Real-Time PCR Detection of SARS-CoV-2 on Food Packaging and Environmental Surfaces

USER GUIDE

for use with:

PrepSEQ™ Nucleic Acid Extraction Kit for Food and Environmental Testing

KingFisher™ Flex Purification System with 96 Deep-Well Head

MagMAX™ Express-96 Deep Well Magnetic Particle Processor

TaqMan™ 2019-nCoV Assay Kit v1

TaqMan™ 2019-nCoV Control Kit v1

RNA UltraSense™ One-Step qPCR Master Mix

Applied Biosystems™ QuantStudio™ Design and Analysis Software v1.5.1 or later

Applied Biosystems™ 7500 Software SDS v.1.4.2 or later

PERFORMANCE TESTED

AOAC

RESEARCH INSTITUTE

LICENSE NUMBER   012103

For testing of Food and Environmental samples only.

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Revision	Date	Description
E.0	22 January 2021	Minor edits from the technical board.
D.0	05 December 2020	Updated the review results topic, and added Appendix B software examples.
C.0	12 October 2020	Addition of RNA UltraSense™ One-Step qPCR Master Mix
B.0	02 October 2020	Correction of sample volume on page 15
A.0	11 September 2020	New document.

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Product information

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

Product description

This guide describes the following *Performance Tested* workflow for detection of SARS-CoV-2 in food packaging and environmental surfaces with the Applied Biosystems™ TaqMan™ 2019-nCoV Assay Kit v1 (Cat. No. A47532). This kit includes three TaqMan™ RT-PCR assays that target SARS-CoV-2 genes, and one positive control assay that targets the Human RNase P RPPH1 gene:

- Assays target three different viral genomic regions, reducing the risk of false negatives.
- Assays have undergone bioinformatic selection and analysis to specifically target sequences that are unique to SARS-CoV-2.
- The RNase P assay is run in duplex with the combined 2019-nCoV assays as an internal positive control.

The kit is used with the TaqMan™ 2019-nCoV Control Kit v1 (Cat. No. A47533) to monitor assay-specific amplification.

For more information about genetic analysis solutions available for SARS-CoV-2, go to **thermofisher.com/coronavirus**.

Required materials

Unless otherwise indicated, all materials are available through the Thermo Fisher Microbiology ordering process or **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Note: Parts may ship separately depending on configuration and storage conditions.

Materials for sample preparation and nucleic acid extraction

Table 1   PrepSEQ™ Nucleic Acid Extraction Kit

Contents	Cat. No. 4480466 (100 reactions)	Cat. No. 4428176 (300 reactions)	Storage[1]
Lysis Buffer	2 × 50 mL	6 × 50 mL	15°C to 30°C
Magnetic Particles	2 × 1.5 mL	6 × 1.5 mL	
Binding Solution (Isopropanol)[2]	1 empty bottle	3 empty bottles	
Wash Buffer Concentrate[3]	2 × 26 mL	6 × 26 mL	
Elution Buffer	25 mL	3 × 25 mL	
Proteinase K (PK) Buffer	50 mL	3 × 50 mL	
Proteinase K, 20 mg/mL	1.25 mL	3 × 1.25 mL	−25°C to −15°C

[1]   Refer to the product label for the expiration date.

[2]   Add ~35 mL of 100% isopropanol to the empty bottle before use.

[3]   Add 74 mL of 95% ethanol before use.

Table 2   Magnetic particle processor

Item	Source
KingFisher™ Flex instrument and accessories	
KingFisher™ Flex Purification System with 96 Deep-Well Head with BindIt™ Software	A32681
KingFisher™ Flex 96 Deep-Well Heating Block	24075430 (included in A32681)
KingFisher™ Deep-Well 96 Plate, V-bottom	95040450
KingFisher™ 96 tip comb for DW magnets	97002534
MagMAX™ Express-96 instrument and accessories	
MagMAX™ Express-96 Deep Well Magnetic Particle Processor	Contact your local sales representative.
MagMAX™ Express-96 Deep Well Plates	
MagMAX™ Express-96 Deep Well Tip Combs	

Materials for PCR detection and analysis

Table 3 TaqMan™ 2019-nCoV Assay Kit v1 (Cat. No. A47532)

Contents	Dye	Amount[1]	Concentration	Storage
2019-nCoV (ORF1ab) (Tube 1)	FAM™ dye	75 µL	20X	−30°C to −10°C
2019-nCoV (S Protein) (Tube 2)	FAM™ dye	75 µL	20X	
2019-nCoV (N Protein) (Tube 3)	FAM™ dye	75 µL	20X	
RNase P Assay (Tube 4)	VIC™ dye	250 µL	20X	

[1] Sufficient for 50 × 25-µL reactions.

Table 4 TaqMan™ 2019-nCoV Control Kit v1 (Cat. No. A47533)

Contents	Target Sequence	Amount[1]	Concentration	Storage
2019-nCoV Control v1	ORF1ab	50 µL	1×10^4 copies/µL	−30°C to −10°C
	S protein			
	N protein			
	Human RNase P RPPH1			

[1] Sufficient for 50 positive-control reactions.

Table 5 Other required materials

Item	Source
Master Mix	
Thermo Scientific™ RNA UltraSense™ One-Step qPCR Master Mix: • 250 µL RNA UltraSense™ Enzyme Mix • 1 mL RNA UltraSense™ 5X Reaction Mix • 300 µL 20X Bovine Serum Albumin (BSA) • 1 mL 50-mM Magnesium Sulfate (MgSO$_4$) • 100 µL ROX™ Reference Dye	11732927
Total RNA Control (Human)	4307281, or equivalent
Real-Time PCR Instrument (one of the following)	
Applied Biosystems™ QuantStudio™ 5 Food Safety Real-Time PCR System	A36328 or A36320
Applied Biosystems™ 7500 Fast Food Safety Real-Time PCR System	A30299 or A30304

Table 5   Other required materials *(continued)*

Item	Source
Real-Time PCR Instrument Software (one of the following)[1]	
Applied Biosystems™ QuantStudio™ Design and Analysis Software v1.5.1 or later	Included with QuantStudio™ 5 Real-Time PCR System
Applied Biosystems™ 7500 Software SDS v.1.4.2 or later	Included with 7500 Real-Time PCR Instrument
Equipment	
Laboratory freezers -30℃ to -10℃ and ≤-70℃	MLS
Centrifuge, with a rotor that accommodates deepwell microplates	
Microcentrifuge	
Laboratory mixer, vortex or equivalent	
Single and multichannel adjustable pipettors (1 µL to 1,000 µL)	
Cold block (96-well or 384-well) or ice	
Tubes, plates, and other consumables	
MicroAmp™ Fast Optical 96-Well Reaction Plate, 0.1 mL	4346907
MicroAmp™ Optical Adhesive Film Kit	4313663
MicroAmp™ Optical 8-Cap Strips	4323032
MicroAmp™ Fast 8-Tube Strip, 0.1 mL	4358293
Disposable gloves	MLS
Micropipette tips, aerosol resistant	
Swabs with synthetic tip and aluminium or plastic shaft, preferably large macrofoam tipped applicator	
Reagents	
Thermo Scientific™ Oxoid™ Phosphate Buffered Saline	BR0014G
Ethanol, 95%	MLS
Isopropanol, 100%	
Nuclease-free water	

[1]   Real-Time PCR Instrument Software is used to control the instrument and to collect instrument run data.

Procedural guidelines

Guidelines for sample collection and storage

Sample collection:

- Pre-moisten a non-cellulose swab with synthetic tip with Phosphate Buffered Saline (PBS).
- When sampling, apply pressure with the wet swab onto the surface, move in at least two different directions while rotating the swab stick. Avoid letting the swab dry completely. Swab an area up to 2 x 2 inches, ensuring that the full area is covered in the sampling.
- Place the swab back into the transport tube containing 2 mL of PBS.

Note: Viral Transport Media (VTM) can be used instead of PBS according to World Health Organization protocol on *Surface sampling of coronavirus disease (COVID-19): A practical "how to" protocol for health care and public health professionals*, Version: 1.1, February 2020 (**www.who.int**).

Storage:

- Swabs should be placed at refrigeration temperature (2-8°C) no more than 15 minutes post-sampling and maintain refrigeration until analysis.
- Analysis of swabs should occur within 24 hours from sample collection (48 hours maximum). If the swabs are not analyzed with the 48 hour maximum, store the swab between -70°C and -80°C and ship on dry ice.

  Note: Analyze the swabs preferably in the coming 24 hours. If the swabs are not analyzed within 48 hours maximum, store the swabs preferably between -70°C and -80°C, and ship on dry ice.

Guidelines for the 2019-nCoV assay

- Use purified, non-degraded total nucleic acid that is free of RNase activity and RT-PCR inhibitors.
- Protect the assays and master mix from light.
- For each research sample, include the primers and probes for all three 2019-nCoV targets (FAM™ assay) in multiplex with the RNase P target (VIC™ assay).
- Before you begin, determine the number of required reactions. In addition to the nucleic acid research samples, include the following reactions for the combined 2019-nCoV assay:
  - One negative extraction control (NEC) per plate
  - One 2019-nCoV Control v1 reaction per plate
  - One no-template control (NTC) per plate

Workflow overview

SARS-CoV-2 Detection on Food Packaging and Environmental Surfaces

Sampling and Transport	Sample Preparation	PCR and Data Analysis
Simple sample collection with non-cellulose swabs and Viral Transport Medium or Phosphate Buffered Saline	Options for automated medium to high-throughput and manual low-throughput	Master mix and Real-Time PCR instrumentation with software designed to run environmental PCR range of products

Prepare the samples and extract RNA using PrepSEQ™ Nucleic Acid Extraction Kit for Food and Environmental Testing

The KingFisher™ Flex Purification System with 96 Deep-Well Head or the MagMAX™ Express-96 Deep Well Magnetic Particle Processor can be used to extract RNA with the PrepSEQ™ Nucleic Acid Extraction Kit for Food and Environmental Testing.

- Ensure that the KingFisher™ Flex Purification System with 96 Deep-Well Head is set up with the KingFisher™ Flex 96 Deep-Well Heating Block.
- Ensure that the **PSNA_Flex_300ul** has been downloaded from the product page and loaded onto the instrument using the BindIt™ software.

Note: Use **PSNA_MagMAX_300ul** if you are using MagMAX™ Express-96 Deep Well Magnetic Particle Processor.

- Add 74 mL 95% ethanol the Wash Buffer Concentrate bottle.
- Add 35 mL 100% isopropanol to an empty Binding Solution bottle.

1. Prepare and label the side of the processing plates according to the following table.

Plate ID	Plate type	Reagent	Volume per well
Sample	KingFisher™ Deep-Well 96 Plates	Binding Bead Mix	700 µL
Wash Plate 1		Wash Buffer	300 µL
Wash Plate 2		Wash Buffer	300 µL
Elution Plate		Elution Buffer	100 µL
Tip Comb Plate	Place a KingFisher™ 96 tip comb for Deep Well magnets in a KingFisher™ 96 microplate		

2. Cover the plates with an adhesive film, then store at room temperature while setting up the sample plate (up to 1 hour).

3. Vortex the Magnetic Beads to ensure the bead mixture is homogenous.

   If a white precipitate has formed, first incubate the tube of Magnetic Particles at 37±1°C for approximately 10 minutes and re-vortex.

4. Prepare the Binding Bead Mix according to the following table.

Component	Volume per well[1]
Proteinase K Buffer	340 µL
Binding Solution	325 µL
Total Nucleic Acid Magnetic Beads	25 µL
Proteinase K	10 µL
Total Binding Bead Mix	700 µL

[1] Include 10% overage.

Prepare only the amount of Binding Bead Mix required for one day of use.

5. Invert the Binding Bead Mix five times gently to mix, then add 700 µL to each sample well and the Negative Extraction Control well in the Sample Plate.

Note: Remix the Binding Bead Mix by inversion frequently during pipetting to ensure even distribution of beads at all times. 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.

6. Add 1 µL of Total Human RNA Control to each sample well and to the Negative Extraction Control well.

7. Vigorously mix each sample tube containing the swab by vortexing for 30 seconds.

8. Add 300 µL from PBS solution in step 7 to each sample well and 300 µL of Nuclease-free Water (not DEPC-treated) to the Negative Extraction Control well in the Sample Plate.

9. Select the **PSNA_Flex_300ul** on the KingFisher™ Flex Purification System with 96 Deep-Well Head.

Note: Use **PSNA_MagMAX_300ul** if you are using MagMAX™ Express-96 Deep Well Magnetic Particle Processor.

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

11. After the run is complete (~40 minutes after start), immediately remove the Elution Plate from the instrument, then cover the plate with an adhesive film.

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

Note: RNA can be stored at -70℃ for long-term storage (up to 1 year if not used immediately).

Perform 1-Step RT-PCR

1. Set up the real-time PCR instrument as indicated in the following table

Stage	Step	Temperature	Time
Hold	Reverse transcription	50°C	15 minutes
Hold	Activation	95°C	2 minutes
Cycling (45 cycles)	Denaturation	95°C	3 seconds
	Anneal/Extension	60°C	30 seconds

2. On ice, prepare a Reaction Mix for the number of reactions required plus 10% overage.

Component	Volume per reaction
RNA UltraSense™ Enzyme Mix	1 µL
RNA UltraSense™ 5X Reaction Mix	4 µL
2019-nCoV ORF1ab Assay (20X)	1 µL
2019-nCoV N Protein Assay (20X)	1 µL
2019-nCoV S Protein Assay (20X)	1 µL
RNase P Assay (20X)	1 µL
$MgSO_4$ (50 mM)	0.25 µL
Total Reaction Mix Volume	9.25 µL

3. For each reaction, combine the following components.

Component	Volume per reaction
Reaction Mix	9.25 µL
Template OR NEC OR 1 µL 2019-nCoV Control v1 + 9.75 µL Nuclease-free Water (not DEPC-Treated) OR NTC (RT-PCR Grade Water)	10.75 µL
Total Reaction Volume	20 µL

4. Cap or seal the reaction vessels, and gently mix to make sure that all components are at the bottom of the amplification tube. Centrifuge briefly if needed.

Note: It is essential that no bubbles remain in the PCR reaction tubes. If bubbles remain after centrifugation, gently tap the reaction plate/tubes on the bench top and then briefly spin again at a higher speed until all bubbles are removed.

5. Place reactions in a preheated thermal cycler programmed as described above. Collect data and analyze results.

   The following software should be used with each instrument.

Instrument	Software
7500 Fast Food Safety Real-Time PCR System	7500 Software SDS v.1.4.2 or later
QuantStudio™ 5 Food Safety Real-Time PCR System	QuantStudio™ Design and Analysis Software v1.5.1 or later

Analyze data

For more information about using a software, see the software user guide or help.

1. Open the data file (EDS) in the same software used to run the PCR.

2. Perform analysis using the following analysis settings.

Target	Baseline	Threshold
2019-nCoV assay (FAM™ dye)	Auto	Auto
RNaseP assay (IPC) (VIC™ dye)		

IMPORTANT! Analyze the run with no reference dye.

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3. For each plate, confirm that the control reactions for each 2019-nCoV assay perform as expected.

Table 6 Expected results of the control reactions for each 2019-nCoV assay

Reaction	Expected C_t value		About unexpected results
	2019-nCoV assay (FAM™ dye)	RNaseP assay (IPC) (VIC™ dye)	
NTC	Undetermined		If the NTC has an amplification curve that crosses the threshold (false positive), sample contamination may have occurred. Repeat the test with new reagents, following good RT-PCR practices.
Negative extraction control	Undetermined	$C_t < 40$	If a C_t value is detected in the 2019-nCoV assay, then contamination may have occurred during extraction. Repeat the test with new reagents.
2019-nCoV Control v1	$C_t \leq 35$	$C_t < 40$	If the C_t value is above the expected result, repeat the test with new reagents.

4. Review all of the results for the 2019-nCoV assay to ensure that all positive results are detected. Evaluate the overall shape of the amplification curves. An exponential and continuous increase in signal amplification indicates positive amplification.

5. Export the results.

Review results

1. Review the amplification plots for the 2019-nCoV assay and classify the result according to the guidelines below:

Signal	Interpretation	Result
Positive	Amplification curves are considered positive if: • they have a sudden increase in fluorescence into an exponential signal OR • they have a shallow or linear continuous increase in fluorescence with little or no flattening of the curve	In both cases, there should be no decrease in fluorescence after the initial increase.
Negative	Amplification curves are considered negative if they have no continuous increase in fluorescence. Any increase in signal is likely to be minor and reach a plateau rapidly or decrease, resulting in no overall continuous increase in fluorescence.	If an increase in fluorescence is present, the signal must be minor and reach a plateau rapidly or decrease to result in no overall continuous increase in fluorescence.

Note: For examples of positive and negative signals, including baseline anomalies and fixes, see Appendix B, "Software examples".

Note: If the amplification signal is weak, the software can be manipulated to allow for simpler interpretation of results, see "Adjust the QuantStudio software to simplify the interpretation" on page 16 or "Adjust the 7500 software to simplify the interpretation" on page 17.

2. Review the amplification plots for the RNase P assay (IPC) and classify according to the following table.

2019-nCoV assay (FAM™ dye)	RNAseP assay (IPC) (VIC™ dye)	2019-nCoV assay result
Clear positive signal	Any value[1]	Positive.
Weak positive signal	$C_t < 40$	Positive.
	C_t = Undetermined *or* $C_t \geq 40$	Invalid. Proceed to "Troubleshooting" on page 18.

(continued)

2019-nCoV assay (FAM™ dye)	RNAseP assay (IPC) (VIC™ dye)	2019-nCoV assay result
No positive signal	$C_t < 40$	Negative.
	C_t = Undetermined *or* $C_t \geq 40$	Invalid. Re-purify the nucleic acid from the sample, then repeat the test.

[1] An RNase P positive result is expected in most reactions. However, when a strong positive signal is detected in the 2019-nCoV assay the RNase P assay can occasionally give a negative result. These samples should be treated as positive if the amplification curve for the 2019-nCoV assay appears normal.

3. For each test sample, interpret the results using the following table. It is recommended that each lab perform accuracy testing with appropriate samples to establish guidelines for interpreting results.

2019-nCoV assay results	Interpretation of results
Clearly positive.	High risk positive. Employ full hygiene intervention strategy.
Weak positive.	Lower risk positive. Use risk assessment to employ appropriate hygiene intervention strategy.
Negative.	Negative. No need for hygiene intervention strategy.

Adjust the QuantStudio software to simplify the interpretation

This can be achieved with the following steps, which also remove baseline anomalies.

1. Navigate to **Show Plot Settings** by clicking the 👁 button. Change the **Graph Type** to **Linear**.

   Note: Ensure the **Plot Type** is set to **ΔRn vs Cycle** and the **Graph Type** is set to **Linear**.

2. Navigate to **Plot Properties** by clicking the 🖼 button.

3. On the **X Axis** tab, ensure **Auto-adjust range** is unchecked. Change the **Range** minimum value to 12, and the maximum value to 45.

4. On the **Y Axis** tab, ensure **Auto-adjust range** is unchecked. Change the **Range** minimum value to -100, and the maximum value to 1,000,000.

5. Navigate to **Analysis ▸ Analysis Settings**.

6. Under **CT Settings for 2019-nCoV Assay**, uncheck the **Default Settings** option and enter the following details:

   • Uncheck the **Automatic Threshold** and **Automatic Baseline** options.

   • Set the threshold to 1 to remove it from view.

   • Set the baseline to remove any anomalous data. For example: Baseline Start Cycle: 12, End Cycle: 25.

   • Click **Apply**.

Adjust the 7500 software to simplify the interpretation

This can be achieved with the following steps, which also remove baseline anomalies.

1. Navigate to the **Amplification Plot** tab. Right-click on the plot and select **Graph Settings**.

2. Under **Post Run Settings**, change the Y-Axis to **Linear** and uncheck **Auto Scale** for both the Y-Axis and X-Axis.

3. Change the Y-Axis minimum value to -100 and the maximum value to 1,000,000.

4. Change the X-Axis minimum value to 12 and the maximum value to 45.

5. Click **Apply**.

6. Navigate to the **Amplification Plot** tab, then under **Analysis Settings**:

7. Select **Manual Ct** and set **Threshold** to 1 to remove it from view.

8. Select **Manual Baseline** and set **Start (cycle)** to 12 and **End (cycle)** to 25.

9. Click **Analyze**.

Troubleshooting

Observation	Possible cause	Recommended action
Inhibition of downstream PCR, indicated by non-detection of IPC reaction	Magnetic Particles were in the Elution Plate.	Avoid disturbing the Magnetic Particles during transfer of eluted RNA to the lyophilized assay.
		Avoid transfer of Magnetic Particles using one of the following methods (optional):
		• Place the Elution Plate on the 96-Well Magnetic Ring Stand during transfer of eluted DNA sample to the lyophilized assay.
		• Spin the plate at maximum speed in a plate centrifuge for the equivalent of approximately 4,000 × g for approximately 30 seconds, to pellet the Magnetic Particles to the bottom of the plate.
	Elution Plate contains other inhibitory substances.	Dilute the eluted RNA 1:5 with Nuclease-free Water to dilute PCR inhibitors, and repeat the assay. If PCR remains inhibited, repeat the sample preparation.
In positive control wells, no target-specific signal is detected.	Positive control was omitted (pipetting error).	Repeat the assay. Make sure to pipet the positive control into all positive control wells.
In positive control wells or unknown sample wells, no IPC signal is detected, but target-specific signal is detected.	A high copy number of target RNA exists in samples, resulting in preferential amplification of the target-specific RNA.	No action is required. The result is considered positive if the target-specific amplification curve appears normal.
In negative extraction control wells, a target-specific signal is detected.	Carryover contamination occurred.	1. Repeat the assay using fresh aliquots of all reagents and clean pipetting equipment. 2. If the negative extraction control continues to show contamination, repeat the assay using a new kit. 3. If the negative extraction control continues to show contamination, contact Technical Support.
Amplification curve appears abnormal (different from an exponential and continuous increase).	Auto baseline settings cannot account for large deviations in C_T values between samples.	Manually adjust the baseline settings for the sample under investigation.
	Inhibitory substances carried over from RNA extraction.	Dilute the eluted RNA 1:5 with Nuclease-free Water to dilute PCR inhibitors, and repeat the assay. If PCR remains inhibited, repeat the sample preparation.

Observation	Possible cause	Recommended action
Amplification curve appears abnormal (different from an exponential and continuous increase). *(continued)*	Primers and probes have degraded.	Repeat the PCR assay using fresh reagents.

Supplemental information

Good laboratory practices for PCR

To avoid amplicon contamination of samples, follow these guidelines when preparing or handling samples for PCR amplification:

- Wear clean gloves and a clean lab coat (not previously worn while handling amplified products or used during sample preparation).
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Do not open reaction tubes after PCR.
- Do not autoclave reaction tubes after PCR.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA*Zap*™ Solutions (Cat. No. AM9890). After cleaning with bleach we recommend a rinse with an ethanol solution because bleach will rust stainless steel.

For additional information, refer to EN ISO 22174:2005 or **www.thermofisher.com/us/en/home/life-science/pcr/real-time-learning-center/real-time-pcr-basics.html**.

Symbol definitions

Symbol	Definition
LOT	BATCH CODE
REF	CATALOG NUMBER
$\nabla\Sigma$	CONTAINS SUFFICIENT FOR <n> TESTS
📖	CONSULT INSTRUCTIONS FOR USE
🏭	MANUFACTURER
🌡	UPPER AND LOWER TEMPERATURE LIMIT (storage temperature)
⧗	USE BY

Software examples

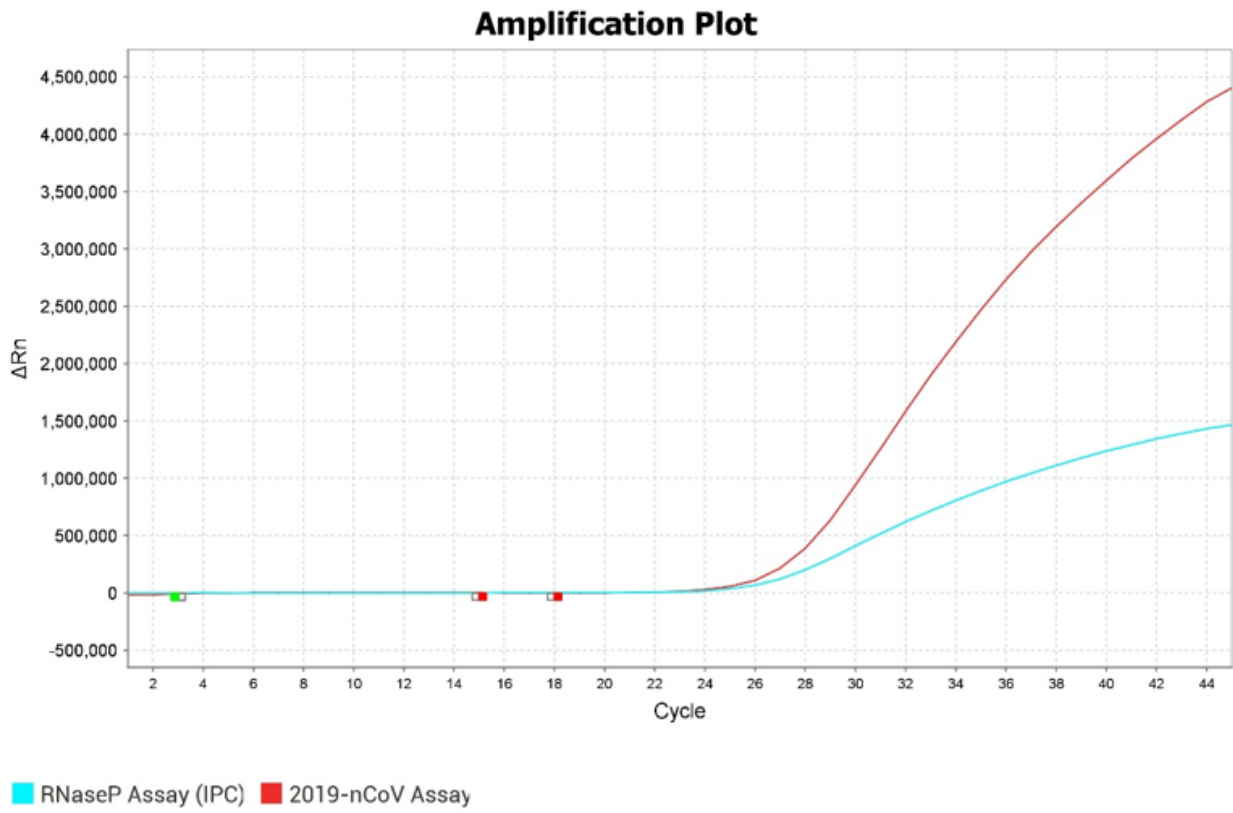## QuantStudio Design and Analysis Software v1.5.1 examples

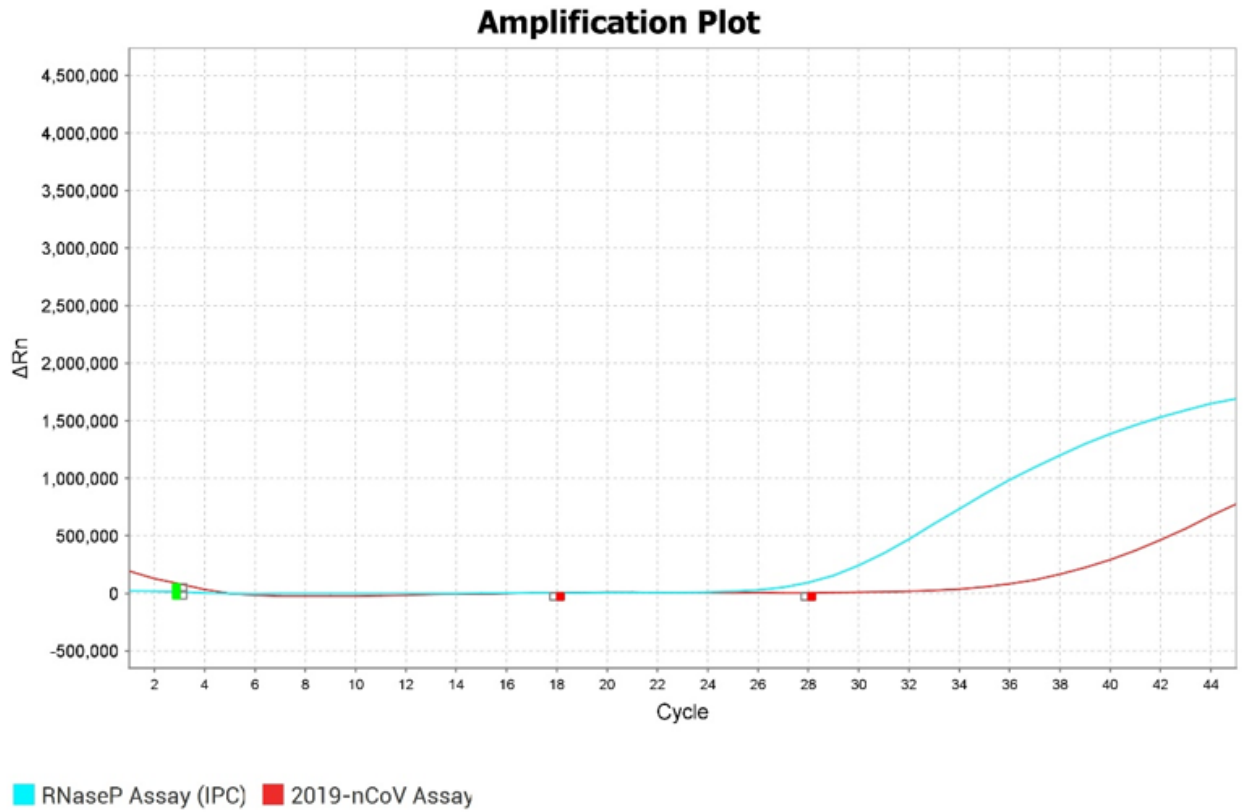**Figure 1** Positive amplification of 2019-nCoV Assay target (strong)

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Amplification Plot

RNaseP Assay (IPC) 2019-nCoV Assay

Figure 2   Positive amplification of 2019-nCoV Assay target (weaker)
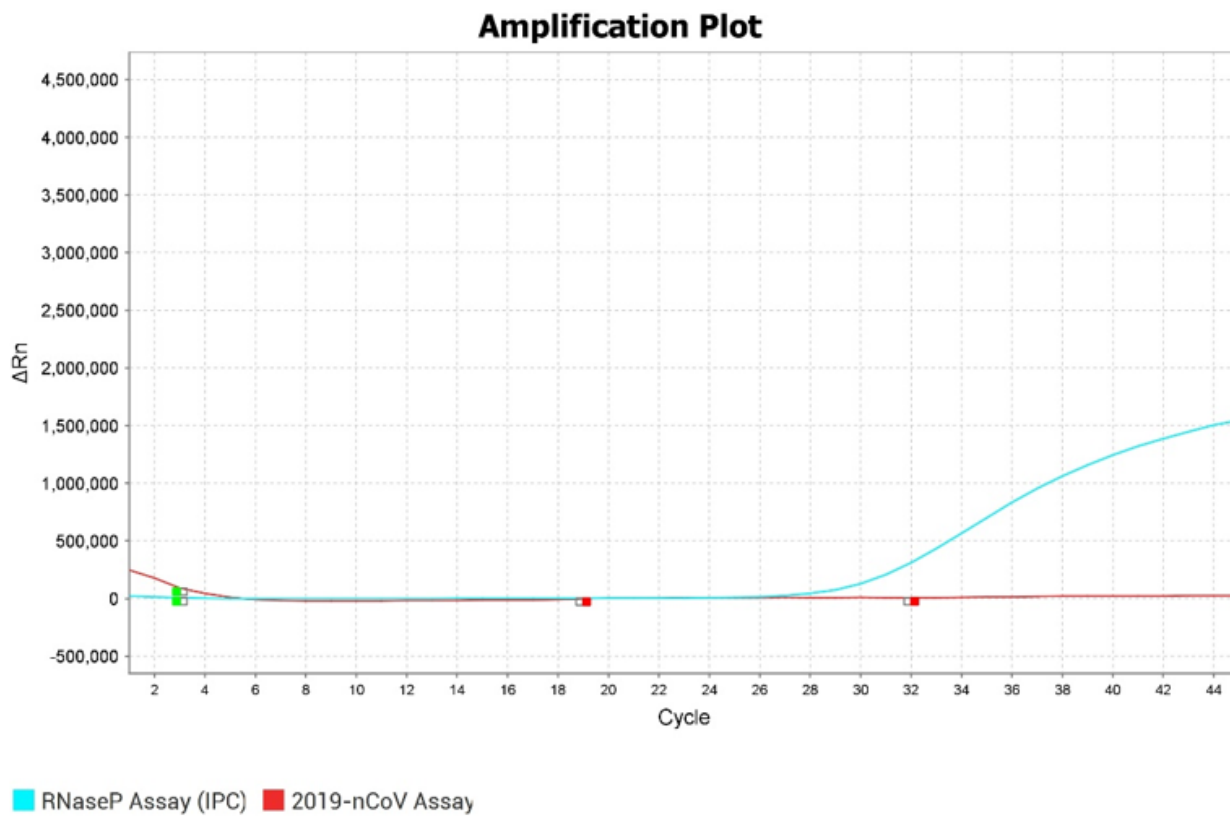
Figure 3   Negative amplification of 2019-nCoV Assay target
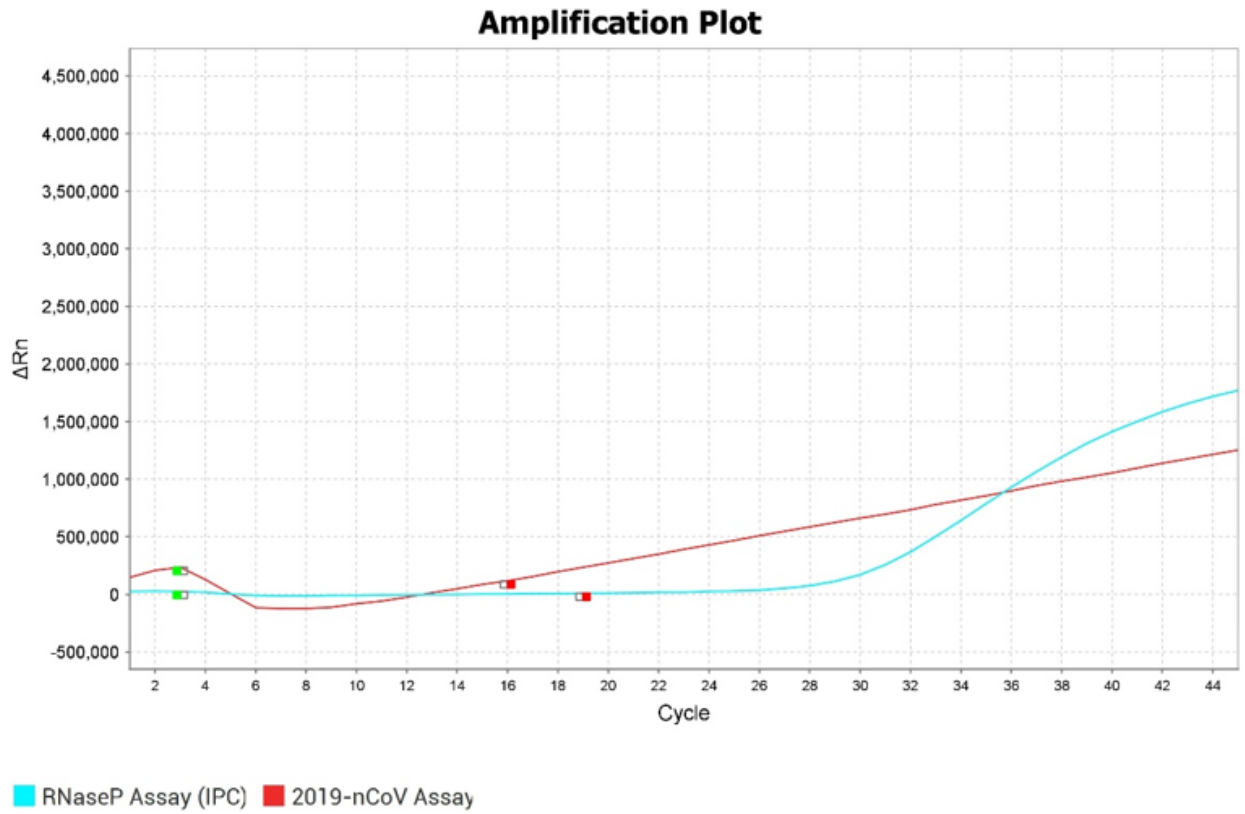
Figure 4   Baseline anomaly for the 2019-nCoV Assay target: Result according to software note in the Review Results section
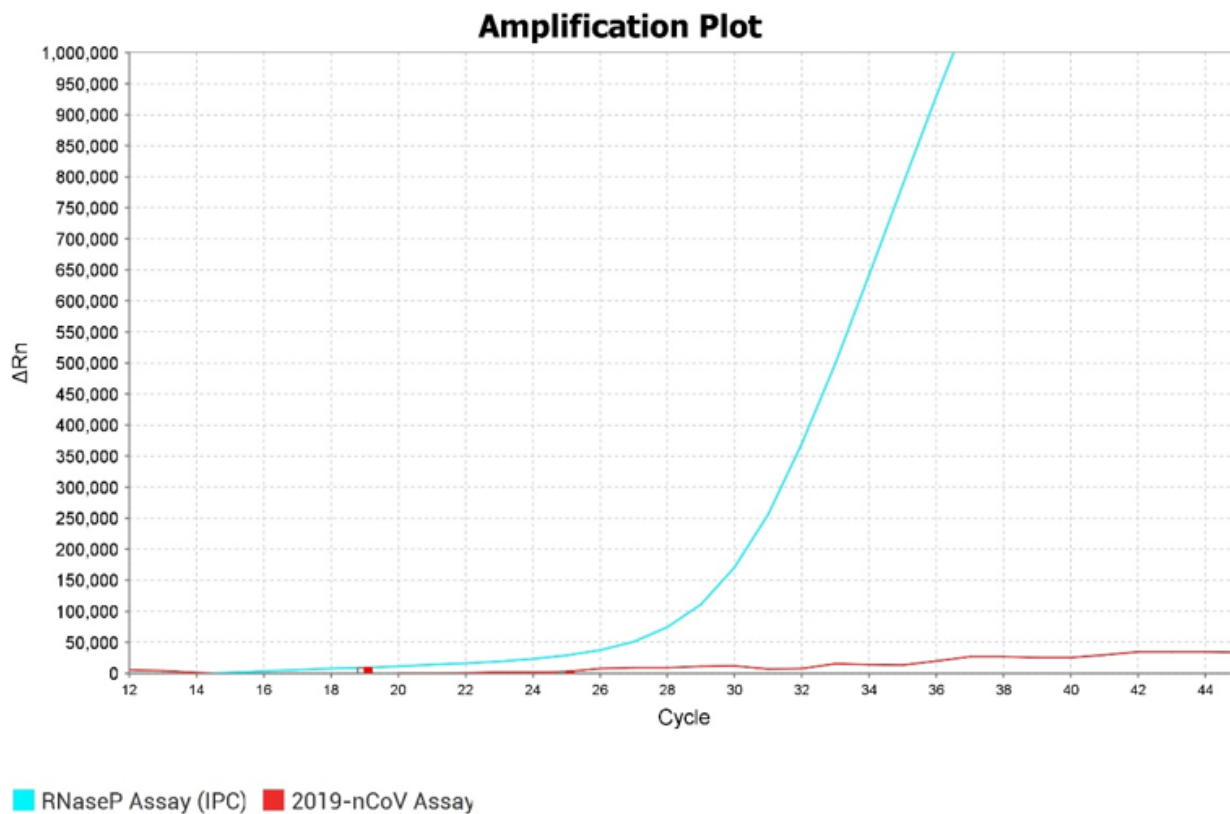
Figure 5   Baseline anomaly for the 2019-nCoV Assay target: Result according to software note in the Review Results section
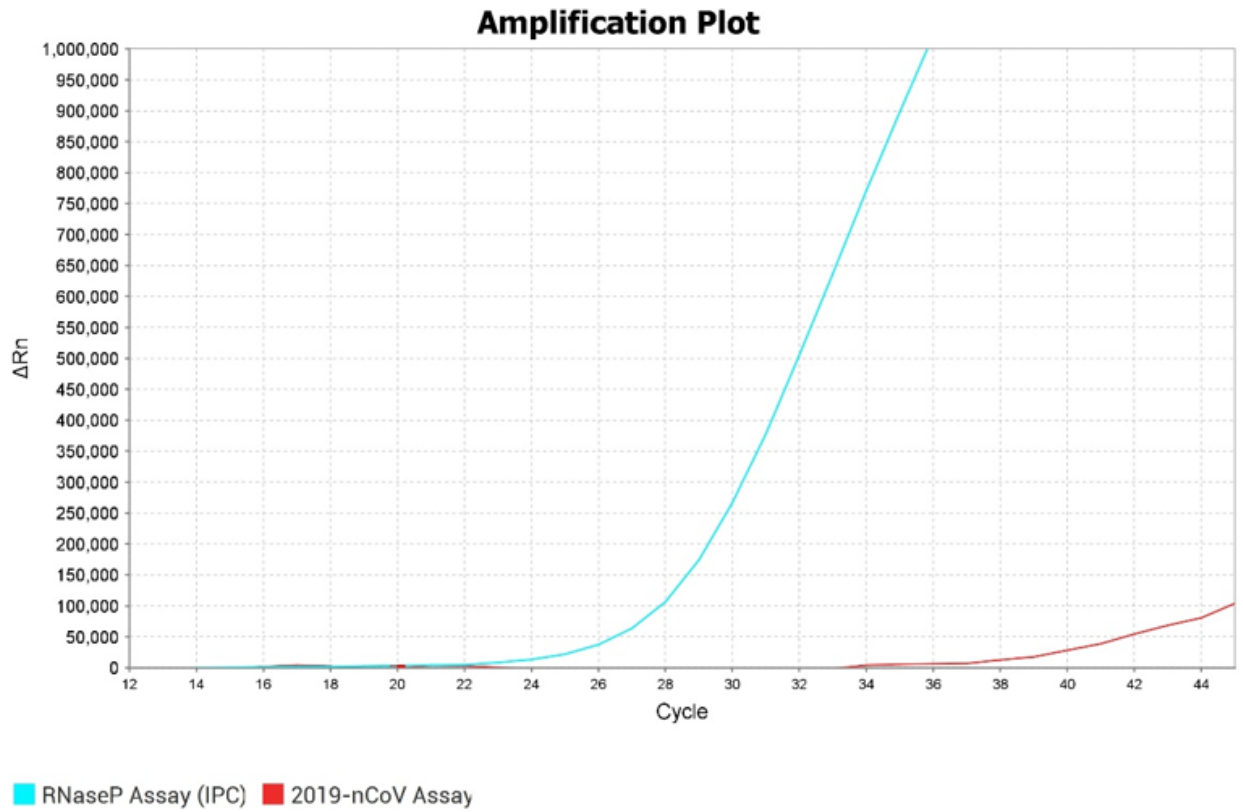
Amplification Plot

RNaseP Assay (IPC)   2019-nCoV Assay

Figure 6   2019-nCoV Assay target result according to software note in the Review Results section with a yellow positive interpretation
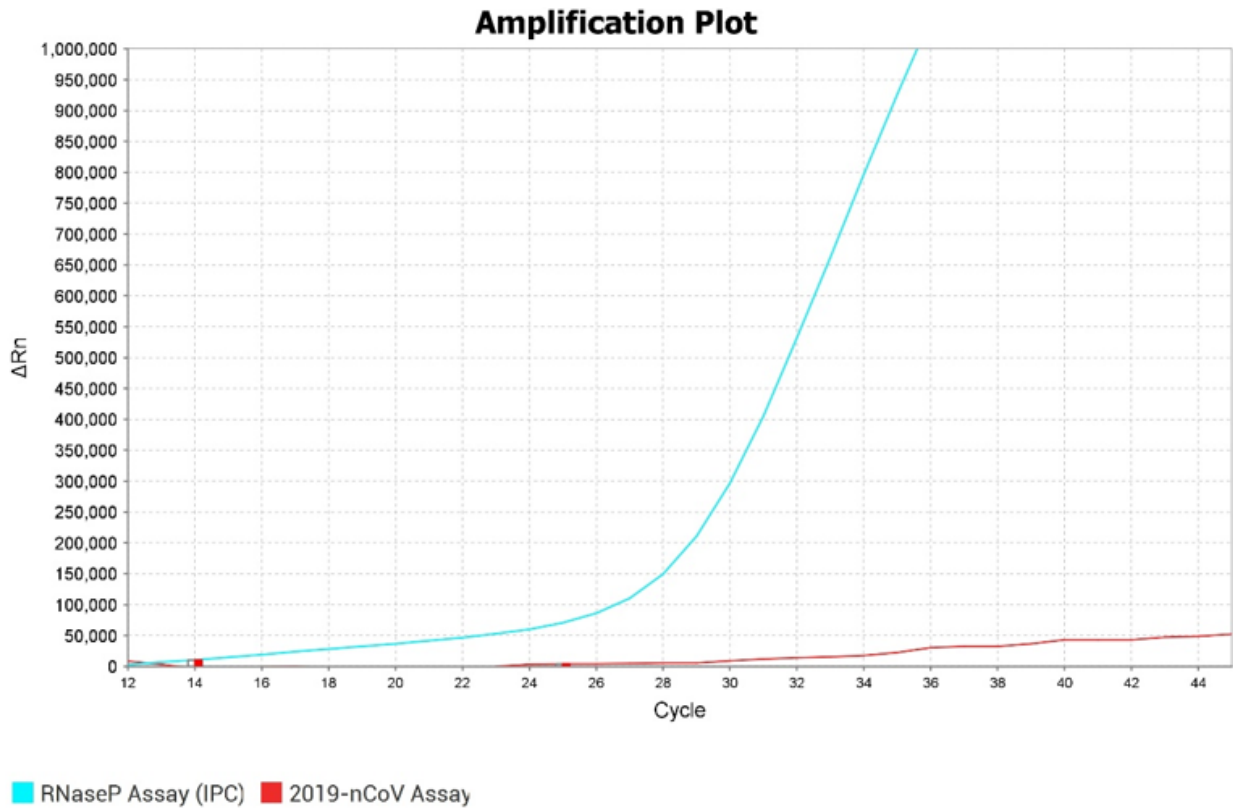
Figure 7 2019-nCoV Assay target result according to software note in the Review Results section with a green negative interpretation

7500 Software SDS v1.4.2 examples

RNaseP Assay (IPC) amplification plots are red, 2019-nCoV Assay amplification plots are green.
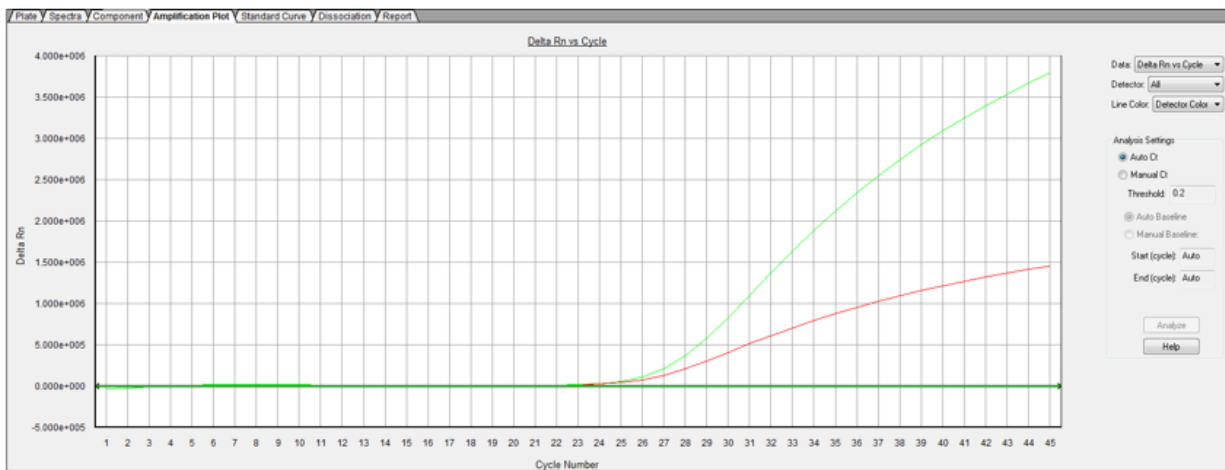

Figure 8 Positive amplification of 2019-nCoV Assay target (strong)
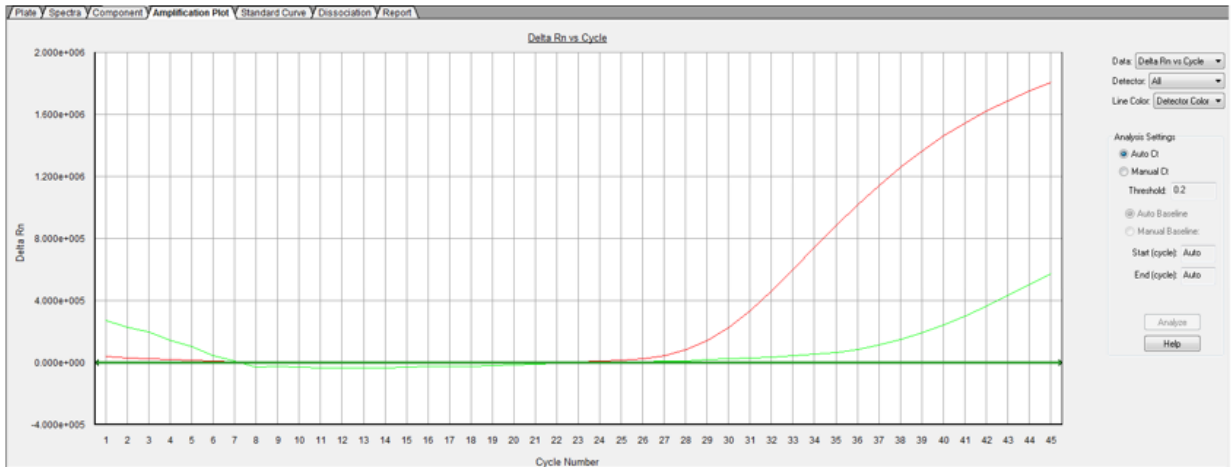
Figure 9   Positive amplification of 2019-nCoV Assay target (weaker)
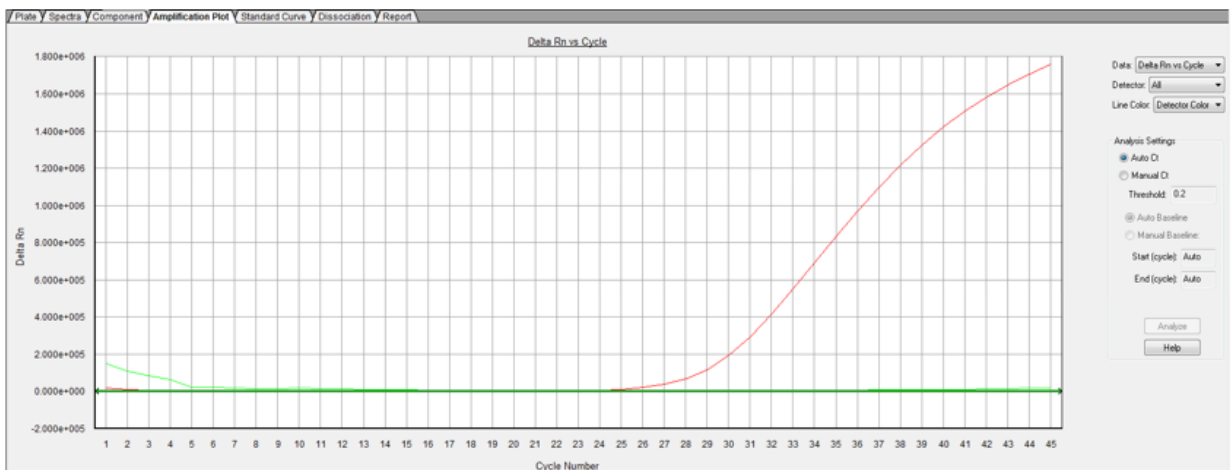

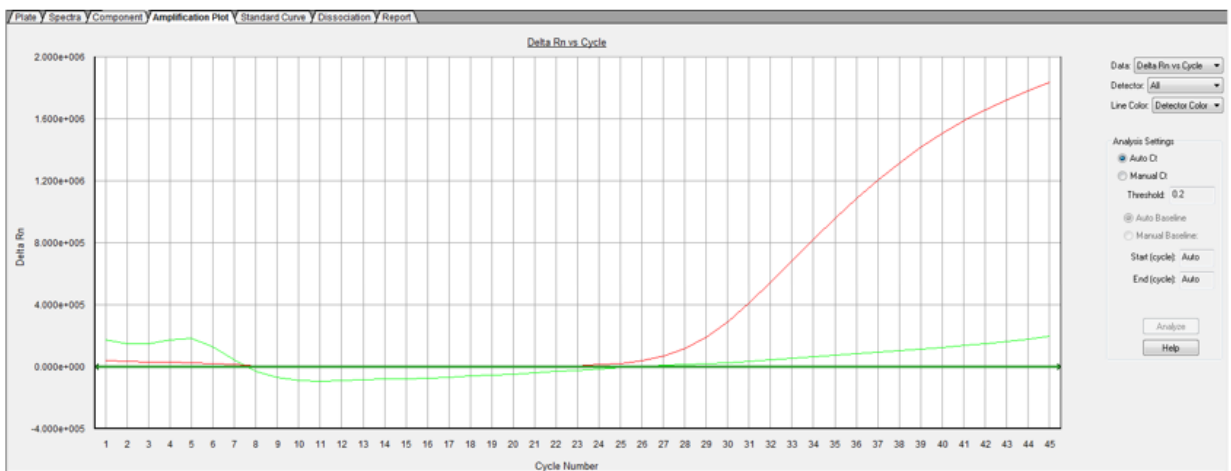Figure 10   Negative amplification of 2019-nCoV Assay target

Figure 11   Baseline anomaly for the 2019-nCoV Assay target: Result according to software note in the Review Results section
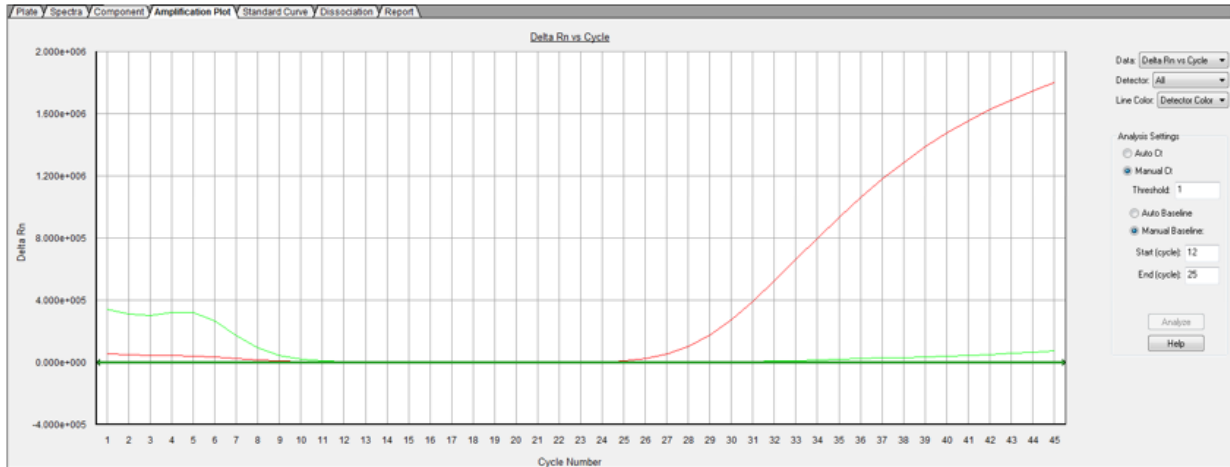
Figure 12   Baseline anomaly for the 2019-nCoV Assay target: Result according to software note in the Review Results section
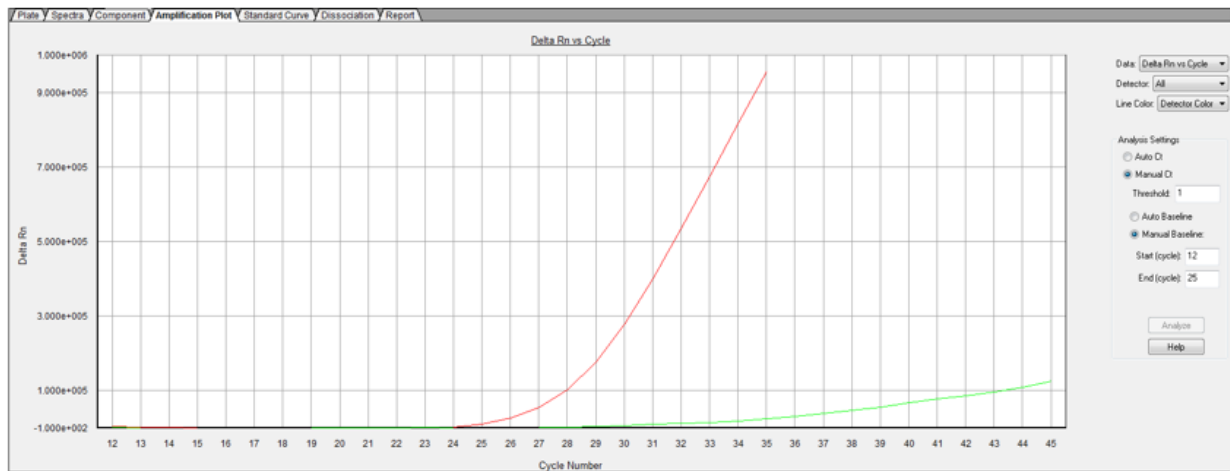

Figure 13   2019-nCoV Assay target result according to software note in the Review Results section with a yellow positive interpretation
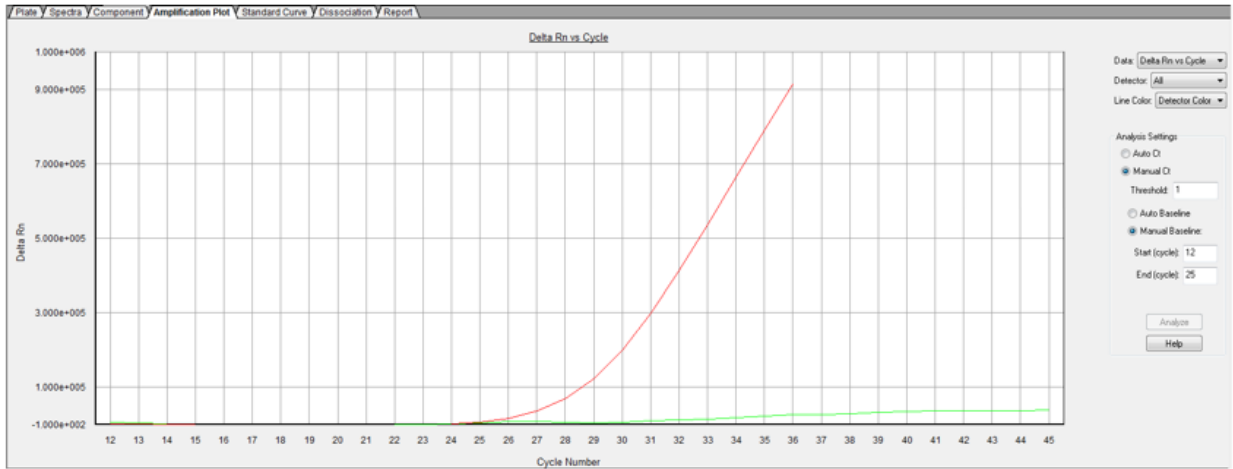
Figure 14 2019-nCoV Assay target result according to software note in the Review Results section with a green negative interpretation

C Safety

> ⚠ **WARNING! GENERAL SAFETY.** Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.
>
> · Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
> · Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

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

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

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

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

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

Documentation and support

Food Safety support

Website: **thermoscientific.com/foodmicro** or **thermofisher.com/foodsafety**

Support email:

- Europe, Middle East, Africa: **microbiology.techsupport.uk@thermofisher.com**
- North America: **microbiology@thermofisher.com**

Phone: Visit **thermofisher.com/support**, select the link for phone support, and select the appropriate country from the dropdown menu.

Related documentation

Document	Publication Number
TaqMan™ 2019-nCoV Control Kit v1 Product Information Sheet	MAN0019097
QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide	MAN0010407
Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide	4378657
Applied Biosystems™ 7500/7500 Fast Real-Time PCR System: Maintenance Guide	4387777

Reference

World Health Organization protocol on *Surface sampling of coronavirus disease (COVID-19): A practical "how to" protocol for health care and public health professionals*, Version: 1.1, February 2020 (**www.who.int**)