# 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 2.0**

| | | |
|---|---|---|
| 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 2.0 | May 16, 2020 | 1.Add QIAGEN QIAamp Viral RNA Mini Kit as an optional RNA extraction method<br>2.Update assay LOD. Add LOD, when QIAamp Viral RNA Mini Kit for RNA is used, for 500 copies/ml<br>3.Provide instructions for QIAamp Virus RNA Mini Kit<br>4.Delete Background section<br>5.New design for the cover page of this IFU |
| R 1.1 | April 24, 2020 | 1.Add page number<br>2.Reformat to keep each table on the same page |
| R 1.0 | April 15, 2020 | New document |

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## 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

Table 1. SARS-CoV-2 Fluorescent PCR Kit

| Component | Description | Storage | Amount Supplied | | |
|---|---|---|---|---|---|
| | | | BUSGN 7101109 (32 Tests) | BUSGN 7102109 (64 Tests) | BUSGN 7103109 (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 (5.0 x$10^3$ 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 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℃ to 8℃ | 500 µL×1 |
| Extraction reagent ② | LysisBuffer | 2℃ to 8℃ | 31 mL×1 |
| Extraction reagent ③ | Magnetic nanoparticles | 2℃ to 8℃ | 250 µL×1 |
| Extraction reagent ④ | Wash Buffer 1 | 2℃ to 8℃ | 41 mL×1 |
| Extraction reagent ⑤ | Wash Buffer 2 | 2℃ to 8℃ | 36 mL×1 |
| Extraction reagent ⑥ | Mineral oil | 2℃ to 8℃ | 5 mL×1 |
| Extraction reagent ⑦ | Elution Buffer | 2℃ to 8℃ | 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℃to -10℃ | 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.

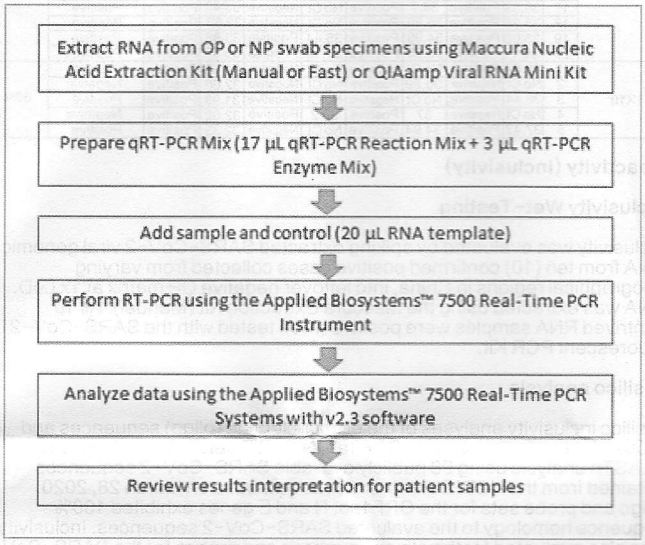
**Specimen Collection and Preparation**

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℃ 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 | 5 µL | N=n+2 |
| | Internal Control | 2 µL | |

**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 5 µL and the actual volume of internal control is N x 2 µL. It is recommended that extra magnetic bead mix is prepared to account for loss during pipetting of mix to individual centrifuge tubes.

4. Add 600 µL of Extraction reagent ② and 7 µL 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 800 µL 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 700 µL of Extraction reagent ⑤ and 100 µL 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 35 µL 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 200 µL of specimen and 2 µL 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 50 µL 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 2 μL 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**

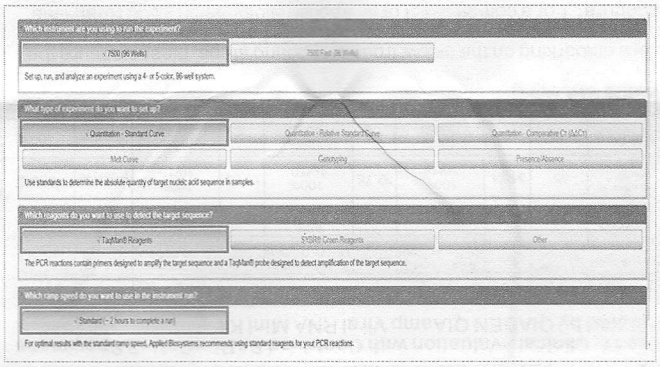
IMPORTANT!
1. The experiment workflow is recommended to be carried out in segmented PCR laboratories.
2. To prevent contamination, prepare reagents in a PCR workstation or equivalent amplicon-free area. Always use aerosol barrier pipette tips when processing samples and positive controls. Do not reuse 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 | 17 μL | N=n+2 |
| | qRT-PCR Enzyme Mix | 3 μL | |

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 17 μL and the actual volume of qRT-PCR Enzyme Mix is N X 3 μL. 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.

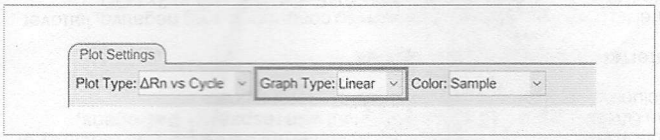
## Chapter 3 DATA ANALYSIS AND RESULTS

**Setting data analysis parameters**

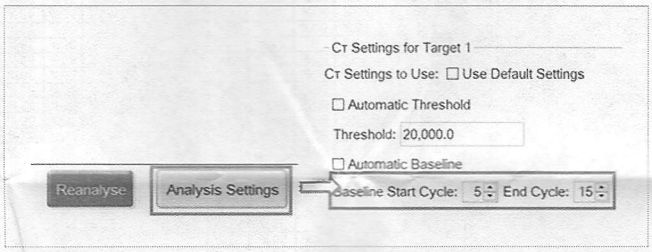
Please save the result immediately after a run is completed. Conduct the following adjustment after each run.

1.Set the Baseline

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

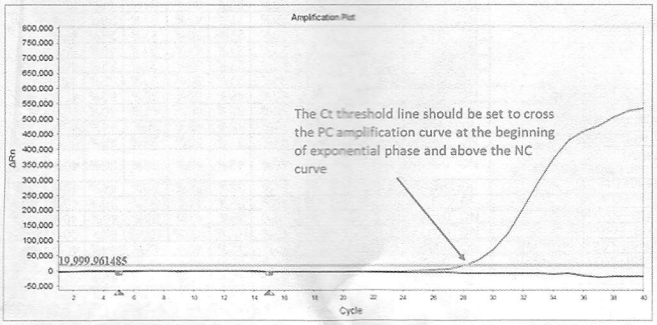



- Set the baseline for each channel, starting point at cycle 5 and ending Point at cycle 15 in **Analysis Setting-Ct Setting** window:




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 | >37or 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.
- 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 $1.0x10^3$ 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 | | |
| 2.0X10³ | 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 | |
| 1.0X10³ | 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 | |
| 5.0X10² | 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 | No Ct | 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 | No Ct | 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 | No Ct | Negative | 38.02 | Negative | 34.98 | Positive | 31.36 | Positive | Negative | |
| | 19 | No Ct | Negative | 37.85 | Negative | 35.25 | Positive | 32.81 | Positive | Negative | |
| | 20 | No Ct | 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 | | |
| 2.0X10³ | 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 | |
| 1.0X10³ | 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 | No Ct | 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 | |
| 5.0X10² | 1 | 31.97 | Positive | No Ct | Negative | 34.14 | Positive | 33.32 | Positive | Positive | 90% |
| | 2 | No Ct | Negative | No Ct | Negative | No Ct | Negative | 32.78 | Positive | Negative | |
| | 3 | 34.22 | Positive | 36.01 | Positive | 35.41 | Positive | 34.37 | Positive | Positive | |
| | 4 | No Ct | Negative | No Ct | 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 | No Ct | 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 | No Ct | Negative | 33.05 | Positive | Positive | |
| | 20 | 34.56 | Positive | No Ct | Negative | No Ct | 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 | | |
| 2X10³ | 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 | |
| 1X10³ | 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 | |
| 5X10² | 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 | |
| 1X10² | 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 in China, 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 229E coronavirus | NC_002645.1 | 41% | 60% | 36% | 46% | 41% | 46% | 52% | 40% | 52% |
| Human OC43 coronavirus | NC_006213.1 | 45% | 55% | 36% | 42% | 45% | 35% | 40% | 40% | 40% |
| Human HKU1 coronavirus | NC_006577.2 | 59% | 45% | 32% | 35% | 41% | 42% | 44% | 40% | 44% |
| HumanNL63 coronavirus | 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 | FJ889955.1 | 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), 100 μg/mL phenylephrine, 100 μg/mL oxymetazoline, 100 μg/mL beclomethasone, 100 μg/mL flunisolide, 100 μg/mL, triamcinolone acetonide, 200 μg/mL budesonide, 100 μg/mL mometasone, 200 μg/mL fluticasone, 200 μg/mL histamine hydrochloride, 10 mg/mL ribavirin, 400 U/μL α- interferon, 0.1mg/mL dexamethasone, 0.568 μg/mL zanamivir, 100 μg/mL oseltamivir, 100 μg/mL peramivir, 100 μg/mL lopinavir, 100 μg/mL ritonavir, 100 μg/mL arbidol, 100 μg/mL levofloxacin, 200 μg/mL azithromycin, 100 μg/mL ceftriaxone, 100 μg/mL meropenem, 100 μg/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 in Chengdu, China). 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 | 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 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 Matrix30 | 30 | NA | 100% 30/30 | NA | 100% 30/30 | NA | 100% 30/30 | Negative | 100% |

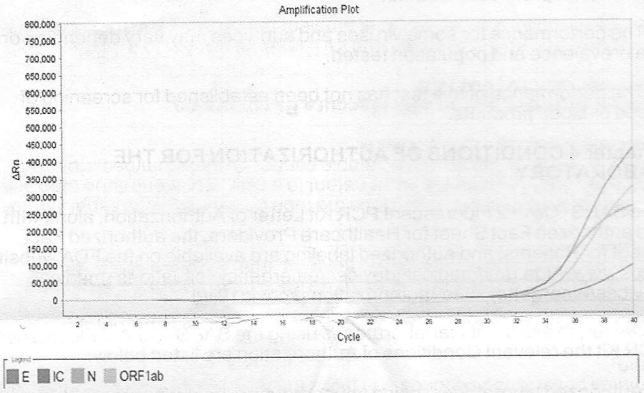
## Trouble Shooting

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.

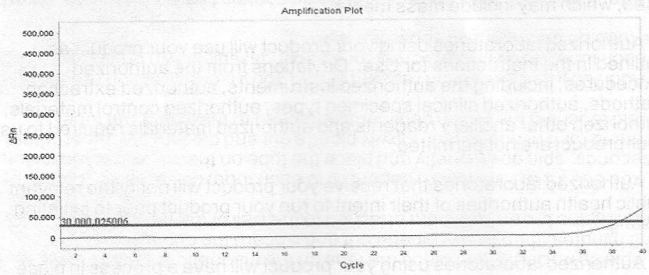
**Table 18 Troubleshooting Table**

| 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 orsample 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 **Illustration 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 theconditions 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 **Illustration 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 |
| IVD | IN VITRO DIAGNOSTIC USE |
| | UPWARD |
| | DISPOSE IN TRASH AFTER USE |
| | RECYCLABLE MATERIAL |
| | CONSULT INSTRUCTIONS FOR USE |
| LOT | BATCH CODE |
| REF | CATALOGUE NUMBER |
| | USE BY |
| | DATE OF MANUFACTURE |
| | MANUFACTURER |
| | SUFFICIENT FOR <N> TESTS |