# RapidScript™ SARS-CoV-2 Assay (1-step RT-qPCR)

Qualitative RT-PCR based detection of SARS-CoV-2

*For In Vitro Diagnostic Use Only. For Professional Use Only*

Store at -20 C.

REF **11MSTP400**

**400 tests**

IVD CE

# RapidScript™ SARS-CoV-2 Assay (1-step RT-qPCR)

## INTENDED USE

The MP Biomedical RapidScript™ SARS-CoV-2 Assay (1-step qPCR) is a real-time reverse transcriptase (RT) polymerase chain reaction (PCR) test intended for the in vitro qualitative detection of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) nucleic acid from nasopharyngeal and oropharyngeal swab specimens collected from individuals suspected of COVID-19 infection. The SARS-CoV-2 primer and probe sets are designed for the specific detection of SARS-CoV-2 according to the Centers for Disease Control and Prevention (United States) Guidelines.

Test results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in nasal, nasopharyngeal, and oropharyngeal swabs during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA. Positive results do not rule out bacterial infections or co-infections with other viruses. The agent detected may not be the primary cause of disease. Negative results do not preclude COVID-19 infection. Samples tested positive should always be confirmed through complementary methods and additional analysis in an independent laboratory.

## TEST PRINCIPLE

The MP RapidScript™ SARS-CoV-2 Assay (1-step qPCR) test is a real-time reverse transcription-polymerase chain reaction (RT-qPCR) test. The methods described here have been adapted from the "CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel" document effective March 30, 2020. The oligonucleotide primers and probes for detection of 2019-nCoV were selected from regions of the virus nucleocapsid (N) gene. The panel is designed for specific detection of the 2019-nCoV (two primer/probe sets). An additional primer/probe set to detect the human RNase P gene (RP) in control samples and clinical specimens is also included in the panel.

The first step in the detection of SARS-CoV-2 is the conversion of viral RNA into complementary DNA (cDNA). Afterward, the target sequences unique for SARS-CoV-2 are specifically amplified with amplification monitored in real-time through the use of fluorescently labeled probes: upon incorporation into the newly amplified DNA strands, the fluorophore (FAM) is released and an increase in fluorescence signal can be observed by a qRT-PCR machine.

Due to the intrinsic mutation rate of coronaviruses, mutations in the target sequence may occur and accumulate over time. This can lead to a false-negative result with a PCR-based approach.

The MP Biomedicals RapidScript™ SARS-CoV-2 Assay (1-step qPCR) test is to be used with Qiagen Viral RNA extraction kit and any Real-Time PCR system such as ThermoFisher: 7900HT, 7500, 7500 Fast, 7500 Fast Dx, 7300, Step One, StepOnePlus, QuantStudio3, QuantStudio5, QuantStudio6 Flex, QuantStudio7 Flex, QuantStudio 12 Flex; Bio-Rad: CFX96, iCycler, MyIQ, IQ5, CFX96 Touch, CFX 384, CFX Connect, Roche: 480 LightCycler, Lightcycler 2.0, LightCycler 1536, Stratagene/Agilent: Mx3000p, Mx3005p, Eppendorf: Mastercycler, Takara: TP800, and Qiagen: Rotorgene 3000A, Rotorgene Q.

Detection of viral RNA not only aids in the diagnosis of illness but also provides epidemiological and surveillance information.

## KIT COMPONENTS

| Component | Specification | Quantity |
|---|---|---|
| RapidScript™ Reverse Transcriptase RNAse Inhibitor Enzyme Mix | 160 uL | 1 |
| RapidScript™ 2X Probe Supermix | 1 mL / tube | 4 |
| RapidScript™ RNase-free water | 1 mL / tube | 4 |
| RapidScript™ nCOV_N1 Primer Probe Mix | 400 reactions | 1 |
| RapidScript™ nCOV_N2 Primer Probe Mix | 400 reactions | 1 |
| RapidScript™ RNaseP Primer Probe Mix | 400 reactions | 1 |
| RapidScript™ High ROX Passive Reference Dye | 160 uL | 1 |
| RapidScript™ Low ROX Passive Reference Dye | 160 uL | 1 |
| RapidScript™ SARS-CoV-2 Assay Instruction for Use | | 1 |

## COMPONENT INGREDIENTS

RapidScript™ 2X Probe Supermix: 2x Buffer (80 mM Tris-HCl, pH 8.4, 0.2 M KCl), 3 mM MgCl, 0.4 mM dNTP (each), 10 nM oligo(dT) 20 ng random hexamer, 19% Trehalose, 0.2% Chapso, 5 units of MMLV(RnaseH-) RT, and 2 units of Taq DNA polymerase.

RapidScript™ Reverse Transcriptase RNAse Inhibitor Enzyme Mix: The reverse transcriptases may include, without limitation, AMV RT, RSV RT, MMLV RT, RNase H-mutants of various reverse transcriptases, HIV RT, EIAV RT, RAV2 RT, TTH DNA polymerase, C. hydrogeno formans DNA polymerase, Superscript II RT, SuperScript RT. ThermoScript RT and mixtures thereof.

RapidScript™ nCOV_N1, N2, RNase P Primer Probe Mix: CDC Primers and Probes in each tube.

RapidScript™ RNase-free wáter: RNAse-free water

RapidScript™ High ROX, Low ROX Passive Reference Dye: ROX Reference Dye

## MATERIALS REQUIRED BUT NOT PROVIDED

- Viral RNA Extraction Kit:
    - Qiagen: QIAmp DSP Viral RNA Mini Kit, QIAamp Viral RNA Mini Kit, EZ1 DSP Virus Kit, EZ1 Virus Mini Kit v2.0
    - Roche: MagNA Pure 24 Total Nucleic Acid Isolation Kit, DNA and Viral NA Small Volume Kit, DNA and Viral NA Small Volume Kit, Total Nucleic Acid Kit
    - ThermoFisher: MagMAX Viral/Pathogen Nucleic Acid Isolation Kit
    - MP Biomedicals: MPure-12™ Automated Nucleic Acid Purification System (117002200), MPure Viral/Pathogen Nucleic Acids Extraction Kit (117022130), MPure™ Viral Nucleic Acid Extraction Kit (117022300).
- Real-Time PCR detection system capable of reading FAM™ or equivalent channels
    - ThermoFisher: 7900HT, 7500, 7500 Fast, 7500 Fast Dx, 7300, Step One, StepOnePlus, QuantStudio3, QuantStudio5, QuantStudio6 Flex, QuantStudio7 Flex, QuantStudio 12 Flex
    - Bio-Rad: CFX96, iCycler, MyIQ, IQ5, CFX96 Touch, CFX 384, CFX Connect
    - Roche: 480 LightCycler, Lightcycler 2.0, LightCycler 1536
    - Stratagene/Agilent: Mx3000p, Mx3005p
    - Eppendorf: Mastercycler
    - Takara: TP800
    - Qiagen: Rotorgene 3000A, Rotorgene Q
- Adjustable pipettes & fitting filtered pipette tips

- Surface decontaminants such as DNA-Off (11QD0500, MP Biomedicals), RNase Erase ® decontamination solution (112440204, MP Biomedicals, LLC), 10% bleach
- Nuclease-free tubes/strips / plates to prepare dilutions and master mixes
- Nuclease-free tubes/strips / plates corresponding to the Real-Time PCR detection system
- Microcentrifuge
- Vortex Mixer
- Suitable storage options for reagents and specimens (-70 °C to 4 °C)

## SUGGESTED INTERNAL CONTROLS BUT NOT PROVIDED

A positive template control (CoV-19 Positive Control) is to be included in each assay plate to ensure the reagents and instruments are performing optimally. The positive control can be a synthetic DNA plasmid containing the entire sequence of the N gene of the COVID-19 virus, in vitro translated COVID-19 RNA, or known positive samples provided by the user. No positive controls are provided in the kit.

RapidScript™ RNaseP Primer Probe Mix consists of Human RNase P (RP) primer/probe combination is included in the kit. The RP serves as an internal, extraction, and amplification control. No human extraction controls are provided with this kit.

## STORAGE AND EXPIRATION

Upon receipt, store all components at -20 °C and avoid repeated free and thaw cycles.

Protect the kits from light as prolonged exposure can diminish the performance of the fluorophores.

Keep the reagents separate from sample material to avoid contamination.

If the kit components have been damaged, please contact MP Biomedicals, LLC directly.

Do not use after the designated expire date.

## WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic only and not intended for self-testing.

Wear appropriate personal protective equipment (gowns, gloves, eye protection) when working with clinical specimens.

To prevent contamination of PCR reactions, clean and decontaminate all working surfaces, centrifuges, pipets, and other equipment with RNase Erase, DNA-Off, or 10% bleach.

To minimize cross-contamination between samples, disposable pipettes and pipette tips are strongly recommended.

Specimen processing should be in a certified class II biological safety cabinet (BSL-2) or higher following its guidelines.

The use of RapidScript™ SARS-CoV-2 Assay (1-step RT-qPCR) is restricted to trained laboratory personnel only.

Decontaminate and dispose of all potentially infectious materials following local, state, and federal regulations.

# SAFETY PRECAUTIONS

Refer to the Materials Data Safety Sheet for information regarding the safe handling, transport, and disposal of this product.

This kit does not contain human-sourced or potentially infectious components.

# SPECIMEN COLLECTION, TRANSPORTATION, and STORAGE

Human nasopharyngeal swabs, oropharyngeal (throat) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal aspirates, nasal washes, bronchoalveolar lavage (BAL) fluid and sputum specimens can be tested with COVID-19 Coronavirus Real Time PCR Kit. Inappropriate sampling, storage and transportation may lead to incorrect detection results. The following are recommended:

**Specimen Collection**
Please refer to https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinicalspecimens.html for information on collection of appropriate specimens to test for SARS-CoV-2. Follow product instructions of instruments and consumables.

**Transportation**
Specimen packaging and transportation should follow https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafety-guidelines.html#specimen

Specimens collected from suspected SARS-CoV-2 cases should be preserved at 2-8°C using ice bags or ≤ -70°C on dry ice and sent to qualified laboratories within 24 hours.

**Storage**
Specimens may be stored at 2-8°C up to 24 hours after receipt.
Specimens may be stored at -70°C or colder if processing is delayed more than 24 hours.
Extracted RNA should be stored at -70°C or colder.

# ASSAY PROTOCOL

The performance of the RT-PCR assay STRONGLY depends on the amount and quality of sample template RNA. It is strongly recommended to qualify and validate RNA extraction procedures for recovery and purity before testing specimens.

### SAMPLE PROCESSING

Use a sample preservation solution for virus inactivation and RNA preservation

Use a viral nucleic acid extraction or purification kit, or a Trizol based extraction to purify viral RNA

### SAMPLE PREPARATION

Thaw assay to 15 - 30 °C. Keep on ice and covered. Probes are sensitive to light conditions. Once thawed reagents, except for the reverse transcriptase enzyme, can be stored at 2 - 4°C for 24 hours. Vortex all tubes each use. Centrifuge tubes briefly or tap tubes on the bench to bring the contents of the tube to the bottom of the tube before use.

### PCR ASSAY SETUP

1) Thaw reagents to room temperature and store on ice. Keep reverse transcriptase on ice at all time and keep primer-probe tubes away from light. Vortex for 5 seconds to mix and centrifuge for 5 seconds to bring all solutions to the bottom of the tube

2) Calculate the reactions needed. Every individual patient sample will require 3 replicate wells. Therefore, the number of samples will be 3 for each patient (using the same RNA sample) x the number of different patients in total. For example, if there were 10 patients that needed to be tested, the number of reactions would be 10 (total number of patients) x 3 (replicates of reactions per patient) = 30 reactions total. Also, for each reaction plate, there will be 3 negative controls (using water as the template) and 3 positive controls (in vitro translated viral RNA, plasmid positive control, or known COVID-19 positive patient sample).

**Reaction mix composition**: Prepare a real-time RT-qPCR master mix. The volumes given are based on a standard 20ul final reaction mix and can be scaled accordingly.

When using primer and probe designs adapted from the "CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT PCR diagnostic Panel" provided in the test kit, the reaction mix composition is as follows:

| Reagent | Volume | Final Concentration |
|---|---|---|
| RapidScript™ 2X Probe Supermix | 10 ul | 1x |
| RapidScript™ Primer Probe Mix (N1, N2, or RNase P) | 1.5 ul | |
| RapidScript™ Reverse Transcriptase RNAse Inhibitor Enzyme Mix | 0.4 ul | |
| RapidScript™ RNase-free water | To 20 ul | |
| RNA Template | | 1pg – 100ng |
| **Final Volume** | 20 ul | |

**Note: Do not add ROX to reaction mix**

# PREPARE PCR MASTER MIX

1) Each reaction should contain 10ul of 2x RapidScript™ 2X Probe Supermix, 0.4ul of RapidScript™ Reverse Transcriptase RNAse Inhibitor Enzyme mix and 1.5ul of RapidScript™ Primer Probe Mix for a 20ul reaction . It is recommended to use **2-4ul** of viral RNA extracted from the patient swab specimen. Then add water to the reaction to a final volume of 20ul.

*For example:* For 10 patient samples, 30 reactions will be needed (because each patient will require 3 replicate reactions for their sample).

| Reagent | 1 test | 30 tests |
|---|---|---|
| RapidScript™ 2X Probe Supermix | 10 uL | 300 uL |
| RapidScript™ Primer Probe Mix | 1.5 uL | 45 uL |
| RapidScript™ Reverse Transcriptase RNAse Inhibitor Enzyme Mix | 0.4 uL | 12 uL |
| Total | 11.9 uL | 357 uL |

There will be a total volume of 357ul. Mix the master mix tube well and centrifuge for 5 seconds to bring the master mix solution to the bottom of the tube. Dispense 11.9ul of master mix per reaction tube. To each well add 8.1ul of a mix of RNA template/sample and RapidScriptTM RNase-free water (depending on the amount of viral RNA that is used) to each reaction tube.

### CONTROL REACTION SET-UP

### INTERNAL EXTRACTION CONTROL (RNase P/ RP)

To set up the Extraction Control for each sample, the reaction well for a 20ul reaction will contain the following. To set up the RNase P control for each plate, 3 replicates will be needed, a total of 60ul total volume for the master mix. Each reaction of 20ul will contain:

- 10ul RapidScript™ 2X Probe Supermix

- 1.5ul of RapidScript™ RNAseP Primer Probe Mix
- 0.4ul RapidScript™ Reverse Transcriptase RNAse Inhibitor Enzyme mix
- 5.1ul of RapidScriptTM RNase-free water
- 3 uL of Viral RNA from patient samples

To make 3 replicate tubes, multiply each volume of each reagent for the 20uL reaction by 3 (See table)

| Reagent | 1 replicate | 3 replicates |
|---|---|---|
| RapidScript™ 2X Probe Supermix | 10 uL | 30uL |
| RapidScript™ RNAseP Primer Probe Mix | 1.5 uL | 4.5uL |
| RapidScript™ Reverse Transcriptase RNAse Inhibitor Enzyme Mix | 0.4 uL | 1.2uL |
| RapidScript™ RNase-free water | 5.1 uL | 15.3uL |
| Viral Template | 3 uL | 9 uL |

## NEGATIVE CONTROL (NTC)

1) 3 reactions will be need for the negative control (NTC) no template control. To set up the NTC for each plate, 3 replicates will be needed, a total of 60ul total volume for the master mix. Each reaction of 20ul will contain:

- 10ul RapidScript™ 2X Probe Supermix
- 1.5ul of RapidScriptTM Primer Probe Mix
- 0.4ul RapidScript™ Reverse Transcriptase RNAse Inhibitor Enzyme mix
- 8.1ul of RapidScriptTM RNase-free water

To make 3 replicate tubes, multiply each volume of reagent for the 20ul reaction by 3 (See table)

| Reagent | 1 replicate | 3 replicates |
|---|---|---|
| RapidScript™ 2X Probe Supermix | 10 uL | 30 uL |
| RapidScript™ Primer Probe Mix | 1.5 uL | 4.5 uL |
| RapidScript™ Reverse Transcriptase RNAse Inhibitor Enzyme Mix | 0.4 uL | 1.2 uL |
| RapidScript™ RNase-free water | 8.1 uL | 24.3 uL |

Mix the master mix well, centrifuge for 5 seconds to bring master mix solution to the bottom of the tube. Dispense 20ul of master mix to each reaction tube.

## POSITIVE CONTROL

1) 3 reactions will be needed for the positive control reaction tubes. To set up the positive controls for each plate, 3 replicates will be needed, a total of 60ul total. Each reaction of 20ul will contain:

- 10ul RapidScript™ 2X Probe Supermix
- 1.5ul of RapidScriptTM Primer Probe Mix
- 0.4ul RapidScript™ Reverse Transcriptase RNAse Inhibitor Enzyme mix
- 7.1ul of RapidScriptTM RNase-free water
- 1ul of COVID-19 RNA or any other COVID-19 positive sample/external positive control

To make 3 replicate tubes, multiply each volume of reagent for the 20ul reaction by 3 (see table)

| Reagent | 1 replicate | 3 replicates |
|---|---|---|
| RapidScript™ 2X Probe Supermix | 10 uL | 30 uL |
| RapidScript™ Primer Probe Mix | 1.5 uL | 4.5 uL |
| RapidScript™ Reverse Transcriptase RNAse Inhibitor Enzyme Mix | 0.4 uL | 1.2 uL |
| RapidScript™ RNase-free water | 7.1 uL | 24.3 uL |
| COVID-19 RNA or External Positive Control | 1 uL | 3 uL |

Mix the master mix well, centrifuge for 5 seconds to bring master mix solution to the bottom of the tube. Dispense 20ul of master mix to each reaction tube.

For all reactions, ROX is not added. However, ROX (in 2 concentrations) is supplied. The use of ROX is not recommended but its use is at the discretion of the user. If the user chooses to use ROX, the following table will help determine with formulation of ROX normalization dye should be used.

| qPCR Machine | ROX |
|---|---|
| Applied Biosystems Prism, Step One, Step One plus, 7900HT, 7900 HT Fast | High |
| Applied Biosystem Prism 7500, Prism 7500 Fast, ViiA7, QuantStudio, Stratagene | Low |
| Roche LightCycler, MJ Research Opticon 2, Takara TP-800, Bio-Rad iCycler, CFX96, C1000, Thermo Scientific Pikoreal 96, Qiagen RotorGene, Eppendorf Mastercycler | Not Recommended |

**SAMPLE REACTION PLATE SET-UP**:

NTC – negative control/no template control; S1-10 are unknown patient samples (S1 is a replicate of 3 for patient 1, S2 is a replicate of 3 for patient 2, S3 is a replicate of 3 for patient 3, etc.).; Pos. Control is the COVID-19 positive RNA sample, positive control plasmid or known positive patient control sample; RP is Human RNase P target control for each patient sample. The target for this reaction plate can be either N1 or N2 or any gene targeting SARS-CoV-2. trac

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | NTC | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | Pos. Control |
| B | NTC | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | Pos. Control |
| C | NTC | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | Pos. Control |
| D | | S1 RP | S2 RP | S3 RP | S4 RP | S5 RP | S6 RP | S7 RP | S8 RP | S9 RP | S10 RP | |
| E | | S1 RP | S2 RP | S3 RP | S4 RP | S5 RP | S6 RP | S7 RP | S8 RP | S9 RP | S10 RP | |
| F | | S1 RP | S2 RP | S3 RP | S4 RP | S5 RP | S6 RP | S7 RP | S8 RP | S9 RP | S10 RP | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |

**REAL-TIME RT-QPCR CONDITIONS:**

1) Set up plate with reactions conditions described on Sample Reaction Plate Set-up section above.
2) Assign each well with the corresponding sample names, targets, and FAM fluorophore.
3) Select FAM™ or equivalent channel as the fluorescence channel for all wells.
4) Select NONE for passive reference
5) Load the plate into the Real-Time PCR instrument and start the PCR protocol

**Real-Time RT-PCR Program**

| Stage | Description | # of Cycles | Temperature | Time |
|---|---|---|---|---|
| Step 1 | Reverse Transcription | 1 | 50°C | 5 minutes |
| Step 2 | Polymerase Activation | 1 | 94°C | 30 seconds |
| Step 3 | Denature | 45 | 94°C | 5 seconds |
| | Annealing, Extension | | 60°C | 30 seconds |
| | Detect Fluorescence/Plate Read | | | |

Real-Time RT-qPCR optimization: Some reactions may require optimization to increase their efficiency:

1) Extend the reverse transcription reaction time, up to 15 minutes

2) The annealing/extension time can be extended up to 60 seconds and/or the temperature of annealing/extension can be increased up to 65°C.

# QUALITY CONTROL

All test controls should be examined prior to the interpretation of the result. If the controls are not valid, the patient results cannot be interpreted. Visually inspect the amplification signals for each control to verify their validity.

Quality control requirements must be performed in conformance with local, state, and federal regulations or accreditation requirements and the user's laboratory's standard quality control procedures.

**POSITIVE CONTROL (PC)**

Positive control will show amplification signals which detect the presence of SARS-CoV-2. If the Positive Control sample are detected after 40 amplification cycles (Ct > 40) for the virus target, the control must be replaced with new aliquot. If this problem is not resolved, the whole kit must be replaced with a new one.

**NEGATIVE / NO TEMPLATE CONTROL (NTC)**

The No Template Control (NTC) will show no amplification for either the virus targets (N1, N2) or RNase P. The No Template Control sample will be considered valid if no amplification occurs with N1, N2, and RNase P Primer Probe Mix. If amplification occurs in any of those targets, contamination of extraction and/or reagents may have occurred, and reagents must be replaced.

Table . Expected Performance of Controls

| Control Type | N1, N2 target | Rnase P target | Expected Ct Values |
|---|---|---|---|
| No Template Control | - | - | No Ct value for any target |
| Positive Control | + | + | Ct of ≤ 40 for all N targets and Rnase P |

# INTERPRETATION OF RESULTS

## INTERNAL EXTRACTION CONTROL (RNase P)

All clinical samples should exhibit fluorescence growth curves in the RNase P reaction within 40 cycles, thus indicated the presence of human RNase P gene. Failure to detect RNase P in any clinical specimens may indicate improper extraction of nucleic acid from clinical materials resulting in loss of RNA and/or RNA degradation, absence of sufficient human cellular material due to poor collection or loss of specimen integrity, improper assay set up and execution, and reagent or equipment malfunction.

If the RP assay does not produce a positive result for human clinical specimens, interpret as follows:

- If the N1 and N2 are positive even in the absence of a positive RP, the result should be considered valid. It is possible, that some samples may fail to exhibit RNase P growth curves due to low cell numbers in the original clinical sample. A negative RP signal does not preclude the presence of SARS-CoV-2 virus RNA in a clinical specimen.
- If all N1, N2 markers AND RNase P are negative for the specimen, the result should be considered invalid for the specimen. If residual specimen is available, repeat the extraction procedure and repeat the test. If all markers remain negative after re-test, report the results as invalid and a new specimen should be collected if possible.

## SARS-CoV-2 Nucleocapsid Markers (N1 and N2)

- When all controls exhibit the expected performance, a specimen is considered negative if all N1 and N2 markers cycle threshold amplification curves DO NOT cross the threshold line within 40.00 cycles (< 40.00 Ct) AND the RNase P growth curve DOES cross the threshold line within 40.00 cycles (< 40.00 Ct).

- When all controls exhibit the expected performance, a specimen is considered positive for SARS-CoV-2 if all N1, N2 marker cycle threshold growth curves cross the threshold line within 40.00 cycles (< 40.00 Ct). The RNase P may or may not be positive as described above, but the result is still valid.

- When all controls exhibit the expected performance and the amplification curves for the SARS-CoV-2 markers (N1, N2) AND the RNase P marker DO NOT cross the cycle threshold growth curve within 40.00 cycles (< 40.00 Ct), the result is invalid. The extracted RNA from the specimen should be retested. If residual RNA is not available, re-extract RNA from residual specimen and re-test. If the
- re-tested sample is negative for all markers and RNase P, the result is invalid and collection of a new specimen from the patient should be considered.

- When all controls exhibit the expected performance and the cycle threshold growth curve for any one marker (N1 or N2, but not both markers) crosses the threshold line within 40.00 cycles (<40.00 Ct) the result is inconclusive. The extracted RNA should be retested. If residual RNA is not available, re-extract RNA from residual specimen and re-test. If the same result is obtained, report the inconclusive result. Consult with your state public health laboratory, as appropriate, to request guidance and/or to coordinate transfer of the specimen for additional analysis.

- If your sample is positive for N1 or N2, then contamination may have occurred during extraction or sample processing. Invalidate all results for specimens extracted alongside the sample. Re-extract specimens and re-test.

- The table below lists the expected results for the RapidScriptTM SARS-CoV-2 Assay (1-step RT-qPCR). If a laboratory obtains unexpected results for assay controls or if inconclusive or invalid results are obtained and cannot be resolved through the recommended re-testing,

**COVID-19 ONE-STEP RT RESULTS INTERPRETATION TABLE**

| COVID-19_N1 | COVID-19_N2 | RP | Interpretation | Report |
|---|---|---|---|---|
| + | + | +/- | SARS-CoV-2 detected | COVID-19 Positive |
| One of 2 targets is positive | One of 2 targets is positive | + | Inconclusive result, repeat assay | Inconclusive |
| - | - | + | SARS-CoV-2 not detected, amplification successful | COVID-19 negative |
| - | - | - | Invalid result, repeat assay | Invalid results |

# LIMITATIONS OF THE PROCEDURE

1) This assay is for in vitro diagnostic use only. All users, analysts, and any person reporting diagnostic results should be trained to perform this procedure by a competent instructor. They should demonstrate their ability to perform the test and interpret the results prior to performing the assay independently.

2) Optimal performance of this test requires appropriate specimen collection, storage and transport to test site

3) Detection of SARS-CoV-2 RNA is dependent on sample collection methods, patient factors (presence of symptoms) and/or stage of infection

4) False-negative results may arise from:

- faulty sample collection, processing, transportation, or low sample concentration
- degradation of RNA during transport
- variation in the target sequences of the 2019-nCoV novel coronavirus by other reasons
- improper reagent storage
- other unverified interferences or PCR inhibitors
- cross-contamination during sample processing

5) As with any molecular test, mutations within the target regions of the MP Biomedicals RapidScript™ SARS-CoV-2 Assay (1-step qPCR) test could affect primer/probe binding failing to detect the presence of virus

6) Performance has been established only with specimen types listed in the Intended Use. Other specimen types have not been evaluated and should not be used with this assay

7) Results should be interpreted by a trained professional in conjunction with the patient's history and clinical signs and symptoms.

8) Negative results do not preclude infection with the SARS-CoV-2 virus and should not be the sole basis of patient treatment or management or public health decisions.

# PERFORMANCE CHARACTERISTICS

**LIMIT OF DETECTION (LOD)**

Analytical sensitivity was confirmed using serial 2-fold serial dilutions of in vitro transcribed viral RNA (10 copies/ul, 5 copies/ul, 2.5 copies/ul and 1.7 copies/ul) with 30 replicates of the n-CoV-2_N1 and n-CoV-2_N2 targets. The final LOD of each test was determined to be the lowest concentration in which positive detection in 100% of all replicates (30/30).

The final LOD for this test was determined to be 2.5 copies/ul.

However, the MP BIO RapidScript™ SARS-CoV-2 Assay (1-step qPCR) kit can detect as low as 1.7 copies/ul with 93.3% confidence.

## Summary of LOD

Confirmation for COVID-19 by One-step RT-PCR using in vitro translated viral RNA

| | **n-CoV_N1** | | | | **n-CoV_N2** | | | |
|---|---|---|---|---|---|---|---|---|
| RNA concentration | 10 copies/ul | 5 copies/ul | 2.5 copies/ul | 1.7 copies/ul | 10 copies/ul | 5 copies/ul | 2.5 copies/ul | 1.7 copies/ul |
| Positive Detection | 30/30 | 30/30 | 30/30 | 28/30 | 30/30 | 30/30 | 30/30 | 28/30 |
| Mean Ct | 32.53 | 33.66 | 34.39 | 35.79 | 32.36 | 33.59 | 34.56 | 34.83 |
| Standard Deviation Ct | 0.26 | 0.46 | 0.46 | 1.3 | 0.38 | 0.65 | 0.69 | 0.94 |

# PROCEDURE NOTES

Read this manual carefully before using the assay.

This test needs to be conducted in a laboratory under proper testing conditions. All samples and materials in the testing process should be handled according to the operational specifications of an infectious disease laboratory.

Protect assay kit from light.

All reagents (with the exception of the reverse transcriptase enzyme) should reach room temperature (15-30 °C) before use. Once thawed store reagents on ice and away from light.

# DATE OF ISSUE

RapidScriptTM SARS-CoV-2 Assay (1-step RT-qPCR) Instruction For Use. Version 1, 17th June 2020.

# BIBLIOGRAPHY

Centers for Disease Control and Prevention (CDC). CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT PCR diagnostic Panel. Available online at https://www.cdc.gov/coronavirus/2019-nCoV/lab/index.html

## EXPLANATION OF THE SYMBOLS USED

| | |
|---|---|
|  | **For in vitro diagnostic use** |
|  | **Catalog Number** |
|  | **Batch code** |
|  | **Manufacturer** |
|  | **Date of Manufacture** |
|  | **Use by YYYY-MM** |
|  | **Consult instruction for use** |
|  | **Upper limit of temperature** |
|   | **Contents sufficient for 400 tests** |

## GENERAL INFORMATION

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