept were calculated for each target (Table 10 on page 45).

**Table 10 C<sub>t</sub> shifts for 3- and 5-sample pools**

Pool type	Target	Slope	Y-intercept
3-sample pool	ORF1ab	1.01	1.54
	N gene	0.99	1.73
	S gene	1.02	1.37
5-sample pool	ORF1ab	1.02	2.09
	N gene	1.02	2.11
	S gene	1.06	1.54

*In silico* analysis was conducted to evaluate the effect of 3-sample and 5-sample pooling on the clinical sensitivity of the TaqPath™ COVID-19 Pooling Kit using historical data consisting of 251 individually tested consecutive positive nasopharyngeal and anterior nasal swab specimens collected from four geographic locations. This analysis used the Passing-Bablok regression coefficients (slope and intercept) generated from a pooling validation study to estimate the C<sub>t</sub> shift for each gene target for individual samples due to N-sample pooling.

For each gene target, the C<sub>t</sub> values at the sample-level limit of detection (95% detection) and at the calling threshold (50% detection) were used to estimate detection in dilution-adjusted samples by generating a piecewise linear approximation to a sigmoid curve that estimates the percent detection for four different C<sub>t</sub> Zones, defined as follows:

- Zone 1: C<sub>t</sub> 40 to Calling Threshold minus Shift
- Zone 2: Calling Threshold minus Shift to LoD
- Zone 3: LoD to LoD minus Shift
- Zone 4: LoD minus Shift to C<sub>t</sub> 0

The estimated percent detection for each C<sub>t</sub> Zone due to pooling was then applied to individual sample testing data collected across four sites (384-well formats), where the observed positivity rate was between 9% and 25%.

**Table 11** *In silico* analysis results using samples collected from various testing sites (United States)

Site	N samples	Total number of Positive samples	PPA for Positive N=3 <sup>[1]</sup>	PPA for Positive N=5 <sup>[1]</sup>
Site 1	368	76	99.9%	99.3%
Site 2	368	93	100.0%	99.5%
Site 3	370	48	100.0%	100.0%
Site 4	376	34	99.7%	99.0%
<b>Total</b>	<b>1,482</b>	<b>251</b>	<b>99.9%</b>	<b>99.4%</b>

<sup>[1]</sup> PPA calculations exclude inconclusive samples. Inconclusive samples should be retested according to Table 5 on page 35.

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# C<sub>t</sub> cutoff values for assay targets

The Applied Biosystems™ COVID-19 Interpretive Software uses the following C<sub>t</sub> cutoff values for assay targets during interpretation of the results.

Table 12 C<sub>t</sub> cutoff values

Sample or Control	Target	C <sub>t</sub> cutoff
Positive Control	MS2	Valid C <sub>t</sub> values are >37 <sup>[1]</sup>
	Viral targets	Valid C <sub>t</sub> values are ≤37
Negative Control	MS2	Valid C <sub>t</sub> values are ≤32
	Viral targets	Valid C <sub>t</sub> values are >37 <sup>[2]</sup>
Clinical samples	MS2	Valid C <sub>t</sub> values are ≤32 <sup>[3]</sup>
	Viral targets	Positive C <sub>t</sub> values are ≤37

<sup>[1]</sup> The software will report Negative for the MS2 target.

<sup>[2]</sup> The software will report Negative for each viral target, and a result of "SARS-CoV-2 Not Detected".

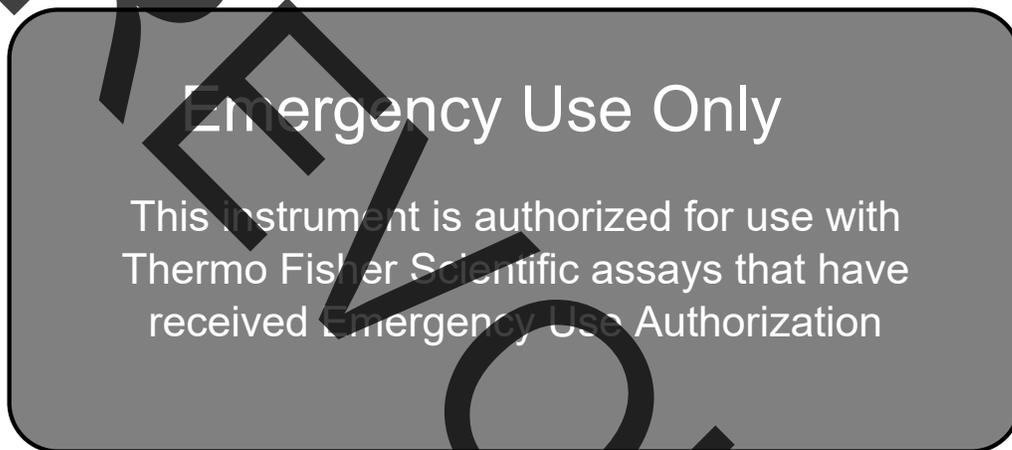
<sup>[3]</sup> If any of the viral targets is positive, the C<sub>t</sub> for MS2 can be >32 (reported as Negative in the software)



# EUO label for RUO instrument

For the Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR Instrument, affix the Emergency Use Only (EUO) label on each instrument and retain this labeling throughout the Emergency Use Authorization (EUA) use of the 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.



**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, visit [thermofisher.com/support](https://www.thermofisher.com/support).

## 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:  
[www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2020-P.pdf](http://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](http://www.who.int/publications/i/item/9789240011311)



# Documentation and support

## Related documentation

Document	Publication Number
<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>MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit Instructions For Use</i>	MAN0019746
<i>MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (automated extraction) User Guide</i>	MAN0018073
<i>MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (manual extraction) User Guide</i>	MAN0018072
<i>Thermo Scientific™ KingFisher™ Flex User Manual</i>	MAN0019870
<i>COVID-19 Interpretive Software Installation Quick Reference</i>	MAN0019257

## Customer and technical support

For additional documentation and information about this kit, visit:

<https://www.thermofisher.com/covid19eua>

For download instructions for the COVID-19 Interpretive Software, see “Obtain the Applied Biosystems™ COVID-19 Interpretive Software” on page 33.

Refer to the Software Release Notes 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)

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

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## 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](http://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](http://www.thermofisher.com/support).

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