

TaqCheck™ SARS-CoV-2 Fast PCR Assay

USER GUIDE

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

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| B.0 | 4 May 2021 | Added new catalog numbers. Updated safety information. |
| A.0 | 27 January 2021 | New document. |

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Product information

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

Product description

The TaqCheck™ 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 1 Assay targets, dyes, and quenchers

| Target | Dye | Quencher |
|---|----------|---------------|
| SARS-CoV-2 N gene | VIC™ dye | QSY™ quencher |
| SARS-CoV-2 S gene | | |
| Human RNase P RPP30 gene ^[1] | FAM™ dye | QSY™ quencher |

^[1] Serves as an internal positive control to monitor sample quality.

The assay requires the following components:

- TaqCheck™ SARS-CoV-2 Control—RNA control that contains SARS-CoV-2 N protein and S protein target regions
- TaqCheck™ SARS-CoV-2 Control Dilution Buffer—Dilution buffer for the control
- TaqPath™ 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™ 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™ SARS-CoV-2 Fast PCR Assay. The items listed are sufficient for 1,200 reactions.

Table 2 TaqCheck™ SARS-CoV-2 Fast PCR Assay Kit with Master Mix (Cat. No. [A50916](#))

| Components | Cat. No. | Amount | Storage |
|--|------------------------|------------|----------------|
| TaqCheck™ SARS-CoV-2 Fast PCR Assay Kit (Cat. No. A50914) | | | |
| TaqCheck™ SARS-CoV-2 Fast PCR Assay | A47693 | 690 µL | -30°C to -10°C |
| TaqCheck™ SARS-CoV-2 Control | 956127 | 3 × 10 µL | ≤ -70°C |
| TaqCheck™ SARS-CoV-2 Control Dilution Buffer | A50486 | 3 × 250 µL | -30°C to -10°C |
| TaqPath™ 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](https://www.thermofisher.com). "MLS" indicates that the material is available from [fisherscientific.com](https://www.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 | |
| <p>An Applied Biosystems™ real-time PCR instrument compatible with the dyes listed in Table 1 on page 4.</p> <p>The assay was tested with the following instruments:</p> <ul style="list-style-type: none"> Applied Biosystems™ 7500 Fast Real-Time PCR Instrument Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument, 384-well block Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR Instrument, 384-well block | Contact your local sales office |
| (Recommended) QuantStudio™ Design and Analysis Software v2.5 or later ^[1] | thermofisher.com/qpcrsoftware |

(continued)

| Item | Source |
|---|--|
| Equipment | |
| Laboratory freezers <ul style="list-style-type: none"> • -30°C to -10°C • $\leq -70^{\circ}\text{C}$ | <ul style="list-style-type: none"> • MLS • thermofisher.com/tsx |
| BSL-2 biological safety cabinet, such as Herasafe™ 2030i Class 2 A2 Biological Safety Cabinets | <ul style="list-style-type: none"> • MLS • thermofisher.com |
| Centrifuge (capable of achieving $1,400 \times g$), such as Megafuge™ 8 Small Benchtop Centrifuge Series or Multifuge™ X4 Pro Centrifuge Series | <ul style="list-style-type: none"> • MLS • thermofisher.com |
| Microcentrifuge, such as Pico™ 17 Microcentrifuge | <ul style="list-style-type: none"> • MLS • thermofisher.com |
| Laboratory mixer, vortex or equivalent, such as Digital Vortex Mixers | <ul style="list-style-type: none"> • MLS • thermofisher.com |
| Single and multichannel adjustable pipettors (2.00 μL to 1,000.0 μL) | <ul style="list-style-type: none"> • 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™ General Purpose Baths | <ul style="list-style-type: none"> • MLS • thermofisher.com |
| Liquid handler (if needed for automation) ^[2] | MLS |
| Kits and reagents | |
| TBE Buffer (Tris-borate-EDTA) (10X) | B52 , or equivalent |
| Tween®-20 Surfact-Amps™ Detergent Solution | 28320 |
| Nuclease-free Water (not DEPC-Treated) | <ul style="list-style-type: none"> • AM9938 (1 x 100 mL) • AM9932 (1 x 1,000 mL) |
| 70% Isopropanol spray or wipes | MLS |

(continued)

| Item | Source |
|---|--|
| Tubes, plates, and other consumables | |
| <p>(Recommended) Sterile tube with leak-proof, screw-top lid for sample collection^[3]</p> <p>IMPORTANT! Do not use tubes that contain preservative.</p> | <p>One of the following, or equivalent:</p> <ul style="list-style-type: none"> • 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™ Fast Optical 96-Well Reaction Plate, 0.1 mL | <ul style="list-style-type: none"> • 4346906 (with barcode) • 4366932 (with barcode) • 4346907 (without barcode) |
| MicroAmp™ EnduraPlate™ Optical 96-Well Fast Clear Reaction Plates, 0.1 mL | <ul style="list-style-type: none"> • 4483485 (with barcode) • 4483494 (with barcode) • A36930 (without barcode) |
| MicroAmp™ Optical 96-Well Reaction Plate, 0.2 mL | <ul style="list-style-type: none"> • 4306737 (with barcode) • 4326659 (with barcode) • N8010560 (without barcode) • 4316813 (without barcode) |
| MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates, 0.2 mL | <ul style="list-style-type: none"> • 4483352 (with barcode) • 4483354 (with barcode) • A36924 (without barcode) |
| MicroAmp™ Optical 384-Well Reaction Plate | <ul style="list-style-type: none"> • 4309849 (with barcode) • 4326270 (with barcode) • 4343814 (with barcode) • 4343370 (without barcode) |
| MicroAmp™ EnduraPlate™ Optical 384-Well Clear Reaction Plates | <ul style="list-style-type: none"> • 4483273 (with barcode) • 4483285 (with barcode) • A36931 (without barcode) |
| MicroAmp™ Clear Adhesive Film | 4306311 |

(continued)

| Item | Source |
|--|---|
| MicroAmp™ Optical Adhesive Film | 4311971, 4360954 |
| MicroAmp™ 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™ General Long-Term Storage Cryogenic Tubes, 0.2 mL | 5000-0020 |
| Nunc™ Biobanking and Cell Culture Cryogenic Tubes, 4.5 mL | 337516 |
| Sterilin™ Certified Universal Containers – RNase, DNase, human DNA and Pyrogen Free, 30 mL | <ul style="list-style-type: none"> • 30APPRN (Unlabelled) • 30BPPRN (Graduated label) |

^[1] Use of QuantStudio™ 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.

^[2] Follow the guidelines provided by the manufacturer.

^[3] 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.



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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 23).

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 ^[1] | Volume per four 96-well plates ^[1] |
|--|-----------------|---|---|
| TBE Buffer (10X) ^[2] | 20 µL | 2.4 mL | 9.6 mL |
| Tween [®] -20 Detergent (10%) ^[3] | 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 |

^[1] Includes 25% overage.

^[2] The TBE Buffer has a final concentration of 2X in the TBE-T mix.

^[3] 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[™] 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™ SARS-CoV-2 Control to a working stock:
 - a. Pipet 95.0 µL of TaqCheck™ SARS-CoV-2 Control Dilution Buffer into a microcentrifuge tube, then add 5.0 µL of TaqCheck™ SARS-CoV-2 Control. Mix well, then centrifuge briefly.
 - b. Pipet 95.0 µL of TaqCheck™ 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 n samples plus 2 controls ^[1] | Volume for 94 samples plus 2 controls ^[1] |
|---|------------------------------|---|--|
| TaqPath™ 1-Step RT-qPCR Master Mix, CG (4X) | 2.5 µL | $2.75 \times (n + 2)$ µL | 264.0 µL |
| TaqCheck™ SARS-CoV-2 Fast PCR Assay | 0.5 µL | $0.55 \times (n + 2)$ µL | 52.8 µL |
| Nuclease-free water | 2.0 µL | $2.2 \times (n + 2)$ µL | 211.2 µL |
| Total Reaction Mix volume | 5.0 µL | — | 528.0 µL |

^[1] All volumes include 10% overage for pipette error.

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

| Component | Volume per reaction | | |
|--|---------------------|---------------------------|------------------------------|
| | 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™ 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 |

- Add 5.0 µL of the Reaction Mix prepared in step 4 to each well of an optical 96-well reaction plate.
- Add 5.0 µL of prepared sample (saliva plus TBE-T) to each sample well of the reaction plate.
- Add 2.0 µL of the diluted TaqCheck™ SARS-CoV-2 Control and 3.0 µL Nuclease-free water to the Positive Control well of the reaction plate.
- Add 5.0 µL of Nuclease-free water to the No Template Control well of the reaction plate.
- 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.

- 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 $\geq 1,400 \times g$ ($\geq 1,400$ 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)

- If frozen, thaw the reagents on ice or on a cold block.
- Gently vortex the reagents, then briefly centrifuge the tube or swirl the bottle to collect the liquid at the bottom of the container.
- Dilute TaqCheck™ SARS-CoV-2 Control to a working stock:
 - Pipet 95.0 μL of TaqCheck™ SARS-CoV-2 Control Dilution Buffer into a microcentrifuge tube, then add 5.0 μL of TaqCheck™ SARS-CoV-2 Control. Mix well, then centrifuge briefly.
 - Pipet 95.0 μL of TaqCheck™ 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.
- Prepare the Reaction Mix:
 - 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 n samples plus 2 controls ^[1] | Volume for 382 samples plus 2 controls ^[1] |
|---|-------------------------------------|---|---|
| TaqPath™ 1-Step RT-qPCR Master Mix, CG (4X) | 2.5 μL | $2.75 \times (n + 2)$ μL | 1,056.0 μL |
| TaqCheck™ SARS-CoV-2 Fast PCR Assay | 0.5 μL | $0.55 \times (n + 2)$ μL | 211.2 μL |
| Nuclease-free water | 2.0 μL | $2.2 \times (n + 2)$ μL | 844.8 μL |
| Total Reaction Mix volume | 5.0 μL | – | 2,112.0 μL |

^[1] All volumes include 10% overage for pipette error.

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

| Component | Volume per reaction | | |
|---|---------------------|---------------------------|------------------------------|
| | 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™ 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 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™ 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™ 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.

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.

7. Centrifuge the reaction plate for 1–2 minutes at $\geq 1,400 \times g$ ($\geq 1,400$ 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™ and VIC™ 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™ 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 | 40 |
| Anneal / extension | 60°C | 100% | 24 seconds | |

Table 4 Applied Biosystems™ QuantStudio™ 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 | 40 |
| Anneal / extension | 60°C | 2.7°C per second | 20 seconds | |

Table 5 Applied Biosystems™ QuantStudio™ 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 | 40 |
| Anneal / extension | 60°C | 1.8°C per second | 20 seconds | |

Table 6 Applied Biosystems™ QuantStudio™ 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 | 40 |
| Anneal / extension | 60°C | 1.98°C per second | 20 seconds | |

4. Load the plate and start the instrument run.

Analyze data

IMPORTANT! It is the responsibility of the laboratories using the TaqCheck™ SARS-CoV-2 Fast PCR Assay 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 23.

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

Note: QuantStudio™ Design and Analysis Software v2 requires data files created on a 7500 Fast Real-Time PCR Instrument, QuantStudio™ 5 Real-Time PCR System, and QuantStudio™ 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™ 5 Real-Time PCR System with the 96-well, 0.2-mL block and 384-well block, and QuantStudio™ 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 ^[1] |
|---|---|
| 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™ 5 Real-Time PCR System with the 96-well, 0.2-mL block and 384-well block, and QuantStudio™ 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™ 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.



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.



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.

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; found at:
<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); found at:
www.who.int/publications/i/item/9789240011311



Documentation and support

Related documentation

| Document | Publication Number |
|---|--------------------|
| <i>Applied Biosystems™ 7500/7500 Fast Real-Time PCR System: Maintenance Guide</i> | 4387777 |
| <i>QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide</i> | MAN0010407 |
| <i>QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Maintenance and Administration Guide</i> | 4489821 |
| <i>QuantStudio™ Design and Analysis Software v2 User Guide</i> | MAN0018200 |
| <i>TaqCheck™ SARS-CoV-2 Fast PCR Assay Quick Reference</i> | MAN0019744 |

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 - 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

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