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TaqMan™ SARS-CoV-2 MS2 Assay 2.0

USER GUIDE

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For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

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Revision	Date	Description
A.0	04 May 2021	New document for the TaqMan™ SARS-CoV-2 MS2 Assay 2.0.

Contents

1 Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product description

The Applied Biosystems™ TaqMan™ SARS-CoV-2 MS2 Assay 2.0 is a multiplexed real-time PCR research testing solution that can detect the RNA from the SARS-CoV-2 virus and MS2 extraction control in a single reaction well.

The TaqMan™ SARS-CoV-2 MS2 Assay 2.0 is a multiplexed assay that includes TaqMan™ MS2 Phage Control and contains primer and probe sets specific to the following targets (see Table 1):

- SARS-CoV-2 N Gene (3 targets)
- SARS-CoV-2 ORF1a (3 targets)
- SARS-CoV-2 ORF1b (2 targets)
- MS2 (1 target)

Table 1 Dyes, quenchers, and targets

Target	Dye	Quencher
SARS-CoV-2 N Gene	VIC™	None[1]
SARS-CoV-2 ORF1a	FAM™	
SARS-CoV-2 ORF1b	ABY™	
MS2	JUN™	

[1] TaqMan™ SARS-CoV-2 MS2 Assay 2.0 probes contain QSY™ quenchers, which do not fluoresce. Select **None** for **Quencher** in the instrument set up procedure (see page 21 and page 24).

In addition to TaqMan™ SARS-CoV-2 MS2 Assay 2.0, the workflow described in this user guide requires the following components, which must be purchased separately (see "Required materials not supplied" on page 6).

- TaqMan™ SARS-CoV-2 Plus Control—*In vitro* transcribed RNA control that contains targets specific to the SARS-CoV-2 regions targeted by the assays
- TaqMan™ Control Dilution Buffer—Dilution buffer for the TaqMan™ SARS-CoV-2 Plus Control
- TaqPath™ 1-Step Multiplex Master Mix (No ROX™)—Ready-to-use PCR mix, including polymerase, dNTPs, and salt buffer

For assay catalog number and storage conditions, see "Contents and storage" on page 6.

IMPORTANT! It is the responsibility of the laboratories using the TaqMan™ SARS-CoV-2 MS2 Assay 2.0 to design and validate their own experimental design and analysis parameters.

Contents and storage

The items listed in the following table are included with the TaqMan™ SARS-CoV-2 MS2 Assay 2.0 (Cat. No. A51327). The items listed are sufficient for 1,000 reactions.

Components	Amount	Storage
TaqMan™ SARS-CoV-2 MS2 TB 2.0 (N Gene, ORF1a, ORF1b, and MS2)	1,500 µL	–30°C to –10°C
TaqMan™ MS2 Phage Control	10 × 1,000 µL	

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.

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

IMPORTANT! The customer is responsible for performing all of the necessary validations to run this assay.

Item	Source
Real-time PCR instrument compatible with the four dyes listed in Table 1 on page 5	
Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block[1] (used with QuantStudio™ Design and Analysis Desktop Software v1.5.1)[2]	Contact your local sales office
Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR Instrument, 384-well block[1] (used with QuantStudio™ Real-Time PCR Software v1.3)	

(continued)

Item	Source
Recommended analysis software	
Applied Biosystems™ QuantStudio™ Design and Analysis Software v2.5 or later	**thermofisher.com/qpcrsoftware**
Equipment	
Laboratory freezers • –30°C to –10°C	MLS
Centrifuge, with a rotor that accommodates standard and deepwell microplates	MLS
Microcentrifuge	MLS
Laboratory mixer, vortex or equivalent	MLS
Single and multichannel adjustable pipettors (1.00 µL to 1,000.0 µL)	MLS
Cold block (96-well or 384-well) or ice	MLS
Automated nucleic acid extraction system and materials[3]	
KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head[1]	5400630
KingFisher™ Flex 96 Deep-Well Heating Block	24075430
KingFisher™ 96 Deep-Well Plate	95040450, A48305, A48424
KingFisher™ 96 tip comb for DW magnets	97002534, A48438, A48414
96-well plate for the tip comb, one of the following:	
KingFisher™ 96 KF microplate	97002540
Tip Comb Presenting Plate for KF 96	267600
Nunc™ MicroWell™ 96-Well Microplate, Flat Bottom	167008
Nunc™ MicroWell™ 96-Well Microplate, barcoded	269787
ABgene™ 96–Well Polypropylene Storage Microplate	AB0796
ABgene™ 96–Well 1.2–mL Polypropylene Deepwell Storage Plate	AB1127
KingFisher™ 96 Deep-Well Plate	95040450, A48305, A48424, 95040455
Kits and reagents	
TaqMan™ SARS-CoV-2 Plus Control (10 × 10 µL)	956129
TaqMan™ Control Dilution Buffer (10 × 250 µL)	A49889
TaqPath™ 1-Step Multiplex Master Mix (No ROX™) (10 mL)	A28523

(continued)

Item	Source
MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit	A48383R
Fisher BioReagents™ Ethanol, Absolute, Molecular Biology Grade, or equivalent	BP2818100, BP2818500, BP28184
Nuclease-free Water (not DEPC-Treated)	MLS
Calibration plates (QuantStudio™ 5 Real-Time PCR Instrument)[4]	
QuantStudio™ 3/5 Spectral Calibration Plate 1 (FAM™, VIC™, ROX™, and SYBR™ dyes), 96-Well 0.2-mL	A26331
QuantStudio™ 3/5 Spectral Calibration Plate 2, 96-Well 0.2-mL (ABY™, JUN™, and MUSTANG PURPLE™ dyes)	A26332
QuantStudio™ 3/5 Spectral Calibration Plate 3, 96-Well 0.2-mL (TAMRA™, NED™, and Cy®5 dyes)	A26333
Calibration plates (QuantStudio™ 7 Flex Real-Time PCR Instrument)[5]	
FAM™ Dye Spectral Calibration Plate, 384-well	4432271
VIC™ Dye Spectral Calibration Plate, 384-well	4432278
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™ 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™ Clear Adhesive Film	4306311
MicroAmp™ Optical Adhesive Film	4311971, 4360954
MicroAmp™ Adhesive Film Applicator	4333183
MicroAmp™ Optical Film Compression Pad[6]	4312639

(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

[1] This instrument was validated for use with the TaqMan™ SARS-CoV-2 MS2 Assay 2.0. For all other instruments, you are responsible for performing all of the necessary validation.

[2] QuantStudio™ Design and Analysis Software v2.5 can also be used, however you are responsible for performing all of the necessary validation.

[3] The nucleic acid extraction procedure described in this user guide was validated using the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head. For other extraction methods, refer to the user guide for the extraction kit used in your laboratory. You are responsible for validating your own experimental RNA extraction procedure.

[4] The QuantStudio™ 5 Real-Time PCR Instrument is factory-calibrated with ABY™, JUN™, and MUSTANG PURPLE™ dyes. Calibration plates are only required if the initial calibration has expired.

[5] A maintained instrument will be calibrated for many dyes. In addition, the instrument operator must calibrate the instrument for ABY™ and JUN™ dyes that are used with this kit. For calibration procedures and maintenance schedule for optimal system performance, see *QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Maintenance and Administration Guide* (Pub. No. 4489821).

[6] Required for use with QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block only.

General laboratory recommendations

WARNING! Do not use bleach or bleached pipette tips with the Binding Bead Mix. The Binding Solution contains guanidium thiocyanate, which produces cyanide gas when combined with bleach.

- Implement standard operating procedures in your laboratory to prevent contamination, such as the following:
  - Frequent glove changes
  - Frequent decontamination of surfaces, equipment, and pipettes with 10% bleach (0.1% V/V sodium hypochlorite) or decontamination solution, followed by 70% ethanol
  - Use of ultraviolet light during biosafety cabinet decontamination (when available)
- To prevent degradation, keep eluted sample RNA, master mixes, assays, and controls on ice or in cold blocks while in use. Limit freeze-thaw cycles.
- Minimize exposure to light for the assay. The probes are light-sensitive.
- Aliquot reagents to prevent stock contamination and reduce the number of freeze-thaw cycles.
- After each run, review the amplification curves in the instrument software according to data QC standard operating procedures for your lab.

Workflow

TaqMan™ SARS-CoV-2 MS2 Assay 2.0

Extract RNA from samples

Perform automated RNA extraction using the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head with a 200-µL sample input volume (see page 11).

Perform RT-PCR

1. Prepare RT-PCR reactions (see page 16).
2. Perform RT-PCR using one of the following real-time PCR instruments:
   - QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block (see page 21)
   - QuantStudio™ 7 Flex Real-Time PCR System, 384-well block (see page 24)

Analyze data

We recommend using QuantStudio™ Design and Analysis Software v2.5 or later for data analysis (see page 27).

2 Extract RNA

IMPORTANT! It is the responsibility of the laboratories to validate their own experimental design, including RNA extraction.

Before you begin

Note: During the wash steps, the Wash Solution may develop inert white or brown particulates that float in solution. This is not a cause for concern and does not negatively affect performance.

- Determine the number of required reactions based on the number of samples, plus one Negative Extraction Control per plate.

  Note: Each plate can accommodate up to 93 samples *plus* one Negative Extraction Control.

- Prepare fresh 80% ethanol solution using Ethanol, Absolute, Molecular Biology Grade and Nuclease-free Water (not DEPC-Treated) for the required number of reactions, sufficient for 1 mL per reaction, plus 10% overage.
- Label the short side of each KingFisher™ 96 Deep-Well Plate (4):

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

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

Label	Number of plates
Tip comb	1

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

- Tip Comb Presenting Plate for KF 96
- Nunc™ MicroWell™ 96-Well Microplate, Flat Bottom
- Nunc™ MicroWell™ 96-Well Microplate, barcoded
- ABgene™ 96–Well Polypropylene Storage Microplate
- ABgene™ 96–Well 1.2–mL Polypropylene Deepwell Storage Plate
- KingFisher™ 96 Deep-Well Plate

- Mark the Negative Extraction Control well on the plate.

Set up the instrument

1. Ensure that the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head is set up with the KingFisher™ Flex 96 Deep-Well Heating Block.

   IMPORTANT! Failure to use the proper magnetic head and heat block results in lower yields and potential harm to the instrument.

2. Ensure that the **MVP_2Wash_200_Flex** program has been downloaded from the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit product page at **www.thermofisher.com** and loaded onto the instrument.

Prepare the processing plates

Prepare the processing plates according to the following table. Cover the plates with a temporary seal (such as MicroAmp™ Clear Adhesive Film), then store at room temperature for up to 1 hour while you set up the sample plate.

Plate ID	Plate position	Plate type	Reagent	Volume per well
Wash 1 Plate	2	KingFisher™ 96 Deep-Well Plate	Wash Solution	500 µL
Wash 2 Plate	3		80% Ethanol	1,000 µL
Elution Plate	4		Elution Solution	50 µL
Tip Comb Plate	5	Place a KingFisher™ 96 tip comb for DW magnets in a KingFisher™ 96 KF microplate		

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

- Tip Comb Presenting Plate for KF 96
- Nunc™ MicroWell™ 96-Well Microplate, Flat Bottom
- Nunc™ MicroWell™ 96-Well Microplate, barcoded
- ABgene™ 96–Well Polypropylene Storage Microplate

- ABgene™ 96–Well 1.2–mL Polypropylene Deepwell Storage Plate
- KingFisher™ 96 Deep-Well Plate

Prepare Binding Bead Mix

WARNING! Do not use bleach or bleached pipette tips with the Binding Bead Mix. The Binding Solution contains guanidium thiocyanate, which produces cyanide gas when combined with bleach.

Prepare the required amount of Binding Bead Mix on each day of use.

1. Vortex the Total Nucleic Acid Magnetic Beads to ensure that the bead mixture is homogeneous.
2. For the number of required reactions, prepare the Binding Bead Mix according to the following table:

Component	Volume per well[1]	Volume per 96-well plate
Binding Solution	265 µL	28.0 mL
Total Nucleic Acid Magnetic Beads	10 µL	1.1 mL
Total volume	**275 µL**	**29.1 mL**

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

3. Invert at least five times to mix, then store at room temperature.

Prepare a Proteinase K and TaqMan™ MS2 Phage Control Mix

Prepare the required amount of the Proteinase K and TaqMan™ MS2 Phage Control Mix on each day of use. Keep on ice.

1. Thaw the vial of TaqMan™ MS2 Phage Control.
2. For the number of required samples plus one Negative Extraction Control, prepare the Proteinase K and TaqMan™ MS2 Phage Control Mix according to the following table:

Component	Volume per well[1]	Volume for 94[2] reactions plus 10% overage
Proteinase K	5 µL	517 µL
TaqMan™ MS2 Phage Control	5 µL	517 µL
Total volume per well	**10 µL**	**1,034 µL**

[1] Include 10% overage when preparing the Proteinase K and TaqMan™ MS2 Phage Control Mix for use with multiple reactions.
[2] 93 samples plus one Negative Extraction Control

3. Mix well by inversion, then store on ice.

Prepare sample plate

WARNING! Do not use bleach or bleached pipette tips with the Binding Bead Mix. The Binding Solution contains guanidium thiocyanate, which produces cyanide gas when combined with bleach.

Each 96-well RT-PCR plate requires three controls:

- Positive Control (PC)
- No Template Control (NTC)
- Negative Extraction Control (NC)

Therefore, each 96-well plate can accommodate 93 samples plus 1 Negative Extraction Control.

Note: NTC is a control on a RT-PCR plate only. It is not derived from the extraction plate.

1. Invert the prepared Binding Bead Mix 5 times gently to mix, then add 275 µL to each sample well and to the Negative Extraction Control well in the Sample Plate.

   Note:
   - Remix the Binding Bead Mix by inversion frequently during pipetting to ensure even distribution of beads to all samples or wells. The Binding Bead Mix is viscous, so pipet slowly to ensure that the correct amount is added. Do NOT reuse pipette tips to add Binding Bead Mix to the samples, as the high viscosity will cause variations in the volumes added.
   - Do NOT vortex the Binding Bead Mix.

2. Add 200 µL of sample to each sample well.

   Note: Change tips between samples to eliminate cross-contamination.

3. Add 200 µL of Nuclease-free Water (not DEPC-Treated) to the Negative Extraction Control well.

4. Add 10 µL of the prepared Proteinase K and TaqMan™ MS2 Phage Control Mix to each well, including the Negative Extraction Control well, in the KingFisher™ 96 Deep-Well Plate labeled ***Sample Plate***.

Process the samples

1. Select the **MVP_2Wash_200_Flex** on the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head.

2. Start the run, then load the prepared plates into position when prompted by the instrument.

3. After the run is complete (~24 minutes after start), immediately remove the Elution Plate from the instrument, then cover the plate with MicroAmp™ Clear Adhesive Film.

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

   The samples are eluted in 50 µL of Elution Solution (see “Prepare the processing plates” on page 12).

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

Note:

- Significant bead carry over may adversely impact RT-PCR performance. If bead carry over is observed, re-extract a new aliquot of the sample.
- To ensure reliable performance of the KingFisher™ Flex Magnetic Particle Processor, perform preventive maintenance as instructed by the manufacturer.

3 Prepare RT-PCR reactions

Guidelines for RT-PCR

IMPORTANT!

- Prepare and keep the run plate on ice (or cold block) 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 a RNase-free environment. Periodically decontaminate surfaces.
- 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 (PC)
  - One No Template Control (NTC)
  - One Negative Extraction Control (NC) from each extraction run.

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

Prepare the RT-PCR reactions (96-well reaction plate)

If frozen, thaw the reagents on ice.

1. Gently vortex the reagents, then centrifuge briefly to collect liquid at the bottom of the tube.

2. Dilute TaqMan™ SARS-CoV-2 Plus Control to a working stock (1/2,675 dilution):

   a. Pipet 98 µL of TaqMan™ Control Dilution Buffer into a microcentrifuge tube, then add 2.0 µL of TaqMan™ SARS-CoV-2 Plus Control. Mix well, then centrifuge briefly.

   b. Pipet 105 µL of TaqMan™ Control Dilution Buffer into a second microcentrifuge tube, then add 2.0 µL of the dilution created in substep 2a. Mix well, then centrifuge briefly.

3. Prepare the Reaction Mix.
   a. For each run, combine the following components sufficient for the number of RNA samples plus one Positive Control, one Negative Extraction Control, and one No Template Control.

IMPORTANT! The volumes in the following table assume that you extracted sample RNA using an original sample input volume of up to 200 uL.

Note: All volumes include 10% overage.

Component	Volume per RNA Sample or Control	Volume for *n* RNA Samples plus 3 Controls[1]	Volume for 93 RNA Samples plus 3 Controls[1]
TaqPath™ 1-Step Multiplex Master Mix (No ROX™) (4X)	6.25 µL	6.875 x (*n* + 3) µL	660 µL
TaqMan™ SARS-CoV-2 MS2 Assay 2.0	1.25 µL	1.375 x (*n* + 3) µL	132 µL
Total Reaction Mix volume	**7.5 µL**	**—**	**792 µL**

[1] 1 NC, 1 NTC, and 1 PC

4. Set up the reaction plate.
   a. Pipet 7.5 µL of the Reaction Mix prepared in step 3 into each well of a MicroAmp™ Optical 96-Well Reaction Plate with Barcode, 0.2 mL.

Note: For other reaction plates that can be used, see "Required materials not supplied" on page 6.

   b. Gently vortex the sealed plate containing the purified sample RNA and Negative Extraction Control from the RNA extraction procedure, then centrifuge briefly to collect liquid at the bottom of the plate.

Note: Do not store the plate. Proceed immediately to substep 4c.

   c. Unseal the plate containing the purified sample RNA and Negative Extraction Control from the RNA extraction procedure. Add either sample RNA, Negative Extraction Control, No Template Control, or Positive Control to each well of the reaction plate according to Table 2.

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

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

   e. Vortex the plate at the highest setting speed for 10–30 seconds with medium pressure. Move the plate around to ensure equal contact on the vortex mixer platform.

IMPORTANT! Vortex for 10–30 seconds to ensure proper mixing. Failure to do so might result in inaccurate sample results.

f. Centrifuge the reaction plate for 1–2 minutes at ≥650 × *g* (≥650 RCF) to remove bubbles and to collect the liquid at the bottom of the reaction plate.

IMPORTANT! Centrifuge the plate for 1–2 minutes to ensure bubbles are removed. Failure to do so might result in false classification of samples.

Table 2 Reaction plate volumes

Component	Volume per reaction			
	RNA Sample	Positive Control	Negative Extraction Control	No Template Control
Reaction Mix (from step 3)	7.5 µL	7.5 µL	7.5 µL	7.5 µL
Purified sample RNA (from RNA extraction)	17.5 µL	—	—	—
Positive Control (diluted TaqMan™ SARS-CoV-2 Plus Control from step 2)	—	17.5 µL	—	—
Negative Extraction Control (from RNA extraction)	—	—	17.5 µL	—
Nuclease-free water	—	—	—	17.5 µL
Total volume	**25.0 µL**	**25.0 µL**	**25.0 µL**	**25.0 µL**

Proceed immediately to set up and run the real-time PCR instrument.

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

If frozen, thaw the reagents on ice.

1. Gently vortex the reagents, then centrifuge briefly to collect liquid at the bottom of the tube.
2. Dilute TaqMan™ SARS-CoV-2 Plus Control to a working stock (1/2,675 dilution):
   a. Pipet 98 µL of TaqMan™ Control Dilution Buffer into a microcentrifuge tube, then add 2.0 µL of TaqMan™ SARS-CoV-2 Plus Control. Mix well, then centrifuge briefly.
   b. Pipet 105 µL of TaqMan™ Control Dilution Buffer into a second microcentrifuge tube, then add 2.0 µL of the dilution created in substep 2a. Mix well, then centrifuge briefly.
3. 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 RT-PCR reaction plate, one No Template Control per 384-well RT-PCR reaction plate, and one Negative Extraction Control from each extraction run.

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

IMPORTANT! The volumes in the following table assume that you extracted sample RNA using an original sample input volume of up to 200 uL.

Note: All volumes include 10% overage.

Component	Volume per RNA sample or control	Volume for *n* RNA samples plus 3 controls[1]	Volume for 378 RNA Samples + 6 Controls[2]
TaqPath™ 1-Step Multiplex Master Mix (No ROX™) (4X)	5 µL	5.5 x (*n* + 3) µL	2,112.0 µL
TaqMan™ SARS-CoV-2 MS2 Assay 2.0	1 µL	1.1 x (*n* + 3) µL	422.4 µL
Total Reaction Mix volume	**6 µL**	**—**	**2,534.4 µL**

[1] 1 NC, 1 NTC, and 1 PC
[2] 4 NCs, 1 NTC, and 1 PC

4. Set up the reaction plate.
   a. Pipet 6 µL of the Reaction Mix prepared in step 3 into each well of a MicroAmp™ Optical 384-Well Reaction Plate with Barcode.

      Note: For other reaction plates that can be used, see "Required materials not supplied" on page 6.

   b. Gently vortex the sealed plate containing the purified sample RNA and Negative Extraction Control from the RNA extraction procedure, then centrifuge briefly to collect liquid at the bottom of the plate.

      Note: Do not store the plates. Proceed immediately to substep 4c.

   c. Unseal the plate containing the purified sample RNA and Negative Extraction Control from the RNA extraction procedure. Add either sample RNA, Negative Extraction Control, No Template Control, or Positive Control to each well of the reaction plate according to Table 3.

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

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

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

   e. Vortex the plate at the highest setting speed for 10–30 seconds with medium pressure. Move the plate around to ensure equal contact on the vortex mixer platform.

      IMPORTANT! Vortex for 10–30 seconds to ensure proper mixing. Failure to do so might result in inaccurate sample results.

f. Centrifuge the reaction plate for 1–2 minutes at ≥650 × *g* (≥650 RCF) to remove bubbles and to collect the liquid at the bottom of the reaction plate.

IMPORTANT! Centrifuge the plate for 1–2 minutes to ensure bubbles are removed. Failure to do so might result in false classification of samples.

Table 3 Reaction plate volumes

Component	Volume per reaction			
	RNA Sample	Positive Control	Negative Extraction Control	No Template Control
Reaction Mix (from step 3)	6.0 µL	6.0 µL	6.0 µL	6.0 µL
Purified sample RNA (from RNA extraction)	14.0 µL	—	—	—
Positive Control (diluted TaqMan™ SARS-CoV-2 Plus Control from step 2)	—	14.0 µL	—	—
Negative Extraction Control (from RNA extraction)	—	—	14.0 µL	—
Nuclease-free water	—	—	—	14.0 µL
Total volume	**20.0 µL**	**20.0 µL**	**20.0 µL**	**20.0 µL**

Proceed immediately to set up and run the real-time PCR instrument.

4 Perform RT-PCR using the QuantStudio™ 5 Real-Time PCR Instrument (96-well, 0.2-mL block)

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.

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

For more information about the instrument, see “Related documentation” on page 31.

Note: For the QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block, use the system defaults for the PCR filters.

1. In the QuantStudio™ Design and Analysis Desktop Software v1.5.1 home screen, in the **New Experiment** box, select **Create New Experiment ▸ Template**.

   For experiment set up without a template, click **Create New Experiment**, then proceed directly to step 3.

2. Browse to, then open the EDT file of interest.

3. In the **Properties** screen, enter or confirm the following information.

   - **Name**: Enter a unique name
   - **Instrument type**: **QuantStudio™ 5 System**
   - **Block type**: **96-Well 0.2-mL Block**
   - **Experiment type**: **Standard Curve**
   - **Chemistry**: **TaqMan™ Reagents**
   - **Run Mode**: **Standard**

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

Step	Temperature[1]	Time	Number of cycles
UNG incubation	25°C	2 minutes	1
Reverse transcription	53°C	10 minutes	1
Preincubation	85°C	10 minutes	1
Activation	95°C	2 minutes	1
Denaturation	95°C	3 seconds	40
Anneal / extension	60°C	30 seconds	

[1] Confirm that the ramp rate for each step is 1.6°C per second.

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

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

7. Click **Advanced Setup**.

8. In the **Targets** table, confirm that the targets, reporter dyes, and quencher are listed correctly.

Target name	Reporter dye	Quencher
N Gene	VIC™	None
ORF1a	FAM™	
ORF1b	ABY™	
MS2	JUN™	

9. In the plate layout pane, confirm that the targets from step 8 are assigned to each well in the plate.

   To assign a target to a well, select the well in the plate layout, then select the targets from the **Targets** table.

10. In the plate layout pane, confirm the labeling of the control wells.

    Example labeling of the control wells:

    - The plate has one Positive Control, one Negative Extraction Control, and one No Template Control assigned to wells for reference.
    - The Positive Control is named ***PC***. If additional characters are included, it can be named ***PC***<>, where <> is defined by the user, for example ***PC1***.
    - The Negative Extraction Control is named ***NC***. If additional characters are included, it can be named ***NC***<>, where <> is defined by the user, for example ***NC1***.
    - The No Template Control is named ***NTC***. If additional characters are included, it can be named ***NTC***<>, where <> is defined by the user, for example ***NTC1***.
    - Move the control well assignments by copying the existing control wells and pasting them according to their location on the physical plate.

11. In the plate layout pane, confirm the **Task** assignments.
    - For wells with a Positive Control (***PC***), confirm that the **Task** is set to **S (Standard)** for all of the targets.
    - For wells with a Negative Extraction Control (***NC***) and No Template Control (***NTC***), confirm that the **Task** is set to **N (Negative Control)** for all of the targets.
    - For the wells with a sample, confirm that the **Task** is set to **U (Unknown)** for all of the targets.

12. In the **Samples** table, click **Add** to define a sample name for each sample. Create a unique sample name for each well in the physical plate.

13. Assign a sample name to each well to match the physical plate.

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

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

14. Load the prepared and sealed RT-PCR reaction plate into the real-time PCR instrument.

15. Place a MicroAmp™ Optical Film Compression Pad gray side down on the surface of the RT-PCR reaction plate, to ensure a proper seal between the thermal cycler and the adhesive film.

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

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

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

18. At the end of the run, remove the MicroAmp™ Optical Film Compression Pad from the plate and store the compression pad inside the pack.

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

5 Perform RT-PCR using the QuantStudio™ 7 Flex Real-Time PCR System (384-well block)

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

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

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

For more information about the instrument, see “Related documentation” on page 31.

1. In the QuantStudio™ Real-Time PCR Software v1.3 home screen, click **Template**.
   For experiment set up without a template, click **Experiment Setup**, then proceed directly to step 3.
2. Browse to, then open the EDT file of interest.
3. In the **Experiment Properties** screen, 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™ Reagents**
   - **Properties**: **Standard**

4. In the **Define** screen, in the **Targets** table, confirm that the targets, reporter dyes, and quencher are listed correctly.

Target name	Reporter dye	Quencher
N Gene	VIC™	None
ORF1a	FAM™	
ORF1b	ABY™	
MS2	JUN™	

5. In the **Define** screen, in the **Samples** table, define a sample name for each sample. Create a unique sample name for each well in the physical plate.

6. In the **Define** screen, confirm that the **Passive Reference** is set to **None**.

7. In the **Assign** screen, confirm that targets from step 4 are assigned to each well in the **Plate Layout** tab.

   To assign a target to a well, select the well in the plate layout, then select the targets from the **Targets** table.

8. In the **Assign** screen, in the **Plate Layout** tab, confirm the labeling of the control wells.

   Example labeling of the control wells:

   - The plate has one Positive Control, one Negative Extraction Control, and one No Template Control assigned to wells for reference.
   - The Positive Control is named ***PC***. If additional characters are included, it can be named ***PC***<>, where <> is defined by the user, for example ***PC1***.
   - The Negative Extraction Control is named ***NC***. If additional characters are included, it can be named ***NC***<>, where <> is defined by the user, for example ***NC1***.
   - The No Template Control is named ***NTC***. If additional characters are included, it can be named ***NTC***<>, where <> is defined by the user, for example ***NTC1***.
   - Move the control well assignments by copying the existing control wells and pasting them according to their location on the physical plate.

9. In the **Assign** screen, confirm the **Task** assignments.
   - For wells with a Positive Control (***PC***), confirm that the **Task** is set to **S (Standard)** for all of the targets.
   - For wells with a Negative Extraction Control (***NC***) and No Template Control (***NTC***), confirm that the **Task** is set to **N (Negative Control)** for all of the targets.
   - For the wells with a sample, confirm that the **Task** is set to **U (Unknown)** for all of the targets.

10. In the **Assign** screen, assign a sample name to each well to match the physical plate.

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

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

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11. In the **Run Method** screen, confirm that the **Reaction Volume Per Well** is 20 µL, then confirm the thermal protocol.

Step	Temperature[1]	Time	Number of cycles
UNG incubation	25°C	2 minutes	1
Reverse transcription	53°C	10 minutes	1
Preincubation	85°C	10 minutes	1
Activation	95°C	2 minutes	1
Denaturation	95°C	3 seconds	40
Anneal / extension	60°C	30 seconds	

[1] Confirm that the ramp rate for each step is 1.6°C per second.

12. In the **Run Method** screen, select **Optical Filters**, then select the 8 PCR filters that are listed in Table 4.

    To enable **Optical Filters**, navigate to **Tools** ▸ **Preferences**, then in the **Defaults** tab, select **Show optical filters for run method**.

    Note: Five filters are pre-selected by default.

Table 4 PCR Filters

		Emission Filter					
		m1(520±15)	m2(558±11)	m3(586±10)	m4(623±14)	m5(682±14)	m6(711±12)
Excitation Filter	x1(470±15)	✔	✔				
	x2(520±10)		✔	✔			
	x3(550±11)			✔	✔		
	x4(580±10)				✔		
	x5(640±10)					✔	
	x6(662±10)						

13. Load the prepared and sealed RT-PCR reaction plate into the real-time PCR instrument.

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

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

6 Analyze data

IMPORTANT! It is the responsibility of the laboratories using the TaqMan™ SARS-CoV-2 MS2 Assay 2.0 to design and validate their own experimental design and analysis parameters.

(Recommended) Use QuantStudio™ Design and Analysis Software v2.5 or later for data analysis. For more information about using the software, see "Related documentation" on page 31.

Note: QuantStudio™ Design and Analysis Desktop Software reports C_q values instead C_t values. The C_q values are equivalent to the C_t values indicated for data analysis and interpretation.

1. In the QuantStudio™ Design and Analysis Desktop Software home screen, open the data file (EDS).
2. In the open data file, click **Actions** ▸ **Save As**, then save the data file with a new name.

   Note: QuantStudio™ Design and Analysis Desktop Software requires data files created on a QuantStudio™ 5 Real-Time PCR System and QuantStudio™ 7 Flex Real-Time PCR System to be saved as a new data file.
3. For Positive Control samples, change the sample **Type** from **Standard** to **Positive Control**.
4. In the analysis settings, select automatic baseline with a start cycle of 5 and an end cycle of auto.
5. Set the appropriate threshold values for each target, as validated by your laboratory.

   IMPORTANT! Do not use automatic threshold values.
6. Determine C_t/C_q cutoff values for each target for samples and controls.
7. Analyze results according to analysis, interpretation, and QC parameters, as validated by your laboratory.

Contact Support for more information.

A 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, see the “Documentation and Support” section in this document.

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.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

WARNING! Do not use bleach or bleached pipette tips with the Binding Bead Mix. The Binding Solution contains guanidium thiocyanate, which produces cyanide gas when combined with bleach.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

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

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

B

Documentation and support

Related documentation

Document	Publication Number
QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide	MAN0010407
QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Maintenance and Administration Guide	4489821
QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Quick Reference	4489826
QuantStudio™ Real-Time PCR Software Getting Started Guide	4489822
QuantStudio™ Design and Analysis Software v2 User Guide	MAN0018200
MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit User Guide	MAN0024756
Thermo Scientific™ KingFisher™ Flex User Manual	N07669

Customer and technical support

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- 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.

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**.