maccura

SARS-CoV-2 Fluorescent PCR Kit

Instructions for Use

REF

IVD For In-vitro Diagnostic (IVD) Use

For Use under the Emergency Use Authorization Only

RX Only

SARS-CoV-2 Fluorescent PCR Kit

For Use under the Emergency Use Authorization Only
For *In-vitro* Diagnostic (IVD) Use
RX Only

Instructions for Use, Revision 3.0

SARS-CoV-2 Fluorescent PCR Kit	**P/N: BUSGN7101109**	**32 tests**
SARS-CoV-2 Fluorescent PCR Kit	**P/N: BUSGN7102109**	**64 tests**
SARS-CoV-2 Fluorescent PCR Kit	**P/N: BUSGN7103109**	**96 tests**
Nucleic Acid Extraction Kit, Manual Version	**P/N: GN7102903**	**48 tests**
Nucleic Acid Extraction Kit, Fast Version	**P/N: GN7101909**	**32 tests**

Revision History

Revision	Date	Description
R 3.0	November 17, 2020	1. Add FDA SARS-CoV-2 Reference Panel Testing Summary with table 18 2. Change Table 18 to Table 19 3. Add "controls should only be used fresh. Avoid multiple freeze-thaw cycles" in "Assay Limitation"
R 2.0	May 16, 2020	1. Add QIAGEN QIAamp Viral RNA Mini Kit as an optional RNA extraction method 2. Update assay LOD. Add LOD, when QIAamp Viral RNA Mini Kit for RNA is used, for 500 copies/ml 3. Provide instructions for QIAamp Virus RNA Mini Kit 4. Delete Background section 5. New design for the cover page of this IFU
R 1.1	April 24, 2020	1. Add page number 2. Reformat to keep each table on the same page
R 1.0	April 15, 2020	New document

Content

Chapter 1 PRODUCT INFORMATION

Intended Use

The SARS-CoV-2 Fluorescent PCR Kit is a real-time RT-PCR test intended for the qualitative detection of SARS-CoV-2 viral nucleic acids in upper respiratory specimens (e.g., oropharyngeal swabs, nasopharyngeal swabs, nasal swabs, and mid-turbinate swabs) from individuals suspected of COVID-19 by their healthcare provider. The SARS-CoV-2 Fluorescent PCR Kit is for use only under Emergency Use Authorization (EUA) in US laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is needed to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The SARS-CoV-2 Fluorescent PCR Kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of reverse transcriptase-PCR and *in vitro* diagnostic procedures. The SARS- CoV-2 Fluorescent PCR Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

Product Description

The SARS-CoV-2 Fluorescent PCR Kit includes the assays and controls for a multiplexed real-time RT-PCR test for the qualitative detection of RNA from SARS-CoV-2 in upper respiratory specimens (e.g., nasopharyngeal swabs, oropharyngeal swabs, nasal swabs, and mid-turbinate swabs) from individuals suspected of COVID-19 by their healthcare providers. The SARS-CoV-2 Fluorescent PCR Kit Package includes the following components:

● SARS-CoV-2 Fluorescent PCR Kit - Multiplexed assays that contain three primer/probe sets specific to different SARS-CoV-2 genomic regions (ORF1ab, N and E genes) and primers/probes for the internal control.

- Controls
  - All controls, including the positive control, negative control, and internal control should go through RNA extraction and PCR amplification to monitor the extraction and PCR amplification processes.

- Internal Control - MS2 based pseudo-virus containing exogenous RNA sequence that serves as an internal process control for nucleic acid extraction and monitors for potential PCR inhibitors in patient specimens.
- Positive Control - MS2 based pseudo-virus containing ORF1ab, N, and E target gene fragments used to monitor extraction and PCR amplification processes.
- Negative Control is DEPC-treated water that is used to monitor non-specific amplification and contamination during the extraction and RT-PCR processes.

- And one of the following two extraction kits:
  - Nucleic Acid Extraction Kit, Manual Version
  - Nucleic Acid Extraction Kit, Fast Version

Contents and Storage

Component	Description	Storage	Amount Supplied		
			BUSGN7101109 (32 Tests)	BUSGN7102109 (64 Tests)	BUSGN7103109 (96 Tests)
qRT-PCR Reaction Mix	Multiplexed assay primers/probes for ORF1ab, N, and E genes	-30℃ to -10℃	544 μL×1	1088 μL×1	816 μL×2
qRT-PCR Enzyme Mix	Reverse transcriptase, Taq polymerase, Uracil-DNA-glycosylase	-30℃ to -10℃	96 μL×1	192 μL×1	288 μL×1
Negative Control	DEPC-treated water	-30℃ to -10℃	450 μL×1	900 μL×1	1350 μL×1
Positive Control	MS2 based pseudo-virus containing ORF1ab, N, and E target gene fragments ($5.0x10^3$ copies/mL)	-30℃ to -10℃	450 μL×1	900 μL×1	1350 μL×1
Internal Control	MS2 based pseudo-virus containing exogenous sequence	-30℃ to -10℃	64 μL×1	128 μL×1	192 μL×1

Table 1. SARS-CoV-2 Fluorescent PCR Kit

Table 2. Nucleic Acid Extraction Kit, Manual Version (Cat. No. GN7102903, 48 tests)

Component	Description	Storage	Amount Supplied (48 Tests)
Extraction reagent ①	Proteinase K	2°C to 8°C	500 μL×1
Extraction reagent ②	Lysis Buffer	2°C to 8°C	31 mL×1
Extraction reagent ③	Magnetic nanoparticles	2°C to 8°C	250 μL×1
Extraction reagent ④	Wash Buffer 1	2°C to 8°C	41 mL×1
Extraction reagent ⑤	Wash Buffer 2	2°C to 8°C	36 mL×1
Extraction reagent ⑥	Mineral oil	2°C to 8°C	5 mL×1

Extraction reagent ⑦	Elution Buffer	2°C to 8°C	1.8 mL×1

Table 3. Nucleic Acid Extraction Kit, Fast Version (Cat. No. GN7101909, 32 tests)

Component	Description	Storage	Amount Supplied (32 Tests)
Extraction reagent	Tris Hydrochloride, Triton X-100, Sodium hydroxide, Carrier RNA, DEPC treated water	-30°C to -10°C	1.8 mL×1

Required Materials - Not Supplied

- Vortex mixer
- Microcentrifuge
- DynaMag-2 Magnet (ThermoFisher, Cat. No. 12321D)
- Micropipettes (2 or 10 µL, 200 µL and 1000 µL)
- Aerosol barrier pipette tips
- Racks for 1.5mL microcentrifuge tubes
- Disposable powder-free gloves and surgical gowns
- 1.5 mL microcentrifuge tubes (DNase/RNase free)
- 0.2 mL PCR reaction plates, or 0.2 mL Flat PCR tube 8-cap strips
- 7500 Real-Time PCR Systems with v2.3 software (Applied Biosystems)
- Phosphate buffer containing 1g/L proteinase K
- Viral Transport Medium (VTM), or equivalent

Optional Materials - Not Supplied

- QIAGEN QIAamp Viral RNA Mini Kit (QIAGEN, Cat. No. 52904 / 52906)

Warnings and Precautions

The SARS-CoV-2 Fluorescent PCR Kit workflow should be performed by qualified and trained staff to avoid the risk of erroneous results. Use separate areas for the preparation of patient samples and controls to prevent false positive results. Samples and reagents must be handled under a laminar airflow hood or a biological safety cabinet.

- The assay is for *in vitro* diagnostic (IVD) use under the FDA Emergency Use Authorization Only.
- For prescription use only.
- Follow standard precautions. All patient specimens and positive controls should be considered infectious and/or biohazardous and handled accordingly with safe laboratory procedures.
- Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.

- Always use pipette tips with aerosol barriers. Tips that are used must be sterile and free from DNases and RNases.
- Modifications to assay reagents, assay protocol, or instrumentation are not permitted, and are in violation of the product Emergency Use Authorization.
- Handle all specimens as of infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2:

https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html.

- Specimen processing should be performed in accordance with national biological safety regulations.
- Dispose of waste in compliance with the local, state, and federal regulations.
- Reagents must be stored and handled as specified in Tables 1-3.
- Do not use the kit after the indicated expiry date.
- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

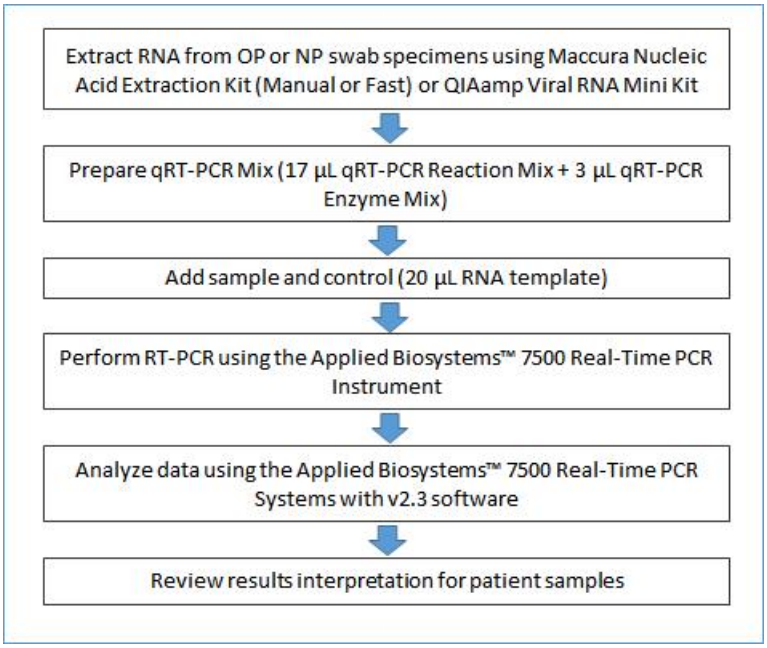
Specimen Collection and Preparation

Follow proper standard operating procedures to collect oropharyngeal and nasopharyngeal swabs.

It is recommended to test the specimen as soon as possible, otherwise specimens should be stored at <-70°C for up to 15 days. Avoid multiple freeze-thaw cycles.

Chapter 2 ASSAY PROCEDURE

Workflow Overview



The workflow begins with nucleic acids extraction from oropharyngeal (OP) swabs or nasopharyngeal swab specimens. Nucleic acid extraction is performed using ONE of three extraction methods: Maccura Nucleic Acid Extraction Kit Fast version OR Manual

(provided) or QIAGEN QIAamp Viral RNA Mini Kit (not provided).

The Fast version extraction kit requires 200 µL sample input and yields approximately 50 µL eluent; The Manual extraction kit requires 200 µL sample input and yields approximately 35 µL nucleic acid eluent. The Qiagen kit requires 140 µL sample and yields about 80 µL nucleic acid eluent.

The purified nucleic acid is reverse transcribed and amplified in a combined reaction process. 20 µL RNA template is added into 20 µL qRT-PCR mix which is prepared from qRT-PCR Reaction Mix (17 µL) and qRT-PCR Enzyme Mix (3 µL). The RT-PCR reaction is performed on Applied Biosystems™ 7500 Real-Time PCR instrument (v2.3). In the RT-PCR reaction, the viral RNA is first converted into cDNA in a reverse transcription reaction. The cDNA is then amplified by the target specific forward and reverse primers in the PCR reaction. During the extension phase of the PCR cycles, the target specific fluorescent probe is degraded, generating a fluorescent signal. The fluorescence intensity signal is collected at each PCR cycle by Applied Biosystems™ 7500 Real-Time PCR Systems. The fluorescent signal plot against PCR cycles is analyzed to yield test results.

Extract RNA with a Validated Nucleic Acid Extraction Kit

Note: Extraction should only be performed using the Maccura Manual Version (Cat. No. GN7102903) or Maccura Fast Version (Cat. No. GN7101909) or QIAGEN QIAamp Viral RNA Mini Kit (Cat. No. 52904/52906).

Remove the extraction reagent components and the Internal Control from the SARS-CoV-2 Fluorescent PCR Kit and place on the bench and allow to equilibrate to ambient temperature. Vortex briefly and move to the sample preparation area.

Nucleic Acids Extraction with Maccura Manual Version (Cat. No. GN7102903)

This step requires a magnetic separator that is not provided. The DynaMag-2 Magnet (ThermoFisher, Cat. No. 12321D) is recommended.

1. For each patient specimen, add 10 µL of Extraction reagent ① to a sterile 1.5 mL or 2.0 mL microcentrifuge tube.

2. Add 200 µL of patient specimen to the microcentrifuge tube, close the lid, vortex for 5 seconds, and spin down briefly with a microcentrifuge.

3. Prepare the Magnetic Beads Mix. Use the following table to calculate the volume of Magnetic Beads Mix to prepare, which is dependent on the number of the tests you plan to run:

Table 4. Preparation of Magnetic Beads Mix.

Name	Reagent	Volume/test	Number of tests
Magnetic beads Mix	Extraction reagent ③	5µL	N=n+2

	Internal Control	2μL	

Note: The number of tests (patient samples) is N = n + 2, where n is the number of samples to be tested, and 2 accounts for the Negative Control and Positive Control. The actual volume of Magnetic Beads Mix needed will be N x 5μL and the actual volume of internal control is N x 2μL. It is recommended that extra magnetic bead mix is prepared to account for loss during pipetting of mix to individual centrifuge tubes.

4. Add 600μL of Extraction reagent ② and 7μL of Magnetic Beads Mix to each microcentrifuge tube, close the lid, vortex for 10 seconds, and incubate for 10 minutes at ambient temperature.

5. Spin down briefly and place the tube on the magnetic separator, let stand for 3 minutes, then slowly pipette out and discard the supernatant (avoid touching the brown sediment attached to the wall of the tube).

6. Add 800μL of Extraction reagent ④ to each tube, close the lid, vortex for 5 seconds, spin down briefly and place the tube on the magnetic separator, let stand for 3 minutes, then slowly pipette out and discard the supernatant (avoid touching the brown sediments attached to the wall of the tube).

7. Add 700μL of Extraction reagent ⑤ and 100μL of Extraction reagent ⑥ to each tube, close the lid, vortex for 5 seconds, spin down briefly and place the tube on the magnetic separator again.

8. After 3 minutes, the supernatant should contain two visible layers. Insert the pipette tip into the bottom of supernatant layer and slowly pipette both layers out and discard supernatant. Then vortex microcentrifuge tube that contains pellet for 30 seconds and place the tube on the magnetic separator again.

9. After 3 minutes, pipette out and discard any residual supernatant.

10. Add 35μL of Extraction reagent ⑦ to each tube, vortex to resuspend brown sediments completely in the solution, then incubate at 60℃ for 10 minutes.

11. Spin down briefly and then place the tube on the magnetic separator for 3 minutes. Next, pipette the supernatant to a new microcentrifuge tube and stored on ice until testing. If the sample cannot be tested immediately, it should be stored at -20℃ or below.

Nucleic Acid Extraction with Maccura Fast Version (Cat. No. GN7101909) Kit

1. Transfer 200μL of specimen and 2μL Internal Control to a 1.5mL microcentrifuge tube, centrifuge at ambient temperature for 10min at 13,000 x g.

2. After centrifugation, remove supernatant by pipetting (Avoid touching the sediment, which may or may not be readily visible).

3. Add 50μL of Extraction Reagent to the pellet, vortex for 10 seconds, and incubate for 10 minutes at ambient temperature. The mix is put on ice and ready for testing. If the sample cannot be tested immediately, it should be stored at -20℃ or below.

Optional Nucleic Acids Extraction with QIAamp Viral RNA Mini Kit (QIAGEN, Cat. No. 52904 / 52906) Kit)

Please refer to the QIAamp Viral RNA Mini Handbook for instructions. Please add 2μL Internal Control to a 1.5mL microcentrifuge tube before the first step.

The sample input and eluent volumes are as shown in Table 5.

Table 5. QIAamp Viral RNA Mini Kit Sample Input and Eluent Volumes

Extraction Reagent	Volume of Sample	Volume of elution buffer
QIAamp Viral RNA Mini Kit	140 μL	80 μL

Perform RT-PCR

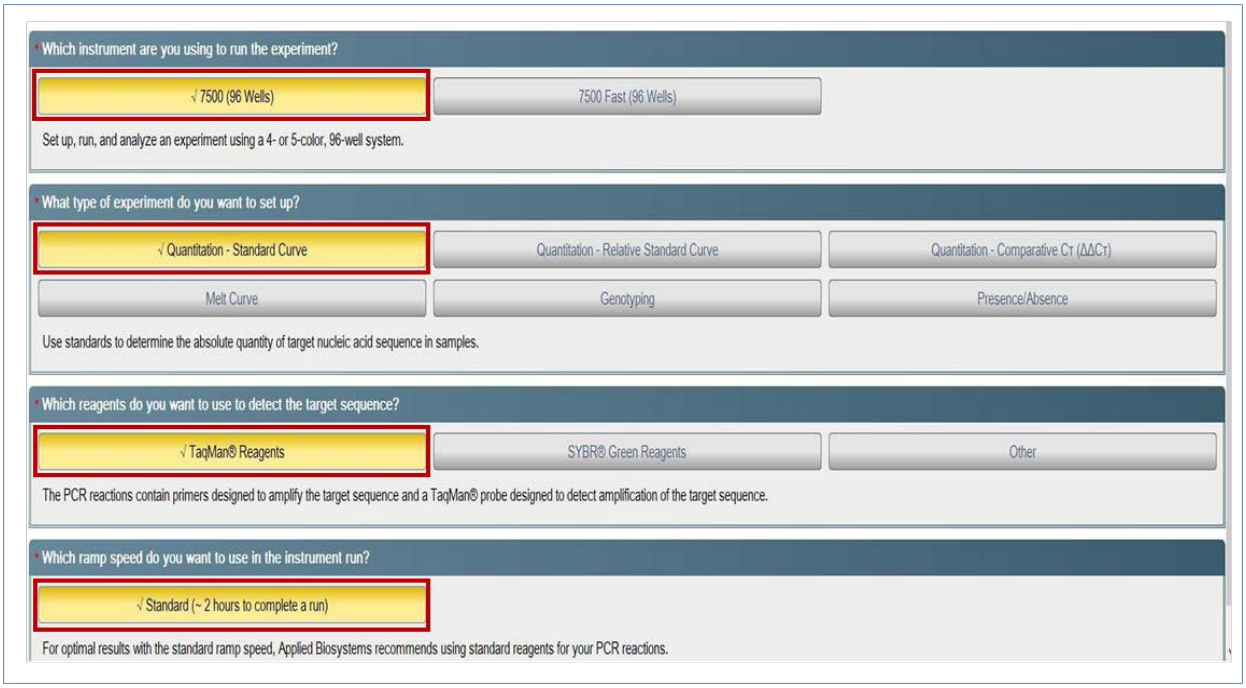
IMPORTANT!

1. The experiment workflow is recommended to be carried out in segmented PCR laboratories.
2. To prevent contamination, prepare reagents in a PCR workstation or equivalent amplicon-free area. Always use aerosol barrier pipette tips when processing samples and positive controls. Do not reuse the pipette.
3. Run PCR within two hours of preparation.
4. Include at least one Positive Control and one Negative Control per run.

Set up the Applied Biosystems™ 7500 Real-Time PCR Instrument (in the thermal-cycling area)

- Set up PCR Machine
  1. Ensure that the Applied Biosystems™ 7500 Real-Time PCR Instrument is set up before you prepare sample for PCR run
  2. Ensure the correct PCR amplification protocol is used
- Passive reference: None

- Assay is set up as follows:



- Set up fluorescence channels:

Table 6. Fluorescence channel setting

Reporter Dye	FAM	VIC / HEX	ROX	CY5
Quencher	None	None	None	None
Detector	ORF1ab	Internal Control	E gene	N gene

- Set up the plate layout by assigning a unique sample name to each well.
- Set and confirm the thermal-cycling protocol:

Table 7. RT-PCT protocol

Step		**Temperature**	**Time**	**Cycles**
1	Reverse transcription	55℃	15min	1
2	Taq polymerase activation, pre-denaturing	95℃	2min	1
3	Denaturation	95℃	15sec	40
	Annealing, extension, fluorescence acquisition	58℃	35sec	
4	Instrument cooling	40℃	10sec	1

- Save file, ready to run.

Reagent preparation (in reagent preparation area)

1. Take out all reagent components and place them on bench to equilibrate until they

reach ambient temperature, vortex and then spin briefly to collect the reagent.

2. Prepare qRT-PCR Mix according to the following table:

Table 8. Preparation of qRT-PCR Mix

Name	Reagent	Volume/test	Number of tests
qRT-PCR Mix	qRT-PCR Reaction Mix	17μL	N=n+2
	qRT-PCR Enzyme Mix	3μL	

Note: The number of tests is N = n + 2, where n is the number of samples to be tested, and 2 accounts for the negative control and the positive control. The actual volume of qRT-PCR Reaction Mix will be N X 17μL and the actual volume of qRT-PCR Enzyme Mix is N X 3μL. It is recommended that extra qRT-PCR Mix is prepared to account for loss that may occur during pipetting of mix to individual tubes/plate wells.

Run PCR

For each specimen, negative and positive control, add 20 μL RNA template (nucleic acid extracted from Negative Control, Positive Control and specimen) to a separate PCR reaction tube or a well on the PCR reaction plate containing qRT-PCR Mix. Final volume should be 40 μL/test.

Close the lid or seal the plate immediately to avoid contamination. Spin down briefly and add plate to the Applied Biosystems 7500 Real-time PCR Instrument. Click run button to start reaction.

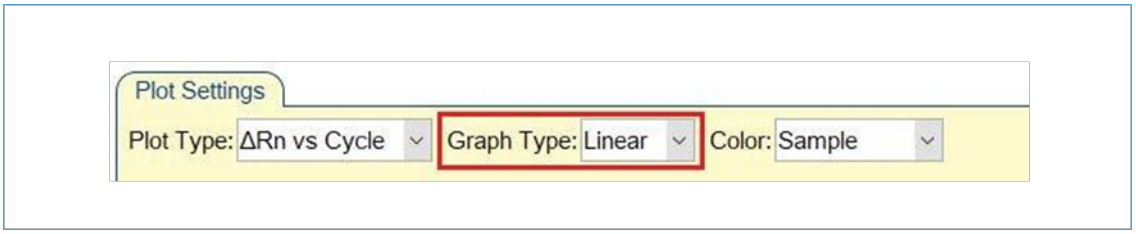
Chapter 3 DATA ANALYSIS AND RESULTS

Setting data analysis parameters

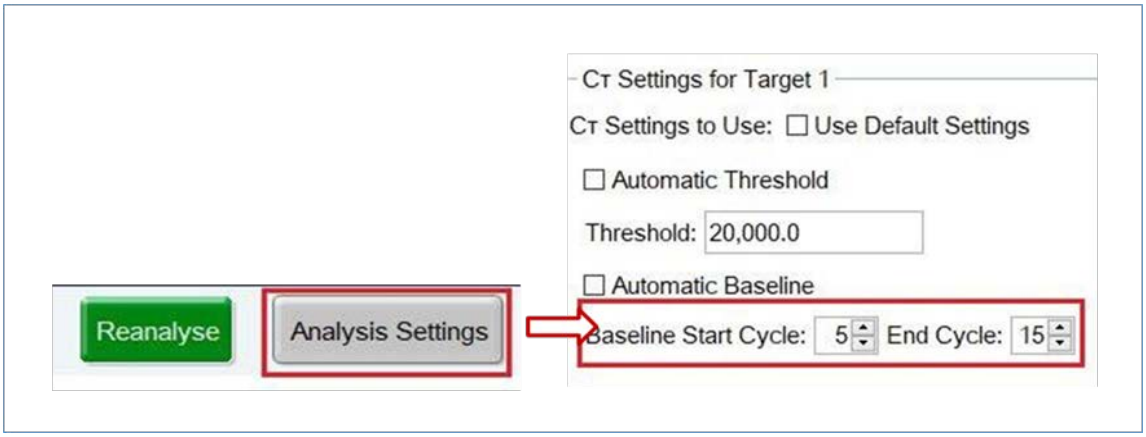
Please save the result immediately after a run is completed. Conduct the following adjustment after each run.

1. Set the Baseline

- In the Analysis screen, select **Linear** for Graph Type:

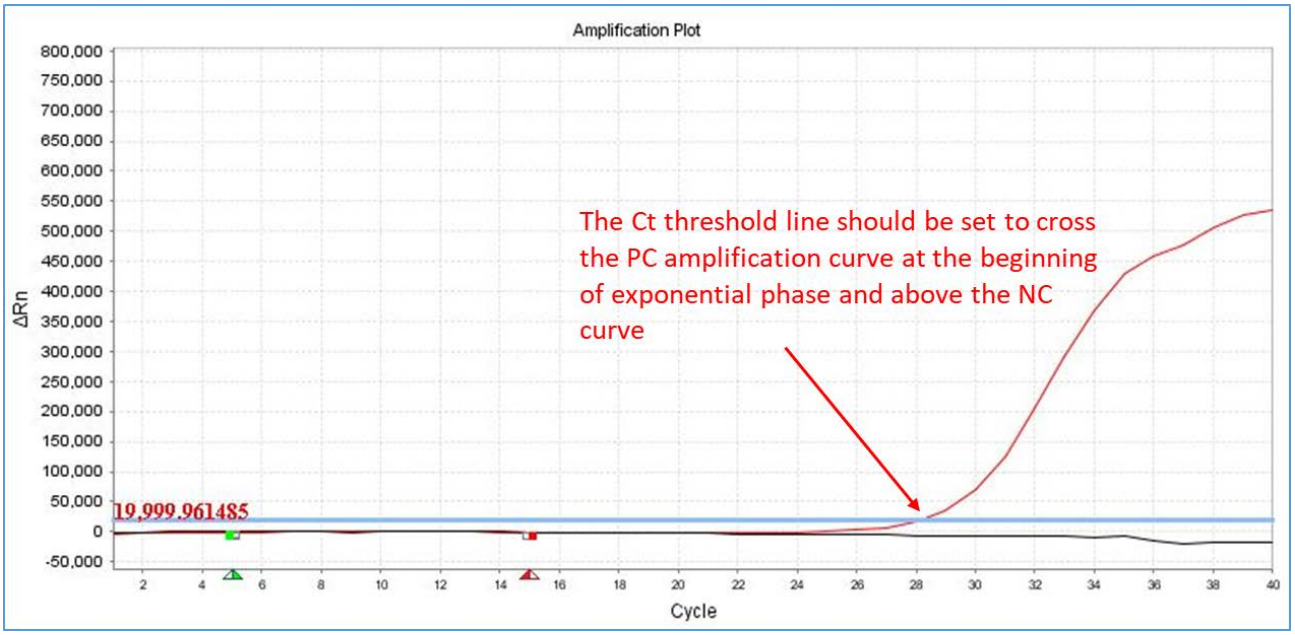


- Set the baseline for each channel, starting point at cycle 5 and ending point at cycle 15 in **Analysis Setting-Ct Setting** window:



2. Set the Ct Threshold

- Manually set the Ct threshold for each channel after each run as follows:
  - o The Ct threshold line should be set just above the NC curve
  - o The Ct threshold should bet set to cross the PC amplification curve at the beginning of exponential phase
- Click “Analyze” and view the results on the Analysis screen.



3. Export data

Select the wells containing patient samples and controls and export the Excel file (XLSX) to a folder.

Analyze the Data

Quality control

The results of any test should meet the following QC requirements, otherwise the test

results are invalid and patient specimen RNA should be re-extracted and re-tested.

Table 9. Acceptance Criteria for Assay Controls

Control Name	FAM	VIC / HEX (Internal Control)	ROX	CY5
Positive Control	≤32	≤38	≤32	≤32
Negative Control	>38 or no Ct value	≤38	>37 or no Ct value	>38 or no Ct value

Ct value interpretation

The Ct result value for each fluorescence channel is called positive or negative based on the following criteria by operators manually after each run:

Table 10. Ct value interpretation

Fluorescence channel	Negative (-)	Positive (+)
FAM channel (ORF1ab)	＞38 or no Ct value	≤38
ROX channel（E gene）	＞37 or no Ct value	≤37
Cy5 channel（N gene）	＞38 or no Ct value	≤38
HEX or VIC channel（Internal Control）	＞38 or no Ct value	≤38

A valid test is a test where the Internal Control result is positive (+), or the internal control is negative (-), but at least one of the three target channels (i.e., FAM, ROX, or Cy5) is positive (+).

Result interpretation for patient samples

Based on the Ct value, the test results are interpreted manually by operators using the following criteria:

Table 11. Result interpretation for patient samples

Test result			Result interpretation*
ORF1ab	N gene/E gene	IC	
Positive (+)	Any	Any	SARS-CoV-2 Positive
Negative (-)	One Positive (+) or both Positive	Positive	SARS-CoV-2 Negative**
Negative (-)	Both Negative	Positive	SARS-CoV-2 Negative
Negative (-)	Both Negative	Negative	Invalid, repeat test

Note: *For diagnostic purposes, results should always be used in combination with other medical findings, such as symptoms, results of other tests, clinical impressions, etc. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.

** ORF1ab(-) N and / E gene (+) result could be caused by 1) a sample at concentrations near or below the limit of detection of the test, 2) a mutation in the ORF1ab target region in the oligo binding sites, or 3) infection with some other human coronavirus (e.g., SARS-CoV or some other human coronavirus previously unknown to infect humans), or 4) other factors.

Assay Limitations

- This device may not be able to differentiate newly emerging SARS-CoV-2 subtypes.

- All results from this and other tests must be considered in conjunction with the clinical history, epidemiological data and other data available to the clinician evaluating the patient.

- The detection of pathogen nucleic acids is dependent upon proper specimen collection, handling, transportation, storage and preparation (including extraction). Failure to observe proper procedures in any one of these steps can lead to incorrect results. There is a risk of false negative values resulting from improperly collected, transported, or handled specimens.

- The primer/probe sequences used in this product exhibit 80-100% homology with SARS-coronavirus. Therefore, the SARS-CoV-2 Fluorescent PCR Kit may cross-react with SARS-coronavirus.

- The performance of the SARS-CoV-2 Fluorescent PCR Kit was established using nasopharyngeal swabs and oropharyngeal swabs, only. Nasal swabs and mid-turbinate nasal swabs are also considered acceptable specimen types for use with the SARS-CoV-2 Fluorescent PCR Kit but performance has not been established. Testing of nasal and mid-turbinate nasal swab (self-collected or collected by a healthcare provider) is limited to patients with symptoms of COVID-19.

- Controls should only be used fresh. Avoid multiple freeze-thaw cycles.

- There is a risk of false positive values resulting from cross-contamination by target organisms, their nucleic acids or amplified product, or from non-specific signals in the assay.

- There is a risk of false negative values due to the presence of sequence variants in the pathogen targets of the assay, procedural errors, amplification inhibitors in specimens, or inadequate numbers of organisms for amplification.

- Analyte targets (viral sequences) may persist in vivo, independent of virus viability. Detection of analyte target(s) does not imply that the corresponding virus(es) are infectious or are the causative agents for clinical symptoms.

- This test is a qualitative test and does not provide the quantitative value of detected organisms present.

- The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

- This device has been evaluated for use with human specimen material only.

- The performance of this device has not been evaluated for patients without signs and symptoms of infection.

- The performance of this device has not been evaluated for monitoring treatment of infection.

- The performance of this assay was not established in immunocompromised patients.

- The performance for some viruses and subtypes may vary depending on the prevalence and population tested.

- The performance of this test has not been established for screening of blood or blood products.

Chapter 4 CONDITIONS OF AUTHORIZATION FOR THE LABORATORY

The SARS-CoV-2 Fluorescent PCR Kit Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd

However, to assist clinical laboratories using the SARS-CoV-2 Fluorescent PCR Kit the relevant Conditions of Authorization are listed below:

A. Authorized laboratories[1] using your product will include with result reports of your product, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

B. Authorized laboratories using your product will use your product as outlined in the Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.

C. Authorized laboratories that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating testing.

D. Authorized laboratories using your product will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E. Authorized laboratories will collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Maccura Biotechnology (USA) LLC (via email: support-USA@ext.maccura.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.

F. All laboratory personnel using your product must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling.

G. Maccura Biotechnology (USA) LLC, authorized distributors, and authorized laboratories using your product will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1]The letter of authorization refers to, “United States (U. S.) laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests” as “authorized laboratories.”

Chapter 5 PERFORMANCE CHARACTERISTICS

Limit of Detection (LoD)

The LoD study established the lowest SARS-CoV-2 viral concentration (copies/mL) that can be detected by the SARS-CoV-2 Fluorescent PCR Kit at least 95% of the time. The LoD of the SARS-CoV-2 Fluorescent PCR Kit was initially estimated by spiking SARS-CoV-2 RNA into leftover, negative OP specimens at concentrations ranging from $1.0x10^9$ copies/mL to $1.0x10^2$ copies/mL. Three samples were prepared at each concentration. The samples were extracted using the provided Maccura Nucleic Acid Extraction Kit (Manual) and tested with SARS-CoV-2 Fluorescent PCR Kit. Based on this study, the LoD was estimated to be $1.0x10^3$ copies/mL.

To confirm the estimated LoD, contrived samples were prepared by spiking viral whole genomic RNA into leftover, negative OP specimens at the following concentrations near the estimated LoD: $2.0x10^3$ copies/mL to $5.0x10^2$ copies/mL. Twenty (20) contrived samples were generated at each concentration. Contrived samples were each independently extracted using the Maccura Nucleic Acid Extraction Kit (Manual) (Table 12), Maccura Nucleic Acid Extraction Kit (Fast) (Table 13), and QIAamp Viral RNA Mini Kit (QIAGEN) (Table 14) and then tested with the SARS-CoV-2 Fluorescent PCR Kit. Results demonstrated that the LoD for the Manual and Fast Maccura Nucleic Acid Extraction Kits was $1.0x10^3$ copies/mL and the LoD for QIAamp Viral RNA Mini Kit was $5.0X10^2$ copies/mL.

Table 12. LoD Confirmation - RNA Extracted with Maccura Nucleic Acid Extraction Kit (Manual)

Concentration (copies/mL)	Rep#	SARS-CoV-2 ORF1ab		SARS-CoV-2 E gene		SARS-CoV-2 N gene		IC		Overall SARS-CoV-2 Result	Overall % Positivity
		Ct	Result	Ct	Result	Ct	Result	Ct	Result		
$2.0X10^3$	1	31.45	Positive	33.73	Positive	31.90	Positive	33.99	Positive	Positive	100%
	2	30.33	Positive	32.11	Positive	31.76	Positive	33.85	Positive	Positive	
	3	31.22	Positive	33.59	Positive	31.83	Positive	33.15	Positive	Positive	
	4	30.27	Positive	32.23	Positive	30.94	Positive	32.50	Positive	Positive	
	5	31.75	Positive	33.59	Positive	31.90	Positive	33.30	Positive	Positive	
	6	30.55	Positive	32.04	Positive	30.62	Positive	33.65	Positive	Positive	
	7	31.19	Positive	33.71	Positive	31.56	Positive	32.83	Positive	Positive	
	8	30.52	Positive	32.93	Positive	31.65	Positive	33.45	Positive	Positive	
	9	30.88	Positive	32.09	Positive	31.16	Positive	32.32	Positive	Positive	
	10	30.45	Positive	33.66	Positive	30.22	Positive	32.84	Positive	Positive	
	11	31.19	Positive	33.64	Positive	30.37	Positive	33.63	Positive	Positive	
	12	31.22	Positive	32.21	Positive	30.20	Positive	32.25	Positive	Positive	
	13	31.42	Positive	33.41	Positive	30.50	Positive	33.39	Positive	Positive	
	14	30.18	Positive	32.74	Positive	30.61	Positive	33.80	Positive	Positive	
	15	30.79	Positive	33.52	Positive	30.04	Positive	33.63	Positive	Positive	
	16	31.92	Positive	33.19	Positive	30.12	Positive	32.27	Positive	Positive	
	17	30.63	Positive	32.07	Positive	31.23	Positive	33.79	Positive	Positive	
	18	30.40	Positive	32.62	Positive	30.57	Positive	32.02	Positive	Positive	
	19	30.04	Positive	32.88	Positive	31.37	Positive	33.34	Positive	Positive	
	20	30.47	Positive	33.11	Positive	30.90	Positive	32.77	Positive	Positive	

$1.0X10^3$	1	33.58	Positive	33.82	Positive	32.93	Positive	31.03	Positive	Positive	100%
	2	34.43	Positive	34.73	Positive	32.36	Positive	32.41	Positive	Positive	
	3	33.97	Positive	34.59	Positive	33.81	Positive	32.79	Positive	Positive	
	4	33.47	Positive	33.08	Positive	33.50	Positive	32.16	Positive	Positive	
	5	33.41	Positive	33.90	Positive	32.78	Positive	32.10	Positive	Positive	
	6	33.99	Positive	34.69	Positive	32.52	Positive	32.55	Positive	Positive	
	7	33.01	Positive	34.08	Positive	32.02	Positive	32.70	Positive	Positive	
	8	33.43	Positive	33.73	Positive	33.39	Positive	31.33	Positive	Positive	
	9	34.88	Positive	34.80	Positive	32.12	Positive	32.66	Positive	Positive	
	10	34.85	Positive	34.97	Positive	33.80	Positive	31.44	Positive	Positive	
	11	34.30	Positive	34.98	Positive	32.40	Positive	32.32	Positive	Positive	
	12	33.21	Positive	33.00	Positive	32.48	Positive	31.40	Positive	Positive	
	13	33.66	Positive	34.77	Positive	33.74	Positive	31.18	Positive	Positive	
	14	34.43	Positive	34.93	Positive	33.81	Positive	31.20	Positive	Positive	
	15	33.93	Positive	34.67	Positive	32.44	Positive	31.17	Positive	Positive	
	16	34.65	Positive	34.84	Positive	33.17	Positive	31.33	Positive	Positive	
	17	34.63	Positive	34.03	Positive	32.72	Positive	32.72	Positive	Positive	
	18	33.16	Positive	33.28	Positive	33.53	Positive	31.16	Positive	Positive	
	19	34.12	Positive	33.49	Positive	32.67	Positive	31.39	Positive	Positive	
	20	33.52	Positive	33.14	Positive	33.54	Positive	31.49	Positive	Positive	
$5.0X10^2$	1	36.27	Positive	36.73	Positive	35.29	Positive	31.61	Positive	Positive	55%
	2	36.51	Positive	36.89	Positive	35.73	Positive	31.84	Positive	Positive	
	3	38.37	Negative	37.9	Negative	35.07	Positive	32.75	Positive	Negative	
	4	36.38	Positive	36.21	Positive	35.66	Positive	31.14	Positive	Positive	
	5	37.27	Positive	36.84	Positive	35.21	Positive	31.09	Positive	Positive	
	6	NoCt	Negative	38.76	Negative	34.17	Positive	31.34	Positive	Negative	
	7	36.80	Positive	36.70	Positive	35.44	Positive	31.35	Positive	Positive	
	8	36.92	Positive	37.09	Negative	34.90	Positive	31.11	Positive	Positive	
	9	38.57	Negative	37.71	Negative	34.38	Positive	31.58	Positive	Negative	
	10	36.07	Positive	36.13	Positive	35.97	Positive	31.39	Positive	Positive	
	11	38.99	Negative	36.05	Positive	34.72	Positive	32.70	Positive	Negative	
	12	37.33	Positive	37.26	Negative	34.30	Positive	32.03	Positive	Positive	
	13	36.73	Positive	36.27	Positive	34.88	Positive	32.38	Positive	Positive	
	14	NoCt	Negative	37.10	Negative	34.30	Positive	32.74	Positive	Negative	
	15	37.49	Positive	36.56	Positive	35.94	Positive	31.98	Positive	Positive	
	16	38.66	Negative	37.35	Negative	34.80	Positive	31.96	Positive	Negative	
	17	36.07	Positive	37.65	Negative	35.81	Positive	31.35	Positive	Positive	
	18	NoCt	Negative	38.02	Negative	34.98	Positive	31.36	Positive	Negative	
	19	NoCt	Negative	37.85	Negative	35.25	Positive	32.81	Positive	Negative	
	20	NoCt	Negative	37.36	Negative	36.00	Positive	32.66	Positive	Negative	

Table 13. LoD Confirmation - RNA Extracted with Maccura Nucleic Acid Extraction Kit (Fast)

Concentration (copies/mL)	Rep#	SARS-CoV-2 ORF1ab		SARS-CoV-2 E gene		SARS-CoV-2 N gene		IC		Overall SARS-CoV-2 Result	Overall % Positivity
		Ct	Result	Ct	Result	Ct	Result	Ct	Result		
$2.0X10^3$	1	30.94	Positive	32.71	Positive	32.58	Positive	33.23	Positive	Positive	100%
	2	28.89	Positive	33.5	Positive	33.69	Positive	33.97	Positive	Positive	
	3	31.28	Positive	33.32	Positive	32.72	Positive	34.35	Positive	Positive	

	4	31.11	Positive	32.46	Positive	33.16	Positive	35.31	Positive	Positive	
	5	30.76	Positive	31.7	Positive	33.53	Positive	34.91	Positive	Positive	
	6	30.59	Positive	32.59	Positive	32.72	Positive	35.38	Positive	Positive	
	7	30.98	Positive	32.17	Positive	33.01	Positive	34.25	Positive	Positive	
	8	30.65	Positive	32.00	Positive	32.29	Positive	34.19	Positive	Positive	
	9	30.43	Positive	32.93	Positive	33.58	Positive	33.56	Positive	Positive	
	10	30.21	Positive	32.43	Positive	32.88	Positive	34.44	Positive	Positive	
	11	30.61	Positive	32.46	Positive	33.31	Positive	34.83	Positive	Positive	
	12	32.28	Positive	33.57	Positive	34.26	Positive	35.85	Positive	Positive	
	13	32.71	Positive	33.00	Positive	33.36	Positive	35.38	Positive	Positive	
	14	30.80	Positive	32.92	Positive	33.66	Positive	35.09	Positive	Positive	
	15	29.63	Positive	30.64	Positive	30.38	Positive	34.86	Positive	Positive	
	16	31.71	Positive	32.63	Positive	32.77	Positive	35.38	Positive	Positive	
	17	31.39	Positive	32.79	Positive	32.13	Positive	35.92	Positive	Positive	
	18	32.16	Positive	33.4	Positive	32.73	Positive	35.14	Positive	Positive	
	19	31.57	Positive	33.16	Positive	33.01	Positive	35.59	Positive	Positive	
	20	33.03	Positive	35.39	Positive	34.86	Positive	35.50	Positive	Positive	
$1.0X10^3$	1	33.94	Positive	35.11	Positive	35.17	Positive	35.90	Positive	Positive	100%
	2	34.53	Positive	35.26	Positive	34.75	Positive	36.25	Positive	Positive	
	3	35.75	Positive	NoCt	Negative	34.33	Positive	36.06	Positive	Positive	
	4	32.03	Positive	33.54	Positive	34.85	Positive	36.48	Positive	Positive	
	5	32.95	Positive	35.80	Positive	37.79	Positive	34.92	Positive	Positive	
	6	33.19	Positive	33.38	Positive	33.9	Positive	35.18	Positive	Positive	
	7	33.27	Positive	36.07	Positive	32.95	Positive	36.31	Positive	Positive	
	8	32.46	Positive	33.72	Positive	33.57	Positive	35.20	Positive	Positive	
	9	33.58	Positive	33.64	Positive	33.92	Positive	34.91	Positive	Positive	
	10	31.79	Positive	32.88	Positive	33.54	Positive	35.93	Positive	Positive	
	11	33.32	Positive	33.54	Positive	34.69	Positive	36.39	Positive	Positive	
	12	32.95	Positive	34.01	Positive	33.79	Positive	35.75	Positive	Positive	
	13	30.23	Positive	32.62	Positive	35.56	Positive	32.45	Positive	Positive	
	14	32.47	Positive	34.06	Positive	34.93	Positive	34.05	Positive	Positive	
	15	32.57	Positive	33.80	Positive	33.92	Positive	33.79	Positive	Positive	
	16	31.18	Positive	32.48	Positive	32.97	Positive	34.89	Positive	Positive	
	17	33.93	Positive	33.83	Positive	34.85	Positive	35.10	Positive	Positive	
	18	31.40	Positive	33.54	Positive	34.81	Positive	35.06	Positive	Positive	
	19	32.80	Positive	33.89	Positive	34.59	Positive	35.68	Positive	Positive	
	20	31.89	Positive	35.25	Positive	33.77	Positive	35.93	Positive	Positive	
$5.0X10^2$	1	31.97	Positive	NoCt	Negative	34.14	Positive	33.32	Positive	Positive	90%
	2	NoCt	Negative	NoCt	Negative	NoCt	Negative	32.78	Positive	Negative	
	3	34.22	Positive	36.01	Positive	35.41	Positive	34.37	Positive	Positive	
	4	NoCt	Negative	NoCt	Negative	35.39	Positive	30.06	Positive	Negative	
	5	33.81	Positive	35.95	Positive	34.61	Positive	34.07	Positive	Positive	
	6	33.55	Positive	34.30	Positive	35.76	Positive	33.76	Positive	Positive	
	7	33.85	Positive	35.32	Positive	34.63	Positive	34.48	Positive	Positive	
	8	33.76	Positive	34.06	Positive	34.54	Positive	35.06	Positive	Positive	
	9	32.59	Positive	34.44	Positive	33.5	Positive	33.37	Positive	Positive	
	10	31.62	Positive	35.54	Positive	35.06	Positive	31.87	Positive	Positive	

	11	31.06	Positive	34.38	Positive	35.06	Positive	33.13	Positive	Positive	
	12	34.41	Positive	33.81	Positive	35.12	Positive	34.00	Positive	Positive	
	13	34.35	Positive	35.11	Positive	35.22	Positive	33.88	Positive	Positive	
	14	31.29	Positive	34.33	Positive	35.29	Positive	33.57	Positive	Positive	
	15	32.82	Positive	34.98	Positive	NoCt	Negative	33.77	Positive	Positive	
	16	32.9	Positive	34.82	Positive	35.37	Positive	33.80	Positive	Positive	
	17	31.46	Positive	34.04	Positive	35.63	Positive	32.88	Positive	Positive	
	18	32.53	Positive	36.38	Positive	32.87	Positive	33.10	Positive	Positive	
	19	30.43	Positive	33.96	Positive	NoCt	Negative	33.05	Positive	Positive	
	20	34.56	Positive	NoCt	Negative	NoCt	Negative	33.75	Positive	Positive	

Table 14. LoD Confirmation - RNA Extracted with QIAamp Viral RNA Mini Kit (QIAGEN)

Concentration (copies/mL)	Replicate	SARS-CoV-2 ORF1ab		SARS-CoV-2 E gene		SARS-CoV-2 N gene		IC		Overall SARS-CoV-2 Result	Overall % Positivity
		Ct	Result	Ct	Result	Ct	Result	Ct	Result		
$2X10^3$	1	32.83	Positive	33.35	Positive	34.32	Positive	32.23	Positive	Positive	100%
	2	33.23	Positive	33.06	Positive	34.91	Positive	32.12	Positive	Positive	
	3	33.33	Positive	33.36	Positive	35.41	Positive	31.71	Positive	Positive	
	4	32.65	Positive	33.06	Positive	34.31	Positive	32.17	Positive	Positive	
	5	33.32	Positive	34.05	Positive	35.24	Positive	32.08	Positive	Positive	
	6	33.47	Positive	34.54	Positive	35.65	Positive	32.11	Positive	Positive	
	7	33.36	Positive	33.28	Positive	34.68	Positive	32.84	Positive	Positive	
	8	33.3	Positive	35.13	Positive	33.9	Positive	32.57	Positive	Positive	
	9	33.43	Positive	34.98	Positive	No Ct	Negative	31.91	Positive	Positive	
	10	33.77	Positive	33.27	Positive	35.54	Positive	32.88	Positive	Positive	
	11	33.91	Positive	34.26	Positive	34.25	Positive	32.51	Positive	Positive	
	12	33.66	Positive	34.84	Positive	34.4	Positive	31.87	Positive	Positive	
	13	33.99	Positive	33.51	Positive	34.54	Positive	32.56	Positive	Positive	
	14	33.32	Positive	34.51	Positive	34.91	Positive	32.09	Positive	Positive	
	15	32.89	Positive	35.28	Positive	35.17	Positive	32.38	Positive	Positive	
	16	33.98	Positive	32.91	Positive	34.59	Positive	32.58	Positive	Positive	
	17	34.95	Positive	35.24	Positive	34.97	Positive	32.81	Positive	Positive	
	18	33.89	Positive	35.55	Positive	35.31	Positive	32.19	Positive	Positive	
	19	34.04	Positive	34.37	Positive	35.32	Positive	32.87	Positive	Positive	
	20	33.92	Positive	33.9	Positive	35.13	Positive	32.70	Positive	Positive	
$1X10^3$	1	35.33	Positive	33.28	Positive	34.38	Positive	31.66	Positive	Positive	100%
	2	34.82	Positive	37.66	Positive	35.47	Positive	31.89	Positive	Positive	
	3	34.2	Positive	37.81	Positive	35.14	Positive	31.76	Positive	Positive	
	4	35.12	Positive	38.73	Positive	35.14	Positive	31.88	Positive	Positive	
	5	34.29	Positive	34.65	Positive	36.11	Positive	31.78	Positive	Positive	
	6	35.99	Positive	35.48	Positive	37.58	Positive	32.03	Positive	Positive	
	7	34.02	Positive	34.49	Positive	37.02	Positive	31.45	Positive	Positive	
	8	34.32	Positive	No Ct	Negative	35.56	Positive	32.33	Positive	Positive	
	9	34.84	Positive	38.47	Positive	36.23	Positive	32.31	Positive	Positive	
	10	35.83	Positive	37.3	Positive	37.3	Positive	31.68	Positive	Positive	
	11	34.98	Positive	34.53	Positive	34.64	Positive	31.96	Positive	Positive	
	12	34.8	Positive	34.67	Positive	36.22	Positive	32.35	Positive	Positive	
	13	35.59	Positive	37.27	Positive	34.61	Positive	31.65	Positive	Positive	

	14	35.26	Positive	35.83	Positive	36.87	Positive	31.52	Positive	Positive	
	15	34.87	Positive	No Ct	Negative	36.19	Positive	31.91	Positive	Positive	
	16	35.21	Positive	34.83	Positive	35.59	Positive	31.64	Positive	Positive	
	17	36.8	Positive	37.59	Positive	34.96	Positive	32.28	Positive	Positive	
	18	34.1	Positive	35.34	Positive	35.76	Positive	32.63	Positive	Positive	
	19	35.15	Positive	34.16	Positive	35.72	Positive	31.89	Positive	Positive	
	20	35.49	Positive	No Ct	Negative	35.14	Positive	31.98	Positive	Positive	
$5X10^2$	1	No Ct	Negative	35.32	Positive	35.18	Positive	33.42	Positive	Negative	95%
	2	35.63	Positive	35.3	Positive	36.4	Positive	33.83	Positive	Positive	
	3	35.57	Positive	37.32	Positive	37.19	Positive	33.18	Positive	Positive	
	4	34.98	Positive	No Ct	Negative	No Ct	Negative	33.24	Positive	Positive	
	5	35.82	Positive	No Ct	Negative	36.79	Positive	33.48	Positive	Positive	
	6	35.90	Positive	38.49	Positive	35.41	Positive	33.60	Positive	Positive	
	7	35.31	Positive	36.42	Positive	37.16	Positive	33.99	Positive	Positive	
	8	35.72	Positive	35.55	Positive	35.53	Positive	33.94	Positive	Positive	
	9	36.77	Positive	35.82	Positive	36.28	Positive	33.95	Positive	Positive	
	10	34.95	Positive	36.59	Positive	35.95	Positive	33.99	Positive	Positive	
	11	37.44	Positive	No Ct	Negative	36.01	Positive	34.08	Positive	Positive	
	12	34.85	Positive	37.52	Negative	37.33	Positive	33.96	Positive	Positive	
	13	35.09	Positive	34.12	Positive	No Ct	Negative	33.75	Positive	Positive	
	14	34.52	Positive	35.56	Positive	No Ct	Negative	34.05	Positive	Positive	
	15	35.66	Positive	35.18	Positive	No Ct	Negative	33.81	Positive	Positive	
	16	34.54	Positive	34.43	Positive	35.66	Positive	33.83	Positive	Positive	
	17	35.53	Positive	36.7	Positive	No Ct	Negative	33.43	Positive	Positive	
	18	35.26	Positive	36.96	Positive	No Ct	Negative	33.66	Positive	Positive	
	19	35.63	Positive	34.99	Positive	35.44	Positive	33.88	Positive	Positive	
	20	35.11	Positive	36.95	Positive	37.16	Positive	33.59	Positive	Positive	
$1X10^2$	1	35.87	Positive	No Ct	Negative	No Ct	Negative	31.58	Positive	Positive	60%
	2	No Ct	Negative	36.89	Positive	No Ct	Negative	32.00	Positive	Negative	
	3	36.44	Positive	No Ct	Negative	No Ct	Negative	31.93	Positive	Positive	
	4	No Ct	Negative	37	Positive	36.2	Positive	32.02	Positive	Negative	
	5	37.43	Positive	34.94	Positive	No Ct	Negative	32.33	Positive	Positive	

Reactivity (Inclusivity)

Inclusivity Wet-Testing

Inclusivity was evaluated by spiking extracted SARS-CoV-2 viral genomic RNA from ten (10) confirmed positive cases collected from varying geographical regions, into leftover negative OP matrix at 1x LoD. RNA was extracted using the Maccura Extraction Kit (Manual). All 10 contrived RNA samples were positive when tested with the SARS-CoV-2 Fluorescent PCR Kit.

In silico analysis

In silico inclusivity analyses of the oligonucleotide (oligo) sequences and probes for the SARS-CoV-2 ORF1ab, N, and E sets was performed by a BLASTn analysis using 98

publicly available SARS-CoV-2 sequences obtained from the NCBI *Betacoronavirus* database on March 28, 2020. Oligo and probe sets for the ORF1ab, N and E genes exhibited 100% sequence homology to the evaluated SARS-CoV-2 sequences. Inclusivity was also evaluated for the oligo sequences and probes for the SARS-CoV-2 ORF1ab, N, and E sets by a MegAlign analysis conducted with 15 SARS-CoV-2 isolate sequences downloaded from NCBI on March 28, 2020 and 36 sequences downloaded from the GISAID database on March 28, 2020. Results of this analysis demonstrated that the primer/probe sets for SARS-CoV-2 ORF1ab and N sets exhibit 100% homology to all evaluated sequences. The primer/probe set for the E gene showed 100% homology to all evaluated sequences except two strains: (GenBank Accession: MT039890; GISAID Accession: EPI_ISL_411929), for which a single nucleotide mismatch was found close to the 3'-end of the probe binding region. The homology of the probe sequence to these two strains were both 96 %. These single nucleotide mismatches are not expected to impact the performance of the SARS-CoV-2 Fluorescent PCR Kit.

Cross-reactivity

To assess cross-reactivity, an *in-silico* analysis was performed using the primer and probe sequences in the SARS-CoV-2 Fluorescent PCR Kit. These sequences were compared against sequences from the NCBI database (available as of March 28, 2020), for the respiratory organisms listed in Table 15.

Results from the *in-silico* cross-reactivity analysis showed the only organism in Table 15 with oligo-hit sequence homology ≥ 80% was SARS-coronavirus. The primers and probe for the E gene have 100% homology to the SARS-coronavirus sequence. The probe for the N gene has 100% homology while the forward and reverse primers exhibit 92% and 90% homology, respectively, to the SARS coronavirus sequence. While the ORF1ab forward primer and probe exhibited >80% homology, the reverse primer exhibited <80% homology and therefore it is not anticipated that SARS coronavirus will be detected by the ORF1ab primer/probe set.

Table 15. *In silico* analysis for cross-reactivity

Microorganism	GenBank Accession	ORF1ab % Homology			E gene % Homology			N gene % Homology		
		F	R	Probe	F	R	Probe	F	R	Probe
Human coronavirus 229E	NC_002645.1	41%	60%	36%	46%	41%	46%	52%	40%	52%
Human coronavirus OC43	NC_006213.1	45%	55%	36%	42%	45%	35%	40%	40%	40%
Human coronavirus HKU1	NC_006577.2	59%	45%	32%	35%	41%	42%	44%	40%	44%
Human coronavirus NL63	NC_005831.2	41%	60%	32%	42%	45%	42%	44%	40%	48%
SARS-coronavirus	NC_004718.3	95%	75%	88%	100%	100%	100%	92%	90%	100%
MERS-coronavirus	NC_019843.3	45%	45%	40%	38%	41%	38%	40%	40%	40%
Adenovirus C1	KF429744.1	45%	50%	36%	38%	41%	38%	36%	45%	40%
Adenovirus 71	KF268207.1	41%	50%	44%	35%	50%	38%	44%	45%	36%
Human Metapneumovirus (hMPV)	NC_039199.1	36%	45%	40%	42%	45%	35%	36%	40%	36%
Parainfluenza virus 1	NC_003461.1	41%	45%	44%	31%	36%	38%	48%	50%	36%

Parainfluenza virus 2	KM190939.1	45%	45%	36%	35%	45%	35%	44%	60%	36%
Parainfluenza virus 3	NC_001796.2	36%	50%	36%	35%	50%	42%	40%	40%	52%
Parainfluenza virus 4	NC_021928.1	45%	45%	40%	46%	59%	35%	52%	45%	40%
Influenza A virus	NC_002023.1	32%	40%	32%	27%	36%	31%	52%	35%	44%
	NC_002022.1	32%	45%	40%	27%	36%	27%	32%	35%	40%
	NC_002021.1	36%	45%	32%	31%	41%	31%	40%	35%	48%
	NC_002020.1	32%	45%	28%	27%	36%	27%	28%	40%	36%
	NC_002019.1	0	55%	28%	27%	41%	31%	28%	35%	28%
	NC_002018.1	32%	35%	28%	27%	36%	31%	44%	35%	32%
	NC_002017.1	32%	40%	32%	31%	32%	38%	32%	40%	32%
	NC_002016.1	32%	35%	32%	27%	32%	27%	28%	35%	32%
Influenza B virus	NC_002204.1	32%	40%	32%	0	41%	27%	56%	40%	36%
	NC_002211.1	32%	35%	28%	27%	0	27%	32%	40%	40%
	NC_002210.1	32%	0	32%	27%	32%	0	32%	40%	40%
	NC_002209.1	45%	40%	28%	0	32%	0	36%	40%	32%
	NC_002208.1	0	40%	36%	27%	32%	31%	36%	40%	28%
	NC_002207.1	36%	35%	32%	35%	45%	27%	32%	40%	32%
	NC_002206.1	36%	35%	36%	0	41%	27%	32%	35%	32%
	NC_002205.1	36%	40%	28%	27%	36%	31%	32%	35%	32%
	NC_002211.1	32%	35%	28%	27%	0	27%	32%	40%	40%
	NC_002210.1	32%	0	32%	27%	32%	0	32%	40%	40%
Enterovirus 68	NC_038308.1	36%	35%	32%	35%	41%	35%	36%	45%	36%
Respiratory syncytial virus	NC_001803.1	41%	60%	36%	50%	36%	31%	36%	35%	36%
Rhinovirus	FJ869955.1	0	0	28%	0	0	0	28%	0	32%
Chlamydia pneumoniae	NC_005043.1	50%	55%	52%	42%	77%	50%	52%	60%	52%
Haemophilus influenzae	NZ_LN831035.1	64%	60%	44%	46%	59%	58%	56%	50%	52%
Legionella pneumophila	NZ_LR134380.1	55%	60%	52%	54%	59%	50%	60%	60%	52%
Mycobacterium tuberculosis	NC_000962.3	59%	65%	48%	38%	64%	50%	48%	55%	56%
Streptococcus pneumoniae	NZ_LN831051.1	50%	60%	56%	50%	55%	54%	52%	65%	52%
Streptococcus pyogenes	LN831034.1	50%	60%	64%	50%	59%	46%	48%	60%	52%
Bordetella pertussis	NC_005357.1	41%	50%	36%	35%	45%	35%	40%	55%	36%
Mycoplasma pneumoniae	AE004092.2	50%	60%	44%	50%	59%	46%	48%	60%	52%
Pneumocystis jirovecii (PJP)	EU979570.1	32%	0	32%	31%	32%	27%	36%	35%	28%
Candida albicans	CM016738.1	55%	60%	52%	46%	59%	46%	68%	55%	52%
Pseudomonas aeruginosa	CP029707.1	59%	60%	60%	50%	77%	50%	44%	55%	48%
Staphylococcus epidermis	MT125873.1	36%	35%	28%	27%	32%	0	28%	35%	28%
Staphylococcus salivarius	CP013216.1	55%	65%	48%	62%	59%	50%	64%	75%	48%

This *in silico* analysis identified no potential unintended cross-reactivity of the SARS-CoV-2 Fluorescent PCR Kit with respiratory pathogens, including coronaviruses, except SARS-coronavirus.

Interference Substances Studies

Interference substances studies were conducted using negative, leftover OP clinical

specimens spiked with SARS-CoV-2 RNA at 2x LOD concentration. Potential interfering substances, at the indicated concentrations, were added to RNA spiked OP specimens. These specimens were then extracted with the Maccura Nucleic Acid Extraction Kit, (Manual) and were tested using the SARS-CoV- 2 Fluorescent PCR Kit. The following interference substances were tested at the stated concentrations in the interference study:

0.9 g/mL sodium chloride (including 1% PC-300), 100µg/mL phenylephrine, 100µg/mL oxymetazoline, 100µg/mL beclomethasone, 100µg/mL flunisolide, 100µg/mL, triamcinolone acetonide, 200µg/mL budesonide, 100µg/mL mometasone, 200µg/mL fluticasone, 200µg/mL histamine hydrochloride, 10 mg/mL ribavirin, 400 U/µL α-interferon, 0.1mg/mL dexamethasone, 0.568µg/mL zanamivir, 100µg/mL oseltamivir, 100µg/mL peramivir, 100µg/mL lopinavir, 100µg/m ritonavir, 100µg/mL arbidol, 100µg/mL levofloxacin, 200µg/mL azithromycin, 100µg/mL ceftriaxone, 100µg/mL meropenem, 100µg/mL tobramycin, 0.25 g/L mucin and 5% (*V/V*) whole blood.

None of the evaluated substances interfered with the SARS-CoV-2 Fluorescent PCR Kit.

Clinical Evaluation

The performance of the SARS-CoV-2 Fluorescent PCR Kit was established using leftover nasopharyngeal swab (NP) and oropharyngeal swab (OP) clinical specimens collected from patients who were suspected of COVID-19. Fifteen (15) individual NP and fifteen (15) individual OP specimens were collected on March 28, 2020 by Great Master Diagnostics (An independent clinical laboratory). All specimens were confirmed negative for SARS-CoV-2 using the SARS-CoV-2 Fluorescent PCR Kit.

To generate contrived positive samples, an aliquot from each leftover negative OP/NP specimen was removed and spiked with SARS-CoV-2 whole genomic RNA. For each matrix (OP or NP), 10 contrived positives were prepared at 2X LoD ($2X10^3$ copies/mL and $1X10^3$ copies/mL for Maccura extraction method and for QIAGEN extraction method, respectively) and 5 contrived positives at 5X LoD, ($5X10^3$ copies/mL and $2.5X10^3$ copies/mL for Maccura extraction method and for QIAGEN extraction method, respectively). The remaining negative leftover OP/NP specimen was used to evaluate the Negative Percent Agreement (NPA) of the SARS-CoV-2 Fluorescent PCR Kit. All positive and negative samples were extracted using the Maccura Nucleic Acid Extraction Kit (Manual Version) and QIAamp Viral RNA Mini Kit, and tested with the SARS-CoV-2 Fluorescent PCR Kit. The positive and negative contrived samples were tested in a blinded and randomized fashion. The results are summarized in the tables below and demonstrated PPA of 100% and NPA of 96.7% for RNA extracted with Maccura RNA extraction Kit (Table 16) and PPA of 96.7% and NPA of 100% for RNA extracted with QIAGEN QIAamp Viral RNA Mini Kit (Table 17).

Table 16. Clinical Evaluation with Contrived SARS-CoV-2 Specimens Extracted by Maccura RNA Extraction Kit

Target RNA Concentration		ORF1ab	E Gene	N Gene	Overall Results	% Positivity

	No of Samples Tested	Mean Ct	% Agreement (#Pos or Neg)/Total	Mean Ct	% Agreement (#Pos or Neg)/Total	Mean Ct	% Agreement (# Pos or Neg)/Total		
SARS-CoV-2 2XLoD $2X10^3$ copies/mL	20	35.08	100% 20/20	35.30	100% 20/20	35.57	100% 20/20	Positive	100%
SARS-CoV-2 5XLoD $5X10^3$ copies/mL	10	33.38	100% 10/10	33.67	100% 10/10	34.01	100% 10/10	Positive	100%
Negative Clinical Matrix	30	NA	100% 30/30	NA	100% 30/30	NA	96.70% 29/30	Negative	3.30%

Table 17. Clinical Evaluation with Contrived SARS-CoV-2 Specimens

Extracted by QIAGEN QIAamp Viral RNA Mini Kit

Target RNA Concentration	No. of Samples Tested	ORF1ab		E Gene		N Gene		Overall Results	% Positivity
		Mean Ct	% Agreement (#Pos or Neg)/Total	Mean Ct	% Agreement (#Pos or Neg)/Total	Mean Ct	% Agreement # Pos or Neg)/Total		
SARS-CoV-2 2x LoD (1000 copies/mL)	20	33.25	95% 19/20	34.22	95% 19/20	34.50	95% 19/20	Positive	95%
SARS-CoV-2 5x LoD (2500 copies/mL)	10	31.71	100% 10/10	32.75	100% 10/10	33.04	100% 10/10	Positive	100%
Negative Clinical Matrix	30	NA	100% 30/30	NA	100% 30/30	NA	100% 30/30	Negative	100%

FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. The extraction method and instrument used were Maccura Nucleic Acid Extraction Kit (Manual Version GN7102903) and Applied Biosystems™ 7500 Real-Time PCR instrument. The results are summarized in Table 18.

Table 18: Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal swabs	$1.8x10^3$ NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL
N/A: Not applicable
ND: Not detected

Trouble Shooting

Before embarking on the below trouble shooting steps, please ensure the RT-PCR protocol is set properly according to **Table 7** in "Chapter 2 Assay Procedure". For a complete list of all Applied Biosystems 7500 Real-time PCR Instrument error messages and a description of the messages, please refer to the Applied Biosystems 7500 Real-time PCR Instrument Operator Manual.

Table 19 Troubleshooting Table

Error Type	Description	Possible Cause	Recommendations
No signal in all channels	No signal was detected in the **Analysis-Amplification Plot** window, but background fluorescence was detected in the **Multicomponent Plot** window.	1. A sample specific error may have occurred; or 2. Extraction failure or sample addition error.	Re-extract and re-run the sample, check IC is added properly according to the IFU, carefully follow the nucleic acid extraction and RT-PCR procedure.
No signal in all channels	No signal was detected in the **Analysis-Amplification Plot** window and **Multicomponent Plot** window.	A sample specific error may have occurred, or this well was not read by the instrument.	Re-extract and re-run the sample, avoid running in the failed well.
IC failure in QC samples	No signal in IC channel, but other targets of PC and NC were detected with acceptable Ct values.	IC failure could be caused by improper handling of IC. A test report will be generated, but QC will be invalid.	Re-extract and re-run the sample and PC, NC, check IC is added properly according to the IFU.
PC failure	The PC did not meet the criteria set for acceptable Ct values. See Amplification Plot of I**llustration for PC failure** below.	A test report will be generated, but PC will be invalid. Possible causes including: 1. Kit was not stored at the recommended conditions; 2. Kit is expired; 3. Operator error during nucleic acid extraction or setting-up of the PCR reaction.	1. Check the storage condition to ensure the conditions meet the product label requirement; 2. Check the expiration date on the product label; 3. Re-extract and re-run the sample, check PC and IC are added properly according to the IFU, carefully follow the nucleic acid extraction and RT-PCR procedure.
NC failure	Fluorescence signal appears late in the NC. See Amplification Plot of I**llustration for NC failure** below	A test report will be generated, but NC will be invalid. 1. The signal is not a true target amplification. It is background noise generated by the instrument software; or 2. The positive signal may be caused by contamination during the PCR set-up.	1.Ignore the Ct value of NC if the curve indicates no amplification.; 2. Re-extract and re-run the sample with new (unused) reagents. Clean all work surfaces and equipment with a 10% household bleach solution or other products that are capable of degrading DNA amplicon contamination. Use fresh gloves and lab coats and follow a direction workflow to avoid introducing post-PCR amplicon contamination.

Illustration for PC failure

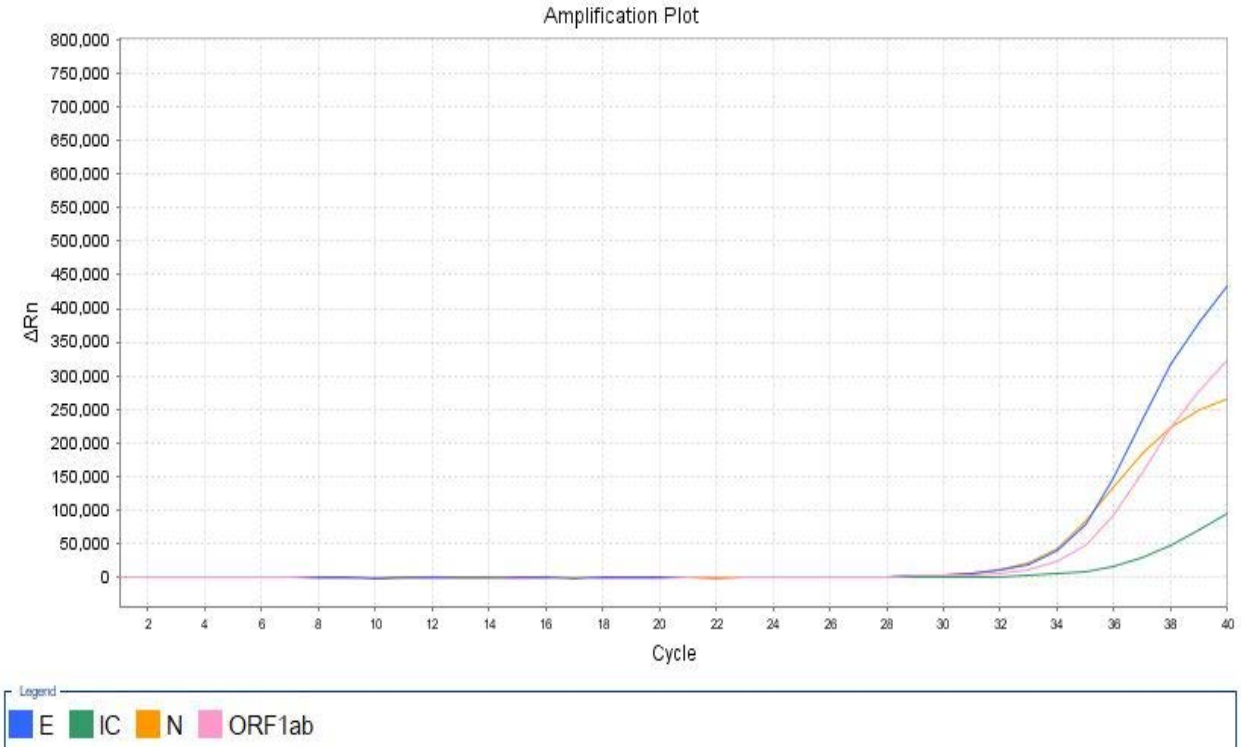
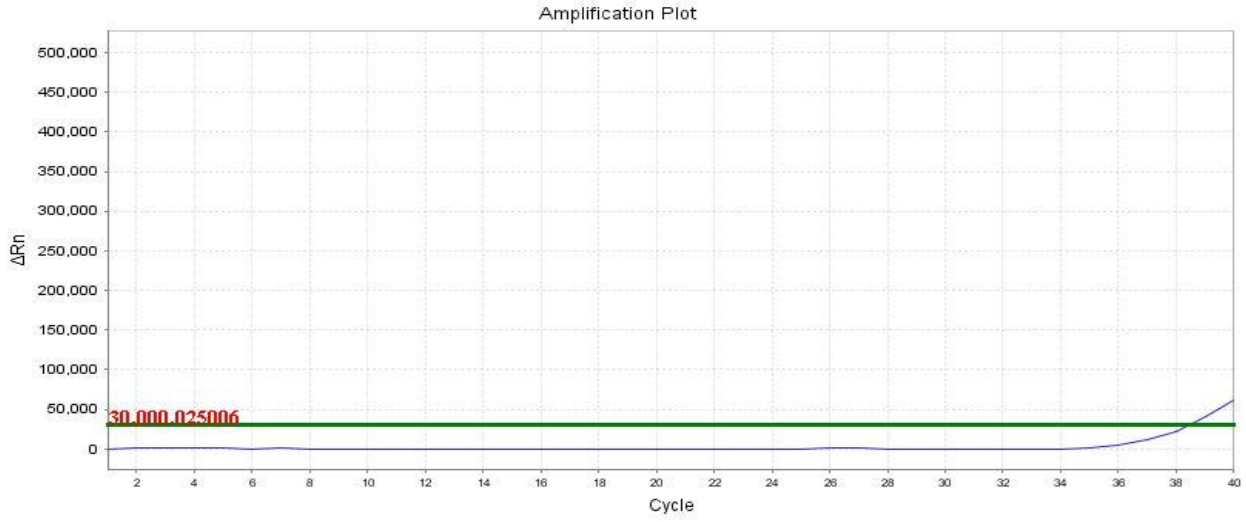


Illustration for NC failure



Disposal

Dispose of hazardous or biologically contaminated materials according to the practices of your institution.

Manufacturer and Distributors

Manufacturer information

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Symbol Glossary

Symbols	Definition
	PROTECT FROM SUNLIGHT
	STORAGE TEMPERATURE LIMITATION
	IN VITRO DIAGNOSTIC USE
	UPWARD
	DISPOSE IN TRASH AFTER USE
	RECYCLABLE MATERIAL
	CONSULT INSTRUCTIONS FOR USE
	BATCH CODE
	CATALOGUE NUMBER
	USE BY
	DATE OF MANUFACTURE
	MANUFACTURER
	SUFFICIENT FOR <N> TESTS