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# TaqCheck<sup>™</sup> SARS-CoV-2 Fast PCR Assay

## Catalog Numbers A47693

Pub. No. MAN0019744 Rev. C.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *TaqCheck<sup>™</sup> SARS-CoV-2 Fast PCR Assay User Guide* (Pub. No. MAN0019745). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

# **Product description**

The TaqCheck<sup>™</sup> SARS-CoV-2 Fast PCR Assay is a multiplex real-time RT-PCR assay for the detection of SARS-CoV-2 viral RNA in human raw saliva samples. The assay has a multi-target design that compensates for emerging SARS-CoV-2 variants and mutations to provide confidence in results. The assay contains primer and probe sets specific to the following targets:

#### Table 1Assay targets, dyes, and quenchers

Target	Dye	Quencher	
SARS-CoV-2 N gene		QSY <sup>™</sup> quencher	
SARS-CoV-2 S gene	VIC™ dye		
Human RNase P RPP30 gene <sup>[1]</sup>	FAM <sup>™</sup> dye	QSY <sup>™</sup> quencher	

<sup>[1]</sup> Serves as an internal positive control to monitor sample quality.

The assay requires the following components:

- TaqCheck<sup>™</sup> SARS-CoV-2 Control—RNA control that contains SARS-CoV-2 N protein and S protein target regions
- TaqCheck<sup>™</sup> SARS-CoV-2 Control Dilution Buffer Dilution buffer for the control
- TaqPath<sup>™</sup> 1-Step RT-qPCR Master Mix, CG

For catalog numbers and storage conditions, see "Contents and storage".

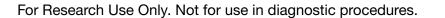
**IMPORTANT!** It is the responsibility of the laboratories using the TaqCheck<sup>™</sup> SARS-CoV-2 Fast PCR Assay to design and validate their own experimental design and analysis parameters.

# Contents and storage

The items listed in the following table are required for the TaqCheck<sup>™</sup> SARS-CoV-2 Fast PCR Assay. The items listed are sufficient for 1,200 reactions.

#### Table 2 TaqCheck<sup>™</sup> SARS-CoV-2 Fast PCR Assay Kit with Master Mix (Cat. No. A50916)

Components	Cat. No.	Amount	Storage	
TaqCheck <sup>™</sup> SARS-CoV-2 Fast PCR Assay Kit (Cat. No. A50914)				
TaqCheck <sup>™</sup> SARS-CoV-2 Fast PCR Assay	A47693	690 µL	–30°C to –10°C	
TaqCheck <sup>™</sup> SARS-CoV-2 Control	956127	3 × 10 μL	≤ -70°C	
TaqCheck <sup>™</sup> SARS-CoV-2 Control Dilution Buffer	A50486	3 × 250 μL	–30°C to –10°C	
TaqPath <sup>™</sup> 1-Step RT-qPCR Master Mix, CG (Cat. No. A15300)				
_	—	1 × 10 mL	–30°C to –10°C	



# 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.

Item	Source		
Real-time PCR instrument and software			
<ul> <li>An Applied Biosystems<sup>™</sup> real-time PCR instrument compatible with the dyes listed in Table 1 on page 1.</li> <li>The assay was tested with the following instruments: <ul> <li>Applied Biosystems<sup>™</sup> 7500 Fast Real-Time PCR Instrument</li> <li>Applied Biosystems<sup>™</sup> QuantStudio<sup>™</sup> 5 Real-Time PCR Instrument, 96-well, 0.2-mL block</li> <li>Applied Biosystems<sup>™</sup> QuantStudio<sup>™</sup> 5 Real-Time PCR Instrument, 384-well block</li> <li>Applied Biosystems<sup>™</sup> QuantStudio<sup>™</sup> 7 Flex Real-Time PCR Instrument, 384-well block</li> </ul> </li> </ul>	Contact your local sales office		
(Recommended) QuantStudio <sup>™</sup> Design and Analysis Software v2.5 or later <sup>[1]</sup>	thermofisher.com/qpcrsoftware		
Equipment			
Laboratory freezers • −30°C to −10°C • ≤ −70°C	MLS     thermofisher.com/tsx		
BSL-2 biological safety cabinet, such as Herasafe <sup>™</sup> 2030i Class 2 A2 Biological Safety Cabinets	MLS     thermofisher.com		
Centrifuge (capable of achieving 1,400 × g), such as Megafuge <sup>™</sup> 8 Small Benchtop Centrifuge Series or Multifuge <sup>™</sup> X4 Pro Centrifuge Series	MLS     thermofisher.com		
Microcentrifuge, such as Pico <sup>™</sup> 17 Microcentrifuge	MLS     thermofisher.com		
Laboratory mixer, vortex or equivalent, such as Digital Vortex Mixers	MLS     thermofisher.com		
Single and multichannel adjustable pipettors (2.00 $\mu L$ to 1,000.0 $\mu L$ )	www.thermofisher.com/cliptip     thermofisher.com/finnpipette		
Cold block (96-well or 384-well) or ice	MLS		
Heat block or water bath (capable of reaching 95°C), such as Touch Screen Dry Bath/Block Heater or Precision <sup>™</sup> General Purpose Baths	MLS     thermofisher.com		
Liquid handler (if needed for automation) <sup>[2]</sup>	MLS		
Kits and reagents			
TBE Buffer (Tris-borate-EDTA) (10X)	B52, or equivalent		
Tween <sup>®</sup> -20 Surfact-Amps <sup>™</sup> Detergent Solution	28320		
Nuclease-free Water (not DEPC-Treated)	<ul> <li>AM9938 (1 x 100 mL)</li> <li>AM9932 (1 x 1,000 mL)</li> </ul>		
70% Isopropanol spray or wipes	MLS		

Item	Source			
Tubes, plates, and other consumables				
( <i>Recommended</i> ) Sterile tube with leak-proof, screw-top lid for sample collection <sup>[3]</sup> <b>IMPORTANT!</b> Do not use tubes that contain preservative.	One of the following, or equivalent: • AM12500 • 339650 • 339651 • 339652 • 339653 • 339658 • 14-959-49B (fisherscientific.com)			
Reservoir for multichannel pipettes	MLS			
Sterile aerosol barrier (filtered) pipette tips	thermofisher.com/pipettetips			
96-well plate (for preparing saliva samples, not for RT-PCR)	AB0796, or equivalent			
MicroAmp <sup>™</sup> Fast Optical 96-Well Reaction Plate, 0.1 mL	<ul> <li>4346906 (with barcode)</li> <li>4366932 (with barcode)</li> <li>4346907 (without barcode)</li> </ul>			
MicroAmp <sup>™</sup> EnduraPlate <sup>™</sup> Optical 96-Well Fast Clear Reaction Plates, 0.1 mL	<ul><li>4483485 (with barcode)</li><li>4483494 (with barcode)</li><li>A36930 (without barcode)</li></ul>			
MicroAmp <sup>™</sup> Optical 96-Well Reaction Plate, 0.2 mL	<ul> <li>4306737 (with barcode)</li> <li>4326659 (with barcode)</li> <li>N8010560 (without barcode)</li> <li>4316813 (without barcode)</li> </ul>			
MicroAmp <sup>™</sup> EnduraPlate <sup>™</sup> Optical 96-Well Clear Reaction Plates, 0.2 mL	<ul> <li>4483352 (with barcode)</li> <li>4483354 (with barcode)</li> <li>A36924 (without barcode)</li> </ul>			
MicroAmp <sup>™</sup> Optical 384-Well Reaction Plate	<ul> <li>4309849 (with barcode)</li> <li>4326270 (with barcode)</li> <li>4343814 (with barcode)</li> <li>4343370 (without barcode)</li> </ul>			
MicroAmp <sup>™</sup> EnduraPlate <sup>™</sup> Optical 384-Well Clear Reaction Plates	<ul> <li>4483273 (with barcode)</li> <li>4483285 (with barcode)</li> <li>A36931 (without barcode)</li> </ul>			
MicroAmp™ Clear Adhesive Film	4306311			
MicroAmp <sup>™</sup> Optical Adhesive Film	4311971, 4360954			
MicroAmp <sup>™</sup> Adhesive Film Applicator	4333183			
Nonstick, RNase-free microcentrifuge tubes (1.5 mL and 2.0 mL)	thermofisher.com/microtubes			
DNase and RNase-free tubes for mixing reagents (capable of mixing 5 mL and 50 mL)	thermofisher.com			
Nunc™ 1.8-mL Externally-Threaded Universal Tubes	374502			
Nalgene <sup>™</sup> General Long-Term Storage Cryogenic Tubes, 0.2 mL	5000-0020			
Nunc <sup>™</sup> Biobanking and Cell Culture Cryogenic Tubes, 4.5 mL	337516			
Sterilin <sup>™</sup> Certified Universal Containers – RNase, DNase, human DNA and Pyrogen Free, 30 mL	<ul><li> 30APPRN (Unlabelled)</li><li> 30BPPRN (Graduated label)</li></ul>			

Use of QuantStudio<sup>™</sup> Design and Analysis Software v2.5 is recommended, but not required. It is the responsibility of the laboratories using the assay to design and validate their own experimental design and analysis parameters.
 Follow the guidelines provided by the manufacturer.

<sup>1</sup> The use of the tubes listed in the table is recommended, but not required. Laboratories are responsible for validating their sample collection and preparation procedures for use with the assay.

# 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)
- Saliva samples should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.
- To prevent degradation, keep 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.
- To ensure reliable performance of the real-time PCR instrument, perform preventive maintenance according to the instructions provided by the manufacturer in the instrument documentation (see "Related documentation" on page 10).

# Guidelines for sample collection and storage

• Collect saliva sample in a collection device with a leak-proof, screw-top lid.

IMPORTANT! Do not collect saliva using a device that contains preservative solution.

- Collect a minimum of 1 mL saliva.
- **IMPORTANT!** Collect saliva samples according to the instructions provided with your collection device. We recommend that you follow best practices to minimize the presence of inhibitors in the saliva:
  - . At least 30 minutes before collection, clean the mouth. Swish water for 10 seconds, then swallow to remove debris.
  - After cleaning the mouth, avoid eating, drinking, smoking, using chewing tobacco, chewing gum, brushing teeth, and using mouthwash or other foreign substances until the sample is collected to ensure reliable results.
  - During collection, allow saliva to passively pool in the mouth, then **DROOL** into the collection device. Do not cough while performing collection, and ensure that the sample is free of phlegm or other debris.

Note: Laboratories are responsible for validation of their sample collection procedure.

• Store raw saliva samples according to the procedure established by your laboratory. For long-term storage, freeze raw saliva samples at -80°C. Avoid multiple freeze-thaw cycles.

# Prepare saliva samples

WARNING! Saliva samples have the potential to transmit infectious diseases. Use safe laboratory procedures, including wearing personal protective equipment (PPE) and handling samples in a BSL-2 biological safety cabinet.

**IMPORTANT!** Saliva samples can contain high amounts of inhibitory compounds that can affect real-time RT-PCR results. Laboratories are responsible for validating their sample collection and preparation procedures for use with the assay.

# Before you begin

- If the raw saliva samples are frozen, thaw completely at room temperature before processing.
- Ensure that the heating block or water bath is at 95°C.

## Prepare 96-well plates with TBE Buffer-Tween®-20 Detergent (TBE-T) mix

1. For the required number of samples, prepare the TBE-T mix in a DNase and RNase-free tube, according to the following table:

Component	Volume per well	Volume per 96-well plate <sup>[1]</sup>	Volume per four 96-well plates <sup>[1]</sup>
TBE Buffer (10X) <sup>[2]</sup>	20 µL	2.4 mL	9.6 mL
Tween®-20 Detergent (10%) <sup>[3]</sup>	10 µL	1.2 mL	4.8 mL
Nuclease-free water	70 µL	8.4 mL	33.6 mL
Total volume	100 µL	12.0 mL	48.0 mL

<sup>[1]</sup> Includes 25% overage.

<sup>[2]</sup> The TBE Buffer has a final concentration of 2X in the TBE-T mix.

<sup>[3]</sup> The Tween®-20 Detergent has a final concentration of 1% in the TBE-T mix.

2. Cap the tube, then mix well by inversion 5–10 times. Do not vortex.

Once mixed, allow bubbles to dissipate naturally.

3. For the required number of samples, add 100 µL of TBE-T mix to each well of a 96-well plate.

Store the plates on ice or at room temperature.

#### Prepare the samples

Keep the saliva samples in the original tubes for the incubation step.

- 1. Incubate the saliva sample tubes in a water bath or heat block at 95°C for 30 minutes.
- 2. Remove the tubes from the water bath or heat block, then allow the samples to equilibrate to room temperature.
- 3. Vortex each sample at maximum speed for a minimum of 10 seconds, or until the sample appears homogenous.

Note: Samples that are particularly viscous or contain high amounts of particulate may require longer vortex times. Some samples may contain particulate that does not fully homogenize.

- 4. Transfer 100 μL of each heat-treated saliva sample to the designated wells in the prepared TBE-T 96-well plates. Gently pipet up and down 10 times to mix. Ensure that you do not generate bubbles while you pipet.
- 5. Seal the plate thoroughly with MicroAmp<sup>™</sup> Clear Adhesive Film.

Store the prepared sample plates on ice or at 4°C for up to 2 hours while setting up the RT-PCR.

# Prepare RT-PCR reactions

## Guidelines for RT-PCR

#### **IMPORTANT!**

- Prepare the RT-PCR plate on ice or a cold block. Keep the RT-PCR plate on ice or a cold block until it is loaded into the real-time PCR instrument.
- Run the RT-PCR plate within an hour after preparation. Failure to do so could result in degraded samples.
- To prevent contamination, prepare reagents in a PCR workstation or equivalent amplicon-free area. Do not use the same pipette for controls and samples, and always use aerosol barrier pipette tips.
- Maintain an RNase-free environment.
- Protect assays from light.
- . Keep samples and components on ice or a cold block during use.
- For each RT-PCR plate, include the following controls:
  - One Positive Control
  - . One No Template Control

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

- 1. If frozen, thaw the reagents on ice or on a cold block.
- 2. Gently vortex the reagents, then briefly centrifuge the tube or swirl the bottle to collect the liquid at the bottom of the container.
- 3. Dilute TaqCheck<sup>™</sup> SARS-CoV-2 Control to a working stock:
  - a. Pipet 95.0 μL of TaqCheck<sup>™</sup> SARS-CoV-2 Control Dilution Buffer into a microcentrifuge tube, then add 5.0 μL of TaqCheck<sup>™</sup> SARS-CoV-2 Control. Mix well, then centrifuge briefly.
  - b. Pipet 95.0 μL of TaqCheck<sup>™</sup> SARS-CoV-2 Control Dilution Buffer into a second microcentrifuge tube, then add 5.0 μL of the dilution created in substep 3a. Mix well, then centrifuge briefly.

#### 4. Prepare the Reaction Mix:

a. For each 96-well plate, combine the following components sufficient for the number of RNA samples plus one Positive Control and one No Template Control.

Component	Volume per sample or control	Volume for <i>n</i> samples plus 2 controls <sup>[1]</sup>	Volume for 94 samples plus 2 controls <sup>[1]</sup>
TaqPath <sup>™</sup> 1-Step RT-qPCR Master Mix, CG (4X)	2.5 µL	2.75 x (n + 2) μL	264.0 µL
TaqCheck <sup>™</sup> SARS-CoV-2 Fast PCR Assay	0.5 µL	0.55 x (n + 2) μL	52.8 μL
Nuclease-free water	2.0 μL	2.2 x (n + 2) μL	211.2 μL
Total Reaction Mix volume	5.0 μL	—	528.0 μL

<sup>[1]</sup> All volumes include 10% overage for pipette error.

#### 5. Set up the reaction plate, according to the following table:

Component	Volume per reaction			
Component	Sample reaction	Positive Control reaction	No Template Control reaction	
Reaction Mix (from step 4)	5.0 µL	5.0 μL	5.0 µL	
Prepared sample (saliva + TBE-T)	5.0 µL	_	_	
Positive Control (diluted TaqCheck <sup>™</sup> SARS-CoV-2 Control from step 3 )	_	2.0 µL	_	
Nuclease-free water	_	3.0 µL	5.0 µL	
Total volume	10.0 μL	10.0 μL	10.0 μL	

- a. Add 5.0 µL of the Reaction Mix prepared in step 4 to each well of an optical 96-well reaction plate.
- b. Add 5.0 µL of prepared sample (saliva plus TBE-T) to each sample well of the reaction plate.

- c. Add 2.0 µL of the diluted TaqCheck<sup>™</sup> SARS-CoV-2 Control and 3.0 µL Nuclease-free water to the Positive Control well of the reaction plate.
- d. Add 5.0 µL of Nuclease-free water to the No Template Control well of the reaction plate.
- e. Seal the plate thoroughly with MicroAmp<sup>™</sup> Optical Adhesive Film.

**IMPORTANT!** When applying the MicroAmp<sup>™</sup> 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.

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

**IMPORTANT!** Failure to vortex the plate for the recommended time can result in inaccurate sample results.

 Centrifuge the reaction plate for 1–2 minutes at ≥1,400 × g (≥1,400 RCF) to remove bubbles and to collect the liquid at the bottom of the reaction plate.

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

- 1. If frozen, thaw the reagents on ice or on a cold block.
- 2. Gently vortex the reagents, then briefly centrifuge the tube or swirl the bottle to collect the liquid at the bottom of the container.
- 3. Dilute TaqCheck<sup>™</sup> SARS-CoV-2 Control to a working stock:
  - a. Pipet 95.0 μL of TaqCheck<sup>™</sup> SARS-CoV-2 Control Dilution Buffer into a microcentrifuge tube, then add 5.0 μL of TaqCheck<sup>™</sup> SARS-CoV-2 Control. Mix well, then centrifuge briefly.
  - b. Pipet 95.0 µL of TaqCheck<sup>™</sup> SARS-CoV-2 Control Dilution Buffer into a second microcentrifuge tube, then add 5.0 µL of the dilution created in substep 3a. Mix well, then centrifuge briefly.

#### 4. Prepare the Reaction Mix:

a. For each 384-well plate, combine the following components sufficient for the number of RNA samples plus one Positive Control and one No Template Control.

Component	Volume per sample or control	Volume for <i>n</i> samples plus 2 controls <sup>[1]</sup>	Volume for 382 samples plus 2 controls <sup>[1]</sup>
TaqPath <sup>™</sup> 1-Step RT-qPCR Master Mix, CG (4X) 2.5 μL		2.75 x (n + 2) μL	1,056.0 µL
TaqCheck <sup>™</sup> SARS-CoV-2 Fast PCR Assay	0.5 µL	0.55 x (n + 2) μL	211.2 µL
Nuclease-free water	2.0 μL	2.2 x (n + 2) μL	844.8 μL
Total Reaction Mix volume	5.0 µL	_	2,112.0 μL

<sup>[1]</sup> All volumes include 10% overage for pipette error.

#### 5. Set up the reaction plate, according to the following table:

Component	Volume per reaction			
Component	Sample reaction	Positive Control reaction	No Template Control reaction	
Reaction Mix (from step 4)	5.0 µL	5.0 µL	5.0 µL	
Prepared sample (saliva + TBE-T)	5.0 µL	_	_	
Positive Control (diluted TaqCheck <sup>™</sup> SARS-CoV-2 Control from step 3 )	_	2.0 μL	_	
Nuclease-free water	_	3.0 µL	5.0 µL	
Total volume	10.0 µL	10.0 µL	10.0 µL	

a. Add 5.0  $\mu$ L of the Reaction Mix prepared in step 4 to each well of an optical 384-well reaction plate.

b. Add 5.0 µL of prepared sample (saliva plus TBE-T) to each sample well of the reaction plate.

- c. Add 2.0 µL of the diluted TaqCheck<sup>™</sup> SARS-CoV-2 Control and 3.0 µL Nuclease-free water to the Positive Control well of the reaction plate.
- d. Add 5.0 µL of Nuclease-free water to the No Template Control well of the reaction plate.
- e. Seal the plate thoroughly with MicroAmp<sup>™</sup> Optical Adhesive Film.

**IMPORTANT!** When applying the MicroAmp<sup>™</sup> 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.

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

**IMPORTANT!** Failure to vortex the plate for the recommended time can result in inaccurate sample results.

 Centrifuge the reaction plate for 1–2 minutes at ≥1,400 × g (≥1,400 RCF) to remove bubbles and to collect the liquid at the bottom of the reaction plate.

#### Set up and run the real-time PCR

A maintained instrument will be calibrated for FAM<sup>™</sup> and VIC<sup>™</sup> dyes. If calibration is required, refer to the standard calibration procedure in the instrument user guide.

- 1. Set up the real-time PCR instrument with the following settings.
  - Analysis type: Standard curve
  - Run mode: Fast
  - Passive reference: ROX
  - Sample volume: 10 µL
- 2. Set up the following reporter dye and detector pairs.

Reporter dye	Detector
FAM	RNAse P
VIC	SARS-CoV-2 N gene and SARS-CoV-2 S gene

3. Set up the thermal protocol for your instrument.

#### Table 3 Applied Biosystems<sup>™</sup> 7500 Fast Real-Time PCR Instrument

Step	Temperature	Ramp rate	Time	Number of cycles
Reverse transcription	50°C	100%	4 minutes	1
Activation	95°C	100%	2 minutes	1
Denaturation	95°C	100%	1 second	10
Anneal / extension	60°C	100%	24 seconds	40

#### Table 4 Applied Biosystems<sup>™</sup> QuantStudio<sup>™</sup> 5 Real-Time PCR Instrument, 96-well, 0.2-mL block

Step	Temperature	Ramp rate	Time	Number of cycles
Reverse transcription	50°C	3.49°C per second	4 minutes	1
Activation	95°C	3.49°C per second	2 minutes	1
Denaturation	95°C	3.49°C per second	1 second	10
Anneal / extension	60°C	2.7°C per second	20 seconds	40

#### Table 5 Applied Biosystems<sup>™</sup> QuantStudio<sup>™</sup> 5 Real-Time PCR Instrument, 384-well block

Step	Temperature	Ramp rate	Time	Number of cycles	
Reverse transcription	50°C	2.2°C per second	4 minutes	1	
Activation	95°C	2.2°C per second	2 minutes	1	
Denaturation	95°C	2.2°C per second 1 second		10	
Anneal / extension	60°C	1.8°C per second	20 seconds	- 40	

#### Table 6 Applied Biosystems<sup>™</sup> QuantStudio<sup>™</sup> 7 Flex Real-Time PCR Instrument, 384-well block

Step	Temperature	Ramp rate	Time	Number of cycles	
Reverse transcription	50°C	2.34°C per second	4 minutes	1	
Activation	95°C	2.34°C per second	2 minutes	1	
Denaturation	95°C	2.34°C per second	1 second	10	
Anneal / extension	60°C	1.98°C per second	20 seconds	- 40	

#### 4. Load the plate and start the instrument run.

# Analyze data

**IMPORTANT!** It is the responsibility of the laboratories using the TaqCheck<sup>™</sup> SARS-CoV-2 Fast PCR Assay to design and validate their own experimental design and analysis parameters.

(*Recommended*) Use QuantStudio<sup>™</sup> Design and Analysis Software v2.5 or later for data analysis. For more information about using the software, see "Related documentation" on page 10.

- 1. In the QuantStudio<sup>™</sup> Design and Analysis Software v2 home screen, open the data file (EDS).
- 2. In the open data file, click **Actions** > **Save As**, the save the data file with a new name.

Note: QuantStudio<sup>™</sup> Design and Analysis Software v2 requires data files created on a 7500 Fast Real-Time PCR Instrument, QuantStudio<sup>™</sup> 5 Real-Time PCR System, and QuantStudio<sup>™</sup> 7 Flex Real-Time PCR System to be saved as a new data file.

- 3. In the analysis settings, select automatic baseline with a start cycle of 5.
- 4. Set the appropriate threshold values for each target, as validated by your laboratory.

**IMPORTANT!** Do not use automatic threshold values.

For the 7500 Fast Real-Time PCR Instrument, QuantStudio<sup>™</sup> 5 Real-Time PCR System with the 96-well, 0.2-mL block and 384-well block, and QuantStudio<sup>™</sup> 7 Flex Real-Time PCR System with the 384-well block, we recommend that you start with the following threshold values, then adjust as needed for optimal performance according to your laboratory processes and validation.

Target	Threshold value guidelines <sup>[1]</sup>	
SARS-CoV-2 N gene and SARS-CoV-2 S gene	Manually set the threshold to 0.1, then adjust as needed.	
RNase P	Manually set the threshold to 0.2, then adjust as needed.	

[1] These threshold settings have not been tested with instruments other than the 7500 Fast Real-Time PCR Instrument, QuantStudio<sup>™</sup> 5 Real-Time PCR System with the 96-well, 0.2-mL block and 384-well block, and QuantStudio<sup>™</sup> 7 Flex Real-Time PCR System with the 384-well block. Other instruments may require different threshold settings. It is the responsibility of the laboratories using the assay to design and validate their own experimental design and analysis parameters.

#### 5. Determine $C_q$ cutoff values for each target for samples and controls.

**Note:** QuantStudio<sup>TM</sup> Design and Analysis Software v2 reports  $C_q$  values instead  $C_t$  values. The  $C_q$  values are equivalent to  $C_t$  values.

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

Contact Support for more information.

# **Related documentation**

Document	Publication Number
Applied Biosystems <sup>™</sup> 7500/7500 Fast Real-Time PCR System: Maintenance Guide	4387777
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™ Design and Analysis Software v2 User Guide	MAN0018200
TaqCheck™ SARS-CoV-2 Fast PCR Assay User Guide	MAN0019745

# Limited product warranty

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

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Revision history: Pub. No. MAN0019744

Revision	Date	Description
C.0	4 May 2021	Added new catalog numbers.
B.0	2	Added Applied Biosystems <sup>™</sup> 7500 Fast Real-Time PCR Instrument, Applied Biosystems <sup>™</sup> QuantStudio <sup>™</sup> 5 Real-Time PCR Instrument, 96-well, 0.2-mL block, and Applied Biosystems <sup>™</sup> QuantStudio <sup>™</sup> 7 Flex Real-Time PCR Instrument, 384-well block.
A.0	4 December 2020	New document.

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