TaqMan™ SARS-CoV-2 Pooling Assay

USER GUIDE

Publication Number MAN0019859

Revision A.0

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ThermoFisher SCIENTIFIC

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

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

Revision history: Pub. No. MAN0019859

Revision	Date	Description
A.0	29 January 2021	New document.

Contents

1 Product information

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

Product description

The TaqMan™ SARS-CoV-2 Pooling Assay contains the assays and the controls for a real-time reverse transcription polymerase chain reaction (RT-PCR) to detect nucleic acid from SARS-CoV-2 in pooled upper respiratory samples.

At least one sample in a pool contains SARS-CoV-2 nucleic acid if the SARS-CoV-2 nucleic acid is detected in the pooled samples. In order to determine which sample contains SARS-CoV-2 nucleic acid, RT-PCR should be performed on each sample in the pool individually.

If SARS-CoV-2 nucleic acid is not detected in the pooled samples, this indicates that none of the samples contain SARS-CoV-2 nucleic acid.

The TaqMan™ SARS-CoV-2 Pooling Assay is performed with the following components:

- TaqMan™ SARS-CoV-2 Pooling Assay Kit
  - TaqMan™ SARS-CoV-2 Pooling Multiplex Assay—Multiplexed assays that contain three primer and probe sets specific to different SARS-CoV-2 genomic regions and primers and probes for bacteriophage MS2 (see Table 1 on page 6)
  - TaqMan™ MS2 Phage Control—Internal process control for nucleic acid extraction
- TaqMan™ SARS-CoV-2 Control—RNA control that contains targets specific to the SARS-CoV-2 genomic regions targeted by the assays
- TaqMan™ Control Dilution Buffer—Dilution buffer for the control
- TaqPath™ 1-Step Multiplex Master Mix

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

Table 1 Dyes, quenchers, and targets

Dye	Quencher	Target
FAM™ dye	QSY™ quencher	ORF1ab
VIC™ dye	QSY™ quencher	N gene
ABY™ dye	QSY™ quencher	S gene
JUN™ dye	QSY™ quencher	MS2

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

Contents and storage

The items listed in the following table are required for the TaqMan™ SARS-CoV-2 Pooling Assay. The items listed are sufficient for 200 reactions.

Kit or product	Cat. No.[1]	Components	Amount	Storage
TaqMan™ SARS-CoV-2 Pooling Assay Kit	A50790	TaqMan™ SARS-CoV-2 Pooling Multiplex Assay	300 µL	–30°C to –10°C
		TaqMan™ MS2 Phage Control	2 × 1 mL	–30°C to –10°C
TaqMan™ SARS-CoV-2 Control	956118	—	2 × 10 µL	≤ –70°C
TaqMan™ Control Dilution Buffer	A49889	—	10 × 250 µL	–30°C to –10°C
TaqPath™ 1-Step Multiplex Master Mix	A28523	—	10 mL	–30°C to –10°C

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

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	
An Applied Biosystems™ real-time PCR instrument compatible with the four dyes listed in Table 1 on page 6. The assay has been tested with the following instruments: Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR Instrument, 384-well block	Contact your local sales office
Software	
QuantStudio™ Design and Analysis Software v2.5 or later	**thermofisher.com/qpcrsoftware**
Equipment	
Laboratory freezers • –30°C to –10°C • ≤ –70°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	
KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head	5400630
KingFisher™ Flex 96 Deep-Well Heating Block	24075430
KingFisher™ Deep-Well 96 Plate	95040450, A48305, A48424, 95040455

(continued)

Item	Source
96-well plate for the tip comb, one of the following: • KingFisher™ 96 KF microplate • Tip Comb Presenting Plate for KF 96 • Nunc™ MicroWell™ 96-Well Microplate, Flat Bottom • Nunc™ MicroWell™ 96-Well Microplate, barcoded • ABgene™ 96–Well Polypropylene Storage Microplate • ABgene™ 96–Well 1.2–mL Polypropylene Deepwell Storage Plate • Nunc™ F96 MicroWell™ Black Polystyrene Plate • Nunc™ F96 MicroWell™ White Polystyrene Plate • KingFisher™ Deep-Well 96 Plate	 • 97002540 • 267600 • 167008 • 269787 • AB0796 • AB1127 • 137101 • 136101 • 95040450, A48305, A48424, 95040455
KingFisher™ 96 tip comb for DW magnets	97002534, A48438, A48414
Kits and reagents	
MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit	A48383R
Fisher BioReagents™ Ethanol, Absolute, Molecular Biology Grade[1], or equivalent	BP2818100, BP2818500, BP28184
Nuclease-free Water (not DEPC-Treated)	MLS
Calibration plates (QuantStudio™ 7 Flex Real-Time PCR Instrument)	
ABY™ Dye Spectral Calibration Plate for Multiplex qPCR, 384-well	A24736
JUN™ Dye Spectral Calibration Plate for Multiplex qPCR, 384-well	A24733
Tubes, plates, and other consumables	
MicroAmp™ 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

(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] Available at fisherscientific.com.

General laboratory recommendations

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

Workflow

Pool samples (page 11)

▼

Extract RNA (page 13)

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Prepare RT-PCR reactions (page 17)

▼

Set up and run the real-time PCR instrument (page 22)

▼

Analyze data (page 24)

(QuantStudio™ Design and Analysis Software v2.5 or later)

▼

Review the results for the pool

▼

For pools with SARS-CoV-2 nucleic acid, extract RNA and perform RT-PCR for each sample to determine which sample contains SARS-CoV-2 nucleic acid

2 Pool samples

Overview of pooling

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

Pooling can lead to a reduction in the ability to detect SARS-CoV-2 nucleic acid in a sample because samples are combined and therefore diluted.

The TaqMan™ SARS-CoV-2 Pooling Assay has been tested with 2 to 5 samples per pool.

Pool samples

IMPORTANT! Samples are diluted, which can result in less viral genetic material available to detect. It is the responsibility of the laboratories to perform all of the necessary validations to run this assay.

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

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

At least one sample in a pool contains SARS-CoV-2 nucleic acid if the SARS-CoV-2 nucleic acid is detected in the pooled samples. In order to determine which sample contains SARS-CoV-2 nucleic acid, RT-PCR should be performed on each sample in the pool individually.

If SARS-CoV-2 nucleic acid is not detected in the pooled samples, this indicates that none of the samples contain SARS-CoV-2 nucleic acid.

1. Combine equal volumes of up to 5 samples into a single tube at a total volume of ≥400 µL. Example volumes are shown in the following table.

Table 2 Example volumes of pooled samples

Sample	Volume		
	N = 5	N = 4	N = 3
1	100 µL	120 µL	150 µL
2	100 µL	120 µL	150 µL
3	100 µL	120 µL	150 µL
4	100 µL	120 µL	—
5	100 µL	—	—
Total volume	**500 µL**	**480 µL**	**450 µL**

2. Store the remaining unpooled volumes of samples for individual testing, as applicable.

3 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.

- Extract RNA from 400 µL of sample (pooled samples or individual samples, see “Pool samples” on page 11).
- Determine the number of required reactions based on the number of samples, plus one Negative Control per plate.
- Prepare fresh 80% Ethanol using Ethanol, Absolute, Molecular Biology Grade and Nuclease-free Water (not DEPC-Treated) for the required number of reactions, sufficient for 1 mL per reaction, plus 10% overage.
- Label the short side of each KingFisher™ Deep-Well 96 Plate (4):

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

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

Label	Number of plates
Tip comb	1

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

- Tip Comb Presenting Plate for KF 96
- Nunc™ MicroWell™ 96-Well Microplate, Flat Bottom
- Nunc™ MicroWell™ 96-Well Microplate, barcoded
- ABgene™ 96–Well Polypropylene Storage Microplate
- ABgene™ 96–Well 1.2–mL Polypropylene Deepwell Storage Plate
- Nunc™ F96 MicroWell™ Black Polystyrene Plate
- Nunc™ F96 MicroWell™ White Polystyrene Plate
- KingFisher™ Deep-Well 96 Plate

- Mark the Negative 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_400_Flex** program has been downloaded from the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit product page at **www.thermofisher.com** and loaded onto the instrument.

Prepare the processing plates

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™ Deep-Well 96 Plate	Wash Solution	1,000 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
- Nunc™ F96 MicroWell™ Black Polystyrene Plate
- Nunc™ F96 MicroWell™ White Polystyrene Plate
- KingFisher™ Deep-Well 96 Plate

Prepare Binding Bead Mix

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	530 µL	56.0 mL
Total Nucleic Acid Magnetic Beads	20 µL	2.1 mL
Total volume per well	**550 µL**	**58.1 mL**

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

3. Mix well by inversion, then store at room temperature.

Prepare sample plate

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

1. Add 10 µL of Proteinase K to each well in the KingFisher™ Deep-Well 96 Plate labeled "Sample Plate".
2. Add 400 µL of individual sample or sample pool to each sample well.
3. Add 400 µL of Nuclease-free Water (not DEPC-Treated) to the Negative Control well.
4. Invert the Binding Bead Mix 5 times gently to mix, then add 550 µL to each sample well and the Negative Control well in the Sample Plate.

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

5. Add 10 µL of TaqMan™ MS2 Phage Control to each sample well and to the Negative Control well.

Process the samples

1. Select the **MVP_2Wash_400_Flex** on the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head.
2. Start the run, then load the prepared plates into position when prompted by the instrument.
3. After the run is complete (~24 minutes after start), immediately remove the Elution Plate from the instrument, then cover the plate with MicroAmp™ Clear Adhesive Film.

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

   The samples are eluted in 50 µL of Elution Solution (see “Prepare the processing plates” on page 15).

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.

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

4 Prepare RT-PCR reactions

Guidelines for RT-PCR

IMPORTANT!

- Prepare the run plate on ice and keep it on ice until it is loaded into the real-time PCR instrument.
- Run the plate immediately after preparation. Failure to do so could result in degraded RNA samples.
- To prevent contamination, prepare reagents in a PCR workstation or equivalent amplicon-free area. Do not use the same pipette for controls and RNA samples, and always use aerosol barrier pipette tips.
- Maintain an RNase-free environment.
- Protect assays from light.
- Keep RNA samples and components on ice during use.
- For each RT-PCR plate, include the following controls:
  - One Positive Control
  - One Negative Control from each extraction run.

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

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

Use this procedure to prepare RT-PCR reactions for instruments that use 96-well reaction plates.

1. If frozen, thaw the reagents on ice.
2. Gently vortex the reagents, then centrifuge briefly to collect liquid at the bottom of the tube.
3. Dilute TaqMan™ SARS-CoV-2 Control to a working stock:
   a. Pipet 98.0 µL of TaqMan™ Control Dilution Buffer into a microcentrifuge tube, then add 2.0 µL of TaqMan™ SARS-CoV-2 Control. Mix well, then centrifuge briefly.

   b. Pipet 87.5 µL of TaqMan™ Control Dilution Buffer into a second microcentrifuge tube, then add 12.5 µL of the previous dilution. Mix well, then centrifuge briefly.

Note: The TaqMan™ SARS-CoV-2 Control does not contain the MS2 template.

4. Prepare the Reaction Mix:
   a. For each run, combine the following components sufficient for the number of RNA samples plus one Positive Control and one Negative Control.

All volumes include 10% overage for pipette error.

Component	Volume per RNA Sample or Control	Volume for *n* RNA Samples plus 2 Controls	Volume for 94 RNA Samples plus 2 Controls
TaqPath™ 1-Step Multiplex Master Mix (No ROX™) (4X)	6.25 µL	6.875 x (*n* + 2) µL	660 µL
TaqMan™ SARS-CoV-2 Pooling Multiplex Assay	1.25 µL	1.375 x (*n* + 2) µL	132 µL
Total Reaction Mix volume	**7.5 µL**	**—**	**792 µL**

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

      Plates without a barcode can be used (see "Required materials not supplied" on page 7).

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

   c. Unseal the plate containing the purified sample RNA and Negative Control from the RNA extraction procedure. Add either sample RNA, Negative Control, or Positive Control to each well of the reaction plate according to Table 3 on page 19.

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

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

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

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

Table 3 Reaction plate volumes

Component	Volume per reaction		
	RNA Sample reaction	Positive Control reaction	Negative Control reaction
Reaction Mix	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 Control from step 3)	—	2.0 µL	—
Negative Control (from RNA extraction)	—	—	17.5 µL
Nuclease-free Water	—	15.5 µL	—
Total volume	**25.0 µL**	**25.0 µL**	**25.0 µL**

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

Use this procedure to prepare RT-PCR reactions for instruments that use 384-well reaction plates.

1. If frozen, thaw the reagents on ice.
2. Gently vortex the reagents, then centrifuge briefly to collect liquid at the bottom of the tube.
3. Dilute TaqMan™ SARS-CoV-2 Control to a working stock:
   a. Pipet 98 µL of TaqMan™ Control Dilution Buffer into a microcentrifuge tube, then add 2 µL of TaqMan™ SARS-CoV-2 Control. Mix well, then centrifuge briefly.
   b. Pipet 87.5 µL of TaqMan™ Control Dilution Buffer into a second microcentrifuge tube, then add 12.5 µL of the previous dilution. Mix well, then centrifuge briefly.

   Note: The TaqMan™ SARS-CoV-2 Control does not contain the MS2 template.
4. Prepare the Reaction Mix.
   a. For each run, combine the following components sufficient for the number of RNA samples, plus one Positive Control per 384-well real-time RT-PCR plate, and one Negative Control from each extraction run.

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

All volumes include 10% overage for pipette error.

Component	Volume per RNA Sample or control	Volume for *n* RNA Samples plus *y* Negative Controls plus 1 Positive Control	Volume for 379 RNA Samples plus 4 Negative Controls plus 1 Positive Control
TaqPath™ 1-Step Multiplex Master Mix (No ROX™) (4X)	5.00 µL	5.50 x (*n* + *y* + 1) µL	2112.0 µL
TaqMan™ SARS-CoV-2 Pooling Multiplex Assay	1.00 µL	1.10 x (*n* + *y* + 1) µL	422.4 µL
Total Reaction Mix volume	**6.0 µL**	**—**	**2534.4 µL**

5. Set up the reaction plate:
   a. Pipet 6.0 µL of the Reaction Mix prepared in step 4 into each well of a MicroAmp™ Optical 384-Well Reaction Plate with Barcode.

      Plates without a barcode can be used (see "Required materials not supplied" on page 7).

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

   c. Unseal the plate containing the purified sample RNA and Negative Control from the RNA extraction procedure. Add either sample RNA, Negative Control, or Positive Control to each well of the reaction plate according to Table 4 on page 21.

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

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

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

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

      IMPORTANT! Vortex for 10–30 seconds to ensure proper mixing. Failure to do so might result in false classification of samples.

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

Table 4 Reaction plate volumes

Component	Volume per reaction		
	RNA Sample reaction	Positive Control reaction	Negative Control reaction
Reaction Mix	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 Control from step 3)	—	2.0 µL	—
Nuclease-free Water	—	12.0 µL	—
Purified Negative Control (from RNA extraction)	—	—	14.0 µL
Total volume	**20.0 µL**	**20.0 µL**	**20.0 µL**

5 Set up and run the real-time PCR instrument

Calibration

Ensure that your real-time PCR instrument is calibrated for the dyes listed in Table 1 on page 6. See your instrument user guide for more information.

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.

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.

Perform RT-PCR

For more information about the instrument, see “Required materials not supplied” on page 7 and “Related documentation” on page 25.

1. Set up and run the real-time PCR instrument with the following settings.
   - Assay: Standard curve
   - Run mode: Standard
   - Passive reference set to **None**
   - Sample volume: 25 µL or 20 µL

   Note: 25 µL is the sample volume for a 96-well plate. 20 µL is the sample volume for 384-well plate.

2. Set up the following reporter dye and detector pairs.

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

3. Set up the thermal protocol.

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

4. Load the plate and start the instrument run.

6 Analyze data

Analyze data

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

Use QuantStudio™ Design and Analysis Software v2.5 or later.

For more information about using the software, see “Related documentation” on page 25.

Note: QuantStudio™ Design and Analysis Software v2 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. Open the data file (EDS or SDS) in the data analysis software.

   Note: QuantStudio™ Design and Analysis Software v2 requires data files created on a QuantStudio™ 5 Real-Time PCR System or a QuantStudio™ 7 Flex Real-Time PCR Instrument to be saved as a new data file. Click **Actions** ▸ **Save As**, then save the data file with a new name.

2. Use automatic baselining with a start cycle of 5.

3. Set the appropriate threshold values for each target, as validated by your laboratory.

   IMPORTANT! Do not use automatic threshold values.

4. Determine C_t/C_q cutoff values for each target for samples and controls.

5. Analyze results according to analysis, interpretation, and QC parameters, as validated by your laboratory.

Contact Support for more information.

A 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

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.

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**.

B

Safety

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)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

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.

AVERTISSEMENT ! **PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES.** Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.

- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

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.