

WHO Emergency Use Assessment SARS-CoV-2 IVDs PUBLIC REPORT

Product: TaqPath COVID-19 CE-IVD RT-PCR Kit

Manufacturer: Thermo Fisher Scientific

EUL Number: EUL 0525-156-00

Outcome: Accepted

The EUL process is intended to expedite the availability of in vitro diagnostics needed in public health emergency situations and to assist interested UN procurement agencies and Member States in determining the acceptability of using specific products in the context of a Public Health Emergency of International Concern (PHEIC), based on an essential set of available quality, safety and performance data. The EUL procedure includes the following:

- Quality Management Systems Review and Plan for Post-Market Surveillance: desk-top review of the manufacturer's Quality Management System documentation and specific manufacturing documents;
- Product Dossier Review: assessment of the documentary evidence of safety and performance.

TaqPath COVID-19 CE-IVD RT-PCR Kit code A48067, CE-mark regulatory version, manufactured by Thermo Fisher Scientific, 6055 Sunol Blvd, Pleasanton CA 94566, United States of America was listed on 14 August 2020.

Report amendments and/or product changes

This public report has since been amended. Amendments may have arisen because of changes to the EUL product for which WHO has been notified and has undertaken a review. Amendments to the report are summarized in the following table, and details of each amendment are provided below.

Version	Summary of amendment	Date of report amendment
2.0	Addition of KingFisher Flex Magnetic Particle Processor with 96 Deep-Well Head (PN 5400630; KingFisher Flex) on the list of validated nucleic acid extraction systems.	4 Nov 2020
3.0	Closing of post EUL commitments.	1 May 2023

Intended use:

According to the claim of intended use from Thermo Fisher Scientific, " *TaqPath COVID-19 CE-IVD RT-PCR Kit contains the reagents and controls for a real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens (such as nasopharyngeal, oropharyngeal,*

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

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory and bronchoalveolar lavage (BAL) specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories may be required to report all positive results to the appropriate Competent Health Authorities.

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

Testing with the TaqPath COVID-19 CE-IVD RT-PCR Kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

Note: The following countries require the CE-marked In Vitro Diagnostics: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, UK, Norway, Iceland, Liechtenstein, Switzerland, Turkey.”

Specimen types that were validated:

nasopharyngeal, oropharyngeal swabs, nasopharyngeal aspirate, and bronchoalveolar lavage (BAL) specimens.

Test kit contents:

Component		1000 tests (product code A48067)
TaqPath COVID-19 RT-PCR Kit	TaqPath COVID-19 Assay Multiplex (Gene ORF1ab, N Protein, S Protein, MS2)	1 tube x 1,500 µL
	MS2 Phage Control	10 tubes x 1 mL
TaqPath COVID-19 Control		10 tubes x 10 µL
TaqPath COVID-19 Control Dilution Buffer		10 tubes x 250 µL
TaqPath 1-Step Multiplex Master Mix (No ROX)		1 bottle x 10 mL

Items required but not provided:

Extraction/Purification kits that were validated together with the product.

- MagMAX Viral/Pathogen Nucleic Acid Isolation Kit.
- MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit.
- KingFisher Flex Magnetic Particle Processor with 96 Deep-Well Head (PN 5400630; KingFisher Flex).

Real-Time PCR equipment:

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

Item	Source
Real-time PCR instrument	
Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument (used with SDS Software v1.4.1)	4406984 (with laptop computer) 4406985 (with tower computer)
Applied Biosystems 7500 Fast Real-Time PCR Instrument (used with SDS Software v1.5.1 or 7500 Software v2.3)	4351106 (with laptop computer) 4351107 (with desktop computer)
Applied Biosystems 7500 Real-Time PCR Instrument (used with 7500 Software v2.3)	4351104 (with laptop computer) 4351105 (with desktop computer)
Applied Biosystems QuantStudio 5 Real-Time PCR Instrument, 0.2-mL block (used with QuantStudio Design and Analysis Desktop Software v1.5.1)	A28569 (with laptop computer) A28574 (with desktop computer) A28139 (instrument only)
Applied Biosystems QuantStudio 5 Real-Time PCR Instrument, 0.1-mL block (used with QuantStudio Design and Analysis Desktop Software v1.5.1)	A28568 (with laptop computer) A28573 (with desktop computer)
Applied Biosystems QuantStudio 5 Real-Time PCR Instrument, 384-well block (used with QuantStudio Design and Analysis Desktop Software v1.5.1)	A28570 (with laptop computer) A28575 (with desktop computer)
Applied Biosystems QuantStudio 5 Dx Real-Time PCR Instrument (used with QuantStudio 5 Dx TD Software v1.0.2 or QuantStudio 5 Dx IVD Software v1.0.2)	A32005 (with laptop computer) A32006 (with tower computer)

Applied Biosystems QuantStudio 7 Flex Real-Time PCR Instrument, 384-well block (used with QuantStudio Real-Time PCR Software v1.3)	4485695 (with laptop computer) 4485701 (with desktop computer)
Equipment	
Laboratory freezers • -30°C to -10°C	MLS
Centrifuge, with a rotor for microplates	MLS

Item	Source
Microcentrifuge	MLS
Laboratory mixer, vortex or equivalent	MLS
Single and multichannel adjustable pipettors (1.00 µL to 1,000.0 µL)	MLS
Cold block (96-well or 384-well) or ice	MLS
Kits and reagents	
Nuclease-free Water (not DEPC-Treated)	MLS
Calibration plates (7500 Real-Time PCR Instrument series)	
ABY Dye Spectral Calibration Plate for Multiplex qPCR, Fast 96-well (0.1-mL)	A24734
JUN Dye Spectral Calibration Plate for Mutliplex qPCR, Fast 96-well (0.1-mL)	A24735
ABY Dye Spectral Calibration Plate for Multiplex qPCR, 96- well (0.2-mL)	A24738
JUN Dye Spectral Calibration Plate for Mutliplex qPCR, 96- well (0.2-mL)	A24737
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 Mutliplex qPCR, 384- well	A24733
Tubes, plates, and other consumables	

MicroAmp Fast Optical 96-Well Reaction Plate with Barcode,	4346906, 4366932
MicroAmp Fast Optical 96-Well Reaction Plate, 0.1 mL	4346907
MicroAmp Optical 96-Well Reaction Plate with Barcode,0.2 mL	4306737, 4326659
MicroAmp Optical 96-Well Reaction Plate, 0.2 mL	N8010560, 4316813
MicroAmp Optical 384-Well Reaction Plate with	4309849, 4326270, 4343814
MicroAmp Optical 384-Well Reaction Plate	4343370
MicroAmp Optical Adhesive Film	4311971, 4360954
MicroAmp Adhesive Film Applicator	4333183

Item	Source
Nonstick, RNase-free microcentrifuge tubes (1.5 mL and	thermofisher.com/plastics
Sterile aerosol barrier (filtered) pipette tips	thermofisher.com/pipette tips

Instrument and software compatibility

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

For information on how to obtain the Applied Biosystems COVID-19 Interpretive Software, see “Obtain the Applied Biosystems COVID-19 Interpretive Software” on page 59.

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

Instrument	Analysis software used with the instrument	Compatible COVID-19 Interpretive Software version
7500 Fast Dx Real-Time PCR Instrument	SDS Software v1.4.1	v1.3
7500 Fast Real-Time PCR Instrument	SDS Software v1.5.1 or 7500 Software v2.3	v1.3
7500 Real-Time PCR Instrument	7500 Software v2.3	v1.3

QuantStudio 5 Real-Time PCR Instrument with instrument firmware v1.3.3 96-well, 0.2-mL lock 96-well, 0.1-mL block	QuantStudio Design and Analysis Desktop Software v1.5.1	v2.3
QuantStudio 5 Real-Time PCR Instrument with instrument firmware v1.3.3 384-well block	QuantStudio Design and Analysis Desktop Software v1.5.1	v2.3
QuantStudio 5 Dx Real-Time PCR Instrument with instrument firmware v1.0.3	QuantStudio 5 Dx TD Software v1.0.2	v2.3

Storage:

Store TaqPath COVID-19 CE-IVD RT-PCR Kit reagents at -30°C to -10°C and TaqPath COVID-19 Controls at $\leq -70^{\circ}\text{C}$.

Shelf-life upon manufacture:

12 months (real time shelf-life studies are ongoing).

Warnings/limitations:

Refer to the instructions for use (IFU)

Product dossier assessment

Thermo Fisher Scientific submitted a product dossier for the TaqPath COVID-19 CE-IVD RT-PCR Kit for detecting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as per the “*Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid (PQDx_0347 version 4)*”. The information (data and documentation) submitted in the product dossier was reviewed by WHO staff and external technical experts (assessors) appointed by WHO.

Post listing Commitments for EUL:

As commitments to listing, the manufacturer is required to;

1. Determine the limit of detection with the WHO international standard when available. The commitment was fulfilled. The issue is closed.
2. Provide matrix equivalency study report by 04 December 2020. Submitted evidence was acceptable and issue was closed.
3. Provide precision study report by 30 November 2020. Submitted evidence was acceptable and issue was closed.
4. Provide a Cross-Reactivity study report by 30 November 2020. Submitted evidence was acceptable and issue was closed.

5. Provide a Robustness study report taking into consideration the likely user and environment in WHO Member States by 04 December 2020. Submitted evidence was acceptable and issue was closed.
6. Provide a Transport Stability study report by 30 October 2020. Real time shelf-life studies are ongoing and interim data will be submitted at 3 months (September 2020) and the final study report within 1 month of completion (August 2021). The commitment was fulfilled. The issue is closed.
7. Provide additional clinical study report by 15 February 2021. Submitted evidence was acceptable and issue was closed.

Risk benefit assessment is acceptable.

Quality Management Systems Review

To establish the eligibility for WHO procurement, Thermo Fisher Scientific was asked to provide up-to-date information about the status of their quality management system.

Based on the review of the submitted quality management system documentation by WHO staff, it was established that sufficient information was provided by Thermo Fisher Scientific to fulfil the requirements described in the *“Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid (PQDx_347)”*.

Quality management documentation assessment conclusion: acceptable.

Plan for Post-Market Surveillance

Post-market surveillance, including monitoring all customer feedback, detecting and acting on adverse events, product problems, non-conforming goods and processes is a critical component of minimizing potential harm of an IVD listed for emergency use.

The following post-EUL activities are required to maintain the EUL listing status:

1. Notification to WHO of any planned changes to a EUL product, in accordance with *“WHO procedure for changes to a WHO prequalified in vitro diagnostic”* (document number PQDx_121); and
2. Post-market surveillance activities, in accordance with *“Guidance for post-market surveillance and market surveillance of medical devices, including in vitro diagnostics”* (ISBN 978-92-4-001531-9).

Thermo Fisher Scientific is also required to submit an annual report that details sales data and all categories of complaints in a summarized form. There are certain categories of complaints and changes to the product that must be notified immediately to WHO, as per the above-mentioned documents.

The manufacturer has committed to ensure that post-emergency use listing safety, quality and performance monitoring activities are in place which are in accordance with WHO guidance “ *Guidance for post-market surveillance and market surveillance of medical devices, including in vitro diagnostics*”¹.

Scope and duration of procurement eligibility

TaqPath COVID-19 CE-IVD RT-PCR Kit, product code A48067 manufactured by Thermo Fisher Scientific is considered to be eligible for WHO procurement for 12 months from the day of listing. The assay may be used for the detection of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA. This listing does not infer that the product meets WHO prequalification requirements and does not mean that the product is listed as WHO prequalified.

As part of the on-going requirements for listing as eligible for WHO procurement, Thermo Fisher Scientific must engage in post-market surveillance activities to ensure that the product continues to meet safety, quality and performance requirements. Thermo Fisher Scientific is required to notify WHO of any complaints, including adverse events related to the use of the product within 7 days.

WHO reserves the right to rescind eligibility for WHO procurement, if additional information on the safety, quality, performance during post-market surveillance activities, and if new data becomes available to WHO that changes the risk benefit balance.

¹ <https://www.who.int/publications/i/item/9789240015319>

Labelling

1.0 Labels

2.0 Instructions for Use (IFU)

1.0 Product labels

TITLE: TaqPath™ COVID-19 CE-IVD RT-PCR Kit

LABELING

The following is a list of product labels for the TaqPath™ COVID-19 CE-IVD RT-PCR Kit, SKU A48067

Box labels:

TaqPath™ COVID-19 RT-PCR Kit, SKU A48102; has two different labels due to dual manufacturing by Pleasanton, USA and Warrington, UK

appliedbiosystems
by Thermo Fisher Scientific

TaqPath™ COVID-19 RT-PCR Kit

Real-time RT-PCR test for in vitro qualitative detection of nucleic acid from the SARS-CoV-2

Part 1 of 4 of TaqPath™ COVID-19 CE-IVD RT-PCR Kit (REF A48067)

REF A48102

QR Code

(01) 1 0190302 01549 3
(17) 000000
(10) 000000
(240) A48102

Σ 1000 **LOT** 00000000 0000-00-00 -30°C -10 °C

CE **IVD** **EC REP** Life Technologies Europe B.V.
Kwartsweg 2, 2665 NN
Bleiswijk, The Netherlands

Read SDS Product of USA

100093305 Rev B

Part Number: 100093305
Rev: B
Label Size: 2.250 x 3.500
Label Stock Part Number: 4465273

TITLE: TaqPath™ COVID-19 CE-IVD RT-PCR Kit

Part Number: 100093686
Rev: B
Label Size: 1.563 X 2.391
Label Stock Part Number:
4412966

appliedbiosystems

by Thermo Fisher Scientific

REF A48102

TaqPath™ COVID-19 RT-PCR Kit

Real-time RT-PCR test for in vitro qualitative
detection of nucleic acid from the SARS-CoV-2



Part 1 of 4 of TaqPath™ COVID-19 CE-IVD RT-PCR Kit (**REF** A48067)

Σ 1000 **LOT** 0000000 **⏰** 0000-00-00

-30°C -10°C

CE **IVD**

EC **REP** Life Technologies Europe B.V.
Kwartsweg 2, 2665 NN
Bleiswijk, The Netherlands

Life Technologies Corporation
6055 Sunol Boulevard
Pleasanton, CA 94566

Read SDS

Made in UK

100093686 Rev B

TaqPath™ COVID-19 Control, SKU A48105

appliedbiosystems

by Thermo Fisher Scientific

REF A48105

TaqPath™ COVID-19 Control

Positive Control for TaqPath™
COVID-19 RT-PCR Kit



Part 2 of 4 of TaqPath™ COVID-19 CE-IVD
RT-PCR Kit (**REF** A48067)

(01) 1 0190302 01550 9

LOT 0000000

(17) 000000

10 Tubes 1x10⁴ copies/μL

-70°C
(10) 00000000
(240) A48105

⏰ 0000-00-00

EC **REP** Life Technologies Europe B.V.
Kwartsweg 2, 2665 NN
Bleiswijk, The Netherlands

CE **IVD**

Life Technologies Corporation
6055 Sunol Boulevard
Pleasanton, CA 94566

Read SDS

Product of USA

100093309 Rev B

Part Number: 100093309

Rev. B

Label Size: 1.875 x 1.875

Label Stock Part Number: 30-0027


TITLE: TaqPath™ COVID-19 CE-IVD RT-PCR Kit



TaqPath™ COVID-19 Control Dilution Buffer, SKU 48099; has two different labels due to dual manufacturing by Austin, USA and Warrington, UK


appliedbiosystems
by Thermo Fisher Scientific

TaqPath™ COVID-19 Control Dilution Buffer
Positive Control Dilution Buffer part of TaqPath™
COVID-19 RT-PCR Kit

Part 3 of 4 of TaqPath COVID-19 CE-IVD RT-PCR Kit
(REF A48067)


10 Tubes **LOT** 0000000  -10°C

CE IVD   0000-00-00 **EC REP** Life Technologies Europe B.V.
Kwartsweg 2, 2665 NN
Bleiswijk, The Netherlands

 Life Technologies Corporation
6055 Sunol Boulevard
Pleasanton, CA 94566

Read SDS Product of USA

REF A48099



(01) 1 0190302 01548 6
(17) 000000
(10) 0000000
(240) A48099

100093293 Rev B

Part Number: 100093293
Rev: B
Label Size: 2.5 x 1.65
Label Stock Part Number: 4444983

appliedbiosystems
by Thermo Fisher Scientific

TaqPath™ COVID-19 Control Dilution Buffer
Positive Control Dilution Buffer part of TaqPath™
COVID-19 RT-PCR Kit

Part 3 of 4 of TaqPath™ COVID-19 CE-IVD RT-PCR Kit
(REF A48067)

10 Tubes **LOT** 0000000  0000-00-00  -10°C

CE IVD  **EC REP** Life Technologies Europe B.V.
Kwartsweg 2, 2665 NN
Bleiswijk, The Netherlands

 Life Technologies Corporation
6055 Sunol Boulevard
Pleasanton, CA 94566

Read SDS Made in UK

REF A48099



100093069 Rev B

Part Number: 100093069
Rev: B
Label Size: 1.563 X 2.391
Label Stock Part Number:
4412966

Life Technologies

TITLE: TaqPath™ COVID-19 CE-IVD RT-PCR Kit

TaqPath™ 1-Step Multiplex Master Mix (No ROX), SKU A48111; has two different labels due to dual manufacturing by Frederick, USA and Vilnius, Lithuania

appliedbiosystems
by Thermo Fisher Scientific

TaqPath™ 1-Step Multiplex Master Mix
No ROX

Part 4 of 4 of TaqPath™ COVID-19 CE-IVD RT-PCR Kit (REF A48067)

10 mL **LOT** 0000000 YYYY-MM-DD (01)10190302015523

CE **IVD** **EC REP** Life Technologies Europe B.V.
Kwartsweg 2, 2665, NN
Bleiswijk, The Netherlands

Read SDS **REF** A48111

(17)000000
(10)0000000
(240)A48111

Product of USA **LBL0019747 Rev B**

Part Number: LBL0019747

Rev: B

Label Size: 2.5 x 1.65

Label Stock Part Number: 56590

appliedbiosystems
by Thermo Fisher Scientific

TaqPath™ 1-Step Multiplex Master Mix
No ROX

Part 4 of 4 of TaqPath™ COVID-19 CE-IVD RT-PCR Kit (REF A48067)

10 mL 0000-00-00 000000000 (01)10190302015523

CE **IVD** **LOT** 000000000 **EC REP** Life Technologies Europe B.V.
Kwartsweg 2, 2665, NN
Bleiswijk, The Netherlands

Read SDS **REF** A48111

(17)000000
(10)0000000
(240)A48111

Made in Lithuania **LBL0019788 Rev. 02.00**

Life Technologies

TITLE: TaqPath™ COVID-19 CE-IVD RT-PCR Kit

Tube labels:

appliedbiosystems
 by Thermo Fisher Scientific

TaqPath™ 1-Step Multiplex Master Mix
 No ROX
 10 mL

REF A48111

LOT XXXXXXXX
 YYYY-MM-DD

CE IVD

EC REP Life Technologies Europe B.V.
 Kwartsweg 2, 2865, NN
 Bleiswijk, The Netherlands

Read SDS Product of USA

Life Technologies Corp.
 6605 Sunol Boulevard
 Pleasanton, CA, 94566

-30°C → -10°C

appliedbiosystems
 by Thermo Fisher Scientific

TaqPath™ 1-Step Multiplex Master Mix
 No ROX
 10 mL

REF A48111

LOT 00000000
 YYYY-MM-DD

CE IVD

EC REP Life Technologies Europe B.V.
 Kwartsweg 2, 2865, NN
 Bleiswijk, The Netherlands

Read SDS Made in Lithuania
 LBL0019789 Rev. 01.00

Life Technologies Corp.
 6605 Sunol Boulevard
 Pleasanton, CA, 94566

-30°C → -10°C

appliedbiosystems
 by Thermo Fisher Scientific

TaqPath™ COVID-19 Control

REF 100093314

10 µL **LOT** 00000000
 YYYY-MM-DD

CE IVD

EC REP Life Technologies Europe B.V.
 Bleiswijk, The Netherlands

Read SDS Product of USA

Life Technologies Corp.
 Pleasanton, CA 94566

-70°C

100093308 Rev A

Part Number: 100093308
 Rev: A
 Label Size: 1.5 x .75
 Label Stock PN: 30-0047

TITLE: TaqPath™ COVID-19 CE-IVD RT-PCR Kit

appliedbiosystems REF 100093291
by Thermo Fisher Scientific

TaqPath™ COVID-19
Control Dilution Buffer

250 µL LOT 0000000

CE IVD YYY-MM-DD

EC REP Life Technologies Europe B.V.
Bleiswijk, The Netherlands

Life Technologies Corp. Read SDS
Pleasanton, CA 94566 Product of USA

OVER LAP AREA

Part Number: 100093292
Rev: A
Label Size: 1.00 x 1.375
Label Stock PN: 4467532
(clear)

appliedbiosystems REF 100093291
by Thermo Fisher Scientific

TaqPath™ COVID-19
Control Dilution Buffer

250 µL LOT 0000000

CE IVD YYY-MM-DD

EC REP Life Technologies Europe B.V.
Bleiswijk, The Netherlands

Life Technologies Corp. Read SDS
Pleasanton, CA 94566 Product of UK

OVER LAP AREA

Part Number: 100093440
Rev: A
Label Size: 1.00 x 1.375
Label Stock PN: 4467532
(clear)

appliedbiosystems REF 100093311
by Thermo Fisher Scientific

TaqPath™ COVID-19
Assay Multiplex

1500 µL LOT 0000000

CE IVD YYY-MM-DD

EC REP Life Technologies Europe B.V.
Bleiswijk, The Netherlands

Life Technologies Corp. Read SDS
Pleasanton, CA 94566 Product of USA

100093306 Rev A OVER LAP AREA

Part Number: 100093306
Rev: A
Label Size: 1.00 x 1.375
Label Stock PN: 4467532
(clear)

appliedbiosystems REF 100093311
by Thermo Fisher Scientific

TaqPath™ COVID-19
Assay Multiplex

1500 µL LOT 0000000

CE IVD YYY-MM-DD

EC REP Life Technologies Europe B.V.
Bleiswijk, The Netherlands

Life Technologies Corp. Read SDS
Pleasanton, CA 94566 Product of UK

OVER LAP AREA

Part Number: 100093684
Rev: A
Label Size: 1.00 x 1.375
Label Stock PN: 4467303
(clear)

TITLE: TaqPath™ COVID-19 CE-IVD RT-PCR Kit

appliedbiosystems by Thermo Fisher Scientific **REF** 100093312
MS2 Phage Control

1000 µL **LOT** 0000000 -30 °C -10 °C YYY-MM-DD

CE IVD **EC REP** Life Technologies Europe B.V.
Life Technologies Corporation Bleiswijk, The Netherlands
Pleasanton, CA 94566 **Read SDS** Product of USA

100093307 Rev A

Part Number: 100093307
Rev: A
Label Size: 1.25 x .625
Label Stock PN: RFSPOLYNBB 13255

appliedbiosystems **REF** 100093312
by Thermo Fisher Scientific

MS2 Phage Control -30 °C -10 °C

1000 µL **LOT** 0000000 YYY-MM-DD

CE IVD **EC REP** Life Technologies Europe B.V.
Bleiswijk, The Netherlands

Life Technologies Corp. **Read SDS**
Pleasanton, CA 94566 Product of UK

OVER LAP AREA

Part Number: 100093685
Rev: A
Label Size: 1.00 x 1.375
Label Stock PN: 4467532
(clear)

2.0 Instructions for use²

² English version of the IFU was the one that was assessed by WHO. It is the responsibility of the manufacturer to ensure correct translation into other languages.

TaqPath™ COVID-19 CE-IVD RT-PCR Kit

INSTRUCTIONS FOR USE

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

Catalog Number A48067

Publication Number MAN0019215

Revision E.0



For In Vitro Diagnostic Use.

ThermoFisher
S C I E N T I F I C



Life Technologies Corporation |
6055 Sunol Blvd |
Pleasanton, CA 94566

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



Life Technologies Europe B.V.
Kwartsweg 2, 2665 NN Bleiswijk
The Netherlands

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

The information in this guide is subject to change without notice.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. MAN0019215

Revision	Date	Description
E.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 v2.1, and Applied Biosystems™ COVID-19 Interpretive Software v2.2. Added new guidelines for sample collection, storage, packaging, and shipping to “Assay limitations” on page 12. Added C_t cutoff information (Appendix A, “Ct cutoff values for assay targets”). Updated instructions to seal, vortex, and centrifuge the RT-PCR plates. Provided additional details for naming negative control wells in 384-well plates with unique names. Updated reactivity (inclusivity) (page 63). Added more information about Limit of Detection experiments to “Limit of detection (LoD)” on page 61.
D.0	2 June 2020	<ul style="list-style-type: none"> Removed instructions to mix by pipetting up and down 10 times when preparing RT-PCR plates. Added instructions to vortex the plates for 10–20 seconds to ensure proper mixing. Added instructions to unseal and reseal one extraction plate at a time when preparing 384-well RT-PCR plates. Updated instructions to create a unique name for each well in the physical plate, not only the wells with a patient sample. Added Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument, 384-well block. Added QuantStudio™ 5 Dx IVD Software for the Applied Biosystems™ QuantStudio™ 5 Dx Real-Time PCR Instrument. Added COVID-19 Interpretive Software v2.2. Updated retesting requirements for samples with inconclusive results.
C.0	1 May 2020	<ul style="list-style-type: none"> Updated specimen types to upper respiratory specimens (such as nasopharyngeal, oropharyngeal, nasal, and mid-turbinate swabs, and nasopharyngeal aspirate) and bronchoalveolar lavage (BAL) specimens (“Intended Use” on page 7, “Assay limitations” on page 12, and “Workflow” on page 14). Added procedures to perform RT-PCR with the following instruments: Applied Biosystems™ 7500 Real-Time PCR Instrument, Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instruments (96-well 0.1-mL block and 96-well 0.2-mL block), Applied Biosystems™ QuantStudio™ 5 Dx Real-Time PCR Instrument, and QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block). Added new software versions: Applied Biosystems™ COVID-19 Interpretive Software v1.2 and Applied Biosystems™ COVID-19 Interpretive Software v2.1. Changed the shelf life of TaqPath™ COVID-19 Control Dilution Buffer from 9 months to 12 months. Updated guidelines extracted RNA (Chapter 3, “Guidelines for the RNA extracted from the sample”). Updated requirements for controls based on the addition of 384-well real-time RT-PCR plates (“Guidelines for RT-PCR” on page 19).

Revision	Date	Description
C.0 <i>(continued)</i>	1 May 2020	<ul style="list-style-type: none"> Added reaction plates without a barcode as an option for real-time RT-PCR (MicroAmp™ Fast Optical 96-Well Reaction Plate, 0.1 mL, MicroAmp™ Optical 96-Well Reaction Plate, 0.2 mL, and MicroAmp™ Optical 384-Well Reaction Plate). Created separate procedures to prepare the RT-PCR reactions based on the volume of sample input ($\leq 200 \mu\text{L}$ or $> 200 \mu\text{L}$), the RT-PCR plate (96-well or 384-well), and added specific instructions to vortex and centrifuge the reaction plate. Specified that retesting must be done with the original sample (“Interpretation of the results” on page 60). Updated control requirements in interpretation of results, based on addition of 384-well real-time RT-PCR plates (“Interpretation of the results” on page 60). Added “Interfering substances” on page 63. Removed references to COVID-19 Interpretive Software v1.1.
B.0	27 March 2020	<ul style="list-style-type: none"> Corrected description of MS2 Phage Control in 'Product Description' from DNA Control to RNA Control. Expanded description of the service and technical support information available at https://www.thermofisher.com/contactus in 'Customer and technical support'. Changed the shelf life of the TaqPath™ COVID-19 Control Dilution Buffer.
A.0	24 March 2020	New document.

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TaqPath™ COVID-19 CE-IVD RT-PCR Kit product information

Intended Use

TaqPath™ COVID-19 CE-IVD RT-PCR Kit contains the reagents and controls for a real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens (such as nasopharyngeal, oropharyngeal, nasal, and mid-turbinate swabs, and nasopharyngeal aspirate) and bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory and bronchoalveolar lavage (BAL) specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories may be required to report all positive results to the appropriate Competent Health Authorities.

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

Testing with the TaqPath™ COVID-19 CE-IVD RT-PCR Kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.

Note: The following countries require the CE-marked In Vitro Diagnostics: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, UK, Norway, Iceland, Liechtenstein, Switzerland, Turkey.

Product description

TaqPath™ COVID-19 CE-IVD RT-PCR Kit includes the following components:

- TaqPath™ COVID-19 RT-PCR Kit, 1000 reactions
 - TaqPath™ COVID-19 Assay Multiplex—Multiplexed assays that contain three primer/probe sets specific to different SARS-CoV-2 genomic regions and primers/probes for bacteriophage MS2.
 - MS2 Phage Control—RNA control to verify the efficacy of the sample preparation and the absence of inhibitors in the PCR reaction. To perform the control, add MS2 Phage Control to the samples before extraction of the RNA.
- TaqPath™ COVID-19 Control—RNA control that contains targets specific to the SARS-CoV-2 genomic regions targeted by the assays.
- TaqPath™ COVID-19 Control Dilution Buffer
- TaqPath™ 1-Step Multiplex Master Mix (No ROX™)
- Package insert—Provides the instructions and the link to download the Instructions for Use.

Contents and storage

Table 1 TaqPath™ COVID-19 CE-IVD RT-PCR Kit, 1,000 reactions (Cat. No. A48067)

Component	Contents	Quantity	Volume per tube or bottle	Unit part number	Storage	Shelf life ^[1]
TaqPath™ COVID-19 RT-PCR Kit	TaqPath™ COVID-19 Assay Multiplex (Gene ORF1ab, N Protein, S Protein, MS2)	1 tube	1,500 µL (1,000 reactions)	100093311	-30°C to -10°C	12 months
	MS2 Phage Control	10 tubes	1 mL	100093312	-30°C to -10°C	12 months
TaqPath™ COVID-19 Control		10 tubes	10 µL (1 x 10 ⁴ copies / µL)	100093314	≤ -70°C	12 months
TaqPath™ COVID-19 Control Dilution Buffer		10 tubes	250 µL	100093291	-30°C to -10°C	12 months
TaqPath™ 1-Step Multiplex Master Mix (No ROX™)		1 bottle	10 mL	A48111	-30°C to -10°C	12 months

^[1] The shelf life of the kit is determined by the component with the shortest shelf life.

Required materials not supplied

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

Item	Source
Real-time PCR instrument	
Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument (used with SDS Software v1.4.1)	4406984 (with laptop computer) 4406985 (with tower computer)
Applied Biosystems™ 7500 Fast Real-Time PCR Instrument (used with SDS Software v1.5.1 or 7500 Software v2.3)	4351106 (with laptop computer) 4351107 (with desktop computer)
Applied Biosystems™ 7500 Real-Time PCR Instrument (used with 7500 Software v2.3)	4351104 (with laptop computer) 4351105 (with desktop computer)
Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument, 0.2-mL block (used with QuantStudio™ Design and Analysis Desktop Software v1.5.1)	A28569 (with laptop computer) A28574 (with desktop computer) A28139 (instrument only)
Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument, 0.1-mL block (used with QuantStudio™ Design and Analysis Desktop Software v1.5.1)	A28568 (with laptop computer) A28573 (with desktop computer) A28138 (instrument only)
Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument, 384-well block (used with QuantStudio™ Design and Analysis Desktop Software v1.5.1)	A28570 (with laptop computer) A28575 (with desktop computer) A28140 (instrument only)
Applied Biosystems™ QuantStudio™ 5 Dx Real-Time PCR Instrument (used with QuantStudio™ 5 Dx TD Software v1.0.2 or QuantStudio™ 5 Dx IVD Software v1.0.2)	A32005 (with laptop computer) A32006 (with tower computer)
Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR Instrument, 384-well block (used with QuantStudio™ Real-Time PCR Software v1.3)	4485695 (with laptop computer) 4485701 (with desktop computer)
Equipment	
Laboratory freezers <ul style="list-style-type: none"> • -30°C to -10°C • ≤ -70°C 	MLS
Centrifuge, with a rotor for microplates	MLS

(continued)

Item	Source
Microcentrifuge	MLS
Laboratory mixer, vortex or equivalent	MLS
Single and multichannel adjustable pipettors (1.00 µL to 1,000.0 µL)	MLS
Cold block (96-well or 384-well) or ice	MLS
Kits and reagents	
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
MicroAmp™ Optical 96-Well Reaction Plate with Barcode, 0.2 mL	4306737, 4326659
MicroAmp™ Optical 96-Well Reaction Plate, 0.2 mL	N8010560, 4316813
MicroAmp™ Optical 384-Well Reaction Plate with Barcode	4309849, 4326270, 4343814
MicroAmp™ Optical 384-Well Reaction Plate	4343370
MicroAmp™ Optical Adhesive Film	4311971, 4360954
MicroAmp™ Adhesive Film Applicator	4333183

(continued)

Item	Source
Nonstick, RNase-free microcentrifuge tubes (1.5 mL and 2.0 mL)	thermofisher.com/plastics
Sterile aerosol barrier (filtered) pipette tips	thermofisher.com/pipettetips

Instrument and software compatibility

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

For information on how to obtain the Applied Biosystems™ COVID-19 Interpretive Software, see “Obtain the Applied Biosystems™ COVID-19 Interpretive Software” on page 59.

To obtain the analysis software or firmware for use with your real-time PCR instrument, go to thermofisher.com/qpcrsoftware, then select your instrument in the **Real-Time PCR** section.

Instrument	Analysis software used with the instrument	Compatible COVID-19 Interpretive Software version
7500 Fast Dx Real-Time PCR Instrument	SDS Software v1.4.1	v1.3
7500 Fast Real-Time PCR Instrument	SDS Software v1.5.1 or 7500 Software v2.3	v1.3
7500 Real-Time PCR Instrument	7500 Software v2.3	v1.3
QuantStudio™ 5 Real-Time PCR Instrument with instrument firmware v1.3.3 96-well, 0.2-mL block 96-well, 0.1-mL block	QuantStudio™ Design and Analysis Desktop Software v1.5.1	v2.3
QuantStudio™ 5 Real-Time PCR Instrument with instrument firmware v1.3.3 384-well block	QuantStudio™ Design and Analysis Desktop Software v1.5.1	v2.3
QuantStudio™ 5 Dx Real-Time PCR Instrument with instrument firmware v1.0.3	QuantStudio™ 5 Dx TD Software v1.0.2	v2.3

(continued)

Instrument	Analysis software used with the instrument	Compatible COVID-19 Interpretive Software version
QuantStudio™ 5 Dx Real-Time PCR Instrument with instrument firmware v1.0.3	QuantStudio™ 5 Dx IVD Software v1.0.2	v2.3
QuantStudio™ 7 Flex Real-Time PCR Instrument with instrument firmware v1.0.4 384-well block	QuantStudio™ Real-Time PCR Software v1.3	v2.3

Assay limitations

- This assay is intended to be used for *in vitro* diagnostic purposes only. Follow good laboratory practices and all precautions and guidelines in these user guides to avoid cross-contamination between samples.
- The TaqPath™ COVID-19 CE-IVD RT-PCR Kit performance was established using nasopharyngeal and oropharyngeal swab, nasopharyngeal aspirate, and bronchoalveolar lavage samples only. Nasal swabs and mid-turbinate swabs are considered acceptable specimen types according to the *Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19)* published by the Centers for Disease Control and Prevention, but performance of the TaqPath™ COVID-19 CE-IVD RT-PCR Kit with these specimen types has not been established. Testing of nasal and mid-turbinate nasal swabs (self-collected under supervision of or collected by a healthcare provider) is limited to patients with symptoms of COVID-19. Specimen types other than nasopharyngeal, oropharyngeal, 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.
- Refer to the *Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19)*, published by the Centers for Disease Control and Prevention, for specimen collection and storage guidelines.
- Specimens must be packaged, shipped, and transported according to the current edition of the *International Air Transport Association (IATA) Dangerous Goods Regulations (iata.org/en/programs/cargo/dgr)*.
- This kit uses purified RNA as a sample for the analysis. The quality of the RNA recovered from biological samples is essential for the quality of the results generated with this kit.

- False-negative results may arise from:
 - Improper sample collection
 - Degradation of the viral RNA during shipping/storage
 - Specimen collection after nucleic acid can no longer be found in the specimen matrix
 - Using poor extraction method
 - 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 CE-IVD 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 may be required to report all positive results to the appropriate Competent Health Authorities.

Samples and controls

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

Include the following controls:

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

Workflow

The workflow begins with nucleic acid purified from upper respiratory specimens (such as nasopharyngeal, oropharyngeal, nasal, and mid-turbinate swabs, and nasopharyngeal aspirate) and bronchoalveolar lavage (BAL) specimens.

The purified nucleic acid is reverse transcribed into cDNA and amplified using the TaqPath™ COVID-19 CE-IVD 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™ 5 Dx Real-Time PCR Instrument
- Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR Instrument, 384-well block

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

- ORF1ab
- N Protein
- S Protein

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

The data are analyzed and interpreted by the Applied Biosystems™ COVID-19 Interpretive Software.



Before you begin

Warnings and precautions

The TaqPath™ COVID-19 CE-IVD 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 under a laminar airflow hood or biological safety cabinet.

- Specimens 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 *In Vitro Diagnostic Directive 98/79/EC*.
- Do not use the kit after the expiry date.
- Dispose of waste in compliance with the local regulations.
- Safety Data Sheets are available upon request.
- Laboratories may be required to report all positive results to the appropriate Competent Health Authorities.

The following countries require the CE-marked In Vitro Diagnostics: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, UK, Norway, Iceland, Liechtenstein, Switzerland, Turkey.

- Positive results are indicative of the presence of SARS-CoV-2 RNA.
- Handle all samples and controls as if they are capable of transmitting infectious agents.
- Reagents must be stored and handled as specified in Table 1.
- The quality of the sample preparation (purified RNA) may influence the quality of the qPCR test. Laboratories shall only use the purification method they have selected. Laboratories that do not have any selected method may use the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit or the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit.

- The qPCR kit includes an RNA phage control to verify the efficacy of the sample preparation and the absence of inhibitors in the PCR reaction. To perform the control, add MS2 Phage Control to the samples before extraction of the RNA, according to the recommendations in Chapter 3, “Guidelines for the RNA extracted from the sample”.

Sample collection, transport, and storage

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



Guidelines for the RNA extracted from the sample

Sample input volumes

To meet the need for a broad range of sample input volumes, 2 RT-PCR protocols are provided, depending on the sample input volume.

The following sample input volumes were tested:

- Low input: 200 μ L
- High input: 400 μ L

Sample input volumes > 400 μ L have not been tested with the TaqPath™ COVID-19 CE-IVD RT-PCR Kit.

The following RNA extraction kits were tested:

- MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit
- MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit

Guidelines for RNA extraction

Extracted RNA can be prepared with any standard RNA extraction procedure or RNA extraction kit. The minimum recommended elution volume is 50 μ L.

The following guidelines are defined as optimal for the use of the TaqPath™ COVID-19 CE-IVD RT-PCR Kit.

- The MS2 Phage Control provided in the kit must be used to verify the efficacy of the sample preparation and the absence of inhibitors in the RT-PCR reaction. It is added to the sample before extraction (see “Add MS2 Phage Control” on page 18).
- Use Nuclease-free Water (not DEPC-Treated) containing MS2 Phage Control as the Negative Control. The purified Negative Control is used as the Negative Control for RT-PCR.
- We recommend that the volume of MS2 Phage Control is 2.5% of the sample input volume.

Add MS2 Phage Control

The MS2 Phage Control must be used to verify the efficacy of the sample preparation and the absence of inhibitors in the RT-PCR reaction.

Add the appropriate volume of MS2 Phage Control to each sample well and to the Negative Control well just before lysis during RNA extraction.

We recommend that the volume of MS2 Phage Control is 2.5% of the sample input volume. Examples are provided in the table below:

Sample input volume	MS2 Phage Control volume
200 μ L	5 μ L MS2 Phage Control
400 μ L	10 μ L MS2 Phage Control



Prepare RT-PCR reactions

■ Guidelines for RT-PCR	19
■ Prepare the RT-PCR reactions (\leq 200- μ L sample input volume, 96-well reaction plate)	20
■ Prepare the RT-PCR reactions (\leq 200- μ L sample input volume, 384-well reaction plate)	22
■ Prepare the RT-PCR reactions ($>$ 200- μ L sample input volume, 96-well reaction plate)	24
■ Prepare the RT-PCR reactions ($>$ 200- μ L sample input volume, 384-well reaction plate)	27

Note: The procedure used to prepare the RT-PCR reactions depends on the original sample input volume that was used during the preparation of RNA (\leq 200 μ L or $>$ 200 μ L). The procedure used to prepare the RT-PCR reactions also depends on whether a 96-well plate or a 384-well plate is used for RT-PCR.

Guidelines for RT-PCR

IMPORTANT!

- Prepare the run plate on ice and keep it on ice until it is loaded into the real-time PCR instrument.
 - Run the plate immediately after preparation. Failure to do so could result in degraded RNA samples.
 - To prevent contamination, prepare reagents in a PCR workstation or equivalent amplicon-free area. Do not use the same pipette for controls and 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.
 - For each RT-PCR 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 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.
-

Prepare the RT-PCR reactions ($\leq 200\text{-}\mu\text{L}$ sample input volume, 96-well reaction plate)

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

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 :
 - 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 dilution created in substep 3a. Mix well, then centrifuge briefly.

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

4. Prepare the Reaction Mix:
 - a. For each run, combine the following components sufficient for the number of 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 up to 200 μL .

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 μL	$6.875 \times (n + 2)$ μL	660 μL
COVID-19 Real Time PCR Assay Multiplex	1.25 μL	$1.375 \times (n + 2)$ μL	132 μL
Nuclease-free Water	7.50 μL	$8.25 \times (n + 2)$ μL	792 μL
Total Reaction Mix volume	15.0 μL	—	1584 μL

5. Set up the reaction plate:
 - a. Pipette $15.0\ \mu\text{L}$ of the Reaction Mix prepared in step 4 into each well of a MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, $0.1\ \text{mL}$ or a MicroAmp™ Optical 96-Well Reaction Plate with Barcode, $0.2\ \text{mL}$.
Plates without a barcode can be used (see “Required materials not supplied” on page 9).
 - 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 22.
 - 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 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

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

Prepare the RT-PCR reactions ($\leq 200\text{-}\mu\text{L}$ sample input volume, 384-well reaction plate)

Use this procedure if you extracted sample RNA using an original sample input volume of up to 200 μL and are using the Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR Instrument with a 384-well block.

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

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

4. Prepare the Reaction Mix.

- a. For each run, combine the following components sufficient for the number of 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 up to 200 μL .

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

5. Set up the reaction plate:

- a. Pipette 10.0 μL of the Reaction Mix prepared in step 4 into each well of a MicroAmp™ Optical 384-Well Reaction Plate with Barcode.
 Plates without a barcode can be used (see “Required materials not supplied” on page 9).
- 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 24.

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 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

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 μ L	20.0 μ L	20.0 μ L

Prepare the RT-PCR reactions (> 200- μ L sample input volume, 96-well reaction plate)

Use this procedure if you extracted sample RNA using an original sample input volume between 201 μ L and 400 μ L. A sample input volume > 400 μ L has not been tested with the TaqPath™ COVID-19 CE-IVD RT-PCR Kit.

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 × 10⁴ copies/µL) to a working stock of 25 copies/µL:
 - a. Pipet 98 µL of TaqPath™ COVID-19 Control Dilution Buffer into a microcentrifuge tube, then add 2 µL of TaqPath™ COVID-19 Control. Mix well, then centrifuge briefly.
 - b. Pipet 87.5 µL of TaqPath™ COVID-19 Control Dilution Buffer into a second microcentrifuge tube, then add 12.5 µL of the dilution created in substep 3a. Mix well, then centrifuge briefly.

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

4. Prepare the Reaction Mix:
 - a. For each run, combine the following components sufficient for the number of 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 between 201 µL and 400 µL.

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

5. Set up the reaction plate:
 - a. Pipette 20.0 µL of the Reaction Mix prepared in step 4 into each well of a MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL or a MicroAmp™ Optical 96-Well Reaction Plate with Barcode, 0.2 mL. Plates without a barcode can be used (see “Required materials not supplied” on page 9).
 - 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 26.

- 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 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

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 (> 200- μ L sample input volume, 384-well reaction plate)

Use this procedure if you extracted sample RNA using an original sample input volume between 201 μ L and 400 μ L and are using the Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR Instrument with a 384-well block. A sample input volume > 400 μ L has not been tested with the TaqPath™ COVID-19 CE-IVD RT-PCR Kit.

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:
 - 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.
 - 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 between 201 μ L and 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:

- a. Pipette 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.

Plates without a barcode can be used (see “Required materials not supplied” on page 9).

- 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 29.

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

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

IMPORTANT! When applying the MicroAmp™ Optical Adhesive Film, ensure that pressure is applied across the entire plate and that there is a tight seal across every individual well. Failure to do so runs the risk of an improperly sealed well, leading to potential well-to-well contamination during vortexing and 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

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



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

- Dye calibration for the 7500 Real-Time PCR Instrument series 30
- Transfer the template (SDT) file for the 7500 Fast Dx Real-Time PCR Instrument 30
- Set up and run the 7500 Fast Dx Real-Time PCR Instrument 31

Dye calibration for the 7500 Real-Time PCR Instrument series

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

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

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

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

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

2. Select the SDT file:

```
TaqPath COVID-19 Kit Template 7500fastDx sds1_4_1 v1-2.sdt
```

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

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

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

For more information about the 7500 Fast Dx Real-Time PCR Instrument, see the documents listed in “Related documentation” on page 69.

- 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 30.

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

- 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 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.
- 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, confirm that **Task** is set to **Unknown** for all detectors.

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

9. Confirm the thermal protocol.

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

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



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

- Dye calibration for the 7500 Real-Time PCR Instrument series 33
- Transfer the template (SDT or EDT) file for the 7500 Fast Real-Time PCR Instrument 34
- Set up and run the 7500 Fast Real-Time PCR Instrument (SDS Software v1.5.1) 34
- Set up and run the 7500 Fast Real-Time PCR Instrument (7500 Software v2.3) 36

Dye calibration for the 7500 Real-Time PCR Instrument series

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

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

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

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

```
<...>\Applied Biosystems\COVID-19 Interpretive Software\Client\docs\User Documents
```
2. 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 v1-2.sdt
7500 Software v2.3	TaqPath COVID-19 Kit Template 7500fast sds2_3 v1-2.edt

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

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

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

This procedure is specific for the 7500 Fast Real-Time PCR Instrument using SDS Software v1.5.1. For more information, see the documents listed in “Related documentation” on page 69.

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 34.

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

2. Confirm the run settings in the template and adjust as necessary.

- **Assay: Standard Curve (Absolute Quantitation)**
- **Run mode: Standard 7500**
- **Passive reference: None**
- **Sample volume: 25 µL**

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

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

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

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

5. 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.

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, ensure that **Task** is set to **Unknown** for all detectors.

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

9. Confirm the thermal protocol.

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

10. Click **Save As**, enter a file name, then click **Save**.
11. Reopen the file, load the plate, then start the run on the instrument.
12. After the instrument run is complete, open the SDS file in SDS Software v1.5.1. Analyze, then save the file.

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

This procedure is specific for the 7500 Fast Real-Time PCR Instrument using 7500 Software v2.3. For more information, see the documents listed in “Related documentation” on page 69.

1. In the 7500 Software v2.3 home page, click **Template**.
2. Browse to, then open the SDT or EDT file that you transferred in “Transfer the template (SDT or EDT) file for the 7500 Fast Real-Time PCR Instrument” on page 34.

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

3. In the **Experiment Properties** window, enter or confirm the following information:
 - **Experiment name:** Enter a 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.
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.** After the instrument run is complete, click **Analyze**, then save the file.



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

- Dye calibration for the 7500 Real-Time PCR Instrument series 39
- Transfer the template (EDT) file for the 7500 Real-Time PCR Instrument 40
- Set up and run the 7500 Real-Time PCR Instrument (7500 Software v2.3) . . . 40

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 9.

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

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

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

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

2. Select the EDT file:

```
TaqPath COVID-19 Kit Template 7500std sds2_3 v1-2.edt
```

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

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

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

This procedure is specific for the 7500 Real-Time PCR Instrument using 7500 Software v2.3. For more information, see the documents listed in “Related documentation” on page 69.

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 40.

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

3. In the **Experiment Properties** window, enter or confirm the following information:
 - **Experiment name:** Enter a 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.
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. 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

- Dye calibration for the QuantStudio™ 5 Real-Time PCR Instrument 43
- Transfer the template (EDT) file for the QuantStudio™ 5 Real-Time PCR Instrument 44
- Set up and run the QuantStudio™ 5 Real-Time PCR Instrument (96-well plates) 44
- Set up and run the QuantStudio™ 5 Real-Time PCR Instrument (384-well plates) 46

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 is installed.

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

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

2. Select the correct EDT file for your instrument.

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

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

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

Set up and run the QuantStudio™ 5 Real-Time PCR Instrument (96-well plates)

For more information about the QuantStudio™ 5 Real-Time PCR Instrument, see the documents listed in “Related documentation” on page 69.

1. In the QuantStudio™ Design and Analysis Desktop Software v1.5.1, in the **New Experiment** box, select **Create New Experiment ▶ Template**.
2. Browse to, then open the EDT file that you transferred in “Transfer the template (EDT) file for the QuantStudio™ 5 Real-Time PCR Instrument” on page 44.

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

3. In the **Properties** tab, enter or confirm the following.
 - **Name:** Enter a 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.
14. To assign a sample to a well, select the well in the plate layout, then select the sample from the **Samples** table.
For all targets in the patient sample wells, confirm that **Task** is set to **U (Unknown)**.
Note: Wells that do not have a sample name will not be analyzed by the software.
15. In the **Run** tab, click **Start Run**, then select your instrument from the drop-down list.
16. Enter 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 (384-well plates)

For more information about the QuantStudio™ 5 Real-Time PCR Instrument, see the documents listed in “Related documentation” on page 69.

1. In the QuantStudio™ Design and Analysis Desktop Software v1.5.1, in the **New Experiment** box, select **Create New Experiment ▶ Template**.
2. Browse to, then open the EDT file that you transferred in “Transfer the template (EDT) file for the QuantStudio™ 5 Real-Time PCR Instrument” on page 44.

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

3. In the **Properties** tab, enter or confirm the following.
 - **Name:** Enter a name
 - **Instrument type:** **QuantStudio™ 5 System**
 - **Block type:** **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 19). 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.
14. To assign a sample to a well, select the well in the plate layout, then select the sample from the **Samples** table.
For all targets in the patient sample wells, confirm that **Task** is set to **U (Unknown)**.
Note: Wells that do not have a sample name will not be analyzed by the software.
15. In the **Run** tab, click **Start Run**, then select your instrument from the drop-down list.
16. Enter a file name in the dialog box that prompts you to save the run file, then save the file.



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

- Dye calibration for the QuantStudio™ 5 Dx Real-Time PCR Instrument 49
- QuantStudio™ 5 Dx TD Software 50
- QuantStudio™ 5 Dx IVD Software 53

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

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

QuantStudio™ 5 Dx TD Software

Follow the procedures in this section if you are using the QuantStudio™ 5 Dx TD Software.

If you are using QuantStudio™ 5 Dx IVD Software, see “QuantStudio™ 5 Dx IVD Software” on page 53.

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

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™ 5 Dx TD Software is installed.

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

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

2. Select the EDT file:

```
TaqPath COVID-19 Kit Template QS5 Dx_da1_0_2 v2-2.edt
```

3. Transfer the EDT file to the computer with the QuantStudio™ 5 Dx TD Software, using a USB drive or other method.

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

Set up and run the QuantStudio™ 5 Dx Real-Time PCR Instrument (QuantStudio™ 5 Dx TD Software)

For more information about the QuantStudio™ 5 Dx Real-Time PCR Instrument, see the documents listed in “Related documentation” on page 69.

1. In the QuantStudio™ 5 Dx TD 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 Dx Real-Time PCR Instrument (QuantStudio™ 5 Dx TD Software)” on page 50.

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

3. In the **Properties** tab, enter or confirm the following.
 - **Name:** Enter a unique name
 - **Instrument type:** **QuantStudio™ 5 Dx System**
 - **Block type:** **96-Well 0.2-mL Block**
 - **Experiment type:** **Standard Curve**
 - **Chemistry:** **TaqMan™ Reagents**
 - **Run Mode:** **Standard**
4. In the **Method** tab, confirm that **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.
14. To assign a sample to a well, select the well in the plate layout, then select the sample from the **Samples** table.
For all targets in the patient sample wells, confirm that **Task** is set to **U (Unknown)**.
Note: Wells that do not have a sample name will not be analyzed by the software.
15. In the **Run** tab, click **Start Run**, then select your instrument from the drop-down list.
16. Enter the a file name in the dialog box that prompts you to save the run file, then save the file.

QuantStudio™ 5 Dx IVD Software

Follow the procedures in this section if you are using the QuantStudio™ 5 Dx IVD Software.

If you are using QuantStudio™ 5 Dx TD Software, see “QuantStudio™ 5 Dx TD Software” on page 50.

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

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™ 5 Dx IVD Software is installed.

1. On the computer with Applied Biosystems™ COVID-19 Interpretive Software, navigate to the following directory (where <...> is the installation directory):
`<...>\Applied Biosystems\COVID-19 Interpretive Software\Client\docs\User Documents`
2. Select the EDT file:
`TaqPath COVID-19 Kit Template QS5 Dx_da1_0_2_CE_IVD v2-2.edt`
3. Transfer the EDT file to the computer with the QuantStudio™ 5 Dx IVD Software, using a USB drive or other method.

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

The template must be installed in the QuantStudio™ 5 Dx IVD Software before the run is started. See “Install the template file in the QuantStudio™ 5 Dx IVD Software” on page 53.

Install the template file in the QuantStudio™ 5 Dx IVD Software

To install a template, the logged-in user must have the SAE permission of **Manage Installed Templates**. For information about SAE permissions, see the *QuantStudio™ 5 Dx TD Software User Guide* (Pub. No. 100049554).

1. Open the QuantStudio™ 5 Dx IVD Software.
2. When prompted, log in with a user account with the appropriate permissions.
3. In the menu bar, select **Tools ▶ Template Menu**.
4. Click **Install**, then click **Yes** to confirm the **Template Installation Agreement**.

5. Navigate template file that was transferred, then click **Open**.
The template is now installed and accessible in the **Template Menu**.
6. Click **Close**.

Set up and run the QuantStudio™ 5 Dx Real-Time PCR Instrument (QuantStudio™ 5 Dx IVD Software)

For more information about the QuantStudio™ 5 Dx Real-Time PCR Instrument, see the documents listed in “Related documentation” on page 69.

IMPORTANT! When the QuantStudio™ 5 Dx IVD Software is used, the positive control must be placed in well A12 and the negative control must be placed in well A1 on the physical plate.

1. In the QuantStudio™ 5 Dx IVD Software, in the **New Experiment** box, select **Experiment Setup**.
2. Select the template file that you installed in “Install the template file in the QuantStudio™ 5 Dx IVD Software” on page 53.
3. Click **Create New Experiment**.
4. In **Samples** table, click **Add** to define the sample names. Create a unique sample name for each well in the physical plate.
5. To assign a sample to a well, select the well in the plate layout, then select sample from the **Samples** table.
Note: Wells that do not have a sample name will not be analyzed by the software.
6. In the **Run** tab, click **Start Run**, then select your instrument from the drop-down list.
The **Enter Reason for Change** dialog box will appear.
7. In the **Enter Reason for Change** dialog box, click **OK**.
8. Enter a file name in the dialog box that prompts you to save the run file, then save the file.



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 55
- Transfer the template (EDT) file for the QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block) 56
- Set up and run the QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block) 56

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.

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

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

2. Select the EDT file:

```
TaqPath COVID-19 Kit Template QS7 384 1_3 v2-2.edt
```

3. Transfer the EDT file to the computer with QuantStudio™ Real-Time PCR Software v1.3, using a USB drive or other method.

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

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

For more information about the QuantStudio™ 7 Flex Real-Time PCR Instrument, see the documents listed in “Related documentation” on page 69.

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 56.

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

3. In the **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

- 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

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

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

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

- In the **Define** tab, confirm that the **Passive Reference** is set to **None**.
- 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.

- 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 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 19). Label each negative control with a unique name, for example, **NC1**, **NC2**, **NC3**, and **NC4**.

- 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.

- In the **Assign** tab, assign a sample name to each well in 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. In the **Run** tab, click **Start Run**, then select your instrument from the drop-down list.
13. 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 support team or look up the local service phone number on the website (see below).

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

For software installation instructions, see the document listed in “Related documentation” on page 69.

Analyze the data

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

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

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

Interpretation of the results

Interpretation of the results is performed by the Applied Biosystems™ COVID-19 Interpretive Software. For information about the C_t values that are used by the software to interpret results, see Table 14.

Quality control and validity of results

A minimum of one Negative Control and one Positive Control must be present for each run. 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 6 Result interpretation for patient samples

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

^[1] Samples with a result of SARS-CoV-2 Inconclusive shall be retested one time. Retesting must be performed from the biological sample originally collected from the patient.

Performance characteristics

The analytical and clinical performance of the TaqPath™ COVID-19 CE-IVD 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 CE-IVD RT-PCR Kit in a particular specimen type at least 95% of the time. Banked Nasopharyngeal swab (NP) and Bronchoalveolar lavage (BAL) samples were obtained from patients in the United States in the years 2015–2019. The NP and BAL samples were pooled, respectively, and spiked with purified SARS-CoV-2 viral genomic RNA at several concentrations, then processed using the 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 7 LoD determination in BAL

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

Table 7 LoD determination in BAL (continued)

Effective Concentration	Replicate	Mean C _t				Interpretation	% Positive
		ORF1ab	N	S	MS2		
10 GCE/ reaction	12	30.1	29.7	29.1	24.6	Positive	100%
	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 8 LoD determination in Nasopharyngeal specimens

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

Table 8 LoD determination in Nasopharyngeal specimens (continued)

Effective Concentration	Replicate	Mean C _t				Interpretation	% Positive
		ORF1ab	N	S	MS2		
10 GCE/ reaction	16	30.5	29.1	35.7	27.0	Positive	100%
	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 9 LoD results

LoD studies were performed by extracting 400 µL of each specimen followed by elution in 50 µL, then 5 µL of eluate was added to the RT-PCR reaction. The LoD of 10 GCE/reaction is calculated from a starting concentration of 250 GCE/mL of specimen.

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

Reactivity (Inclusivity)

In silico analysis was updated on June 3, 2020, using 25,998 complete SARS-CoV-2 genomes in GISAID and GenBank databases. Based upon BLAST analysis, the TaqPath™ COVID-19 CE-IVD RT-PCR Kit maps with 100% homology to >99.99% of known SARS-CoV-2 isolates in GISAID and 100% of known isolates in GenBank databases. Mapping was deemed successful for a given isolate if at least two of the three targets (ORF1ab, S gene, and N gene) showed 100% identity.

Interfering substances

Pooled SARS-CoV-2-negative nasopharyngeal swab and bronchoalveolar lavage specimens were spiked with purified SARS-CoV-2 viral RNA at 3X the Limit of Detection (30 GCE/reaction) and potential interfering substances at the concentrations above. Each substance was tested with triplicate extractions. The results are presented in Table 10.

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 10 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:

Table 11 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 risk of the 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 CE-IVD 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 CE-IVD 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 12 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 13 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.



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 14 C_t cutoff values for assay targets

Sample	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
	Viral targets	Positive C _t values are ≤37



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™ 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide</i>	4347828
<i>QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide</i>	MAN0010407
<i>QuantStudio™ 5 Dx Real-Time PCR Instrument Maintenance and Administration User Guide</i>	100042186
<i>QuantStudio™ 5 Dx TD Software User Guide</i>	100049554
<i>QuantStudio™ 5 Dx IVD Software User Guide</i>	100049556
<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>COVID-19 Interpretive Software CE-IVD Edition Installation Quick Reference</i>	MAN0019230

Customer and technical support

For additional documentation and information about this kit, visit:

<https://www.thermofisher.com/covid19ceivd>

For download instructions for the COVID-19 Interpretive Software, see “Obtain the Applied Biosystems™ COVID-19 Interpretive Software” on page 59.

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

Visit: **<https://www.thermofisher.com/contactus>** for service and support information for this kit, including the following:

- Worldwide contact telephone numbers
- Product support information
- Order and web support



- Product documentation such as:
 - Certificates of Analysis
 - Safety Data Sheets (SDSs; also known as MSDSs)

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

Limited product warranty

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

