

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 M.0

IVD

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

ThermoFisher
S C I E N T I F I C



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

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Revision history: Pub. No. MAN0019181

Revision	Date	Description
M.0	31 August 2022	<ul style="list-style-type: none">Reactivity/inclusivity data were updated (“Reactivity (Inclusivity)” on page 110).Compatible firmware and software versions were updated (“Instrument, assay, and software compatibility” on page 16).
L.0	18 May 2022	<ul style="list-style-type: none">The version numbers of the COVID-19 Interpretive Software were updated to v1.6 and v2.6.References to the TaqPath™ COVID-19 Combo Kit Advanced were removed throughout.Reactivity/inclusivity data were updated (“Reactivity (Inclusivity)” on page 110).
K.0	5 October 2021	<ul style="list-style-type: none">The Intended Use and warnings and precautions were updated (“Intended Use” on page 10 and “Warnings and precautions” on page 17).References to nasal swabs were modified throughout to specify anterior nasal swabs.Additional guidance was provided on obtaining the correct software or firmware version (“Instrument, assay, and software compatibility” on page 16).The impact of mutations on performance was added to the assay limitations (“Assay limitations” on page 18).In-use stability information was added for the TaqPath™ COVID-19 Control (“In-use stability for the TaqPath™ COVID-19 Control” on page 11).Preparing RT-PCR reactions was added as a step in the workflow diagram (page 20).A note was added that the Binding Bead Mix in the RNA extraction procedures is not compatible with bleach.Language was added that the steps for preparing RT-PCR reactions should be done on ice.HIPAA (Health Insurance Portability and Accountability Act) information was added to two topics (“Samples and controls” on page 19 and “Warnings and precautions” on page 17) and to the procedures to set up and run real-time PCR instruments.Reactivity/inclusivity data were updated (page 110).The following label information was added: Appendix C, “EUO label for RUO instruments”.A step was added to specify loading the plate into the real-time PCR instrument for the following instruments:<ul style="list-style-type: none">7500 Fast Real-Time PCR Instrument (used with 7500 Software v2.3)7500 Real-Time PCR InstrumentQuantStudio™ 5 Real-Time PCR Instrument (96-well 0.2-mL block, 96-well 0.1-mL block, and 384-well block)QuantStudio™ 7 Flex Real-Time PCR InstrumentThe <i>MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit Instructions For Use</i> was added to the list of related documents.The Software Release Notes were referenced for additional software information, and the support URL was corrected (“Customer and technical support” on page 124).Use of a MicroAmp™ Optical Film Compression Pad was added for the QuantStudio™ 5 Real-Time PCR Instrument (96-well, 0.2-mL block) and a MicroAmp™ Optical 96-Well Reaction Plate (with or without barcode).A safety appendix was added (“Safety” on page 121).Results for reference material testing using an FDA protocol were added (“FDA SARS-CoV-2 Reference Panel Testing” on page 115).
J.0	22 February 2021	Provided new options for RT-PCR plates.

Revision	Date	Description
H.01	10 December 2020	Internal document control update only, with no changes to Instructions for Use rev. H.0 released on DCO1199965 and posted to the thermofisher.com website. Update to align the 20 Nov 2020 FDA-released Instructions for Use with removal of the licensing statement covering Limited Use Label Licenses on page 2.
H.0	10 November 2020	<ul style="list-style-type: none"> The instructions for using the TaqPath™ COVID-19 Combo Kit Advanced were updated to indicate that the kit is now compatible with all instruments. The compatible COVID-19 Interpretive Software versions were updated to v1.5 and 2.5, which include amplification curves. Instructions were added on what to do in the event of bead carry over from RNA extraction. The licensing statement covering Limited Use Label Licenses was removed from page 2.
G.0	9 October 2020	<ul style="list-style-type: none"> Guidance and protocols were added for samples collected using the Everlywell™ COVID-19 Test Home Collection Kit, including use of the new TaqMan™ SARS-CoV-2 RNase P Assay Kit. Information and protocols were added for the TaqPath™ COVID-19 Combo Kit Advanced (Cat. No. A47813), which supports preparation of RT-PCR reactions from 17.5 µL and 14.0 µL of purified sample RNA. “Intended Use” on page 10 and Chapter 12, “Conditions of authorization for labs” were updated with information for the Everlywell™ COVID-19 Test Home Collection Kit and the TaqPath™ COVID-19 Combo Kit Advanced. COVID-19 Interpretive Software v1.4 and v.2.4 were added to support use of the RNase P assay, and instructions for obtaining the software were updated. Minor corrections were made to the kit configuration information for the TaqPath™ COVID-19 Combo Kit. General laboratory recommendations were added (see page 19). The name of the Wash Buffer was changed to Wash Solution in the RNA extraction procedures. A note was added that the Wash Solution can develop particulates but this does not affect performance. “Interpretation of the results” on page 103 was updated to include results for the RNase P assay and guidance on reporting results to the appropriate public health authorities. Minor updates were made to the Intended Use.
F.0	15 July 2020	<ul style="list-style-type: none"> Added Applied Biosystems™ COVID-19 Interpretive Software v1.3 and Applied Biosystems™ COVID-19 Interpretive Software v2.3. Removed Applied Biosystems™ COVID-19 Interpretive Software v1.2, Applied Biosystems™ COVID-19 Interpretive Software v2.0, and Applied Biosystems™ COVID-19 Interpretive Software v2.2. Added C_t cutoff information (Appendix B, “Ct cutoff values for assay targets”). Removed instructions to mix by pipetting up and down 10 times when preparing RT-PCR plates. Added instructions to vortex the plates to ensure proper mixing. Updated centrifuge and sealing instructions when preparing the RT-PCR plates. Updated instructions to create a unique name for each well in the physical plate, not just the wells with a patient sample. Updated reactivity (inclusivity) (page 110) and the warnings and precautions (page 17). Specified that retesting must be performed by re-extracting the original sample and repeating the RT-PCR (“Interpretation of the results” on page 103).
E.0	12 May 2020	<p>Added the following products as an alternative to the KingFisher™ 96 KF microplate for the tip comb plate:</p> <ul style="list-style-type: none"> Tip Comb Presenting Plate for KF 96 Nunc™ MicroWell™ 96-Well Microplate, barcoded Nunc™ MicroWell™ 96-Well Microplate, Flat Bottom Nunc™ F96 MicroWell™ Black Polystyrene Plate KingFisher™ 96 Deep-Well Plate

Revision	Date	Description
D.0	11 May 2020	<ul style="list-style-type: none"> • Updated the list of acceptable sample types and added specimen storage conditions. • Removed catalog numbers for combo kit components. • Updated materials listed in “Required materials not supplied” on page 12. • Updated the automated RNA extraction procedure with new plastics, new instrument programs, and revised wash and plate handling steps. • Updated the manual RNA extraction procedure with an optional ethanol volume for 200-µL sample input volumes, and an option to use the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit for 400-µL sample input volumes. • Updated the real-time RT-PCR preparation procedure with new instructions for 384-well plates, clarified guidelines for negative controls, and optional plates without barcodes. • Added procedures for the QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block) and the QuantStudio™ 5 Real-Time PCR Instrument (384-well block). • Added instrument firmware requirements and removed calibration plates for the QuantStudio™ 5 Real-Time PCR Instrument. • Added Applied Biosystems™ COVID-19 Interpretive Software v2.2. • Updated control requirements in interpretation of results, based on addition of 384-well plates.
C.0	20 April 2020	<ul style="list-style-type: none"> • Removed 100-reaction kit. • Added a catalog number for the KingFisher™ 96 Deep-Well 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, 96-well reaction plate, COVID-19 assay only)” on page 41 and “Prepare the RT-PCR reactions (200-µL sample input, 96-well reaction plate, COVID-19 assay only)” on page 37). • Specified that retesting must be done with the original sample (“Interpretation of the results” on page 103). • Reorganized the content to perform RT-PCR based on the real-time PCR instrument. • Added “Interfering substances” on page 111. • Added information to customer and technical support (page 124).
B.0	24 March 2020	<ul style="list-style-type: none"> • 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).

Revision	Date	Description
B.0 <i>(continued)</i>	24 March 2020	<ul style="list-style-type: none"> • 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™ COVID-19 RT-PCR 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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TaqPath™ COVID-19 Combo Kit product information

Intended Use

The 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 nasopharyngeal, oropharyngeal, anterior nasal, and mid-turbinate swabs, nasopharyngeal aspirates, and bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

This test is also authorized for use with the Everlywell™ COVID-19 Test Home Collection Kit when used consistent with its authorization.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory and 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 test results to the appropriate public health authorities.

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

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

Product description

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, anterior nasal, and mid-turbinate swabs, and nasopharyngeal aspirate) and bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19 by their healthcare provider.

Each kit includes the following components:

- 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 as an internal process control for nucleic acid extraction
- TaqPath™ COVID-19 Control as a positive 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)

Box	Components	Amount	Storage
TaqPath™ COVID-19 RT-PCR Kit	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 (1 × 10 ⁴ copies/µL)		2 × 10 µL per box; 5 boxes per kit	≤ -70°C
TaqPath™ COVID-19 Control Dilution Buffer		2 × 250 µL per box; 5 boxes per kit	-30°C to -10°C

In-use stability for the TaqPath™ COVID-19 Control

TaqPath™ COVID-19 Control	Temperature	Stability
TaqPath™ COVID-19 Control, not diluted (1 × 10 ⁴ copies/µL)	3.6–4.6°C ^[1]	48 hours
TaqPath™ COVID-19 Control, working concentration (25 copies/µL)	3.6–4.6°C (including in open tubes) ^[1]	48 hours

^[1] RNA will not be impacted by lower temperature as long as it is not frozen.

Required materials not supplied

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

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

Item	Source
Real-time PCR instrument (for firmware and software compatibility for each instrument, see “Instrument, assay, and software compatibility” on page 16)	
Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument	4406984 (with laptop computer) 4406985 (with tower computer)
Applied Biosystems™ 7500 Fast Real-Time PCR Instrument	4351106 (with laptop computer) 4351107 (with desktop computer)
Applied Biosystems™ 7500 Real-Time PCR Instrument	4351104 (with laptop computer) 4351105 (with desktop computer)
Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block	A28569 (with laptop computer) A28574 (with desktop computer) A28139 (instrument only)
Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.1-mL block	A28568 (with laptop computer) A28573 (with desktop computer) A28138 (instrument only)
Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument, 384-well block	A28570 (with laptop computer) A28575 (with desktop computer) A28140 (instrument only)
Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR Instrument, 384-well block	4485695 (with laptop computer) 4485701 (with desktop computer)
Equipment	
Laboratory freezers <ul style="list-style-type: none"> • -30°C to -10°C • $\leq -70^{\circ}\text{C}$ 	MLS
Centrifuge, with a rotor that accommodates standard and deepwell microplates	MLS
Microcentrifuge	MLS
Laboratory mixer, vortex or equivalent	MLS

(continued)

Item	Source
Single and multichannel adjustable pipettors (1.00 µL to 1,000.0 µL)	MLS
Cold block (96-well or 384-well) 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
KingFisher™ 96 Deep-Well Plate	95040450 , A48305 , A48424 , 95040455
96-well plate for the tip comb, one of the following: <ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • 97002540 • 267600 • 167008 • 269787 • AB0796 • AB1127 • 137101 • 136101 • 95040450, A48305, A48424, 95040455
KingFisher™ 96 tip comb for deep-well magnets	97002534 , A48438 , A48414
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™ 96 Deep-Well Plate	95040450 , A48305 , A48424 , 95040455
Standard 96-well plate for the eluate, one of the following: <ul style="list-style-type: none"> • KingFisher™ 96 KF microplate • MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL • MicroAmp™ Fast Optical 96-Well Reaction Plate, 0.1 mL • MicroAmp™ Optical 96-Well Reaction Plate with Barcode, 0.2 mL • MicroAmp™ Optical 96-Well Reaction Plate, 0.2 mL 	<ul style="list-style-type: none"> • 97002540 • 4346906, 4366932 • 4346907 • 4306737, 4326659 • N8010560, 4316813

(continued)

Item	Source
MicroAmp™ Clear Adhesive Film	4306311
Kits and reagents	
Required if specimens were collected using the Everlywell™ COVID-19 Test Home Collection Kit: TaqMan™ SARS-CoV-2 RNase P Assay Kit	A49564
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
Fisher BioReagents™ Ethanol, Absolute, Molecular Biology Grade ^[1] , or equivalent	BP2818100, BP2818500, BP28184
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 Multiplex 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 Multiplex qPCR, 96-well (0.2-mL)	A24737
Calibration plates (QuantStudio™ 7 Flex Real-Time PCR Instrument)	
ABY™ Dye Spectral Calibration Plate for Multiplex qPCR, 384-well	A24736
JUN™ Dye Spectral Calibration Plate for Multiplex qPCR, 384-well	A24733
Tubes, plates, and other consumables	
MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL	4346906, 4366932
MicroAmp™ Fast Optical 96-Well Reaction Plate, 0.1 mL	4346907

(continued)

Item	Source
MicroAmp™ EnduraPlate™ Optical 96-Well Fast Clear Reaction Plates with Barcode ^[2]	4483485, 4483494
MicroAmp™ EnduraPlate™ Optical 96-Well Fast Clear Reaction Plates ^[2]	A36930
MicroAmp™ Optical 96-Well Reaction Plate with Barcode, 0.2 mL	4306737, 4326659
MicroAmp™ Optical 96-Well Reaction Plate, 0.2 mL	N8010560, 4316813
MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode ^[2]	4483354, 4483352
MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates ^[2]	A36924
MicroAmp™ Optical 384-Well Reaction Plate with Barcode	4309849, 4326270, 4343814
MicroAmp™ Optical 384-Well Reaction Plate	4343370
MicroAmp™ EnduraPlate™ Optical 384-Well Clear Reaction Plates with Barcode ^[2]	4483285, 4483273
MicroAmp™ EnduraPlate™ Optical 384-Well Clear Reaction Plates ^[2]	A36931
MicroAmp™ Clear Adhesive Film	4306311
MicroAmp™ Optical Adhesive Film	4311971, 4360954
MicroAmp™ Adhesive Film Applicator	4333183
MicroAmp™ Optical Film Compression Pad ^[3]	4312639
Nonstick, RNase-free microcentrifuge tubes (1.5 mL and 2.0 mL)	thermofisher.com/plastics
Sterile aerosol barrier (filtered) pipette tips	thermofisher.com/pipettetips

^[1] Available at fisherscientific.com.

^[2] Not compatible with the Everywell™ COVID-19 Test Home Collection Kit procedure.

^[3] Required for QuantStudio™ 5 Real-Time PCR Instrument (96-well, 0.2-mL block) and a MicroAmp™ Optical 96-Well Reaction Plate (with or without barcode).

Instrument, assay, and software compatibility

The following table lists the version of the Applied Biosystems™ COVID-19 Interpretive Software that is compatible with your instrument, its associated data collection software, and whether the TaqMan™ SARS-CoV-2 RNase P Assay is used in the test procedure.

Note: The TaqMan™ SARS-CoV-2 RNase P Assay is required for specimens collected using the Everlywell™ COVID-19 Test Home Collection Kit.

For information on how to obtain the Applied Biosystems™ COVID-19 Interpretive Software, see “Obtain the Applied Biosystems™ COVID-19 Interpretive Software” on page 102.

To obtain the correct data collection software or firmware version for your real-time PCR instrument, go to [thermofisher.com/validatedfirmware](https://www.thermofisher.com/validatedfirmware), then select your instrument in the **Real-Time PCR** section. If you have questions or problems finding the correct version, contact technical support (see Appendix D, “Documentation and support”).

Instrument	Data collection software used with the instrument	Minimum compatible COVID-19 Interpretive Software version
7500 Fast Dx Real-Time PCR Instrument	SDS Software v1.4.1	v1.6
7500 Fast Real-Time PCR Instrument	SDS Software v1.5.1 or 7500 Software v2.3	v1.6
7500 Real-Time PCR Instrument	7500 Software v2.3	v1.6
QuantStudio™ 5 Real-Time PCR Instrument (96-well 0.2-mL or 0.1-mL block, or 384-well block) with firmware v1.3.3	QuantStudio™ Design and Analysis Desktop Software v1.5.1 or v1.5.2	v2.6
QuantStudio™ 5 Real-Time PCR Instrument (96-well 0.2-mL or 0.1-mL block, or 384-well block) with firmware v1.5.1	QuantStudio™ Design and Analysis Desktop Software v1.5.2	v2.6
QuantStudio™ 7 Flex Real-Time PCR Instrument with instrument firmware v1.0.4 (384-well block)	QuantStudio™ Real-Time PCR Software v1.3	v2.6

Warnings and precautions

The TaqPath™ COVID-19 RT-PCR 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.

- Caution: Federal law restricts this device to sale by or on the order of a licensed practitioner.
- For *in vitro* diagnostic use.
- For use under Emergency Use Authorization Only.
- This product has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or 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 “Contents and storage” on page 11.
- Do not use the kits 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 test results to the appropriate public health authorities.
- Positive results are indicative of the presence of SARS-CoV-2 RNA.
- Encrypt, pseudonymize, or anonymize personal data where possible following the requirements of HIPAA (Health Insurance Portability and Accountability Act).

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.
- TaqPath™ COVID-19 RT-PCR Kit performance was established using nasopharyngeal and oropharyngeal swab, nasopharyngeal aspirate, and bronchoalveolar lavage samples only. Anterior nasal swabs and mid-turbinate swabs are considered acceptable specimen types for use with the TaqPath™ COVID-19 RT-PCR 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, oropharyngeal, anterior nasal and mid-turbinate nasal swabs, nasopharyngeal aspirate, and bronchoalveolar lavage should not be tested with this assay.
- 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.
- Specimens submitted using the Everlywell™ COVID-19 Test Home Collection Kit must be tested using the TaqMan™ SARS-CoV-2 RNase P Assay Kit and the TaqPath™ COVID-19 RT-PCR Kit.
- 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™ COVID-19 RT-PCR 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 test results 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. For mutations associated with specific variants that may potentially have an impact on performance of the TaqPath™ COVID-19 RT-PCR Kit, see [fda.gov/medical-devices/letters-health-care-providers/genetic-variants-sars-cov-2-may-lead-false-negative-results-molecular-tests-detection-sars-cov-2](https://www.fda.gov/medical-devices/letters-health-care-providers/genetic-variants-sars-cov-2-may-lead-false-negative-results-molecular-tests-detection-sars-cov-2).
- Detection of RNase P indicates that human nucleic acid is present and implies that human biological material was collected and successfully extracted and amplified. It does not necessarily indicate that the specimen is of appropriate quality to enable detection of SARS-CoV-2.
- MicroAmp™ EnduraPlate™ products are not compatible with the Everlywell™ COVID-19 Test Home Collection Kit procedure.

General laboratory recommendations

- Follow standard operating procedures in your laboratory to prevent contamination, such as the following:
 - Frequent glove changes
 - Frequent decontamination of surfaces, equipment, and pipettes with fresh 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 for signs of inadequate vortexing or centrifugation.

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>.

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

Include the following controls:

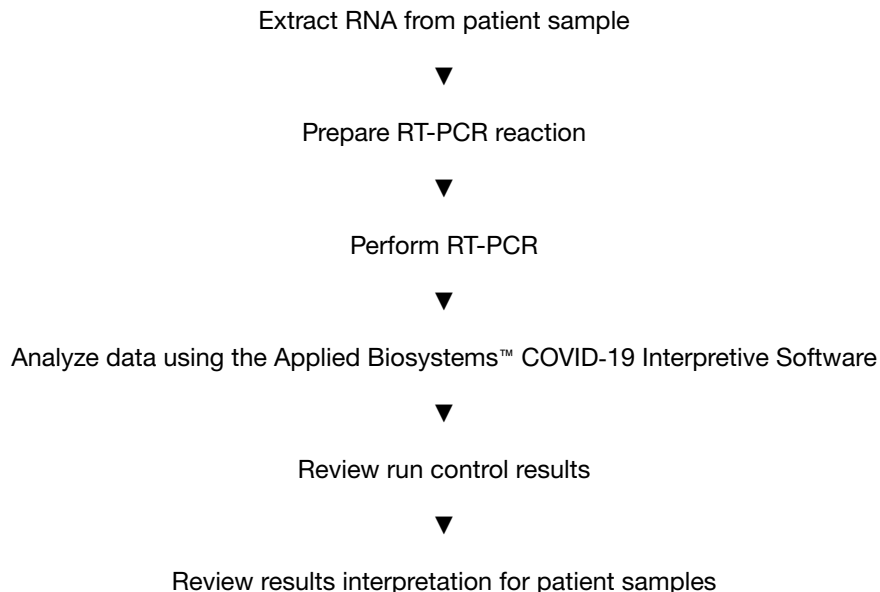
Control	Used to monitor	Assays
Positive Control (TaqPath™ COVID-19 Control Kit)	RT-PCR reaction setup and reagent integrity	All three SARS-CoV-2 assays
MS2 Phage Control	RNA extraction	MS2 assay
Negative Control	Cross-contamination during RNA extraction and reaction setup	All three SARS-CoV-2 assays
		MS2 assay
TaqMan™ SARS-CoV-2 RNase P Assay control ^[1]	Sample adequacy	RNase P assay

^[1] Used with the Everlywell™ COVID-19 Test Home Collection Kit workflow.

Sample collection, transport, and storage

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

Workflow



The workflow begins with nucleic acid extraction from upper respiratory specimens (such as nasopharyngeal, oropharyngeal, anterior 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 123.

The RT-PCR reactions are prepared. For specimens collected using the Everlywell™ COVID-19 Test Home Collection Kit, the TaqMan™ SARS-CoV-2 RNase P Assay Kit is included as an additional control.

The nucleic acid is reverse transcribed into cDNA and amplified using the TaqPath™ COVID-19 RT-PCR 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, 96-well, 0.2-mL block
- Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.1-mL block
- Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument, 384-well block
- 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.

The workflow options have been updated to reduce the number of consumables. Previous versions of the workflow in previous revisions of this document are still validated to run the TaqPath™ COVID-19 RT-PCR Kit.

2

Extract RNA (automated method)

- Before you begin 22
- Extract RNA—Automated method (200-µL sample input volume) 23
- Extract RNA—Automated method (400-µL sample input volume) 26

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

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 patient samples to be processed, plus one Negative Control per plate.
- Prepare fresh 80% Ethanol using Ethanol, Absolute, Molecular Biology Grade and Nuclease-Free Water (not DEPC-Treated) for the required number of reactions, sufficient for 1 mL per reaction, plus 10% overage.
- 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 (200- μ 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 (200- μ 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_200_Flex** program has been downloaded from the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit product page at www.thermofisher.com and loaded onto the instrument.

Prepare the processing plates (200- μ 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	500 μ L
Wash 2 Plate	3		80% Ethanol	1,000 μ L
Elution Plate	4		Elution Solution	50 μ 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 (200- μ 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 ^[1]
Binding Solution	265 μ L
Total Nucleic Acid Magnetic Beads	10 μ L
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- μ L sample input volume)

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

IMPORTANT! Samples collected using the Everlywell™ COVID-19 Test Home Collection Kit have special plate layout considerations. See Appendix A, “Example extraction plate and RT-PCR reaction plate layouts (Everlywell™ COVID-19 Test Home Collection Kit samples)”.

1. Add 5 μ L of Proteinase K to each well in the KingFisher™ 96 Deep-Well Plate labeled "Sample Plate".
2. Add 200 μ L of sample to each sample well.
For Everlywell™ COVID-19 Test Home Collection Kit samples, not all sample wells are used. See Appendix A, “Example extraction plate and RT-PCR reaction plate layouts (Everlywell™ COVID-19 Test Home Collection Kit samples)”.
3. Add 200 μ L of Nuclease-Free Water (not DEPC-Treated) to the Negative Control well.
For Everlywell™ COVID-19 Test Home Collection Kit samples, prepare 2 Negative Control wells on the extraction plate if you are splitting a single extraction plate into two 96-well RT-PCR plates. See Appendix A, “Example extraction plate and RT-PCR reaction plate layouts (Everlywell™ COVID-19 Test Home Collection Kit samples)”.
4. Invert the Binding Bead Mix 5 times gently to mix, then add 275 μ L 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 μ L of MS2 Phage Control to each sample well and Negative Control well.

Process the samples (200- μ L sample input volume)

1. Select the **MVP_2Wash_200_Flex** on the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head.
2. Start the run, then load the prepared plates into position when prompted by the instrument.
3. After the run is complete (~22 minutes after start), immediately remove the Elution Plate from the instrument, then cover the plate with MicroAmp™ Clear Adhesive Film.

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

The samples are eluted in 50 μ L of Elution Solution (see “Prepare the processing plates (200- μ L sample input volume)” on page 24).

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.

Extract RNA—Automated method (400- μ 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- μ 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 and loaded onto the instrument.

Prepare the processing plates (400- μ 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 μ L
Wash 2 Plate	3		80% Ethanol	1,000 μ L
Elution Plate	4		Elution Solution	50 μ 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- μ 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 ^[1]
Binding Solution	530 μ L
Total Nucleic Acid Magnetic Beads	20 μ L
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- μ L sample input volume)

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

IMPORTANT! Samples collected using the Everlywell™ COVID-19 Test Home Collection Kit have special plate layout considerations. See Appendix A, “Example extraction plate and RT-PCR reaction plate layouts (Everlywell™ COVID-19 Test Home Collection Kit samples)”.

1. Add 10 μ L of Proteinase K to each well in the KingFisher™ 96 Deep-Well Plate labeled "Sample Plate".
2. Add 400 μ L of sample to each sample well.
For Everlywell™ COVID-19 Test Home Collection Kit samples, not all sample wells may be used. See Appendix A, “Example extraction plate and RT-PCR reaction plate layouts (Everlywell™ COVID-19 Test Home Collection Kit samples)”.
3. Add 400 μ L of Nuclease-Free Water (not DEPC-Treated) to the Negative Control well.
For Everlywell™ COVID-19 Test Home Collection Kit samples, prepare 2 Negative Control wells on the extraction plate if you are splitting a single extraction plate into two 96-well RT-PCR plates. See Appendix A, “Example extraction plate and RT-PCR reaction plate layouts (Everlywell™ COVID-19 Test Home Collection Kit samples)”.
4. Invert the Binding Bead Mix 5 times gently to mix, then add 550 μ 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 μ L of MS2 Phage Control to each sample well and to the Negative Control well.

Process the samples (400- μ 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 μ L of Elution Solution (see “Prepare the processing plates (400- μ L sample input volume)” on page 27).

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.

3

Extract RNA (manual method)

- Before you begin 30
- Extract RNA—Manual method (200- μ L sample input volume) 30
- Extract RNA—Manual method (400- μ L sample input volume) 33

Manual RNA extraction can be performed from a sample input volume of 200 μ L or 400 μ 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 patient samples to be processed, plus one Negative Control per plate.
- Prepare fresh 80% Ethanol using Ethanol, Absolute, Molecular Biology Grade and Nuclease-Free Water (not DEPC-Treated) for the required number of reactions, plus 10% overage.

Sample input volume	Volume of 80% Ethanol per reaction
200 μ L	0.75 mL
400 μ L	1.5 mL

- Mark the Negative Control well on the plate.

Extract RNA—Manual method (200- μ 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 (200- μ 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 ^[1]
Binding Solution	265 μ L
Total Nucleic Acid Magnetic Beads	10 μ L
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- μ L sample input volume)

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

IMPORTANT! Samples collected using the Everlywell™ COVID-19 Test Home Collection Kit have special plate layout considerations. See Appendix A, “Example extraction plate and RT-PCR reaction plate layouts (Everlywell™ COVID-19 Test Home Collection Kit samples)”.

1. Add 5 μ L of Proteinase K to each well of a KingFisher™ 96 Deep-Well Plate.
2. Add 200 μ L of sample to each sample well.
 For Everlywell™ COVID-19 Test Home Collection Kit samples, not all sample wells may be used. See Appendix A, “Example extraction plate and RT-PCR reaction plate layouts (Everlywell™ COVID-19 Test Home Collection Kit samples)”.
3. Add 200 μ L of Nuclease-Free Water (not DEPC-Treated) to the Negative Control well.
 For Everlywell™ COVID-19 Test Home Collection Kit samples, prepare 2 Negative Control wells on the extraction plate if you are splitting a single extraction plate into two 96-well RT-PCR plates. See Appendix A, “Example extraction plate and RT-PCR reaction plate layouts (Everlywell™ COVID-19 Test Home Collection Kit samples)”.
4. Invert the Binding Bead Mix 5 times gently to mix, then add 275 μ 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 5 μ 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 (200- μ 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 500 μ L 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 500 μ L of 80% Ethanol.
7. Repeat step 2 to step 5 using 250 μ L of 80% Ethanol.
8. Dry the beads by shaking the plate (uncovered) at 1,050 rpm for 2 minutes.

Elute the nucleic acid (200- μ L sample input volume)

1. Add 50 μ 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.

Extract RNA—Manual 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.

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)

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

IMPORTANT! Samples collected using the Everlywell™ COVID-19 Test Home Collection Kit have special plate layout considerations. See Appendix A, “Example extraction plate and RT-PCR reaction plate layouts (Everlywell™ COVID-19 Test Home Collection Kit samples)”.

1. Add 10 µL of Proteinase K to each well of a KingFisher™ 96 Deep-Well Plate.
2. Add 400 µL of sample to each sample well.
 For Everlywell™ COVID-19 Test Home Collection Kit samples, not all sample wells may be used. See Appendix A, “Example extraction plate and RT-PCR reaction plate layouts (Everlywell™ COVID-19 Test Home Collection Kit samples)”.

3. Add 400 μ L of Nuclease-Free Water (not DEPC-Treated) to the Negative Control well.
For Everlywell™ COVID-19 Test Home Collection Kit samples, prepare 2 Negative Control wells on the extraction plate if you are splitting a single extraction plate into two 96-well RT-PCR plates. See Appendix A, “Example extraction plate and RT-PCR reaction plate layouts (Everlywell™ COVID-19 Test Home Collection Kit samples)”.
4. Invert the Binding Bead Mix 5 times gently to mix, then add 550 μ 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 μ 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- μ 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 μ 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- μ L sample input volume)

1. Add 50 μ 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.

4

Prepare RT-PCR reactions— COVID-19 assay only

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Protocols in this chapter

This chapter covers preparation of RT-PCR reaction plates for samples that were collected using methods other than the Everlywell™ COVID-19 Test Home Collection Kit. The RT-PCR reactions in this chapter do not include the RNase P assay.

Note: For samples collected using the Everlywell™ COVID-19 Test Home Collection Kit, see Chapter 5, “Prepare RT-PCR reactions—COVID-19 assay plus RNase P assay (Everlywell™ COVID-19 Test Home Collection Kit)”.

IMPORTANT! Do not combine samples collected using the Everlywell™ COVID-19 Test Home Collection Kit with samples collected via other methods on the same RT-PCR plate. The plate setup and software template files are different.

In this chapter, select the appropriate RT-PCR preparation procedure based on the following criteria:

- The original sample input volume used for RNA extraction (200 μ L or 400 μ L)
- The size of the RT-PCR reaction plate (96-well or a 384-well)
- The volume of purified sample RNA that is added to the reaction

Guidelines for RT-PCR

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 the RT-PCR reactions (200- μ L sample input, 96-well reaction plate, COVID-19 assay only)

Use this procedure under the following conditions:

- Original sample input volume of 200 μ L was used for extraction
- Instrument is compatible with 96-well RT-PCR reaction plates
- Sample was not collected with the Everlywell™ COVID-19 Test Home Collection Kit (no RNase P assay required)

For information about the stability of the TaqPath™ COVID-19 Control, see “In-use stability for the TaqPath™ COVID-19 Control” on page 11.

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 (1×10^4 copies/ μ L) to a working stock of 25 copies/ μ L on ice:
 - a. Pipet 98 μ L of TaqPath™ COVID-19 Control Dilution Buffer into a microcentrifuge tube, then add 2 μ L of TaqPath™ COVID-19 Control. Mix well, then centrifuge briefly.
 - b. Pipet 87.5 μ L of TaqPath™ COVID-19 Control Dilution Buffer into a second microcentrifuge tube, then add 12.5 μ L 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 on ice:
 - a. For each run, combine the following components sufficient for the number of RNA samples to be tested 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 μ L.

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

5. Set up the reaction plate on ice:
 - a. Pipet 15.0 μ L 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.
 For other reaction plates that can be used, see “Required materials not supplied” on page 12.
 - 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 2 on page 39.
 - 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 $\geq 650 \times g$ (≥ 650 RCF) to remove bubbles and to collect the liquid at the bottom of the reaction plate.

Table 2 Reaction plate volumes

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

Prepare the RT-PCR reactions (200- μ L sample input, 384-well reaction plate, COVID-19 assay only)

Use this procedure under the following conditions:

- Original sample input volume of 200 μ L was used for extraction
- Instrument is compatible with 384-well RT-PCR reaction plates
- Sample was not collected with the Everlywell™ COVID-19 Test Home Collection Kit (no RNase P assay required)

For information about the stability of the TaqPath™ COVID-19 Control, see “In-use stability for the TaqPath™ COVID-19 Control” on page 11.

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 (1×10^4 copies/ μ L) to a working stock of 25 copies/ μ L on ice:
 - a. Pipet 98 μ L of TaqPath™ COVID-19 Control Dilution Buffer into a microcentrifuge tube, then add 2 μ L of TaqPath™ COVID-19 Control. Mix well, then centrifuge briefly.
 - b. Pipet 87.5 μ L of TaqPath™ COVID-19 Control Dilution Buffer into a second microcentrifuge tube, then add 12.5 μ L 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 on ice.

- 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.

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

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 μ L	$5.50 \times (n + y + 1)$ μ L	2112.0 μ L
COVID-19 Real Time PCR Assay Multiplex	1.00 μ L	$1.10 \times (n + y + 1)$ μ L	422.4 μ L
Nuclease-free water	4.00 μ L	$4.40 \times (n + y + 1)$ μ L	1690.0 μ L
Total Reaction Mix volume	10.0 μ L	—	4224.4 μ L

5. Set up the reaction plate on ice:

- a. Pipet 10.0 μ L of the Reaction Mix prepared in step 4 into each well of a MicroAmp™ Optical 384-Well Reaction Plate with Barcode.

For other reaction plates that can be used, see “Required materials not supplied” on page 12.

- 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 3 on page 41.

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 $\geq 650 \times g$ (≥ 650 RCF) to remove bubbles and to collect the liquid at the bottom of the reaction plate.

Table 3 Reaction plate volumes

Component	Volume per reaction		
	RNA Sample reaction	Positive Control reaction	Negative Control reaction
Reaction Mix	10.0 μ L	10.0 μ L	10.0 μ L
Purified sample RNA (from RNA extraction)	10.0 μ L	—	—
Positive Control (diluted TaqPath™ COVID-19 Control from step 3)	—	2.0 μ L	—
Nuclease-free water	—	8.0 μ L	—
Purified Negative Control (from RNA extraction)	—	—	10.0 μ L
Total volume	20.0 μL	20.0 μL	20.0 μL

Prepare the RT-PCR reactions (400- μ L sample input, 96-well reaction plate, COVID-19 assay only)

Use this procedure under the following conditions:

- Original sample input volume of 400 μ L was used for extraction
- Instrument is compatible with 96-well RT-PCR reaction plates
- Sample was not collected with the Everlywell™ COVID-19 Test Home Collection Kit (no RNase P assay required)

For information about the stability of the TaqPath™ COVID-19 Control, see “In-use stability for the TaqPath™ COVID-19 Control” on page 11.

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 (1×10^4 copies/ μ L) to a working stock of 25 copies/ μ L on ice:
 - a. Pipet 98 μ L of TaqPath™ COVID-19 Control Dilution Buffer into a microcentrifuge tube, then add 2 μ L of TaqPath™ COVID-19 Control. Mix well, then centrifuge briefly.

- b. Pipet 87.5 μ L of TaqPath™ COVID-19 Control Dilution Buffer into a second microcentrifuge tube, then add 12.5 μ L 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 on ice:
- a. For each run, combine the following components sufficient for the number of RNA samples to be tested 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 μ L.

Component	Volume per RNA Sample or control	Volume for n RNA Samples plus 2 controls	Volume for 94 RNA Samples plus 2 controls
TaqPath™ 1-Step Multiplex Master Mix (No ROX™) (4X)	6.25 μ L	$6.875 \times (n + 2)$ μ L	660 μ L
COVID-19 Real Time PCR Assay Multiplex	1.25 μ L	$1.375 \times (n + 2)$ μ L	132 μ L
Nuclease-free water	12.50 μ L	$13.75 \times (n + 2)$ μ L	1320 μ L
Total Reaction Mix volume	20.0 μL	—	2112 μL

5. Set up the reaction plate on ice:
- a. Pipet 20.0 μ L 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.
 For other reaction plates that can be used, see “Required materials not supplied” on page 12.
- 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 43.
- 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 $\geq 650 \times g$ (≥ 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	20.0 μ L	20.0 μ L	20.0 μ L
Purified sample RNA (from RNA extraction)	5.0 μ L	—	—
Positive Control (diluted TaqPath™ COVID-19 Control from step 3)	—	2.0 μ L	—
Nuclease-free water	—	3.0 μ L	—
Purified Negative Control (from RNA extraction)	—	—	5.0 μ L
Total volume	25.0 μL	25.0 μL	25.0 μL

Prepare the RT-PCR reactions (400- μ L sample input, 384-well reaction plate, COVID-19 assay only)

Use this procedure under the following conditions:

- Original sample input volume of 400 μ L was used for extraction
- Instrument is compatible with 384-well RT-PCR reaction plates
- Sample was not collected with the Everlywell™ COVID-19 Test Home Collection Kit (no RNase P assay required)

For information about the stability of the TaqPath™ COVID-19 Control, see “In-use stability for the TaqPath™ COVID-19 Control” on page 11.

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 (1×10^4 copies/ μ L) to a working stock of 25 copies/ μ L on ice:
 - a. Pipet 98 μ L of TaqPath™ COVID-19 Control Dilution Buffer into a microcentrifuge tube, then add 2 μ L of TaqPath™ COVID-19 Control. Mix well, then centrifuge briefly.

- b. Pipet 87.5 μ L of TaqPath™ COVID-19 Control Dilution Buffer into a second microcentrifuge tube, then add 12.5 μ L 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 on ice.
- 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.

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

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 μ L	$5.50 \times (n + y + 1)$ μ L	2112.0 μ L
COVID-19 Real Time PCR Assay Multiplex	1.00 μ L	$1.10 \times (n + y + 1)$ μ L	422.4 μ L
Nuclease-free water	9.00 μ L	$9.90 \times (n + y + 1)$ μ L	3802.0 μ L
Total Reaction Mix volume	15.0 μL	—	6336.4 μL

5. Set up the reaction plate on ice:
- a. Pipet 15.0 μ L of the Reaction Mix prepared in step 4 into each well of a MicroAmp™ Optical 384-Well Reaction Plate with Barcode.
- For other reaction plates that can be used, see “Required materials not supplied” on page 12.
- 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 5 on page 45.

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 $\geq 650 \times g$ (≥ 650 RCF) to remove bubbles and to collect the liquid at the bottom of the reaction plate.

Table 5 Reaction plate volumes

Component	Volume per reaction		
	RNA Sample reaction	Positive Control reaction	Negative Control reaction
Reaction Mix	15.0 μ L	15.0 μ L	15.0 μ L
Purified sample RNA (from RNA extraction)	5.0 μ L	—	—
Positive Control (diluted TaqPath™ COVID-19 Control from step 3)	—	2.0 μ L	—
Nuclease-free water	—	3.0 μ L	—
Purified Negative Control (from RNA extraction)	—	—	5.0 μ L
Total volume	20.0 μL	20.0 μL	20.0 μL



Prepare RT-PCR reactions— COVID-19 assay plus RNase P assay (Everlywell™ COVID-19 Test Home Collection Kit)

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- Prepare the RT-PCR reactions (200-µL sample input, 96-well reaction plate, includes RNase P assay) 47
- Prepare the RT-PCR reactions (200-µL sample input, 384-well reaction plate, includes RNase P assay) 51
- Prepare the RT-PCR reactions (400-µL sample input, 96-well reaction plate, includes RNase P assay) 55
- Prepare the RT-PCR reactions (400-µL sample input, 384-well reaction plate, includes RNase P assay) 58

Protocols in this chapter

This chapter covers preparation of RT-PCR reaction plates for samples that were collected using the Everlywell™ COVID-19 Test Home Collection Kit. These RT-PCR reactions must contain both the COVID-19 Real Time PCR Assay Multiplex and the TaqMan™ SARS-CoV-2 RNase P Assay.

Note: For samples that were collected using a method other than the Everlywell™ COVID-19 Test Home Collection Kit, see Chapter 4, “Prepare RT-PCR reactions—COVID-19 assay only”.

IMPORTANT! Do not combine samples collected using the Everlywell™ COVID-19 Test Home Collection Kit with samples collected via other methods on the same RT-PCR plate. The plate setup and software template files are different.

In this chapter, select the appropriate RT-PCR preparation procedure based on the following criteria:

- The original sample input volume used for RNA extraction (200 µL or 400 µL)
- The size of the RT-PCR reaction plate (96-well or a 384-well)

Guidelines for RT-PCR

IMPORTANT!

- Prepare two RT-PCR reaction plate wells for each sample and Negative Control—one for the COVID-19 assay and one for the RNase P assay—plus a single RT-PCR reaction plate well for the Positive Control.
 - 96-well reaction plates can contain up to 46 samples (2 wells each), one Negative Control (2 wells), and one Positive Control (1 well).
 - 384-well reaction plates can contain up to 189 samples (2 wells each), 2 Negative Controls (one from each extraction plate; 4 wells total), and one Positive Control (1 well).
 - For example plate layouts, see Appendix A, “Example extraction plate and RT-PCR reaction plate layouts (Everlywell™ COVID-19 Test Home Collection Kit samples)”.
 - Prepare the 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 the RT-PCR reactions (200- μ L sample input, 96-well reaction plate, includes RNase P assay)

Use this procedure under the following conditions:

- Original sample input volume of 200 μ L was used for extraction
- Instrument is compatible with 96-well RT-PCR reaction plates
- Sample was collected with the Everlywell™ COVID-19 Test Home Collection Kit (RNase P assay required)

IMPORTANT! In the following procedure, each sample or Negative Control on the 96-well extraction plate is added to two wells on the 96-well reaction plate (one for the COVID-19 assay and one for the RNase P assay). For this reason, only half of the extraction plate is used per reaction plate. The extraction plate can be stored at 2–8°C for up to 48 hours.

For information about the stability of the TaqPath™ COVID-19 Control, see “In-use stability for the TaqPath™ COVID-19 Control” on page 11.

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 (1×10^4 copies/ μ L) to a working stock of 25 copies/ μ L on ice:
 - a. Pipet 98 μ L of TaqPath™ COVID-19 Control Dilution Buffer into a microcentrifuge tube, then add 2 μ L of TaqPath™ COVID-19 Control. Mix well, then centrifuge briefly.
 - b. Pipet 87.5 μ L of TaqPath™ COVID-19 Control Dilution Buffer into a second microcentrifuge tube, then add 12.5 μ L of the previous dilution. Mix well, then centrifuge briefly.

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

4. Prepare separate reaction mixes for the COVID-19 assay and RNase P assay on ice, according to the following tables:
 - a. Prepare sufficient reaction mix based on the number of RNA samples, plus one Positive Control and 2 Negative Controls per reaction plate.

All volumes include 10% overage for pipette error.

IMPORTANT! The volumes in these tables assume that you extracted sample RNA using an original sample input volume of 200 μ L.

Table 6 COVID-19 Reaction Mix

Component	Volume per RNA sample or control	Volume for n RNA samples plus 2 controls	Volume for 46 RNA samples plus 2 controls
TaqPath™ 1-Step Multiplex Master Mix (No ROX™) (4X)	6.25 μ L	$6.875 \times (n + 2)$ μ L	330 μ L
COVID-19 Real Time PCR Assay Multiplex	1.25 μ L	$1.375 \times (n + 2)$ μ L	66 μ L
Nuclease-free water	7.50 μ L	$8.25 \times (n + 2)$ μ L	396 μ L
Total Reaction Mix volume	15.0 μL	—	792 μL

Table 7 RNase P Reaction Mix

Component	Volume per RNA sample or control	Volume for n RNA samples plus 1 Negative Control	Volume for 46 RNA samples plus 1 Negative Control
TaqPath™ 1-Step Multiplex Master Mix (No ROX™) (4X)	6.25 μ L	$6.875 \times (n + 1)$ μ L	324 μ L
TaqMan™ SARS-CoV-2 RNase P Assay	1.25 μ L	$1.375 \times (n + 1)$ μ L	65 μ L
Nuclease-free water	7.50 μ L	$8.25 \times (n + 1)$ μ L	388 μ L
Total Reaction Mix volume	15.0 μL	—	777 μL

Prepare the RT-PCR reactions (200- μ L sample input, 96-well reaction plate, includes RNase P assay)

5. Add each reaction mix to a reaction plate on ice:
 - a. Starting with row A, pipet 15.0 μ L per well of the COVID-19 Reaction Mix into every other row 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. See Figure 1 on page 49.

A MicroAmp™ Fast Optical 96-Well Reaction Plate, 0.1 mL or a MicroAmp™ Optical 96-Well Reaction Plate, 0.2 mL can be used (see “Required materials not supplied” on page 12).

IMPORTANT! The following plates are not compatible with this procedure:

- MicroAmp™ EnduraPlate™ Optical 96-Well Fast Clear Reaction Plates with Barcode
- MicroAmp™ EnduraPlate™ Optical 96-Well Fast Clear Reaction Plates
- MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode
- MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates

- b. Pipet 15.0 μ L per well of the RNase P Reaction Mix into the remaining rows of the plate.

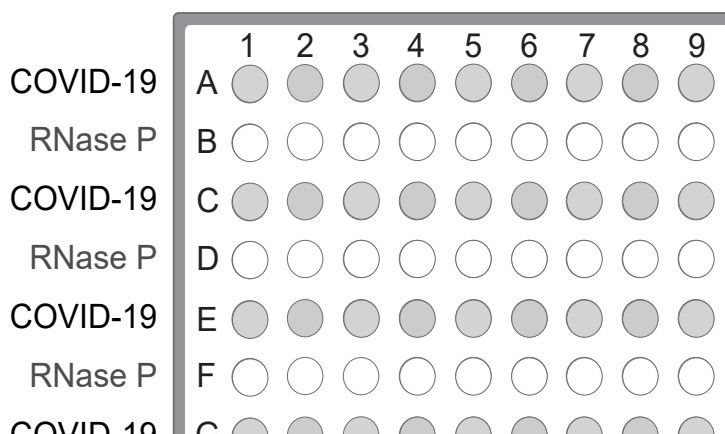


Figure 1 Assays in alternating plate rows

6. Add samples and controls to the reaction plate on ice, using the volumes in Table 8 on page 50:
 - a. 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.
 - b. Unseal the extraction plate, then add the Negative Control from the single well of the extraction plate to wells A1 and B1 of the reaction plate, as shown in Figure 2 on page 50.
 - c. Add the Positive Control (from step 3) and Nuclease-free water to an appropriate well of the reaction plate (containing the COVID-19 assay).
There is no RNase P well for the Positive Control.

- d. Add each RNA sample from the extraction plate to adjacent COVID-19 assay and RNase P assay wells of the reaction plate, as shown in the following figure.

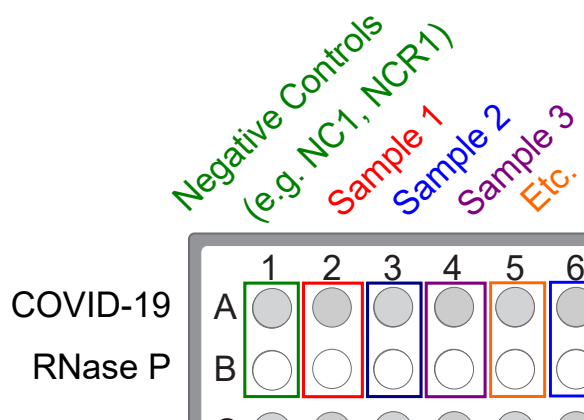


Figure 2 Paired sample and Negative Control wells for each assay

Table 8 Reaction plate volumes

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

7. Seal, vortex, and centrifuge the reaction plate:
- Seal the reaction 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.

- Vortex the reaction 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.

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

Prepare the RT-PCR reactions (200- μ L sample input, 384-well reaction plate, includes RNase P assay)

Use this procedure under the following conditions:

- Original sample input volume of 200 μ L was used for extraction
- Instrument is compatible with 384-well RT-PCR reaction plates
- Sample was collected with the Everlywell™ COVID-19 Test Home Collection Kit (RNase P assay required)

Note: You can combine multiple 96-well extraction plates on a single 384-well RT-PCR reaction plate.

For information about the stability of the TaqPath™ COVID-19 Control, see “In-use stability for the TaqPath™ COVID-19 Control” on page 11.

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 (1×10^4 copies/ μ L) to a working stock of 25 copies/ μ L on ice:
 - a. Pipet 98 μ L of TaqPath™ COVID-19 Control Dilution Buffer into a microcentrifuge tube, then add 2 μ L of TaqPath™ COVID-19 Control. Mix well, then centrifuge briefly.
 - b. Pipet 87.5 μ L of TaqPath™ COVID-19 Control Dilution Buffer into a second microcentrifuge tube, then add 12.5 μ L of the previous dilution. Mix well, then centrifuge briefly.

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

4. Prepare separate reaction mixes for the COVID-19 assay and RNase P assay on ice, according to the following tables:
 - a. Prepare sufficient reaction mix based on the number of RNA samples, plus one Positive Control per 384-well reaction plate, and 2 Negative Controls from each extraction run.
For example, if RNA samples from 2 96-well extraction runs are being combined on one 384-well reaction plate, then prepare 4 Negative Control wells on that reaction plate (2 extraction plates \times 2 assay types).

All volumes include 10% overage for pipette error.

IMPORTANT! The volumes in these tables assume that you extracted sample RNA using an original sample input volume of 200 μ L.

Table 9 COVID-19 Reaction Mix

Component	Volume per RNA Sample or Control	Volume for n RNA Samples plus y Negative Controls plus 1 Positive Control	Volume for 189 RNA Samples plus 2 Negative Controls plus 1 Positive Control
TaqPath™ 1-Step Multiplex Master Mix (No ROX™) (4X)	5.00 μ L	$5.50 \times (n + y + 1)$ μ L	1056 μ L
COVID-19 Real Time PCR Assay Multiplex	1.00 μ L	$1.10 \times (n + y + 1)$ μ L	211 μ L
Nuclease-free water	4.00 μ L	$4.40 \times (n + y + 1)$ μ L	845 μ L
Total Reaction Mix volume	10.0 μL	—	2112 μL

Table 10 RNase P Reaction Mix

Component	Volume per RNA Sample or control	Volume for n RNA Samples plus y Negative Controls	Volume for 189 RNA Samples plus 2 Negative Controls
TaqPath™ 1-Step Multiplex Master Mix (No ROX™) (4X)	5.00 μ L	$5.50 \times (n + y)$ μ L	1050.5 μ L
TaqMan™ SARS-CoV-2 RNase P Assay	1.00 μ L	$1.10 \times (n + y)$ μ L	210.1 μ L
Nuclease-free water	4.00 μ L	$4.40 \times (n + y)$ μ L	840.4 μ L
Total Reaction Mix volume	10.0 μL	—	2101.0 μL

5. Add each reaction mix to a reaction plate on ice:
 - a. Starting with row A, pipet 10.0 μ L per well of the COVID-19 Reaction Mix into every other row of a MicroAmp™ Optical 384-Well Reaction Plate with Barcode. See Figure 3 on page 53. A MicroAmp™ Optical 384-Well Reaction Plate can be used (see “Required materials not supplied” on page 12).

IMPORTANT! MicroAmp™ EnduraPlate™ Optical 384-Well Clear Reaction Plates with Barcode and MicroAmp™ EnduraPlate™ Optical 384-Well Clear Reaction Plates are not compatible with this procedure.

Prepare the RT-PCR reactions (200- μ L sample input, 384-well reaction plate, includes RNase P assay)

- b. Pipet 10.0 μ L per well of the RNase P Reaction Mix into the remaining rows of the plate.

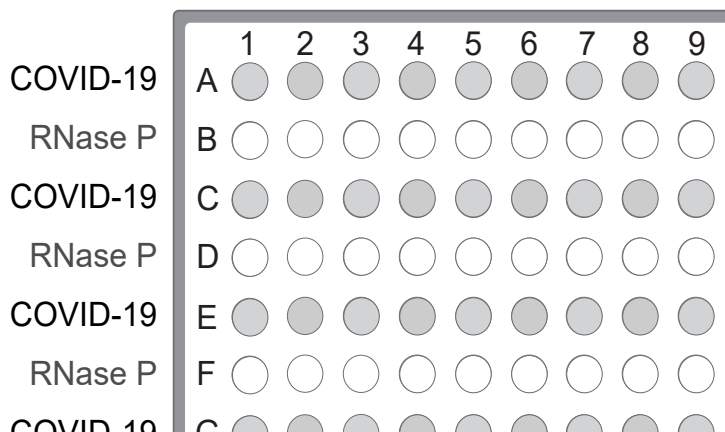


Figure 3 Assays in alternating plate rows

6. Add samples and controls to the reaction plate on ice, using the volumes in Table 11 on page 54:
- a. 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.

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

- b. Unseal the extraction plate, then add the first Negative Control from the single well of the extraction plate to wells A1 and B1 of the reaction plate, as shown in Figure 4 on page 54.

Note: If you combine multiple extraction plates on the reaction plate, add the Negative Control from each extraction plate to adjacent COVID-19 assay and RNase P assay wells.

- c. Add the Positive Control (from step 3) and Nuclease-free water to an appropriate well of the reaction plate (containing the COVID-19 assay).

Note:

- There is no RNase P well for the Positive Control.
 - Only one Positive Control is required per 384-well plate, even if you combine multiple extraction plates.
-

- d. Add each RNA sample from the extraction plate to adjacent COVID-19 assay and RNase P assay wells of the reaction plate, as shown in the following figure.

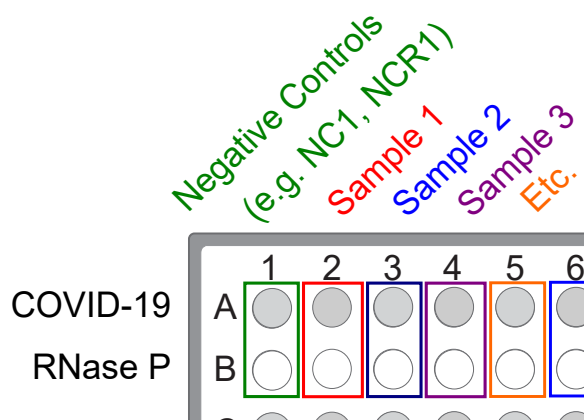


Figure 4 Paired sample and Negative Control wells for each assay

Table 11 Reaction plate volumes

Component	Volume per reaction		
	RNA Sample reaction	Positive Control reaction	Negative Control reaction
Reaction Mix (COVID-19 or RNase P)	10.0 μ L	10.0 μ L	10.0 μ L
Purified sample RNA (from RNA extraction)	10.0 μ L	—	—
Positive Control (diluted TaqPath™ COVID-19 Control from step 3)	—	2.0 μ L	—
Nuclease-free water	—	8.0 μ L	—
Purified Negative Control (from RNA extraction)	—	—	10.0 μ L
Total volume	20.0 μL	20.0 μL	20.0 μL

7. Seal, vortex, and centrifuge the reaction plate:

- a. Seal the reaction 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.

- b. Vortex the reaction 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.

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

Prepare the RT-PCR reactions (400- μ L sample input, 96-well reaction plate, includes RNase P assay)

Use this procedure under the following conditions:

- Original sample input volume of 400 μ L was used for extraction
- Instrument is compatible with 96-well RT-PCR reaction plates
- Sample was collected with the Everlywell™ COVID-19 Test Home Collection Kit (RNase P assay required)

IMPORTANT! In the following procedure, each sample or Negative Control on the 96-well extraction plate is added to two wells on the 96-well reaction plate (one for the COVID-19 assay and one for the RNase P assay). For this reason, only half of the extraction plate is used per reaction plate. The extraction plate can be stored at 2–8°C for up to 48 hours.

For information about the stability of the TaqPath™ COVID-19 Control, see “In-use stability for the TaqPath™ COVID-19 Control” on page 11.

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 (1×10^4 copies/ μ L) to a working stock of 25 copies/ μ L on ice:
 - a. Pipet 98 μ L of TaqPath™ COVID-19 Control Dilution Buffer into a microcentrifuge tube, then add 2 μ L of TaqPath™ COVID-19 Control. Mix well, then centrifuge briefly.
 - b. Pipet 87.5 μ L of TaqPath™ COVID-19 Control Dilution Buffer into a second microcentrifuge tube, then add 12.5 μ L of the previous dilution. Mix well, then centrifuge briefly.

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

4. Prepare separate reaction mixes for the COVID-19 assay and RNase P assay on ice, according to the following tables:
 - a. Prepare sufficient reaction mix based on the number of RNA samples, plus one Positive Control and 2 Negative Controls per reaction plate.

All volumes include 10% overage for pipette error.

IMPORTANT! The volumes in these tables assume that you extracted sample RNA using an original sample input volume of 400 μ L.

Table 12 COVID-19 Reaction Mix

Component	Volume per RNA Sample or control	Volume for n RNA Samples plus 2 controls	Volume for 46 RNA samples plus 2 controls
TaqPath™ 1-Step Multiplex Master Mix (No ROX™) (4X)	6.25 μ L	$6.875 \times (n + 2)$ μ L	330 μ L
COVID-19 Real Time PCR Assay Multiplex	1.25 μ L	$1.375 \times (n + 2)$ μ L	66 μ L
Nuclease-free water	12.50 μ L	$13.75 \times (n + 2)$ μ L	660 μ L
Total Reaction Mix volume	20.0 μL	—	1056 μL

Table 13 RNase P Reaction Mix

Component	Volume per RNA sample or control	Volume for n RNA samples plus 1 Negative Control	Volume for 46 RNA samples plus 1 Negative Control
TaqPath™ 1-Step Multiplex Master Mix (No ROX™) (4X)	6.25 μ L	$6.875 \times (n + 1)$ μ L	323.1 μ L
TaqMan™ SARS-CoV-2 RNase P Assay	1.25 μ L	$1.375 \times (n + 1)$ μ L	64.6 μ L
Nuclease-free water	12.50 μ L	$13.75 \times (n + 1)$ μ L	646.3 μ L
Total Reaction Mix volume	20.0 μL	—	1034.0 μL

5. Add each reaction mix to a reaction plate on ice:

- a. Starting with row A, pipet 20.0 μ L per well of the COVID-19 Reaction Mix into every other row 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. See Figure 5 on page 57.

A MicroAmp™ Fast Optical 96-Well Reaction Plate, 0.1 mL or a MicroAmp™ Optical 96-Well Reaction Plate, 0.2 mL can be used (see “Required materials not supplied” on page 12).

IMPORTANT! The following plates are not compatible with this procedure:

- MicroAmp™ EnduraPlate™ Optical 96-Well Fast Clear Reaction Plates with Barcode
- MicroAmp™ EnduraPlate™ Optical 96-Well Fast Clear Reaction Plates
- MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode
- MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates

Prepare the RT-PCR reactions (400- μ L sample input, 96-well reaction plate, includes RNase P assay)

- b. Pipet 20.0 μ L per well of the RNase P Reaction Mix into the remaining rows of the plate.

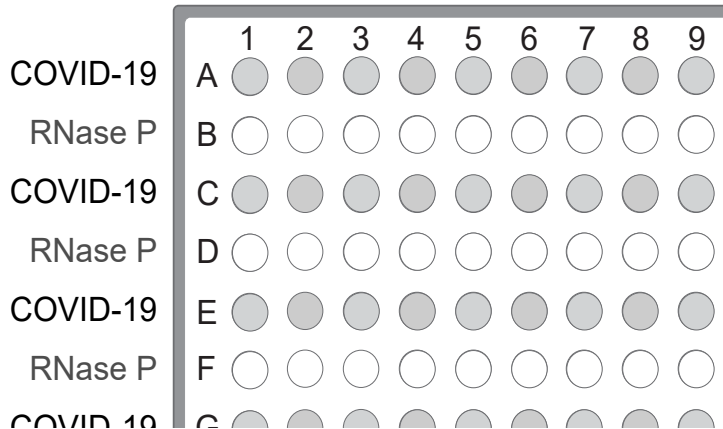


Figure 5 Assays in alternating plate rows

6. Add samples and controls to the reaction plate on ice, using the volumes in Table 14 on page 58:
- 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.
 - Unseal the extraction plate, then add Negative Control from the single well of the extraction plate to wells A1 and B1 of the reaction plate, as shown in Figure 6 on page 57.
 - Add the Positive Control (from step 3) and Nuclease-free water to an appropriate well of the reaction plate (containing the COVID-19 assay).
There is no RNase P well for the Positive Control.
 - Add each RNA sample from the extraction plate to adjacent COVID-19 assay and RNase P assay wells of the reaction plate, as shown in the following figure.

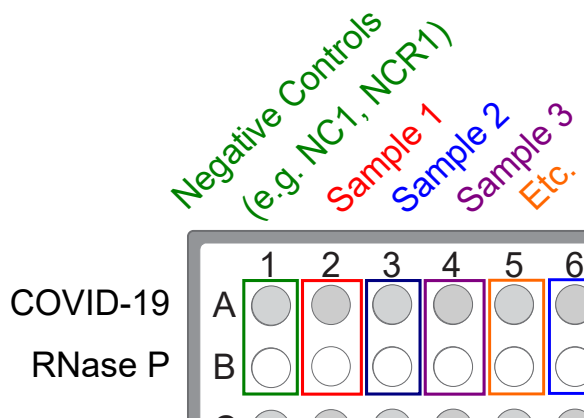


Figure 6 Paired sample and Negative Control wells for each assay

Table 14 Reaction plate volumes

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

7. Seal, vortex, and centrifuge the reaction plate:

- a. Seal the reaction 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.

- b. Vortex the reaction 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.

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

Prepare the RT-PCR reactions (400- μ L sample input, 384-well reaction plate, includes RNase P assay)

Use this procedure under the following conditions:

- Original sample input volume of 400 μ L was used for extraction
- Instrument is compatible with 384-well RT-PCR reaction plates
- Sample was collected with the Everlywell™ COVID-19 Test Home Collection Kit (RNase P assay required)

Note: You can combine multiple 96-well extraction plates on a single 384-well RT-PCR reaction plate.

Prepare the RT-PCR reactions (400-µL sample input, 384-well reaction plate, includes RNase P assay)

For information about the stability of the TaqPath™ COVID-19 Control, see “In-use stability for the TaqPath™ COVID-19 Control” on page 11.

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 (1×10^4 copies/µL) to a working stock of 25 copies/µL on ice:
 - a. Pipet 98 µL of TaqPath™ COVID-19 Control Dilution Buffer into a microcentrifuge tube, then add 2 µL of 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 previous dilution. Mix well, then centrifuge briefly.

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

4. Prepare separate reaction mixes for the COVID-19 assay and RNase P assay on ice, according to the following tables:
 - a. Prepare sufficient reaction mix based on the number of RNA samples, plus one Positive Control per 384-well reaction plate, and 2 Negative Controls from each extraction run.
For example, if RNA samples from 2 96-well extraction runs are being combined on one 384-well reaction plate, then prepare 4 Negative Control wells on that reaction plate (2 extraction plates × 2 assay types).

All volumes include 10% overage for pipette error.

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

Table 15 COVID-19 Reaction Mix

Component	Volume per RNA Sample or control	Volume for n RNA Samples plus y Negative Controls plus 1 Positive Control	Volume for 189 RNA Samples plus 2 Negative Controls plus 1 Positive Control
TaqPath™ 1-Step Multiplex Master Mix (No ROX™) (4X)	5.00 µL	$5.50 \times (n + y + 1)$ µL	1056 µL
COVID-19 Real Time PCR Assay Multiplex	1.00 µL	$1.10 \times (n + y + 1)$ µL	211 µL
Nuclease-free water	9.00 µL	$9.90 \times (n + y + 1)$ µL	1901 µL
Total Reaction Mix volume	15.0 µL	—	3168 µL

Table 16 RNase P Reaction Mix

Component	Volume per RNA Sample or control	Volume for n RNA Samples plus y Negative Controls	Volume for 189 RNA Samples plus 2 Negative Controls
TaqPath™ 1-Step Multiplex Master Mix (No ROX™) (4X)	5.00 μ L	$5.50 \times (n + y)$ μ L	1050.5 μ L
TaqMan™ SARS-CoV-2 RNase P Assay	1.00 μ L	$1.10 \times (n + y)$ μ L	210.1 μ L
Nuclease-free water	9.00 μ L	$9.90 \times (n + y + 1)$ μ L	1890.9 μ L
Total Reaction Mix volume	15.0 μL	—	3151.5 μL

5. Add each reaction mix to a reaction plate on ice:
- Starting with row A, pipet 15.0 μ L per well of the COVID-19 Reaction Mix into every other row of a MicroAmp™ Optical 384-Well Reaction Plate with Barcode. See Figure 7 on page 60. A MicroAmp™ Optical 384-Well Reaction Plate can be used (see “Required materials not supplied” on page 12).

IMPORTANT! MicroAmp™ EnduraPlate™ Optical 384-Well Clear Reaction Plates with Barcode and MicroAmp™ EnduraPlate™ Optical 384-Well Clear Reaction Plates are not compatible with this procedure.

- Pipet 15.0 μ L per well of the RNase P Reaction Mix into the remaining rows of the plate.

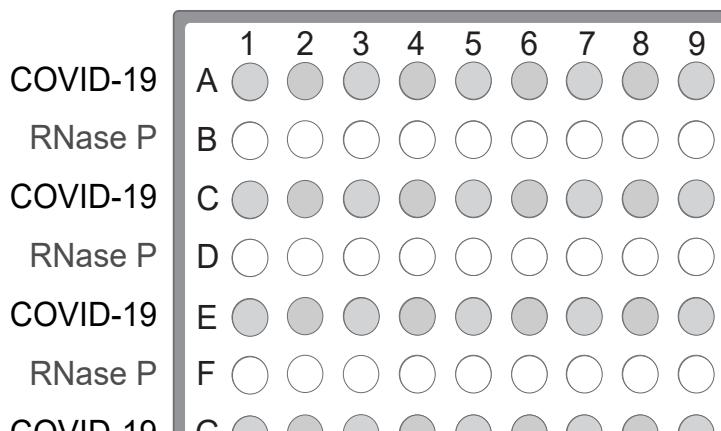


Figure 7 Assays in alternating plate rows

6. Add samples and controls to the reaction plate on ice, using the volumes in Table 17 on page 62:
- 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.

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

Prepare the RT-PCR reactions (400- μ L sample input, 384-well reaction plate, includes RNase P assay)

- b. Unseal the extraction plate, then add the Negative Control from the single well of the extraction plate to wells A1 and B1 of the reaction plate, as shown in Figure 8 on page 61.

Note: If you combine multiple extraction plates on the reaction plate, add the Negative Control from each extraction plate to adjacent COVID-19 assay and RNase P assay wells.

- c. Add the Positive Control (from step 3) and Nuclease-free water to an appropriate well of the reaction plate (containing the COVID-19 assay).

Note:

- There is no RNase P well for the Positive Control.
 - Only one Positive Control is required per 384-well plate, even if you combine multiple extraction plates.
-

- d. Add each RNA sample from the extraction plate to adjacent COVID-19 assay and RNase P assay wells of the reaction plate, as shown in the following figure.

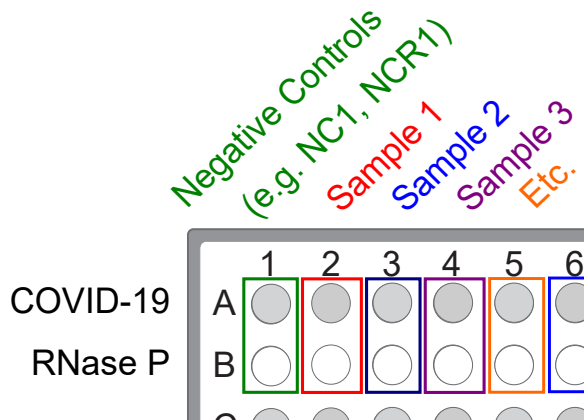


Figure 8 Paired sample and Negative Control wells for each assay

Table 17 Reaction plate volumes

Component	Volume per reaction		
	RNA Sample reaction	Positive Control reaction	Negative Control reaction
Reaction Mix (COVID-19 or RNase P)	15.0 μ L	15.0 μ L	15.0 μ L
Purified sample RNA (from RNA extraction)	5.0 μ L	—	—
Positive Control (diluted TaqPath™ COVID-19 Control from step 3)	—	2.0 μ L	—
Nuclease-free water	—	3.0 μ L	—
Purified Negative Control (from RNA extraction)	—	—	5.0 μ L
Total volume	20.0 μL	20.0 μL	20.0 μL

7. Seal, vortex, and centrifuge the reaction plate:

- a. Seal the reaction 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.

- b. Vortex the reaction 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.

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



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

- Dye calibration for the 7500 Real-Time PCR Instrument series 63
- Transfer the template (SDT) file for the 7500 Fast Dx Real-Time PCR Instrument 63
- Set up and run the 7500 Fast Dx Real-Time PCR Instrument—COVID-19 assay only 64
- Set up and run the 7500 Fast Dx Real-Time PCR Instrument—COVID-19 plus RNase P assay .. 66

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.

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

1. On the computer with Applied Biosystems™ COVID-19 Interpretive Software, navigate to one of the following directories, based on whether the RT-PCR reaction contains only the COVID-19 assay, or both the COVID-19 assay and the RNase P assay (for Everlywell™ COVID-19 Test Home Collection Kit samples):

- <...>\Applied Biosystems\COVID-19 Interpretive Software\Client\docs\User Documents\COVID-19
- <...>\Applied Biosystems\COVID-19 Interpretive Software\Client\docs\User Documents\COVID-19 + RNase P

<...> is the installation directory

2. Select the correct SDT file, based on whether the RT-PCR reaction contains only the COVID-19 assay, or both the COVID-19 assay and the RNase P assay (for Everlywell™ COVID-19 Test Home Collection Kit samples):

Assay type	Template name
COVID-19 only	TaqPath COVID-19 Kit Template 7500fastDx sds1_4_1 v1-4.sdt
COVID-19 and RNase P	TaqPath COVID-19 and RNaseP Template 7500fastDx sds1_4_1 v1-0.sdt

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

Set up and run the 7500 Fast Dx Real-Time PCR Instrument—COVID-19 assay only

This section covers instrument set-up and operation for reaction plates that contain only the COVID-19 assay.

For plates that contain both the COVID-19 assay and RNase P assay, see “Set up and run the 7500 Fast Dx Real-Time PCR Instrument—COVID-19 plus RNase P assay” on page 66.

For more information about the instrument, see the documents listed in “Related documentation” on page 123.

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 63.

IMPORTANT! Be careful to select the appropriate template file for your instrument, software version, and assay type. 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. Confirm the labeling of the control wells.
 - 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 wells with a positive control, confirm that **Task** is set to **Standard**.
7. For wells with a negative control, confirm that **Task** is set to **NTC**.
8. Edit the plate layout to assign a unique sample name to each well 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.

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

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 to connect the computer to the instrument, 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.

Set up and run the 7500 Fast Dx Real-Time PCR Instrument—COVID-19 plus RNase P assay

This section covers instrument set-up and operation for reaction plates that contain both the COVID-19 assay and the RNase P assay. Both assays are required for samples collected using the Everlywell™ COVID-19 Test Home Collection Kit.

For plates that contain only the COVID-19 assay, see “Set up and run the 7500 Fast Dx Real-Time PCR Instrument—COVID-19 assay only” on page 64.

For more information about the instrument, see the documents listed in “Related documentation” on page 123.

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 63.

IMPORTANT! Be careful to select the appropriate template file for your instrument, software version, and assay type. 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
COVID-19 assay	
FAM	ORF1ab
VIC	N gene
ABY	S gene
JUN	MS2
RNase P assay	
VIC	RNaseP

4. Confirm that the detectors above are assigned to the appropriate wells in the plate layout.

Note:

- Wells containing the RNase P assay have only the RNaseP detector assigned.
 - Wells containing the COVID-19 assay have all other detectors assigned.
-

5. Confirm the labeling of the control wells.
 - The template has one positive control and 2 negative controls (one each for the COVID-19 assay and RNase P assay) assigned to wells.
 - Move the control well assignments by copying the existing control wells and pasting them according to their location on the physical plate.
6. For wells with a positive control, confirm that **Task** is set to **Standard**.
7. For wells with a negative control, confirm that **Task** is set to **NTC**.
8. Edit the plate layout to assign the same sample name to each paired COVID-19 assay well and RNase P assay well in the physical plate.

IMPORTANT! Paired sample assay wells that do not have the same sample name will not be analyzed by the software.

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

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

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 to connect the computer to the instrument, load the plate, then start the run on the instrument.
12. After the instrument run is complete, open the SDS file in SDS Software v1.4.1. Analyze, then save the file.



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

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

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

1. On the computer with Applied Biosystems™ COVID-19 Interpretive Software, navigate to one of the following directories, based on whether the RT-PCR reaction contains only the COVID-19 assay, or both the COVID-19 assay and the RNase P assay (for Everlywell™ COVID-19 Test Home Collection Kit samples):
 - <...>\Applied Biosystems\COVID-19 Interpretive Software\Client\docs\User Documents\COVID-19
 - <...>\Applied Biosystems\COVID-19 Interpretive Software\Client\docs\User Documents\COVID-19 + RNase P

<...> is the installation directory
2. Select the correct template file, based on whether the RT-PCR reaction contains only the COVID-19 assay, or both the COVID-19 assay and RNase P assay (for Everlywell™ COVID-19 Test Home Collection Kit samples):

Table 18 COVID-19 assay only

Data collection software version	Template file
SDS Software v1.5.1	TaqPath COVID-19 Kit Template 7500fast sds1_5_1 v1-4.sdt
7500 Software v2.3	TaqPath COVID-19 Kit Template 7500fast sds2_3 v1-2.edt

Table 19 COVID-19 assay and RNase P assay

Data collection software version	Template file
SDS Software v1.5.1	TaqPath COVID-19 and RNaseP Template 7500fast sds1_5_1 v1-0.sdt
7500 Software v2.3	TaqPath COVID-19 and RNaseP Template 7500fast sds2_3 v1-0.edt

3. Select the correct SDT or EDT file for the version of the instrument software that you are using:

Data collection software version	Template file
SDS Software v1.5.1	TaqPath COVID-19 Kit Template 7500fast sds1_5_1 v1-4.sdt
7500 Software v2.3	TaqPath COVID-19 Kit Template 7500fast sds2_3 v1-2.edt

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

Set up and run the 7500 Fast Real-Time PCR Instrument (SDS Software v1.5.1)—COVID-19 assay only

This section covers instrument set-up and operation for reaction plates that contain only the COVID-19 assay, and is specific for the 7500 Fast Real-Time PCR Instrument using SDS Software v1.5.1.

For plates that contain both the COVID-19 assay and RNase P assay, see “Set up and run the 7500 Fast Real-Time PCR Instrument (SDS Software v1.5.1)—COVID-19 plus RNase P assay” on page 74.

For more information about the instrument, see the documents listed in “Related documentation” on page 123.

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 69.

IMPORTANT! Be careful to select the appropriate template file for your instrument, software version, and assay type. 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**.

- 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

- Confirm that the targets above are assigned to each well in the plate layout.
- 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.
- For wells with a positive control, confirm that **Task** is set to **Standard**.
- For wells with a negative control, confirm that **Task** is set to **NTC**.
- Edit the plate layout to assign a unique sample name to each well 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.

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

- 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	

- Click **Save As**, enter a file name, then click **Save**.
- Reopen the file, load the plate, then start the run on the instrument.
- 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)—COVID-19 assay only

This section covers instrument set-up and operation for reaction plates that contain only the COVID-19 assay, and is specific for the 7500 Fast Real-Time PCR Instrument using SDS Software v2.3.

For plates that contain both the COVID-19 assay and RNase P assay, see “Set up and run the 7500 Fast Real-Time PCR Instrument (7500 Software v2.3)—COVID-19 plus RNase P assay” on page 76.

For more information about the instrument, see the documents listed in “Related documentation” on page 123.

1. In the 7500 Software v2.3 home page, click **Template**.
2. Browse to, then open the EDT file that you transferred in “Transfer the template (SDT or EDT) file for the 7500 Fast Real-Time PCR Instrument” on page 69.

IMPORTANT! Be careful to select the appropriate template file for your instrument, software version, and assay type. 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 unique 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
ORF1ab	FAM	None
N gene	VIC	None
S gene	ABY	None
MS2	JUN	None

5. In the **Plate Setup** window, in the **Define Targets and Samples** tab and the **Define 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.
 - To include additional controls, select **Add New Sample**.

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

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

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 the **Passive Reference** is set to **None**.
12. In the **Run Method** window, confirm that the **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. Load the plate, then start the run.
15. After the instrument run is complete, click **Analyze**, then save the file.

Set up and run the 7500 Fast Real-Time PCR Instrument (SDS Software v1.5.1)—COVID-19 plus RNase P assay

This section covers instrument set-up and operation for reaction plates that contain both the COVID-19 assay and the RNase P assay, and is specific for the 7500 Fast Real-Time PCR Instrument using SDS Software v1.5.1.

Both assays are required for samples collected using the Everlywell™ COVID-19 Test Home Collection Kit.

For plates that contain only the COVID-19 assay, see “Set up and run the 7500 Fast Real-Time PCR Instrument (SDS Software v1.5.1)—COVID-19 assay only” on page 70.

For more information about the instrument, see the documents listed in “Related documentation” on page 123.

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 69.

IMPORTANT! Be careful to select the appropriate template file for your instrument, software version, and assay type. 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
COVID-19 assay	
FAM	ORF1ab
VIC	N gene
ABY	S gene
JUN	MS2
RNase P assay	
VIC	RNaseP

- Confirm that the detectors above are assigned to each well in the plate layout.

Note:

- Wells containing the RNase P assay have only the RNaseP detector assigned.
- Wells containing the COVID-19 assay have all other detectors assigned.

- Confirm the labeling of the control wells.
 - The template has one positive control and 2 negative controls (one each for the COVID-19 assay and RNase P assay) 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.
- For wells with a positive control, confirm that **Task** is set to **Standard**.
- For wells with a negative control, confirm that **Task** is set to **NTC**.
- Edit the plate layout to assign the same sample name to each paired COVID-19 assay well and RNase P assay well in the physical plate.

IMPORTANT! Paired sample assay wells that do not have the same sample name will not be analyzed by the software.

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

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

- 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	

- Click **Save As**, enter a file name, then click **Save**.
- Reopen the file, load the plate, then start the run on the instrument.
- 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)—COVID-19 plus RNase P assay

This section covers instrument set-up and operation for reaction plates that contain both the COVID-19 assay and the RNase P assay, and is specific for the 7500 Fast Real-Time PCR Instrument using SDS Software v2.3.

Both assays are required for samples collected using the Everlywell™ COVID-19 Test Home Collection Kit.

For plates that contain only the COVID-19 assay, see “Set up and run the 7500 Fast Real-Time PCR Instrument (7500 Software v2.3)—COVID-19 assay only” on page 72.

For more information about the instrument, see the documents listed in “Related documentation” on page 123.

1. In the 7500 Software v2.3 home page, click **Template**.
2. Browse to, then open the EDT file that you transferred in “Transfer the template (SDT or EDT) file for the 7500 Fast Real-Time PCR Instrument” on page 69.

IMPORTANT! Be careful to select the appropriate template file for your instrument, software version, and assay type. 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 unique 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
COVID-19 assay targets		
ORF1ab	FAM	None
N gene	VIC	None
S gene	ABY	None
MS2	JUN	None
RNase P assay target		
RNaseP	VIC	None

5. In the **Plate Setup** window, in the **Define Targets and Samples** tab and the **Define Samples** pane, confirm the labeling of the control wells.
 - The template has one positive control and 2 negative controls (one each for the COVID-19 assay and RNase P assay) 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.
 - To include additional controls, select **Add New Sample**.
6. Select **Add New Sample** to create a unique sample name.

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

7. In the **Plate Setup** window, in the **Assign Targets and Samples** tab, confirm the following:
 - Wells containing the RNase P assay have only the RNaseP target assigned.
 - Wells containing the COVID-19 assay have all other targets assigned.
8. To assign a target or sample to a well, select the well, then check the **Assign** checkbox.

IMPORTANT! Paired sample assay wells that do not have the same sample name will not be analyzed.

9. For wells with a positive control, confirm that **Task** is set to **S** for Standard.
10. For wells with a negative control, confirm that **Task** is set to **N** for Negative.
11. For wells with a patient sample, confirm that **Task** is set to **U** for Unknown.
12. Confirm that the **Passive Reference** is set to **None**.
13. In the **Run Method** window, confirm that the **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	

14. Select **Start Run**, enter a file name, then click **Save**.
15. Load the plate, then start the run.
16. After the instrument run is complete, click **Analyze**, then save the file.



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

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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.

IMPORTANT! Use only the calibration plates listed in “Required materials not supplied” on page 12.

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.

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

1. On the computer with Applied Biosystems™ COVID-19 Interpretive Software, navigate to one of the following directories, based on whether the RT-PCR reaction contains only the COVID-19 assay, or both the COVID-19 assay and the RNase P assay (for Everlywell™ COVID-19 Test Home Collection Kit samples):

- <...>\Applied Biosystems\COVID-19 Interpretive Software\Client\docs\User Documents\COVID-19
- <...>\Applied Biosystems\COVID-19 Interpretive Software\Client\docs\User Documents\COVID-19 + RNase P

<...> is the installation directory

2. Select the correct EDT file, based on whether the RT-PCR reaction contains only the COVID-19 assay, or both the COVID-19 assay and the RNase P assay (for Everlywell™ COVID-19 Test Home Collection Kit samples):

Assay type	Template name
COVID-19 only	TaqPath COVID-19 Kit Template 7500std sds2_3 v1-2.edt
COVID-19 and RNase P	TaqPath COVID-19 and RNaseP Template 7500std sds2_3 v1-0.edt

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

Set up and run the 7500 Real-Time PCR Instrument— COVID-19 assay only

This section covers instrument set-up and operation for reaction plates that contain only the COVID-19 assay.

For plates that contain both the COVID-19 assay and RNase P assay, see “Set up and run the 7500 Fast Real-Time PCR Instrument (7500 Software v2.3)—COVID-19 plus RNase P assay” on page 76.

For more information about the instrument, see the documents listed in “Related documentation” on page 123.

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 79.

IMPORTANT! Be careful to select the appropriate template file for your instrument, software version, and assay type. 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 unique 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
ORF1ab	FAM	None
N gene	VIC	None
S gene	ABY	None
MS2	JUN	None

5. In the **Plate Setup** window, in the **Define Targets and Samples** tab and the **Define 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.

To include additional controls, select **Add New Sample**.

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

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

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 the **Passive Reference** is set to **None**.
12. In the **Run Method** window, confirm that the **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. Load the plate, then start the run.
15. After the instrument run is complete, click **Analyze**, then save the file.

Set up and run the 7500 Real-Time PCR Instrument—COVID-19 and RNase P assay

This section covers instrument set-up and operation for reaction plates that contain both the COVID-19 assay and the RNase P assay. Both assays are required for samples collected using the Everlywell™ COVID-19 Test Home Collection Kit.

For plates that contain only the COVID-19 assay, see “Set up and run the 7500 Real-Time PCR Instrument—COVID-19 assay only” on page 80.

For more information about the instrument, see the documents listed in “Related documentation” on page 123.

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 79.

IMPORTANT! Be careful to select the appropriate template file for your instrument, software version, and assay type. 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 unique 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
COVID-19 assay targets		
ORF1ab	FAM	None
N gene	VIC	None
S gene	ABY	None
MS2	JUN	None
RNase P assay target		
RNaseP	VIC	None

5. In the **Plate Setup** window, in the **Define Targets and Samples** tab and the **Define Samples** pane, confirm the labeling of the control wells.
 - The template has one positive control and 2 negative controls (one each for the COVID-19 assay and RNase P assay) 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.
 - To include additional controls, select **Add New Sample**.

6. Select **Add New Sample** to create a unique sample name.

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

7. In the **Plate Setup** window, in the **Assign Targets and Samples** tab, confirm the following:
 - Wells containing the RNase P assay have only the RNaseP target assigned.
 - Wells containing the COVID-19 assay have all other targets assigned.

8. To assign a target or sample to a well, select the well, then check the **Assign** checkbox.

IMPORTANT! Each paired COVID-19 assay well and RNase P assay well must have the same sample name, or they will not be correctly analyzed by the software.

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

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

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

12. Confirm that the **Passive Reference** is set to **None**.

13. In the **Run Method** window, confirm that the **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	

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

15. Load the plate, then start the run.

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



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

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COVID-19 and RNase P assay 93

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. If calibration is required, refer to the standard calibration process in the instrument user guide.

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 or v1.5.2 is installed.

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

1. On the computer with Applied Biosystems™ COVID-19 Interpretive Software, navigate to one of the following directories, based on whether the RT-PCR reaction contains only the COVID-19 assay, or both the COVID-19 assay and the RNase P assay (for Everlywell™ COVID-19 Test Home Collection Kit samples):

- <...>\Applied Biosystems\COVID-19 Interpretive Software\Client\docs\User Documents\COVID-19
- <...>\Applied Biosystems\COVID-19 Interpretive Software\Client\docs\User Documents\COVID-19 + RNase P

<...> is the installation directory

2. Select the correct template file, based on whether the RT-PCR reaction contains only the COVID-19 assay, or both the COVID-19 assay and RNase P assay (for Everlywell™ COVID-19 Test Home Collection Kit samples):

Note: The same template file is used for QuantStudio™ Design and Analysis Desktop Software v1.5.1 and v1.5.2.

Table 20 COVID-19 assay only

Instrument	Template file
QuantStudio™ 5 Real-Time PCR Instrument (96-well, 0.1-mL block)	TaqPath COVID-19 Kit Template QS5 0_1ml_da1_5_1 v2-2.edt
QuantStudio™ 5 Real-Time PCR Instrument (96-well, 0.2-mL block)	TaqPath COVID-19 Kit Template QS5 0_2ml_da1_5_1 v2-2.edt
QuantStudio™ 5 Real-Time PCR Instrument (384-well block)	TaqPath COVID-19 Kit Template QS5 384_da1_5_1 v2-2.edt

Table 21 COVID-19 assay and RNase P assay

Instrument	Template file
QuantStudio™ 5 Real-Time PCR Instrument (96-well, 0.1-mL block)	TaqPath COVID-19 and RNaseP Template QS5 0_1ml_da1_5_1 v1-0.edt
QuantStudio™ 5 Real-Time PCR Instrument (96-well, 0.2-mL block)	TaqPath COVID-19 and RNaseP Template QS5 0_2ml_da1_5_1 v1-0.edt
QuantStudio™ 5 Real-Time PCR Instrument (384-well block)	TaqPath COVID-19 and RNaseP Template QS5 384_da1_5_1 v1-0.edt

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

Set up and run the QuantStudio™ 5 Real-Time PCR Instrument (96-well plates)—COVID-19 assay only

This section covers instrument set-up and operation for reaction plates that contain only the COVID-19 assay.

For 96-well plates that contain both the COVID-19 assay and RNase P assay, see “Set up and run the QuantStudio™ 5 Real-Time PCR Instrument (96-well plates)—COVID-19 and RNase P assay” on page 91.

For more information about the instrument, see the documents listed in “Related documentation” on page 123.

1. In the QuantStudio™ Design and Analysis Desktop Software, 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 85.

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

3. In the **Properties** tab, enter or confirm the following.
 - **Name:** Enter a unique 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 the **Passive Reference** is 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	Quencher
FAM	ORF1ab	None
VIC	N gene	None
ABY	S gene	None
JUN	MS2	None

9. Confirm that the targets above are assigned to each well in the plate layout.
10. In the plate layout 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.
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 (Negative Control)**.
13. In the **Samples** table, click **Add** to define the sample names. Create a unique sample name for each well in the physical plate.

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

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. Load the plate into the instrument.
16. *Perform the following step only when using a 0.2-mL block and a MicroAmp™ Optical 96-Well Reaction Plate (with or without barcode). Do not use a compression pad with a 0.1-mL block or other plate types.* Place a MicroAmp™ Optical Film Compression Pad gray side down on the surface of the RT-PCR reaction plate, to ensure a proper seal between the thermal cycler and the adhesive film.

IMPORTANT!

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

17. In the **Run** tab, click **Start Run**, then select your instrument from the drop-down list.
18. Enter a file name in the dialog box that prompts you to save the run file, then save the file.
19. If you are using a MicroAmp™ Optical Film Compression Pad, at the end of the run, remove the pad from the plate and store the pad inside the pack.

IMPORTANT!

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

Set up and run the QuantStudio™ 5 Real-Time PCR Instrument (384-well plates)—COVID-19 assay only

This section covers instrument set-up and operation for reaction plates that contain only the COVID-19 assay.

For plates that contain both the COVID-19 assay and RNase P assay, see “Set up and run the QuantStudio™ 5 Real-Time PCR Instrument (384-well plates)—COVID-19 and RNase P assay” on page 93.

For more information about the instrument, see the documents listed in “Related documentation” on page 123.

1. In the QuantStudio™ Design and Analysis Desktop Software, 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 85.

IMPORTANT! Be careful to select the appropriate template file for your instrument, block type, and assay 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:** 384-well Block
- **Experiment type:** Standard Curve
- **Chemistry:** TaqMan™ Reagents
- **Run Mode:** Standard

4. In the **Method** tab, confirm that the **Volume** 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	

5. In the **Plate** tab, click **Quick Setup**.
6. In the **Plate Attributes** pane, confirm that the **Passive Reference** is 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	Quencher
FAM	ORF1ab	None
VIC	N gene	None
ABY	S gene	None
JUN	MS2	None

9. Confirm that the targets above are assigned to each well in the plate layout.
10. In the plate layout 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 samples from more than one RNA extraction run are being included on the same RT-PCR 384-well plate, then there must be one negative control for each RNA extraction run included on the RT-PCR 384-well plate (see “Guidelines for RT-PCR” on page 37). Label each negative control with a unique name, for example, **NC1**, **NC2**, **NC3**, and **NC4**.

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 (Negative Control)**.
13. In the **Samples** table, click **Add** to define the names. Create a unique sample name for each well in the physical plate.

The template has one positive control and one negative control assigned to wells for reference. If additional control wells are required, each control well must have a unique name.

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

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. Load the plate into the instrument.
16. In the **Run** tab, click **Start Run**, then select your instrument from the drop-down list.
17. Enter a file name in the dialog box that prompts you to save the run file, then save the file.

Set up and run the QuantStudio™ 5 Real-Time PCR Instrument (96-well plates)—COVID-19 and RNase P assay

This section covers instrument set-up and operation for 96-well reaction plates that contain both the COVID-19 assay and the RNase P assay. Both assays are required for samples collected using the Everlywell™ COVID-19 Test Home Collection Kit.

For 96-well plates that contain only the COVID-19 assay, see “Set up and run the QuantStudio™ 5 Real-Time PCR Instrument (96-well plates)—COVID-19 assay only” on page 86.

For more information about the instrument, see the documents listed in “Related documentation” on page 123.

1. In the QuantStudio™ Design and Analysis Desktop Software, 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 85.

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

3. In the **Properties** tab, enter or confirm the following.
 - **Name:** Enter a unique 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 the **Passive Reference** is 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	Quencher
COVID-19 assay targets		
FAM	ORF1ab	None
VIC	N gene	None
ABY	S gene	None
JUN	MS2	None
RNase P assay target		
VIC	RNaseP	None

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

Note:

- Wells containing the RNase P assay have only the RNaseP detector assigned.
- Wells containing the COVID-19 assay have all other detectors assigned.

10. In the plate layout pane, confirm the labeling of the control wells.
- The template has one positive control and 2 negative controls (one each for the COVID-19 assay and RNase P assay) 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.
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 (Negative Control)**.
13. In the **Samples** table, click **Add** to define the sample names. The same sample name is used for each paired COVID-19 assay well and RNase P assay well in the physical plate.

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

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

IMPORTANT! Paired sample assay wells that do not have the same sample name will not be analyzed by the software.

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

15. Load the plate into the instrument.

16. Perform the following step only when using a 0.2-mL block and a MicroAmp™ Optical 96-Well Reaction Plate (with or without barcode). Do not use a compression pad with a 0.1-mL block or other plate types. Place a MicroAmp™ Optical Film Compression Pad gray side down on the surface of the RT-PCR reaction plate, to ensure a proper seal between the thermal cycler and the adhesive film.

IMPORTANT!

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

17. In the **Run** tab, click **Start Run**, then select your instrument from the drop-down list.
18. Enter a file name in the dialog box that prompts you to save the run file, then save the file.
19. If you are using a MicroAmp™ Optical Film Compression Pad, at the end of the run, remove the pad from the plate and store the pad inside the pack.

IMPORTANT!

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

Set up and run the QuantStudio™ 5 Real-Time PCR Instrument (384-well plates)—COVID-19 and RNase P assay

This section covers instrument set-up and operation for 384-well reaction plates that contain both the COVID-19 assay and the RNase P assay. Both assays are required for samples collected using the Everlywell™ COVID-19 Test Home Collection Kit.

For 384-well plates that contain only the COVID-19 assay, see “Set up and run the QuantStudio™ 5 Real-Time PCR Instrument (384-well plates)—COVID-19 assay only” on page 89.

For more information about the instrument, see the documents listed in “Related documentation” on page 123.

1. In the QuantStudio™ Design and Analysis Desktop Software, 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 85.

IMPORTANT! Be careful to select the appropriate template file for your instrument, block type, and assay 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:** 384-well Block
- **Experiment type:** Standard Curve
- **Chemistry:** TaqMan™ Reagents
- **Run Mode:** Standard

4. In the **Method** tab, confirm that the **Volume** 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	

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

6. In the **Plate Attributes** pane, confirm that the **Passive Reference** is 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	Quencher
COVID-19 assay targets		
FAM	ORF1ab	None
VIC	N gene	None
ABY	S gene	None
JUN	MS2	None
RNase P assay target		
VIC	RNaseP	None

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

Note:

- Wells containing the RNase P assay have only the RNaseP detector assigned.
- Wells containing the COVID-19 assay have all other detectors assigned.

10. In the plate layout pane, confirm the labeling of the control wells.
 - The template has one positive control and 2 negative controls (one each for the COVID-19 assay and RNase P assay) assigned. To assign additional negative control wells, see step 15.
 - 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 each negative control well, confirm that **Task** is set to **N (Negative Control)**.
13. In the **Samples** table, click **Add** to define the sample names.

The same sample name is used for each paired COVID-19 assay well and RNase P assay well in the physical plate.

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

14. To assign a sample to paired COVID-19 and RNase P assay wells, select each well in the plate layout, then select the sample from the **Samples** table.

IMPORTANT! Paired sample assay wells that do not have the same name will not be analyzed by the software.

For all targets in the patient sample wells, confirm that **Task** is set to **U (Unknown)**.
15. If the 384-well plate includes additional negative controls (from combined extraction runs), define each additional negative control and assign it to paired control wells (COVID-19 and RNase P) to match the physical plate.

Give each negative control a unique name (for example, **NC1**, **NC2**, and so forth).
16. In the **Run** tab, click **Start Run**, then select your instrument from the drop-down list.
17. Load the plate into the instrument.
18. Enter a file name in the dialog box that prompts you to save the run file, then save the file.

10

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

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 appropriate template file for your instrument, block type, and assay type. Failure to do so can cause errors in the analysis.

1. On the computer with Applied Biosystems™ COVID-19 Interpretive Software, navigate to one of the following directories, based on whether the RT-PCR reaction contains only the COVID-19 assay, or both the COVID-19 assay and the RNase P assay (for Everlywell™ COVID-19 Test Home Collection Kit samples):

- <...>\Applied Biosystems\COVID-19 Interpretive Software\Client\docs\User Documents\COVID-19
- <...>\Applied Biosystems\COVID-19 Interpretive Software\Client\docs\User Documents\COVID-19 + RNase P

<...> is the installation directory

2. Select the correct EDT file, based on whether the RT-PCR reaction contains only the COVID-19 assay, or both the COVID-19 assay and the RNase P assay (for Everlywell™ COVID-19 Test Home Collection Kit samples):

Assay type	Template name
COVID-19 only	TaqPath COVID-19 Kit Template QS7 384 1_3 v2-2.edt
COVID-19 and RNase P	TaqPath COVID-19 and RNaseP Template QS7 384 1_3 v1-0.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)—COVID-19 assay only

This section covers instrument set-up and operation for reaction plates that contain only the COVID-19 assay.

For plates that contain both the COVID-19 assay and RNase P assay, see “Set up and run the QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block)—COVID-19 and RNase P assay” on page 99.

For more information about the instrument, see the documents listed in “Related documentation” on page 123.

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 96.

IMPORTANT! Be careful to select the appropriate template file for your instrument, block type, and assay type. 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! Encrypt, pseudonymize, or anonymize personal data where possible following the requirements of HIPAA (Health Insurance Portability and Accountability Act).

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.

- 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	

- Load the plate into the instrument.
- In the **Run** tab, click **Start Run**, then select your instrument from the drop-down list.
- Enter a file name in the dialog box that prompts you to save the run file, then save the file.

Set up and run the QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block)—COVID-19 and RNase P assay

This section covers instrument set-up and operation for 384-well reaction plates that contain both the COVID-19 assay and the RNase P assay. Both assays are required for samples collected using the Everlywell™ COVID-19 Test Home Collection Kit.

For 384-well plates that contain only the COVID-19 assay, see “Set up and run the QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block)—COVID-19 assay only” on page 97.

For more information about the instrument, see the documents listed in “Related documentation” on page 123.

- In the QuantStudio™ Real-Time PCR Software v1.3 home screen, click **Template**.
- 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 96.

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

- 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
COVID-19 assay targets		
ORF1ab	FAM	None
N gene	VIC	None
S gene	ABY	None
MS2	JUN	None
RNase P assay target		
RNaseP	VIC	None

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

The same sample name is used for each paired COVID-19 assay well and RNase P assay well in the physical plate.

IMPORTANT! Paired sample assay wells that do not have the same name will not be analyzed by the software.

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

6. In the **Define** tab, confirm that the **Passive Reference** is set to **None**.
7. In the **Assign** tab, confirm that targets are assigned to each well in the plate layout.

Note:

- Wells containing the RNase P assay have only the RNaseP target assigned.
 - Wells containing the COVID-19 assay have all other targets assigned.
-

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 and 2 negative controls (one each for the COVID-19 assay and RNase P assay) 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 paired control wells (COVID-19 and RNase P) to match the physical plate.

Give each negative control a unique name (for example, **NC1**, **NC2**, and so forth).

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 paired wells (COVID-19 and RNase P) 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 plate into the 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 the button that is appropriate for your test:
 - **COVID-19 Test**
 - **COVID-19 + RNase P Test**
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.

Interpretation of the results

Interpretation of the results is performed by the Applied Biosystems™ COVID-19 Interpretive Software. For information about the C_t values that are used by the software to interpret results, see Appendix B, “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 for the COVID-19 assay.

A minimum of two Negative Controls (one for the COVID-19 assay and one for the RNase P assay) and one Positive Control must be present for each run for the COVID-19 plus RNase P assay.

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

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

Table 22 COVID-19 Test—Patient samples

ORF1ab	N gene	S gene	MS2	Status	Result	Action
NEG	NEG	NEG	NEG	INVALID	NA	Repeat test by re-extracting the original sample and repeating the RT-PCR. If the repeat result remains invalid, consider collecting a new specimen.
NEG	NEG	NEG	POS	VALID	SARS-CoV-2 Not Detected	Report results to the healthcare provider and appropriate public health authorities. Consider testing for other viruses.
Only one SARS-CoV-2 target = POS			POS or NEG	VALID	SARS-CoV-2 Inconclusive	<ol style="list-style-type: none"> 1. Repeat test by re-extracting the original sample and repeating the RT-PCR. 2. After retesting one time, report results to the healthcare provider and appropriate public health authorities. <p>IMPORTANT! Samples with a result of SARS-CoV-2 Inconclusive shall be retested one time.</p> <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	Report results to the healthcare provider and appropriate public health authorities.

Table 23 COVID-19 + RNase P Test—Patient samples

ORF1ab	N gene	S gene	MS2	RNaseP	Status	Result	Action
NEG	NEG	NEG	NEG	NEG	INVALID	NA	Repeat test by re-extracting the original sample and repeating the RT-PCR. If the repeat result remains invalid, consider collecting a new specimen.
NEG	NEG	NEG	NEG	POS	INVALID	NA	Repeat test by re-extracting the original sample and repeating the RT-PCR. If the repeat result remains invalid, consider collecting a new specimen.
NEG	NEG	NEG	POS	POS	VALID	SARS-CoV-2 Not Detected	Report results to the healthcare provider and appropriate public health authorities. Consider testing for other viruses.
NEG	NEG	NEG	POS	NEG	INVALID	NA	Repeat test by re-extracting the original sample and repeating the RT-PCR. If the repeat result remains invalid, consider collecting a new specimen.

Table 23 COVID-19 + RNase P Test—Patient samples (continued)

ORF1ab	N gene	S gene	MS2	RNaseP	Status	Result	Action
Only one SARS-CoV-2 target = POS			POS or NEG		VALID	SARS-CoV-2 Inconclusive ^[1]	<ol style="list-style-type: none"> Repeat test by re-extracting the original sample and repeating the RT-PCR. After retesting one time, report results to the healthcare provider and appropriate public health authorities. <p>IMPORTANT! Samples with a result of SARS-CoV-2 Inconclusive shall be retested one time.</p> <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	Report results to the healthcare provider and appropriate public health authorities.

^[1] Samples with a result of SARS-CoV-2 Inconclusive shall be retested one time.

Conditions of authorization for labs

The TaqPath™ COVID-19 RT-PCR 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: [fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas](https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas).

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

- Authorized laboratories^[1] using the TaqPath™ COVID-19 Combo Kit must 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 must use the 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 must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories testing anterior nasal swab specimens self-collected using the Everlywell™ COVID-19 Test Home Collection Kit must be laboratories designated by Everlywell, Inc. and follow the *Receiving and Processing Everlywell Samples* standard operating procedure when accepting specimens for testing.
- Authorized laboratories must collect information on the performance of the test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Thermo Fisher Scientific (techservices@thermofisher.com; 1 800 955 6288) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.
- All laboratory personnel using the test must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the test in accordance with the authorized labeling.
- Thermo Fisher Scientific, its authorized distributor(s), and authorized laboratories using the TaqPath™ COVID-19 Combo Kit must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records must be made available to FDA for inspection upon request.

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

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Analytical performance of the TaqPath™ COVID-19 RT-PCR 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 24 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	

Table 24 LoD determination in BAL (continued)

Effective Concentration	Replicate	Mean C _t				Interpretation	% Positive
		ORF1ab	N	S	MS2		
10 GCE/reaction	8	30.2	29.8	29.2	23.8	Positive	100%
	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	
	13	30.7	30.1	28.4	25.1	Positive	
	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 25 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	

Table 25 LoD determination in Nasopharyngeal specimens (continued)

Effective Concentration	Replicate	Mean C _t				Interpretation	% Positive
		ORF1ab	N	S	MS2		
10 GCE/reaction	12	31.6	29.7	35.0	27.6	Positive	100%
	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	
	17	30.7	29.4	34.8	27.4	Positive	
	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 26 LoD results

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

Reactivity (Inclusivity)

In silico analysis was executed using 58,081 complete or near complete SARS-CoV-2 genomes in the GISAID database, from 12 April 2022 to 9 May 2022, which includes the following variants of concern:

- 57,840 genomes of the Omicron variant
- 134 genomes of the XE variant
- 7 genomes of the Delta variant
- 100 genomes of variants without a WHO label

Mismatch melting temperature analysis was performed in which a positive result was called when the primers and probe for at least 2 of the 3 assay targets (ORF1ab, S gene, and N gene) exhibited melting temperatures from the template strand that were higher than the annealing temperature for the PCR. Based on the melting temperature analysis, the TaqPath™ COVID-19 Combo Kit correctly identified 58,044 of 58,081 (99.9%) 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, therefore the COVID-19 test is expected to be highly inclusive for SARS-CoV-2 strain detection.

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 listed in Table 27 on page 112. Each substance was tested with triplicate extractions. The results are presented in Table 27 on page 112.

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 27 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% ^[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 was performed.

Table 28 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 clinical evaluation study was performed to evaluate the performance of the TaqPath™ COVID-19 RT-PCR 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™ COVID-19 RT-PCR 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 29 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 30 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.

FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples, and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to corroborate the LoD. All runs were performed using the TaqPath™ COVID-19 Combo Kit and the 7500 Fast Real-Time PCR Instrument (Table 31) or the QuantStudio™ 7 Flex Real-Time PCR Instrument (Table 32).

Table 31 Summary of LoD confirmation result using the FDA SARS-CoV-2 Reference Panel with the TaqPath™ COVID-19 Combo Kit and the 7500 Fast Real-Time PCR Instrument

Reference materials provided by FDA	Specimen type	Product LoD	Cross-reactivity
SARS-CoV-2	Nasopharyngeal swab (5.0-μL PCR input volume)	1.8 × 10 ⁵ NDU/mL ^[1]	N/A ^[2]
MERS-CoV		N/A ^[2]	ND ^[3]

^[1] RNA NAAT detectable units/mL

^[2] Not Applicable

^[3] Not Detected

Table 32 Summary of LoD confirmation result using the FDA SARS-CoV-2 Reference Panel with the TaqPath™ COVID-19 Combo Kit and the QuantStudio™ 7 Flex Real-Time PCR Instrument

Reference materials provided by FDA	Specimen type	Product LoD	Cross-reactivity
SARS-CoV-2	Nasopharyngeal swab (5.0-μL PCR input volume)	1.98 × 10 ³ NDU/mL ^[1]	N/A ^[2]
MERS-CoV		N/A ^[2]	ND ^[3]

^[1] RNA NAAT detectable units/mL

^[2] Not Applicable

^[3] Not Detected



Example extraction plate and RT-PCR reaction plate layouts (Everlywell™ COVID-19 Test Home Collection Kit samples)

Everlywell™ COVID-19 Test Home Collection Kit samples require that users prepare two RT-PCR plate wells for each sample and Negative Control—one for the COVID-19 assay and one for the RNase P assay—plus a single RT-PCR plate well for the Positive Control. A single 96-well extraction plate contains enough samples for two 96-well RT-PCR reaction plates, or may be combined with another extraction plate onto a single 384-well RT-PCR reaction plate.

The following diagrams provide example layouts for extraction plates and RT-PCR plates, assuming that you want to fit the maximum number of samples onto each plate.

Layout of RNA extraction plate

92 RNA samples, 2 Negative Controls

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC1	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
B	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23
C	S24	S25	S26	S27	S28	S29	S30	S31	S32	S33	S34	S35
D	S36	S37	S38	S39	S40	S41	S42	S43	S44	S45	S46	EMPTY
E	NC2	S47	S48	S49	S50	S51	S52	S53	S54	S55	S56	S57
F	S58	S59	S60	S61	S62	S63	S64	S65	S66	S67	S68	S69
G	S70	S71	S72	S73	S74	S75	S76	S77	S78	S79	S80	S81
H	S82	S83	S84	S85	S86	S87	S88	S89	S90	S91	S92	EMPTY



Layout of 2 96-well RT-PCR plates from 1 RNA extraction plate

Each RT-PCR plate contains the following:

46 RNA samples

1 Negative Control into 2 wells (COVID-19 and RNase P assay wells)

1 Positive Control (COVID-19 assay well)

RT-PCR plate #1

	1	2	3	4	5	6	7	8	9	10	11	12	
COVID-19	A	NC1	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
RNase P	B	NCR1	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
COVID-19	C	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23
RNase P	D	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23
COVID-19	E	S24	S25	S26	S27	S28	S29	S30	S31	S32	S33	S34	S35
RNase P	F	S24	S25	S26	S27	S28	S29	S30	S31	S32	S33	S34	S35
COVID-19	G	S36	S37	S38	S39	S40	S41	S42	S43	S44	S45	S46	PC
RNase P	H	S36	S37	S38	S39	S40	S41	S42	S43	S44	S45	S46	EMPTY

RT-PCR plate #2

	1	2	3	4	5	6	7	8	9	10	11	12	
COVID-19	A	NC2	S47	S48	S49	S50	S51	S52	S53	S54	S55	S56	S57
RNase P	B	NCR2	S47	S48	S49	S50	S51	S52	S53	S54	S55	S56	S57
COVID-19	C	S58	S59	S60	S61	S62	S63	S64	S65	S66	S67	S68	S69
RNase P	D	S58	S59	S60	S61	S62	S63	S64	S65	S66	S67	S68	S69
COVID-19	E	S70	S71	S72	S73	S74	S75	S76	S77	S78	S79	S80	S81
RNase P	F	S70	S71	S72	S73	S74	S75	S76	S77	S78	S79	S80	S81
COVID-19	G	S82	S83	S84	S85	S86	S87	S88	S89	S90	S91	S92	PC
RNase P	H	S82	S83	S84	S85	S86	S87	S88	S89	S90	S91	S92	EMPTY

Figure 9 Example plate layouts for 96-well RT-PCR reactions (Everlywell™ COVID-19 Test Home Collection Kit samples)



Layout of 2 RNA extraction plates

RNA extraction plate #1

95 RNA samples, 1 Negative Control

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC1	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
B	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23
C	S24	S25	S26	S27	S28	S29	S30	S31	S32	S33	S34	S35
D	S36	S37	S38	S39	S40	S41	S42	S43	S44	S45	S46	S47
E	S48	S49	S50	S51	S52	S53	S54	S55	S56	S57	S58	S59
F	S60	S61	S62	S63	S64	S65	S66	S67	S68	S69	S70	S71
G	S72	S73	S74	S75	S76	S77	S78	S79	S80	S81	S82	S83
H	S84	S85	S86	S87	S88	S89	S90	S91	S92	S93	S94	S95

RNA extraction plate #2

94 RNA samples, 1 Negative Control

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC2	S96	S97	S98	S99	S100	S101	S102	S103	S104	S105	S106
B	S107	S108	S109	S110	S111	S112	S113	S114	S115	S116	S117	S118
C	S119	S120	S121	S122	S123	S124	S125	S126	S127	S128	S129	S130
D	S131	S132	S133	S134	S135	S136	S137	S138	S139	S140	S141	S142
E	S143	S144	S145	S146	S147	S148	S149	S150	S151	S152	S153	S154
F	S155	S156	S157	S158	S159	S160	S161	S162	S163	S164	S165	S166
G	S167	S168	S169	S170	S171	S172	S173	S174	S175	S176	S177	S178
H	S179	S180	S181	S182	S183	S184	S185	S186	S187	S188	S189	EMPTY



Layout of 1 384-well RT-PCR plate from 2 RNA extraction plates

RT-PCR plate contains the following:

189 RNA samples

2 Negative Controls into 4 wells (2 COVID-19 and 2 RNase P assay wells)

1 Positive Control (COVID-19 assay well)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
COVID-19	A	NC1	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S48	S49	S50	S51	S52	S53	S54	S55	S56	S57	S58	S59
RNase P	B	NC1	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S48	S49	S50	S51	S52	S53	S54	S55	S56	S57	S58	S59
COVID-19	C	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S60	S61	S62	S63	S64	S65	S66	S67	S68	S69	S70	S71
RNase P	D	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S60	S61	S62	S63	S64	S65	S66	S67	S68	S69	S70	S71
COVID-19	E	S24	S25	S26	S27	S28	S29	S30	S31	S32	S33	S34	S35	S72	S73	S74	S75	S76	S77	S78	S79	S80	S81	S82	S83
RNase P	F	S24	S25	S26	S27	S28	S29	S30	S31	S32	S33	S34	S35	S72	S73	S74	S75	S76	S77	S78	S79	S80	S81	S82	S83
COVID-19	G	S36	S37	S38	S39	S40	S41	S42	S43	S44	S45	S46	S47	S84	S85	S86	S87	S88	S89	S90	S91	S92	S93	S94	S95
RNase P	H	S36	S37	S38	S39	S40	S41	S42	S43	S44	S45	S46	S47	S84	S85	S86	S87	S88	S89	S90	S91	S92	S93	S94	S95
COVID-19	I	NC2	S96	S97	S98	S99	S100	S101	S102	S103	S104	S105	S106	S143	S144	S145	S146	S147	S148	S149	S150	S151	S152	S153	S154
RNase P	J	NC2	S96	S97	S98	S99	S100	S101	S102	S103	S104	S105	S106	S143	S144	S145	S146	S147	S148	S149	S150	S151	S152	S153	S154
COVID-19	K	S107	S108	S109	S110	S111	S112	S113	S114	S115	S116	S117	S118	S155	S156	S157	S158	S159	S160	S161	S162	S163	S164	S165	S166
RNase P	L	S107	S108	S109	S110	S111	S112	S113	S114	S115	S116	S117	S118	S155	S156	S157	S158	S159	S160	S161	S162	S163	S164	S165	S166
COVID-19	M	S119	S120	S121	S122	S123	S124	S125	S126	S127	S128	S129	S130	S167	S168	S169	S170	S171	S172	S173	S174	S175	S176	S177	S178
RNase P	N	S119	S120	S121	S122	S123	S124	S125	S126	S127	S128	S129	S130	S167	S168	S169	S170	S171	S172	S173	S174	S175	S176	S177	S178
COVID-19	O	S131	S132	S133	S134	S135	S136	S137	S138	S139	S140	S141	S142	S179	S180	S181	S182	S183	S184	S185	S186	S187	S188	S189	PC
RNase P	P	S131	S132	S133	S134	S135	S136	S137	S138	S139	S140	S141	S142	S179	S180	S181	S182	S183	S184	S185	S186	S187	S188	S189	EMPTY

Figure 10 Example plate layouts for 384-well RT-PCR reactions (Everlywell™ COVID-19 Test Home Collection Kit samples)



C_t cutoff values for assay targets

The Applied Biosystems™ COVID-19 Interpretive Software uses the following C_t cutoff values for assay targets during interpretation of the results.

Table 33 COVID-19 Assay C_t cutoff values

Sample or Control	Target	C _t cutoff
Positive Control	MS2	Valid C _t values are >37
	Viral targets	Valid C _t values are ≤37
Negative Control	MS2	Valid C _t values are ≤32
	Viral targets	Valid C _t values are >37
Clinical samples	MS2	Valid C _t values are ≤32 ^[1]
	Viral targets	Positive C _t values are ≤37

^[1] If any of the viral targets is positive, the C_t for MS2 can be >32.

Table 34 RNase P Assay C_t cutoff values

Sample or Control	Target	C _t cutoff
Negative Control	RNase P	Valid C _t values are >33
Clinical samples	RNase P	Valid C _t values are ≤33



EUO label for RUO instruments

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

- Applied Biosystems™ 7500 Fast Real-Time PCR Instrument
- Applied Biosystems™ 7500 Real-Time PCR Instrument
- Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument
- Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR Instrument

1. 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.

Biological hazard safety



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
<https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf>
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
www.who.int/publications/i/item/9789240011311

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.



Documentation and support

Related documentation

Document	Publication Number
<i>Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument Reference Guide</i>	4406991
<i>Applied Biosystems™ 7500/7500 Fast Real-Time PCR System: Maintenance Guide</i>	4387777
<i>QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide</i>	MAN0010407
<i>QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Maintenance and Administration Guide</i>	4489821
<i>QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Quick Reference</i>	4489826
<i>QuantStudio™ Real-Time PCR Software Getting Started Guide</i>	4489822
<i>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>MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit Instructions For Use</i>	MAN0019746
<i>Thermo Scientific™ KingFisher™ Flex User Manual</i>	N07669
<i>COVID-19 Interpretive Software Installation Quick Reference</i>	MAN0019257
<i>TaqMan™ SARS-CoV-2 RNase P Assay Kit Product Information Sheet</i>	MAN0019524



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 102.

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)

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.

TaqPath™ COVID-19 Combo Kit

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

Catalog Number A47814

Pub. No. 100092995 Rev. F

For Emergency Use Authorization Only

Intended Use

The 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 nasopharyngeal, oropharyngeal, anterior nasal, and mid-turbinate swabs, nasopharyngeal aspirates, and bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

This test is also authorized for use with the Everlywell™ COVID-19 Test Home Collection Kit when used consistent with its authorization.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory and 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 test results to the appropriate public health authorities.

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

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

Instructions for Use/Interpretation/Limitations

The *TaqPath™ COVID-19 Combo Kit Instructions for Use* (Pub. No. MAN0019181) can be downloaded from the following link:

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

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

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>.

Warnings and precautions

- This product has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- This product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or the authorization is revoked sooner.

IVD

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

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Contents and storage

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

Box	Components	Amount	Storage
TaqPath™ COVID-19 RT-PCR Kit	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 (1 × 10 ⁴ copies/µL)		2 × 10 µL per box; 5 boxes per kit	≤ -70°C
TaqPath™ COVID-19 Control Dilution Buffer		2 × 250 µL per box; 5 boxes per kit	-30°C to -10°C

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 1. 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 worldwide contact telephone numbers, product support information, order and web support, Certificates of Analysis, and Safety Data Sheets (SDSs; also known as MSDSs).

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

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.



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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision history: Pub. No. 100092995

Revision	Date	Description
F	18 May 2022	References to the TaqPath™ COVID-19 Combo Kit Advanced were removed throughout.
E	5 October 2021	The Intended Use was updated to specify anterior nasal swabs, plus additional wording changes. The warnings and precautions were updated with revised wording. The download link for the Instructions for Use and the contact team for obtaining the software were updated. Licensing information was removed. The Software Release Notes were referenced for additional software information.
D	9 October 2020	Added new Warnings and Precautions. Minor updates to Intended Use.
C	2 October 2020	Updated the Intended Use to include the Everlywell™ COVID-19 Test Home Collection Kit and TaqPath™ COVID-19 Combo Kit Advanced. Updated the instructions for obtaining the software.
B	25 March 2020	Removed information related to the procedure and directed users to the EUA website for the instructions for use. Removed 100-reaction kit size.
A	13 March 2020	New document.

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