applied biosystems

TaqPath™ COVID-19 HT Kit

INSTRUCTIONS FOR USE

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

Catalog Number A50883

Publication Number MAN0025745

Revision C.0

CE IVD

For In Vitro Diagnostic Use.

ThermoFisher SCIENTIFIC

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

Revision history: Pub. No. MAN0025745

Revision	Date	Description
C.0	30 August 2022	• Amplitude™ Interpretive Software Driver version was updated to 1.3. • Added barcode scanning instructions in "Load the Tecan™ Fluent™ 1080 Automation Workstation deck" on page 16 and "Module 2 required sample input, reagents, and consumables" on page 20.
B.0	1 June 2022	• The workflow was updated to account for partial runs (<4 plates per run), Amplitude™ Interpretive Software Driver to version 1.2, and Tecan™ FluentControl™ Software update to version 3.0. • HT Water for Dilution was removed from "Product description" on page 6 and "Contents and storage" on page 6. • "In-use reagent stability" on page 9 was updated. • "Warnings and precautions" on page 10 was updated. • Appendix A, "Performance characteristics for Amplitude™ High-Throughput COVID-19 Testing Solution" was updated. • "Related documentation" on page 43 was added.
A.0	19 October 2022	New *TaqPath™ COVID-19 HT Kit Instructions for Use*.

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

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

Australian Sponsor Information: Thermo Fisher Scientific Australia Pty Ltd | 5 Caribbean Drive | Scoresby, VIC 3179 | Australia

Contents

1 TaqPath™ COVID-19 HT Kit product information

Intended Use

Applied Biosystems™ TaqPath™ COVID-19 HT Kit is the high-throughput format of the TaqPath™ COVID-19 Combo Kit and TaqPath™ COVID-19 CE-IVD RT-PCR Kit. The kit is intended for use with the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit, High-Throughput and is compatible with the Amplitude™ Platform. The 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 nasopharyngeal swab and nasopharyngeal aspirate from individuals suspected of COVID-19 by their healthcare provider.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in nasopharyngeal specimens during the acute phase of infection. Results should not be used as the sole basis for diagnosis and patient management decisions. Clinical correlation with patient history and other diagnostic and epidemiological information should be used to evaluate the significance of the results in order to assist the clinician or epidemiologist to determine patient infection status and the appropriate treatment regime. 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.

Testing with the TaqPath™ COVID-19 HT Kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The TaqPath™ COVID-19 HT Kit is intended for use in a qualified laboratory environment according to the sample type.

Product description

The TaqPath™ COVID-19 HT Kit includes the following components:

- TaqPath™ COVID-19 Module 1 MS2 Phage Control—An internal process control for RNA extraction.
- TaqPath™ COVID-19 Module 2 Assay Kit—Multiplexed assays that contain three primer/probe sets specific to 3 different SARS-CoV-2 genomic regions (ORF1ab, N gene, and S gene) and primers/probes for bacteriophage MS2.
- TaqPath™ COVID-19 Module 2 IVT RNA Control—An RNA control that contains targets specific to the SARS-CoV-2 genomic regions targeted by the assays.
- TaqPath™ COVID-19 Module 2 Control Dilution Buffer
- TaqPath™ HT Module 2 1-Step Multiplex Master Mix (No ROX)
- TaqPath™ HT Module 2 Empty 5 mL Mixing Tubes

The TaqPath™ COVID-19 HT Kit is compatible with the Amplitude™ Platform. As part of the Amplitude™ High-Throughput COVID-19 Testing Solution, the Amplitude™ Platform is used with the following products to detect SARS-CoV-2 RNA in human nasopharyngeal specimens.

- TaqPath™ COVID-19 HT Kit (Cat. No. A50883)
- MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit, High-Throughput (Cat. No. A50884)

The results are automatically analyzed with the pre-installed Amplitude™ Interpretive Software Driver v1.3. For more information on the automated results process, see Chapter 5, “Review results (automated workflow)”.

Contents and storage

The TaqPath™ COVID-19 HT Kit contains sufficient reagents for 20,000 reactions.

Table 1 TaqPath™ COVID-19 HT Kit (REF. A50883)

Amplitude™ Platform module	Component	REF.	Quantity	Amount	Storage[1]
TaqPath™ COVID-19 Module 1 MS2 Phage Control (Cat. No. A50749)					
Module 1	TaqPath™ COVID-19 HT MS2 Phage Control	100099158	3	6 × 10 mL	–30°C to –10°C
TaqPath™ COVID-19 Module 2 Assay Kit (Cat. No. A50750)					
Module 2	TaqPath™ COVID-19 HT qPCR Assay	100099159	3	6 × 1,600 µL	–30°C to –10°C
	2-mL mixing tubes[2]	4339337	3	6 tubes	15°C to 30°C
TaqPath™ COVID-19 Module 2 IVT RNA Control (Cat. No. 966128)					
Module 2	TaqPath™ COVID-19 HT IVT RNA Control	91-6128	3	3 × 25 µL	≤–70°C

Table 1 TaqPath COVID-19 HT Kit (REF. A50883) *(continued)*

Amplitude™ Platform module	Component	REF.	Quantity	Amount	Storage[1]
TaqPath™ COVID-19 Module 2 Control Dilution Buffer (Cat. No. A50751)					
Module 2	TaqPath™ HT Control Dilution Buffer	100099160	3	3 × 750 µL	–30°C to –10°C
TaqPath™ HT Module 2 1-Step Multiplex Master Mix (No ROX) (Cat. No. A50772)					
Module 2	TaqPath™ 1-Step Multiplex Master Mix (No ROX)	100032397F4	3	6 × 10 mL	–30°C to –10°C
TaqPath™ HT Module 2 Empty 5 mL Mixing Tubes (Cat. No. A50893)					
Module 2	5-mL mixing tubes	—	3	25 tubes	15°C to 25°C

[1] See label for expiration date.

[2] Empty plastic tubes used for the serial dilution of the TaqPath™ COVID-19 Module 2 IVT RNA Control in the TaqPath™ COVID-19 Module 2 Control Dilution Buffer.

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
Amplitude™ Platform Module 1 and Module 2 equipment	See the user guide for the Amplitude™ Platform (Pub. No. MAN0019842)[1]
MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit, High-Throughput	
80% Ethanol	
Silicone oil	
Amplitude™ High-Throughput Consumable Package 3 Plastics	

[1] See also “Module 1 required samples, reagents, and consumables” on page 13 and “Module 2 required sample input, reagents, and consumables” on page 20.

Assay limitations

- The TaqPath™ COVID-19 HT Kit performance was established using nasopharyngeal swab and nasopharyngeal aspirate samples only. Specimen types other than nasopharyngeal swab or nasopharyngeal aspirate 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.
- Chemical inactivation and other known clinically relevant interfering substances and techniques can lead to inappropriate primary samples and interfere with test results.
- The Amplitude™ Platform workflow must be performed according to the specified methods described in the user guide for the system (see Appendix D, “Documentation and support”).
- The quality of the 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 SARS-CoV-2 RNA during shipping/storage
  - Specimen collection after SARS-CoV-2 RNA can no longer be found in the specimen matrix
  - Using unauthorized extraction or assay reagents
  - The presence of RT-PCR inhibitors
  - Mutation in the SARS-CoV-2 virus
  - Failure to follow instructions for use
- False-positive results may arise from:
  - Cross contamination during specimen handling or preparation
  - Cross contamination between patient samples
  - Specimen mix-up
  - RNA contamination during product handling
- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated. The TaqPath™ COVID-19 HT 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.
- Certain mutations may affect detection of individual targets with the TaqPath™ COVID-19 HT Kit, including the B.1.1.7 variant of SARS-CoV-2 which may exhibit positive results for the N and ORF1ab targets and negative results for the S-gene target. If such a pattern of detection is observed, further characterization of the specimen by sequence analysis should be considered.
- Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
- Laboratories are required to report all test results to the appropriate public health authorities.

In-use reagent stability

For stability information for reagents stored at room temperature, refer to the packaging of each reagent.

Reagent	Stability information
TaqPath™ COVID-19 HT MS2 Phage Control	Once thawed, the TaqPath™ COVID-19 HT MS2 Phage Control is stable for up to 14 days at room temperature (up to 30°C) or in the Tecan™ Fluent™ 1080 Automation Workstation reagent carrier. Perform a maximum of 5 freeze-thaw cycles.
TaqPath™ COVID-19 HT qPCR Assay	The assay is stable for up to 14 days at room temperature (up to 30°C) or in the Tecan™ Fluent™ 780 Automation Workstation reagent carrier (9°C).
TaqPath™ COVID-19 HT IVT RNA Control (undiluted)	The undiluted positive control reagent is stable for up to 48 hours at room temperature (up to 30°C) or in the Tecan™ Fluent™ 780 Automation Workstation reagent carrier (9°C). Perform a maximum of 5 freeze-thaw cycles.
Diluted TaqPath™ COVID-19 HT IVT RNA Control	Once diluted as part of the Module 2 process in the Tecan™ Fluent™ 780 Automation Workstation reagent carrier, the dilute positive control is stable for up to 14 days at up to 30°C or in the Tecan™ Fluent™ 780 Automation Workstation reagent carrier (9°C).
TaqPath™ HT Control Dilution Buffer	The dilution buffer is stable for up to 14 days at room temperature (up to 30°C) or in the Tecan™ Fluent™ 780 Automation Workstation reagent carrier (9°C).
TaqPath™ 1-Step Multiplex Master Mix (No ROX)	The master mix is stable for up to 14 days at room temperature (up to 30°C) and in the Tecan™ Fluent™ 780 Automation Workstation reagent carrier (9°C).
Purified sample RNA	Once extracted, purified sample RNA is stable at room temperature (up to 30°C) for 1 hour, 4°C for 48 hours, and at –30°C to –10°C or at ≤ –70°C for up to 8 weeks. Perform a maximum of 3 freeze-thaw cycles.
Assembled sample extraction plate	After the completion of the Module 1 process, the assembled sample extraction plate is stable for up to 7 hours at room temperature (up to 30°C).
Assembled RT-PCR plate	After completion of the compression process, the assembled RT-PCR plate is stable for up to 25 minutes at room temperature (up to 30°C).

2 Before you begin

Warnings and precautions

The system and assay should only be used in a qualified laboratory environment according to the sample type by qualified and trained staff to avoid cross-contamination and the risk of erroneous results. For information about operator roles and requirements, see the user guide for your system.

- Before using this product, read and understand the information provided in this guide and the user guide for your system.
- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Samples and controls should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.
- Follow necessary precautions when handling specimens. Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples.
- Always use pipette tips with aerosol barriers. Visually inspect tips to ensure filters are present. 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.
- Reagents must be stored and handled as specified in “Contents and storage” on page 6.
- Do not use system consumables or reagents after the indicated expiry date.
- Dispose of waste in compliance with local regulations.
- If product primary packaging is damaged, vials are unintentionally opened before use, or product is exposed to temperatures outside the stability conditions outlined in the instructions for use, discard the product.

Incident reporting

Report any serious incident affecting patient or user safety to Thermo Fisher Scientific (for contact details, see “Customer and technical support” on page 43) and to the authority having jurisdiction in your locale.

Sample collection, transport, and storage

CAUTION! Handle all samples and controls as if they are capable of transmitting infectious agents.

- Patient samples must be collected according to appropriate laboratory guidelines. The Amplitude™ Platform can be used with patient samples collected via nasopharyngeal swab or nasopharyngeal aspirate.
- Store samples at 2–8°C for up to 72 hours after collection. If a delay in testing is expected, store samples at ≤-70°C.

Nucleic acid isolation guidelines

For information on isolation and purification of viral nucleic acid from human respiratory specimens, see the *MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit, High-Throughput Instructions for Use* (Pub. No. MAN0019941).

3 Perform an Amplitude™ Platform Module 1 run

Module 1 overview

Module 1 operation is controlled by the Tecan™ FluentControl™ Software v3.0, via the Tecan™ Fluent™ 1080 Automation Workstation touchscreen. For more information, see the user guide for the Amplitude™ Platform (Pub. No. MAN0019842).

During Module 1 operation:

- Technicians load prepared sample tube runners, reagents, and consumables on the Tecan™ Fluent™ 1080 Automation Workstation deck and start runs.
- Depending on the number of samples in a run, the instrument prepares 1–4 sample extraction plates containing the following components:
  - 200 µL of sample from the sample tubes
  - MagMAX™ MVPII HT Proteinase K
  - TaqPath™ COVID-19 HT MS2 Phage Control
  - Binding Bead Mix
  - Silicone oil
- The Tecan™ Fluent™ 1080 Automation Workstation scans the barcodes on sample tubes and sample extraction plates, then registers the information with the SampleManager LIMS™ Software Version 12.2 for sample tracking.

Module 1 required samples, reagents, and consumables

For a complete list of required samples, reagents, and consumables, see the user guide for the Amplitude™ Platform (Pub. No. MAN0019842).

Required samples, reagents, and consumables	Usage (Reload frequency)
Sample input	
Barcoded sample tubes in sample carrier racks	94, 188, 282, or 376 samples per run
Tips and Plates	
KingFisher™ Deep-Well 96 Plate with Barcode (sample extraction plates)	1–4 plates per run
1,000 µL Flexible Channel Arm™ (FCA) Disposable Tips Rack	15 racks per 12 plates
200 µL Flexible Channel Arm™ (FCA) Disposable Tips Rack	3 racks per 18–24 plates
Axygen™ Multi Well Low Profile Reagent Reservoir (for MagMAX™ MVPII HT Proteinase K/TaqPath™ COVID-19 HT MS2 Phage Control)	1 reservoir per 18–24 plates
Axygen™ Single Well High Profile Reagent Reservoir (for Binding Bead Mix)	1 reservoir per 18–24 plates[1]
Axygen™ Single Well High Profile Reagent Reservoir (for silicone oil)	1 reservoir per 18–24 plates[1]
Reagents[2,3]	
MagMAX™ MVPII HT Binding Beads	1 bottle per 18–24 plates
MagMAX™ MVPII HT Binding Solution	1 bottle per 18–24 plates
TaqPath™ COVID-19 HT MS2 Phage Control	2 bottles per 18–24 plates
MagMAX™ MVPII HT Proteinase K	1 bottle per 18–24 plates
HT Water for Dilution	1 bottle per 18–24 plates
Silicone oil	1 bottle per 18–24 plates

[1] Replaced daily, if running <18–24 plates.

[2] See “In-use reagent stability” on page 9.

[3] Reagent consumption and reload frequency depend on the number of sample extraction plates used per run and vary from system to system.

Procedural guidelines

Guidelines for controls

Include the following positive and negative test controls to ensure that test results are interpreted accurately:

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

Guidelines for sample tubes

- Only use sample tubes meeting the specifications listed in the table below:

Tube runner type	Tube diameter range		Maximum tube height	Tube bottom type
	Minimum	Maximum		
13 mm, 32-position	12 mm	14 mm	140 mm	Skirted, internal conical only
16 mm, 26-position	15 mm	17 mm	140 mm	Skirted, internal conical only
17 mm, 24-position	16 mm	18 mm	140 mm	Non-skirted, external conical *or* Skirted, internal conical

IMPORTANT!

- For each Module 1 run, all 376 sample tubes must be within the same diameter range. Individual tube heights can vary, but should not exceed 140 mm.
- For each Module 1 run, all 376 sample tubes must be loaded in the same type of tube runner. Tube runner types cannot be mixed within a run.

- Ensure that you have the minimum volume of sample required for the sample tube size.

Sample tube size	Minimum volume required
12–14 mm	0.5 mL
15–17 mm	1 mL
16–18 mm	1 mL

- All sample tubes must be barcoded with a unique ID. For sample tube barcode requirements, see the user guide for the Amplitude™ Platform (Pub. No. MAN0019842).

  Note: The Amplitude™ Platform does not perform sample accessioning. If required, perform sample accessioning according to guidelines established by your laboratory prior to processing the samples on Module 1. After testing, the results can be ported into your laboratory system.

- For sample collection devices containing swabs, the swabs must be removed from sample tube before loading into the tube runners. Alternatively, the sample can be transferred to a fresh, barcoded sample tube that is then loaded into an appropriate runner type.
- The Amplitude™ Platform can only run samples in multiples of 94. If there is an instance where there are fewer samples than 94, 188, 282, or 376, you must include additional empty, barcodes tubes to fill any empty positions in the tube runners.

Guidelines for sample inactivation

Follow the sample inactivation guidelines and requirements established by your laboratory and local regulatory bodies. If your laboratory inactivates samples, do so before you begin the Module 1 workflow.

Chemical inactivation is not recommended and may interfere with test results.

Before you begin—Module 1

Follow the procedure as described in the user guide for the Amplitude™ Platform (Pub. No. MAN0019842), starting with powering the system on through preparing the Tecan™ Fluent™ 1080 Automation Workstation.

Thaw TaqPath™ COVID-19 HT MS2 Phage Control

1. For every 24 sample extraction plates, thaw 2 bottles of the TaqPath™ COVID-19 HT MS2 Phage Control at room temperature (up to 30°C).
2. Perform the following procedures as described in the user guide for the Amplitude™ Platform.
   a. Prepare the Binding Bead Mix
   b. View the SampleManager Module 1 Load Indicator
   c. Start the method on the Tecan™ Fluent™ 1080 Automation Workstation

Load the Tecan™ Fluent™ 1080 Automation Workstation deck

IMPORTANT! Start the method on the Tecan™ Fluent™ 1080 Automation Workstation, then load reagents and consumables in the correct deck positions when prompted by the instrument touchscreen. Failure to do so can result in reduced throughput.

The following procedure describes the process for loading consumables and reagents for the TaqPath™ COVID-19 HT Kit only. For a complete list of materials that are loaded in the instrument, see the user guide for the Amplitude™ Platform (Pub. No. MAN0019842).

1. Open the Tecan™ Fluent™ 1080 Automation Workstation access door.

2. Follow the touchscreen prompts to fill the empty deck.

   The order in which the consumables and reagents are refilled and reload frequency is summarized in Table 2. The reload frequency is dependent on the number of sample extraction plates used per run and varies from system to system. When prompted to scan reagents, refer to Table 3 to scan the correct barcode for each reagent.

Table 2 Reagents and consumables

Load the empty deck	Reload frequency			
	Every run	Every shift	Every 12 plates	Every 18–24 plates
Load 1,000 µL FCA Disposable Tips Rack × 15			X	X
Load 200 µL FCA Disposable Tips Rack × 3				X
Add new Binding Bead Mix trough[1] and Bead Mix Baffle		X		
Fill Binding Bead Mix trough	X		X	X
Add new silicone oil trough[1] and Oil Level Sensor		X		
Fill silicone oil trough	X		X	X
Add new MagMAX™ MVPII HT Proteinase K/TaqPath™ COVID-19 HT MS2 Phage Control mixing trough[2]				X[3]
Load MagMAX™ MVPII HT Proteinase K in reagent carrier (see Figure 2)				X
Load HT Water for Dilution in reagent carrier (see Figure 2)				X
Load TaqPath™ COVID-19 HT MS2 Phage Control × 2 in reagent carrier (see Figure 2)				X
Load KingFisher™ Deep-Well 96 Plates with Barcodes × 4	X		X	X

[1] Axygen™ Single Well High Profile Reagent Reservoir
[2] Axygen™ Multi Well Low Profile Reagent Reservoir
[3] If running <18–24 plates per day, replace daily.

Table 3 Barcode scanning

Reagent	Scan barcode from:
TaqPath™ COVID-19 HT MS2 Phage Control	Bottle
MagMAX™ MVPII HT Binding Solution	Bottle
MagMAX™ MVPII HT Binding Beads	Bottle
Silicone oil	Bottle
HT Water for Dilution	Bottle
MagMAX™ MVPII HT Proteinase K	Bottle

a. (**Every shift**) In the **Load reagents and mixing trough** screen, follow the prompts to replace both the used Binding Bead Mix trough and the silicone oil trough with a new Axygen™ Single Well High Profile Reagent Reservoir. Make sure to assemble the troughs with the appropriate inserts before loading onto the instrument deck:

- Insert the Bead Mix Baffle into the Binding Bead Mix trough.
- Insert the Oil Level Sensor into the ***left*** side of the silicone oil trough.

IMPORTANT! When assembled correctly with the troughs, the text stamped on the inserts should be legible and oriented the correct side up (see Figure 1).

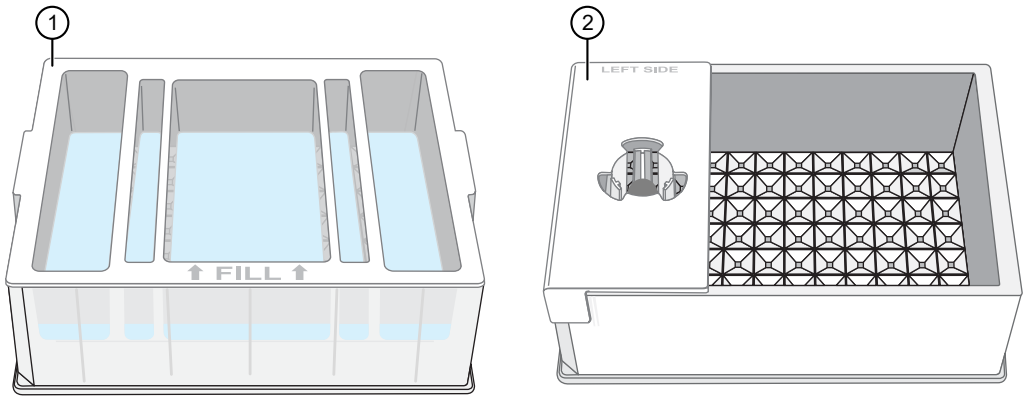

Figure 1

① Bead Mix Baffle ② Oil Level Sensor

b. (**Every run**) In the **Load reagents and mixing trough** screen, follow the prompts to invert the bottle of Binding Bead Mix 10 times to ensure that the mixture is homogenous, then pour the Binding Bead Mix into the middle compartment of the trough.

- For full-capacity back-to-back runs (6 runs, consisting of 4 sample extraction plates each), fill the trough up to the fill line.
- For all other plate and run configurations, fill the trough with volume specified in the user guide for the Amplitude™ Platform (Pub. No. MAN0019842).

Note: If there is a significant delay between Module 1 runs (e.g., >2 hours), we recommend using a new Binding Bead Mix trough to prevent aggregation of the MagMAX™ MVPII HT Binding Solution, which can lead to liquid detection errors.

WARNING!

- Binding Bead Mix serves to inactivate virus in the extraction plate. Any misuse that leads to failure to add the Binding Bead Mix to the extraction plate wells can lead to increased risk of exposure to infectious material.
- Improper or overly vigorous mixing of the Binding Bead Mix can lead to invalid results or increased risk of exposure to infectious material. Minimize foaming during the mixing process.

CAUTION! Ensure that you add the Binding Bead Mix to the correct trough. Adding the reagent to the incorrect trough can lead to invalid results.

c. (**Every run**) In the **Load reagents and mixing trough** screen, follow the prompts to pour the silicone oil to the trough fill line.

CAUTION! Ensure that you add the silicone oil to the correct trough. Adding the reagent to the incorrect trough can lead to invalid results.

d. (**Every 18–24 plates, or every 6 full-capacity runs of 4 plates/run**) Follow the prompts to replace the used MagMAX™ MVPII HT Proteinase K/TaqPath™ COVID-19 HT MS2 Phage Control mixing trough with a new Axygen™ Multi Well Low Profile Reagent Reservoir.

e. (**Every 18–24 plates, or every 6 full-capacity runs of 4 plates/run**) Follow the prompts to scan the reagents, then load the reagent carrier.

Note: Before removing the reagent bottle cap, gently tap the reagent bottle on the bench to ensure the liquid is collected at the bottom of the container.

CAUTION! Only scan when loading new, full bottles of reagents.

Reagent	Amount
MagMAX™ MVPII HT Proteinase K	1 bottle
HT Water for Dilution (for Negative Control)	1 bottle
TaqPath™ COVID-19 HT MS2 Phage Control	2 bottles

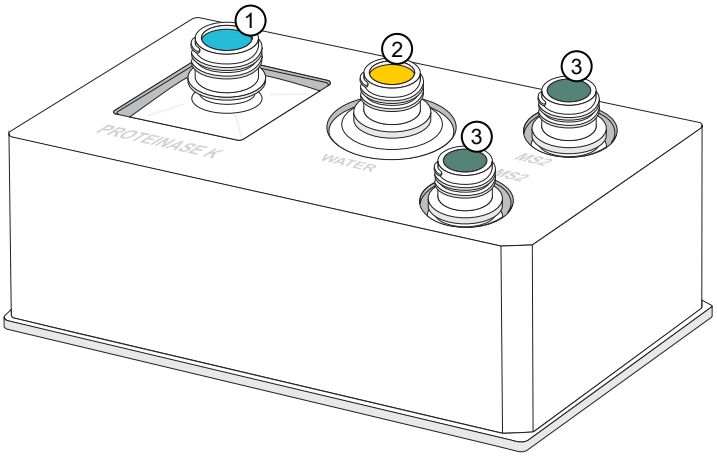

Figure 2 Module 1 reagent carrier positions

① MagMAX™ MVPII HT Proteinase K
② HT Water for Dilution
③ TaqPath™ COVID-19 HT MS2 Phage Control × 2

Perform Module 1 run—After all of the reagents and consumables are reloaded, follow the touchscreen prompts to load the tube runners, then complete the remaining procedures for Module 1 as described in the user guide for the Amplitude™ Platform (Pub. No. MAN0019842).

During a Module 1 run, the Tecan™ Fluent™ 1080 Automation Workstation performs the following steps:

1. Picks up equal parts of MagMAX™ MVPII HT Proteinase K and TaqPath™ COVID-19 HT MS2 Phage Control, then mixes them in the MagMAX™ MVPII HT Proteinase K/TaqPath™ COVID-19 HT MS2 Phage Control mixing trough.
2. Transfers 200 µL of each sample to a well in a KingFisher™ Deep-Well 96 Plate with Barcode.
3. Transfers 275 µL of Binding Bead Mix and 10 µL of MagMAX™ MVPII HT Proteinase K/TaqPath™ COVID-19 HT MS2 Phage Control mix to every well in each KingFisher™ Deep-Well 96 Plates with Barcodes used in a run.
4. Adds 200 µL of silicone oil to every well in each KingFisher™ Deep-Well 96 Plates with Barcodes used in a run.

4 Perform an Amplitude™ Platform Module 2 run

Module 2 overview

Module 2 operation is controlled via the Momentum™ Workflow Scheduler Software 6.1 on the Module 2 computer station. For more information, see the user guide for the Amplitude™ Platform (Pub. No. MAN0019842).

During Module 2 operation, the following tasks are performed:

- Technicians load sample extraction plates, replenish reagents and consumables, and unload the outgoing RNA elution plates.
- 1–4 sample extraction plates are processed, with an output of one RNA elution plate that contains purified sample RNA for each sample extraction plate used (up to 4 plates maximum).
- The Tecan™ Fluent™ 780 Automation Workstation prepares the real-time RT-PCR plates, including 10 µL of purified sample RNA transferred from the RNA elution plates.
- Real-time RT-PCR is performed, then the results are automatically analyzed.

Module 2 required sample input, reagents, and consumables

The following tables list the required sample input, reagents, and consumables based on the action being completed in the Momentum™ Workflow Scheduler Software 6.1: **Check in**, **Replenish reagents**, or **Replenish consumables**.

Momentum™ Software prompts to reload reagents and consumables as required. The required amount of reagents and consumables as well as reload frequency is dependant on the number of sample extraction plates used. When prompted to scan reagents, refer to Table 7 to scan the correct barcode for each reagent.

For a complete list of required samples, reagents, and consumables, see the user guide for the Amplitude™ Platform (Pub. No. MAN0019842).

Table 4 Check in—Required sample input

Required sample input	Usage (Reload frequency)
Hotel Input (Position 1)	
Sample extraction plates from Module 1	1–4 plates per run

Table 5 Replenish reagents—Required reagents and consumables

Required reagents and consumables	Usage (Reload frequency)
Tecan™ Fluent™ 780 Automation Workstation (Position 2)	
TaqPath™ COVID-19 HT qPCR Assay	1 tube/3 runs or 1 tube/12 plates
TaqPath™ COVID-19 HT IVT RNA Control	1 tube/22 plates
TaqPath™ HT Control Dilution Buffer	1 tube/22 plates
TaqPath™ 1-Step Multiplex Master Mix (No ROX)	1 tube/3 runs or 1 tube/12 plates
HT Water for Dilution	1 bottle/24 plates
1,000 µL Flexible Channel Arm™ (FCA) Disposable Tips Rack	3 racks/18 runs
200 µL Flexible Channel Arm™ (FCA) Disposable Tips Rack	3 racks/18 runs
150 µL MultiChannel Arm™ (MCA) Disposable Tips Rack	12 racks/12 plates
2-mL mixing tubes	2 tubes/6 runs
TaqPath™ HT Module 2 Empty 5 mL Mixing Tubes	6 tubes/6 runs
Multidrop™ Combi Reagent Dispenser × 3 (Positions 6-8)	
MagMAX™ MVPII HT Elution Solution (used with Combi 1)	1 bottle/72 plates
MagMAX™ MVPII HT Wash Solution (Wash 1, used with Combi 2)	3 bottles/72 plates
80% Ethanol (Wash 2, used with Combi 3)	2 bottles/72 plates

Table 6 Replenish consumables—Required consumables

Required consumables	Usage (Reload frequency)
Tecan™ Fluent™ 780 Automation Workstation (Position 2)	
MicroAmp™ EnduraPlate™ Optical 384-Well Clear Reaction Plate with Barcode	6 plates/6 runs

Table 6 Replenish consumables—Required consumables *(continued)*

Required consumables	Usage (Reload frequency)
RNA Elution Plate Stackers (Position 3)	
KingFisher™ Deep-Well 96 Plate with Barcode (RNA elution plates)	10–40 plates/10 runs[1]
KingFisher™ 96 tip comb for deep-well magnets (for RNA elution plates)	10–40 tip combs/10 runs[2]
Wash Plate Stacker Carousel (Position 5)	
KingFisher™ Deep-Well 96 Plates with Barcodes (wash plates)	20–80 plates/10 runs[3]

[1] 1 RNA elution plate per sample extraction plate per run
[2] 1 KingFisher™ 96 tip comb for deep-well magnets for each KingFisher™ Deep-Well 96 Plate with Barcode
[3] 2 wash plates per sample extraction plate per run

Table 7 Barcode scanning

Reagent	Scan barcode from:
TaqPath™ COVID-19 Module 2 Assay Kit	Box
TaqPath™ COVID-19 HT IVT RNA Control	Box
TaqPath™ HT Control Dilution Buffer	Box
MagMAX™ MVPII HT Wash Solution	Bottle
MagMAX™ MVPII HT Elution Solution	Bottle
HT Water for Dilution	Bottle
TaqPath™ HT Module 2 Empty 5 mL Mixing Tubes	Box
TaqPath™ 1-Step Multiplex Master Mix (No ROX)	Box
80% Ethanol	Bottle

Before you begin—Module 2

Follow the procedure as described in the user guide for the Amplitude™ Platform (Pub. No. MAN0019842).

Thaw Module 2 reagents

Thaw Module 2 reagents as needed.

Thaw the following reagents at room temperature (up to 30°C):

IMPORTANT! Ensure that thaw time plus use time remain within the specified stability window (see “In-use reagent stability” on page 9).

- 2 tubes of TaqPath™ COVID-19 HT qPCR Assay
- 1 tube of TaqPath™ HT Control Dilution Buffer
- 1 tube of TaqPath™ COVID-19 HT IVT RNA Control
- 2 bottles of TaqPath™ 1-Step Multiplex Master Mix (No ROX)

Note:

- Gently invert or mix the reagents prior to use.
- Ensure that each reagent is localized to the bottom of the tube or bottle.

Perform the following procedures as described in the user guide for the Amplitude™ Platform (Pub. No. MAN0019842).

1. Prepare the RNA elution plates
2. Reload Module 2 consumables

Load reagents and consumables on the Tecan™ Fluent™ 780 Automation Workstation deck

The following procedure describes the process for loading consumables and reagents for the TaqPath™ COVID-19 HT Kit only. For a complete list of materials that are loaded in the instrument, see the user guide for the Amplitude™ Platform (Pub. No. MAN0019842).

IMPORTANT! Before you load the deck, ensure that the current temperature of the A25 Refrigerated Circulator water bath is 9°C. Failure to maintain the water bath at 9°C can affect results.

Refer to the deck layout in the user guide for the Amplitude™ Platform when loading the deck.

1. Follow the procedure to replenish reagents in the Momentum™ Workflow Scheduler Software 6.1 as described in the user guide for the Amplitude™ Platform.

2. Open the Tecan™ Fluent™ 780 Automation Workstation access door.

3. (**Every 6 runs**) Load a MicroAmp™ EnduraPlate™ Optical 384-Well Clear Reaction Plate with Barcode (× 6) in the top 6 nests of the hotel at **Position 6**.

CAUTION! Ensure that each MicroAmp™ EnduraPlate™ Optical 384-Well Clear Reaction Plate with Barcode is seated properly in the grooves of the hotel nests with the plate barcodes facing in toward the Tecan™ Fluent™ 780 Automation Workstation deck.

WARNING! Dry any moisture from the Tecan™ Fluent™ 780 Automation Workstation deck and reagent block to prevent fluid accumulation and potential mold growth.

4. (**Every 6 runs**) Discard empty reagent vials, then load the reagent carrier according to the diagram. Make sure to uncap the reagent vials after loading.

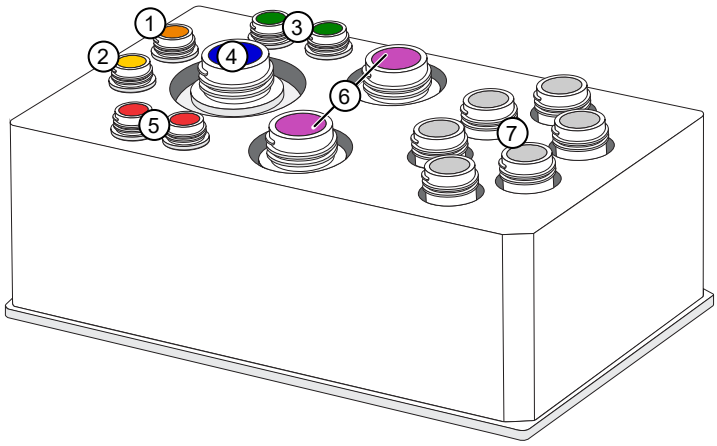

Figure 3 Module 2 reagent carrier layout

① TaqPath™ COVID-19 HT IVT RNA Control
② TaqPath™ HT Control Dilution Buffer
③ 2 × 2-mL mixing tubes (used to dilute theTaqPath™ COVID-19 HT IVT RNA Control in the TaqPath™ HT Control Dilution Buffer)
④ HT Water for Dilution
⑤ 2 × TaqPath™ COVID-19 HT qPCR Assay
⑥ 2 × TaqPath™ 1-Step Multiplex Master Mix (No ROX)
⑦ 6 × TaqPath™ HT Module 2 Empty 5 mL Mixing Tubes (used to mix the PCR reaction)

CAUTION!

- If removing partially used reagents from the Tecan™ Fluent™ 780 Automation Workstation deck for later reuse, make sure to place colored reagent caps on the correct vials. Failure to do so can lead to invalid results, incorrect reagent placement, and/or reagent contamination when vials are replaced in the carrier.
- If replacing partially used reagents on the Tecan™ Fluent™ 780 Automation Workstation deck, verify the correct reagent positioning based on the vial labels. Placing vials in incorrect positions can lead to invalid results and damage to the instrument.

5. After all of the reagents and consumables are loaded on the deck, close the Tecan™ Fluent™ 780 Automation Workstation access door.

Perform Module 2 run—Follow the remaining procedures for Module 2 as described in the user guide for the Amplitude™ Platform. The workflow processes 200-µL sample input volumes.

During a Module 2 run, instruments perform the following steps:

1. Four KingFisher™ Presto Purification Systems extract RNA from the samples.
2. A Tecan™ Fluent™ 780 Automation Workstation transfers sample RNA, controls, and reagents to MicroAmp™ EnduraPlate™ Optical 384-Well Clear Reaction Plates with Barcode.
3. Real-time RT-PCR is performed using reagents and assays from the TaqPath™ COVID-19 HT Kit and two Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR Systems.

5 Review results (automated workflow)

Automated results process

1. The Amplitude™ Interpretive Software Driver v1.3 is pre-installed on the Amplitude™ Platform.
2. The real-time RT-PCR data is automatically analyzed by the Amplitude™ Interpretive Software Driver.

   Note: The operator does not interact directly with the Amplitude™ Interpretive Software Driver. The Amplitude™ Interpretive Software Driver is pre-installed and updated appropriately by the Thermo Fisher™ Technical Support team.

3. The results are transferred as a CSV file to the designated location.
4. Review the results in the CSV file (see “Interpretation of the results” on page 27).

   Note:
   - If a batch is **INVALID**, an email notification is sent to users that the plate failed.
   - Samples for which Amplitude™ Platform system fails to aspirate the full specified sample volume do not appear in the CSV results file. Instead, an email notification is sent to users to re-test the failed samples.

Interpretation of the results

Quality control and validity of results

All control wells must pass for the real-time RT-PCR plate results to be considered valid. Validation of results is performed automatically based on the performance of the Positive and Negative Controls.

The following C_t cutoff values for assay targets are used during interpretation of the results:

Table 8 C_t cutoff values for assay targets

Sample	Target C_t cutoff	Result
Positive Control	MS2—C_t values are >37	Valid Positive Control.
	Viral targets—C_t values are ≤37	
Negative Control	MS2—C_t values are ≤32	Valid Negative Control.
	Viral targets—C_t values are >37	
Clinical samples	MS2—C_t values are ≤32	For interpretation of the test results, see Table 9.
	Viral targets—Positive C_t values are ≤37	

Interpretation of test results

The results for each assay target are used to determine the status and result for the sample, as indicated in the table:

Table 9 Interpretation of test results

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 a healthcare provider. Consider testing for other viruses.
Only one SARS-CoV-2 target = POS			POS or NEG	VALID	SARS-CoV-2 Inconclusive	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 a healthcare provider.

A Performance characteristics for Amplitude™ High-Throughput COVID-19 Testing Solution

Analytical performance of the Amplitude™ High-Throughput COVID-19 Testing Solution was evaluated as described in the following sections.

Note: The Amplitude™ High-Throughput COVID-19 Testing Solution uses the same real-time PCR reagents and multiplex assay as the previously-qualified TaqPath™ COVID-19 CE-IVD RT-PCR Kit. For information about the performance characteristics of the TaqPath™ COVID-19 CE-IVD RT-PCR Kit, see *TaqPath™ COVID-19 CE-IVD RT-PCR Kit Instructions for Use* (Pub. No. MAN0019215).

Limit of detection study

A limit of detection (LoD) study was conducted according to the design outlined in CLSI EP17, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, 2nd Edition, with two reagent lots and two lots of virus stock. Results were analyzed via a probit analysis. Because of concerns around mutations in the SARS-CoV-2 S gene, results were reported as-measured and with all S gene amplification results artificially set to negative. The overall worst-case LoDs across viral and reagent lots were 134 GCE/mL as measured and 233 GCE/mL assuming all S gene amplification were negative. Due to variation in concentrations across quantified materials, an overall LoD of 250 GCE/mL is claimed.

LoD was additionally evaluated using the WHO International Standard for SARS-CoV-2. Banked, pooled COVID-19 negative swab samples were spiked with the WHO International Standard at several concentrations, then processed using the Amplitude™ High-Throughput COVID-19 Testing Solution

workflow. A second level of testing was then performed to ensure ≥95% detection at the estimated LoD across multiple days and runs, establishing an LoD of 150 IU/mL.

Day	Replicates	% positive
1	40	100%
2	40	100%
3	40	100%

Cross-contamination study

A cross-contamination study was performed with a surrogate assay to assess the potential for well-to-well cross contamination when using the Amplitude™ High-Throughput COVID-19 Testing Solution workflow. Alternating positive (10^6 × LoD) and negative samples were set up across the extraction plates and then processed through the full assay workflow to evaluate cross contamination.

Results from the combined data points of four runs show that 100% of contrived positive sample replicates (752/752) produced a positive result and 99.7% of contrived negative sample replicates (562/564) produced a negative result.

Table 10 Cross-contamination study results

Run	Sample	Number of Replicates	Number of Replicates with Expected Results	Average C_t	Standard Deviation C_t	% Passed
1	Positive	188	188	18.717	0.411	100%
	Negative	188	187	35.5	N/A[1]	99.5%
2	Positive	188	188	18.976	0.329	100%
	Negative	188	188	N/A[1]	N/A[1]	100%
3	Positive	188	188	18.637	0.393	100%
	Negative	188	188	N/A[1]	N/A[1]	100%
4	Positive	188	188	18.239	0.442	100%
	Negative	188	187	35.8	N/A[1]	99.5%

[1] Not Applicable

Reactivity (Inclusivity)

In silico analysis was executed using 1,116,456 complete SARS-CoV-2 genomes in the GISAID database as of 7 May 2021. 1,116,456 strains were evaluated based on melting temperature analysis. A positive is called when at least two assays show a melting temperature higher than the annealing temperature. A positive was called for 1,098,871 SARS-CoV-2 strains out of 1,116,456 (98.4%). Evaluation of assay components that do not match 100% to target sequences indicated that most of the primer or probe mismatches are unlikely to impact assay function.

In silico analysis was updated using 773,796 sequenced genomes in the GISAID database from 1 May 2021 to 27 August 2021, which includes the following variants of concern:

- 216,636 genomes of the Alpha variant
- 3,889 genomes of the Beta variant
- 28,283 genomes of the Gamma variant
- 320,133 genomes of the Delta variant

Mismatch melting temperature analysis was performed in which a positive result was called when the primers and probe for at least 2 of the 3 assay targets (ORF1ab, S gene, and N gene) exhibited melting temperatures from the template strand that were higher than the annealing temperature for the PCR. Based on the melting temperature analysis, the TaqPath™ COVID-19 HT Kit correctly identified 761,641 (98.4%) of known SARS-CoV-2 strains/isolates in GISAID. Evaluation of assay components that did not match 100% to target sequences indicated that most of the primer or probe mismatches are unlikely to impact assay function.

Analysis was updated using SARS-CoV-2 database entries from 1 November 2021 to 16 December 2021 containing 518,387 sequenced genomes, including 2320 genomes of the Omicron variant. Mismatch and melting temperature analysis was performed as described above. Based on the melting temperature analysis, the TaqPath™ COVID-19 HT Kit correctly identified 99.6% of SARS-CoV-2 strains. An analysis of mutations in variants of concern and variants of interest with respect to the TaqPath™ COVID-19 HT Kit assay primer and probe locations concludes that these variants pose no significant impact to ORF1ab and N gene performance. However, B1.1.529 and BA.1 variants are likely to show a negative result in the S gene channel.

Based on the analysis described above, the TaqPath™ COVID-19 HT Kit is expected to be highly inclusive for SARS-CoV-2 strain detection.

Cross-reactivity

In silico analysis of the following forty-three (43) organisms was performed.

Table 11 Organisms used for *in silico* cross-reactivity analysis

Human coronavirus 229E	Rhinovirus/Enterovirus
Human coronavirus OC43	Parechovirus
Human coronavirus HKU1	*Candida albicans*
Human coronavirus NL63	*Corynebacterium diphtheriae*

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

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

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

Additionally, cross-reactivity was functionally assessed across the pathogens listed in Table 12. For the evaluated pathogens, bacteria and fungi were evaluated at 10^6 CFU/mL equivalence or higher, and viruses at 10^5 $TCID_{50}$/mL equivalence or higher.

Table 12 Organisms used for functional cross-reactivity analysis

Organism	% negative
Legionella pneumophila	100%
Bordetella pertussis	100%
Streptococcus pneumonia	100%
Parainfluenza 1[1]	100%
Parainfluenza 2	100%
Parainfluenza 3	100%
Parainfluenza 4A	100%
Rhinovirus 1A	100%
Pneumocystis jirovecii (PJP)	100%
Haemophilus influenzae	100%
Chlamydophila pneumoniae	100%
Human Metapneumovirus (hMPV-9)[2]	100%
Coronavirus 229E[1]	100%
Coronavirus NL63[3]	100%
Coronavirus OC43	100%
MERS-CoV[1]	100%
Adenovirus Type 1	100%
Mycoplasma pneumoniae[1]	100%
Mycobacterium tuberculosis[1]	100%
Streptococcus pyogenes	100%
Epstein-Barr Virus	100%
Influenza A (Brisbane/59/07)	100%
Influenza B (Florida/04/06)	100%
RSV A	100%
RSV B	100%

[1] Tested at less that the referenced values due to limited stock or available starting concentration; all tested at >10^4 units/mL.
[2] Tested at less that the referenced values due to limited stock or available starting concentration; all tested at >10^3 units/mL.
[3] Tested at less that the referenced values due to limited stock or available starting concentration; all tested at >10^2 units/mL.

Interfering substances

Potential interfering substances were evaluated for impact on pooled, SARS-CoV-2-negative clinical matrix spiked as well as matrix spiked to 3X LoD with SARS-CoV-2 virus (isolate England/02/2020, WHO International Standard). The following substances were observed to have no impact on clinical call: Tobramycin, Mupirocin, Oseltamivir, whole blood, mucin (submaxillary gland, type I-S), Wal-Four® Nasal Spray (contains phenylephrine), Nasacort® (contains triamcinolone), Zicam® (contains oxymetazoline hydrochloride), Chloraseptic® Throat Lozenge, and Chloraseptic® Sore Throat Spray. NeilMed™ Nasogel™ was found to have an inhibitory impact at concentrations >5%.

Table 13 Interfering substances

Potential interfering substance	Tested concentration	# of negative calls at 0X LoD	# of positive calls at 3X LoD
Antibacterial, systemic—Tobramycin	2.43 mg/mL	3/3	3/3
Antibiotic, nasal ointment—Mupirocin	4.3 mg/mL	3/3	3/3
Anti-viral drugs—Oseltamivir	33 µg/mL	3/3	3/3
Blood—human (whole blood)	1%	3/3	3/3
Mucin: bovine submaxillary gland, type I-S	0.0625%	3/3	3/3
Nasal sprays or drops—Wal-Four® Nasal Spray Nasal Spray (Phenylphrine)	20%	3/3	3/3
Nasal corticosteroids—Nasacort® (Triamcinolone)	0.31 mg/mL	3/3	3/3
Nasal gel—Zicam®	20%	3/3	3/3
	10%	3/3	3/3
	5%	3/3	3/3
Nasal gel—NeilMed™ Nasogel™	20%	0/3	0/3
	10%	0/3	3/3
	5%	3/3	3/3
Throat lozenges, oral anaesthetic and analgesic—Chloraseptic® Throat Lozenge	20%	3/3	3/3
Throat lozenges, oral anaesthetic and analgesic—Chloraseptic® Sore Throat Spray	20%	3/3	3/3

Linearity and Precision

Contrived samples using pooled negative clinical oropharyngeal/nasal specimens spiked with SARS-CoV-2 virus (isolate England/02/2020, WHO International Standard) were tested over a dilution series of four $\log_{10}$ dilution steps, from 1X LoD to 1,000X LoD, with 5 replicates at each concentration, using the Amplitude™ High-Throughput COVID-19 Testing Solution. Testing was conducted executing one run per day over 5 days, to determine the linearity or PCR efficiency and intra- and inter-assay precision (see Table 14).

Table 14 Linearity results

Viral target	Test level (X LoD)	Concentration (IU/mL)	Average C_t	Test levels: 1X–1,000X LoD		
				Slope	Efficiency (%)	R^2
N gene (VIC™)	1,000X	150,000	24.02	-3.07	111.9	0.998
	100X	15,000	27.46			
	10X	1,500	30.4			
	1X	150	33.26			
ORF1a (FAM™)	1,000X	150,000	23.2	-3.17	106.8	0.999
	100X	15,000	26.54			
	10X	1,500	29.79			
	1X	150	32.68			
S gene (ABY™)	1,000X	150,000	23.64	-3.43	95.6	1
	100X	15,000	27.25			
	10X	1,500	30.66			
	1X	150	33.95			

The intra-assay precision was evaluated for each gene at each concentration and day as documented in Table 15. Additionally, inter-assay precision was evaluated for each gene at each concentration across the 5 days (see Table 14).

Table 15 Results of intra-assay precision test (acceptance criteria: <10% for %CV)

Viral target	Test level (X LoD)	Day 1			Day 2			Day 3			Day 4			Day 5		
		Avg. C_t	SD C_t	%CV	Avg. C_t	SD C_t	%CV	Avg. C_t	SD C_t	%CV	Avg. C_t	SD C_t	%CV	Avg. C_t	SD C_t	%CV
N gene (VIC™)	1,000X	23.70	0.15	0.65	23.66	0.14	0.61	24.04	0.30	1.25	24.00	0.13	0.52	27.70	0.57	2.41
	100X	27.25	0.21	0.77	27.04	0.24	0.90	27.41	0.03	0.11	27.41	0.11	0.39	26.94	0.22	0.80
	10X	30.15	0.21	0.69	29.97	0.23	0.76	30.35	0.17	0.56	30.45	0.08	0.26	30.03	0.24	0.80
	1X	33.10	0.77	2.34	33.30	1.24	3.71	33.70	0.44	1.30	32.83	0.15	0.46	32.86	0.45	1.38
ORF1a (FAM™)	1,000X	22.86	0.30	1.31	22.77	0.39	1.71	23.16	0.33	1.43	23.24	0.15	0.63	22.83	0.82	3.59
	100X	26.39	0.49	1.86	26.00	0.36	1.37	26.39	0.44	1.65	26.68	0.08	0.31	25.24	0.83	3.30
	10X	29.53	0.24	0.80	29.53	0.17	0.58	29.67	0.16	0.54	29.91	0.17	0.58	29.12	0.87	2.99
	1X	32.47	0.29	0.90	32.65	0.91	2.80	32.58	0.54	1.64	32.78	0.70	2.13	32.34	0.54	1.67
S gene (ABY™)	1,000X	23.66	0.18	0.74	23.51	0.10	0.43	23.94	0.24	0.01	23.85	0.16	0.67	23.76	0.41	1.71
	100X	27.18	0.23	0.83	27.33	0.22	0.80	27.45	0.07	0.24	27.36	0.08	0.30	26.96	0.32	1.18
	10X	30.57	0.19	0.63	30.78	0.14	0.46	30.77	0.29	0.94	30.82	0.09	0.28	30.61	0.18	0.60
	1X	33.91	0.71	2.11	33.91	0.51	1.50	33.92	0.71	2.08	33.59	0.37	1.11	34.02	0.53	1.55

Table 16 Results of inter-assay precision test (acceptance criteria: <15% for %CV)

Viral target	Test level (X LoD)	Average C_t	SD C_t	%CV
N gene (VIC™)	1,000X	23.82	0.33	1.38
	100X	27.23	0.28	1.01
	10X	30.19	0.26	0.85
	1X	33.16	0.73	2.20
ORF1a (FAM™)	1,000X	22.97	0.46	2.00
	100X	26.14	0.69	2.63
	10X	29.55	0.47	1.58
	1X	32.56	0.60	1.83
S gene (ABY™)	1,000X	23.75	0.27	1.13
	100X	27.31	0.27	0.98
	10X	30.71	0.20	0.66
	1X	33.87	0.55	1.63

Amplitude™ High-Throughput COVID-19 Testing Solution comparator study

The performance of the Amplitude™ High-Throughput COVID-19 Testing Solution was compared against a third-party CE-IVD marked test and a CE-marked COVID-19 test.

Comparator study with a CE-IVD marked test

The performance of the Amplitude™ High-Throughput COVID-19 Testing Solution was compared to a third-party RT-PCR CE-IVD marked test.

A total of 117 mixed positive and negative SARS-CoV-2 nasopharyngeal swab samples were evaluated across the two workflows: Amplitude™ High-Throughput COVID-19 Testing Solution and a third-party CE-IVD test. The 117 samples were run, with 114 valid results. Three samples were excluded due to invalid results that were obtained with the comparator assay.

When comparing the Amplitude™ Solution to a third-party CE-IVD test, the PPA was 100% and the NPA was 100%.

Table 17 Concordance results: Amplitude™ High-Throughput COVID-19 Testing Solution vs. third-party CE-IVD test

Call	Number of samples	Concordance results[1,2,3,4]
True Positive	62	PPA: 100% (95% CI: [94.2%–100%])
False Negative	0	
True Negative	52	NPA: 100% (95% CI: [93.1%–100%])
False Positive	0	

[1] One Amplitude™ High-Throughput COVID-19 Testing Solution sample was initially reported as 'Inconclusive'. Upon rerun, the result was 'SARS-CoV-2 Not Detected', which was the final reported clinical call.

[2] Three 3rd party comparator samples were reported as 'Invalid'; these samples were omitted from analysis.

[3] Two 3rd party comparator samples were reported as 'Presumptive Positive'; these were assigned as 'Positive' for purposes of analysis.

[4] CI—Confidence Interval.

Comparator study with a CE-marked test

The performance of theAmplitude™ High-Throughput COVID-19 Testing Solution was compared against a CE-marked COVID-19 test. A total of 554 retrospective oropharyngeal (OP)/nasal specimens were evaluated across the two workflows. One sample was excluded due to repeated ‘Inconclusive’ results that were obtained with the Amplitude™ High-Throughput COVID-19 Testing Solution workflow.

Table 18 Agreement calculations between testing methods

Amplitude™ High-Throughput COVID-19 Testing Solution	CE-marked comparator device		
	Positive	Negative	Total
Positive	151	5	156
Negative[1]	1	396	397
Total[2]	152	401	553
Agreement calculations			**95% CI**[3]
Sensitivity	151/152	99.34%	96.39–99.98
Specificity	396/401	98.75%	97.11–99.59

[1] One Amplitude™ High-Throughput COVID-19 Testing Solution sample was initially reported as 'Inconclusive'. Upon rerun, the result was 'SARS-CoV-2 Not Detected', which was the final reported clinical call.

[2] One Amplitude™ High-Throughput COVID-19 Testing Solution sample was initially reported as 'Inconclusive'. Upon rerun, the result was ' 'Inconclusive' and was omitted from the study due to lack of a conclusive clinical call.

[3] CI—Confidence Interval.

Robustness

Performance robustness was evaluated by spiking 32 SARS-CoV-2-negative oropharyngeal (OP)/nasal retrospectively-collected individual specimens to 450 IU/mL (3X LoD) with SARS-CoV-2 virus (isolate England/02/2020, WHO International Standard). All samples were called 'SARS-CoV-2 Positive' at the tested level.

B

Amplitude™ Platform system components

Component	Manufacturer
Instruments	
Tecan™ Fluent™ 1080 Automation Workstation (base unit)	Tecan Switzerland | Seestrasse 103 | 8708 Maennedorf | Switzerland
Tecan™ Fluent™ 780 Automation Workstation (base unit)	Tecan Switzerland | Seestrasse 103 | 8708 Maennedorf | Switzerland
Thermo Fisher Scientific Spinnaker™ Microplate Robot with BenchTrak	Thermo CRS Ltd. | 5250 Mainway | Burlington, Ontario L7L 5Z1 | Canada
Spinnaker™ Microplate Robot Mini-Hub Control Puck	Thermo CRS Ltd. | 5250 Mainway | Burlington, Ontario L7L 5Z1 | Canada
Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR System (× 2)	Life Technologies Holdings Pte Ltd | Block 33 | Marsiling Industrial Estate Road 3 | #07-06, Singapore 739256
Thermo Scientific™ Multidrop™ Combi Reagent Dispenser (× 3)	Life Technologies Holdings Pte Ltd | Block 33 | Marsiling Industrial Estate Road 3 | #07-06, Singapore 739256
ALPS™ 3000 Automated Microplate Heat Sealer	Abgene™ UK Ltd | Units 4-7 Suffolk Drive | Fairwood Industrial Park | Ashford | Kent | TN23 4FD | UK
Thermo Scientific™ KingFisher™ Presto Purification System (× 4)	Life Technologies Holdings Pte Ltd | Block 33 | Marsiling Industrial Estate Road 3 | #07-06, Singapore 739256
Agilent™ Microplate Centrifuge	Agilent Technologies Singapore (International) Pte Ltd | 1 Yishun Avenue 7 | Singapore 768923
BioTek™ ELx405™ Select Microplate Washer	BioTek Instruments, Inc. | 100 Tigan Street | Winooski, Vermont 05404 USA
Thermo Scientific™ Arctic™ A25 Refrigerated Circulator (115V or 230V)	Thermo Fisher Scientific (Asheville) LLC | 275 Aiken Road | Asheville, North Carolina 28804 USA
Lab Automation Robotic Testing Platform	Thermo CRS Ltd. | 5250 Mainway | Burlington, Ontario L7L 5Z1 | Canada
Software	
Tecan™ FluentControl™ Software v3.0	Tecan Switzerland | Seestrasse 103 | 8708 Maennedorf | Switzerland

(continued)

Component	Manufacturer
SampleManager LIMS™ Software Version 12.2	Thermo Electron Ltd. | 1 St. George's Court, Hanover Business Park | Altrincham, Cheshire WA14 5TP | United Kingdom
Momentum™ Workflow Scheduler Software 6.1	Thermo CRS Ltd. | 5250 Mainway | Burlington, Ontario L7L 5Z1 | Canada
Amplitude™ Interpretive Software Driver v1.3[1] (part of Cat. No. A50883)	Life Technologies Corporation | 6055 Sunol Blvd | Pleasanton, California 94566 USA
BioTek™ Liquid Handling Control Software 2.2	BioTek Instruments, Inc. | 100 Tigan Street | Winooski, Vermont 05404 USA
FILLit™ Software 2.0[1]	Thermo Fisher Scientific Oy | Ratastie 2 | FI-01620 Vantaa | Finland
BindIt™ Software 4.0[1]	Life Technologies Holdings Pte Ltd | Block 33 | Marsiling Industrial Estate Road 3 | #07-06, Singapore 739256
QuantStudio™ Real-Time PCR Software v1.7	Life Technologies Holdings Pte Ltd | Block 33 | Marsiling Industrial Estate Road 3 | #07-06, Singapore 739256

[1] The operator does not interact directly with the Amplitude™ Interpretive Software Driver v1.3, FILLit™ Software 2.0, or BindIt™ Software 4.0.

C

Safety

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

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Chemical safety

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

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

Biological hazard safety

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

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

WARNING! Potential Biohazard. If you use the kit with the automated nucleic acid extraction workflow, the surface of the KingFisher™ purification system may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

D Documentation and support

Related documentation

Document	Publication Number
Amplitude™ High-Throughput COVID-19 Testing Solution User Guide	MAN0019842
MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit, High-Throughput Instructions for Use	MAN0019941

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

Download a Safety Data Sheet (SDS)

1. Go to **thermofisher.com**, then select SDS from the **Search All** dropdown list.
2. Search for the product catalog number or REF.
3. Select the product catalog number or REF of interest, select the region and language, then download the SDS.

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**.