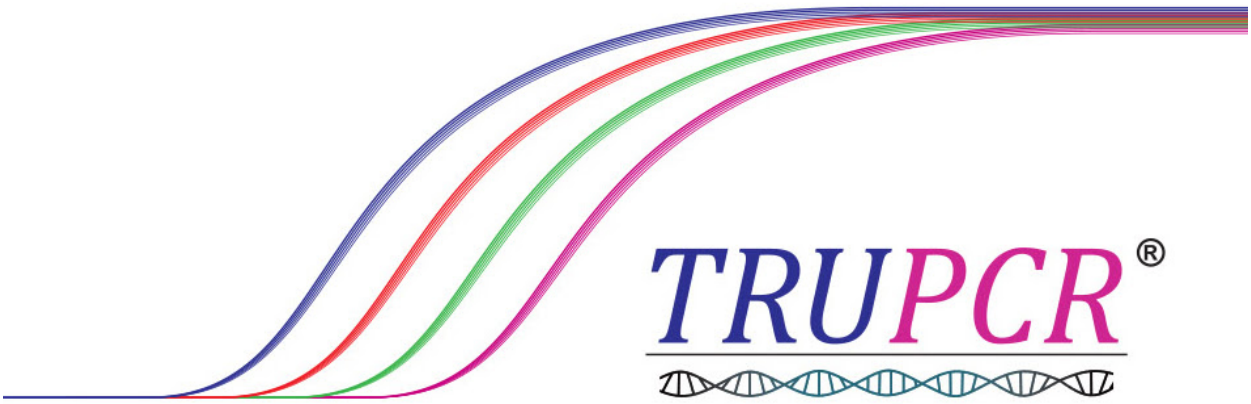

TRUPCR® SARS-CoV-2 KIT

(Real-Time PCR based detection of SARS-CoV-2 virus)

Version 2.0

Instructions for Use

FOR IN VITRO DIAGNOSTIC USE
FOR RX USE ONLY
FOR EMERGENCY USE AUTHORIZATION ONLY

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TRUPCR® SARS-CoV-2 KIT

(Real-Time PCR based detection of SARS-CoV-2 virus)

Version 2.0

IVD

Lic. No.-
MFG/IVD/2020/000024

FOR IN VITRO DIAGNOSTIC USE
FOR RX USE ONLY
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REF Product No.: 3B304

100 tests

Temperature limitation

April 2020

Kilpest India Ltd. DBA 3B Blackbio Biotech
7-C Industrial Area, Govindpura, Bhopal-462023 (M.P)

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INTENDED USE

TRUPCR® SARS-CoV-2 Kit is a real-time RT-PCR assay intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal and oropharyngeal swabs, anterior nasal swab and mid-turbinate nasal swabs, nasopharyngeal aspirates/washes or nasal aspirates, and bronchoalveolar lavage (BAL) specimens from individuals who are suspected of COVID-19 by their healthcare provider.

Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C.§263a, that meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2; clinical correlation with patient history and other diagnostic information is necessary 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.

Testing with the TRUPCR® SARS-CoV-2 kit is intended for use by qualified laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The TRUPCR® SARS-CoV-2 Kit is intended for use only under the Food and Drug Administration's Emergency Use Authorization.

PRINCIPLE

The TRUPCR SARS-Co V-2 Kit is a real-time reverse transcription polymerase chain reaction (RT - PCR) test. The RdRP/N primer and probe set(s) are designed to detect RNA from SARS-CoV-2 in nasopharyngeal or oropharyngeal swabs, anterior nasal swab and mid-turbinate nasal swabs, nasopharyngeal aspirate/wash or nasal aspirates, and bronchoalveolar lavage (BAL) specimens from patients who are suspected of COVID-19 by their healthcare provider.

The TRUPCR® SARS-CoV-2 Kit is RT-qPCR assay based on the principle of oligonucleotide hydrolysis. Real-time RT-PCR technology utilizes reverse-transcription (RT) to convert extracted RNA into complementary DNA (cDNA). In real-time PCR, the fluorescent signal is generated from the presence of an oligonucleotide probe specific to a target DNA sequence. The probe contains a fluorescent dye molecule on its 5’ end and a quencher molecule on its 3’ end. When the probe is intact, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence primarily by Fluorescence resonance energy transfer (FRET). The probe hybridizes with one of the chains of the amplified fragment. During synthesis of a complementary chain, Taq DNA polymerase which possesses 5' - 3' exonuclease activity cleaves the probe. As a result, the fluorescent dye and quencher dye are separated, and the total fluorescence of reaction volume increases in direct proportion to the number of amplicon copies synthesized during PCR. This process occurs in every cycle and does not interfere with the exponential accumulation of product. The fluorescent signal is measured in each cycle of reaction, and the threshold cycle value is determined from the obtained curve. The threshold cycle is proportional to the initial number of RNA copies in a sample and its value allows qualitative comparisons of analyzed and control samples.

The TRUPCR® SARS-CoV-2 Kit is composed of 2 tube assays. Tube 1 contains primers and probes specific to the E gene (FAM) for the detection of the Sarbecovirus (of Genus B-betacoronavirus (B-βCoV)) and the endogenous internal control RNaseP (HEX); tube 2 contains primers and probes specific to the RdRP gene (FAM) and N gene (FAM) for the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) along with the endogenous internal control

RNaseP (HEX). Internal control gene is included in both the tubes, to verify the extracted RNA quality, amplification procedure and possible presence of inhibitors, which may cause false negative results.

In an effort to conserve reagent resources, different workflows can be used with the TRUPCR SARS-CoV-2 Kit. The user can run the Kit without the Tube 1 assay (containing primers and probes specific to the E gene for the detection of the Sarbecovirus (of Genus B-betacoronavirus (B-βCoV)) and the endogenous internal control RNaseP). In addition, the TRUPCR SARS-CoV-2 Kit can also be used to screen for the presence of Sarbecovirus RNA by using the Tube 1 assay (E-gene). If positive, the Tube 2 assay can be used to assess for the presence of SARS-CoV-2 RNA.

GENERAL PRECAUTIONS AND WARNINGS

- For Emergency Use Authorization (EUA) only
- For in vitro diagnostic use
- For Prescription Use Only (Rx)
- This test has not been FDA cleared or approved
- The test has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high complexity tests.
- This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

- After the thawing of reagents, make sure to put back within 15-20 minutes or as soon as possible to -20°C freezers.
- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Thaw all components thoroughly at room temperature before starting detection.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all samples and unused reagents in compliance with local authorities' requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices. Infected material and disposable plasticware that was in contact with infected material must be treated with chlorine-containing solutions.
- Clean and disinfect all sample or reagent spills using a disinfectant, such as 0.5 % sodium hypochlorite or other suitable disinfectant.
- Avoid contact with the skin, eyes and mucosa. If skin, eyes and mucosa contact, immediately flush with water, seek medical attention.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- The laboratory process must be uni-directional; it should begin in the Extraction Area and then move to the Amplification and Detection Areas. Do not return samples, equipment and reagents to the area in which the previous step was performed.

REAGENTS

The kit contains amplification reagents for performance of 100 reactions. Thaw and handle reagents on ice. Do not freeze/thaw kit vials repeatedly. In case of frequent use, we recommend to aliquot the contents of the vials into 10 reactions each. *See photos of each kit component at the end of this document.*

Reagent	Description	Volume in 100 reactions pack size
Master Mix	• Hot-start DNA polymerase • Reaction Buffer • dNTPs (dATP, dCTP, dGTP, dTTP) • MgCl2 and stabilizers	1000 µL X 2
Enzyme Mix	• Enzyme Mix for RT	35 µL X 2
Primer Probe Mix-1	• Primer Probe mix for E gene and human RNaseP gene detection[a,b]	233 µL X 2
Primer Probe Mix-2	• Primer Probe mix for RdRP gene and N gene and human RNaseP gene detection[b]	233 µL X 2
Water (RNase free)	• Sterilized water	500 µL X 2
Negative Control	• Sterilized water	500 µL X 2
High Positive Control	• High copy number Positive control	100 µL X 2
Low Positive control	• Low copy number positive control	100 µL X 2

[a] The TRUPCR SARS-CoV-2 Assay can be used without the E gene Primer-Probe Mix 1
[b] The TRUPCR SARS-CoV-2 Assay can use the E gene Primer Probe Mix-1 as a Sarbecovirus screening assay, followed by use of the RdRP/N Primer Probe Mix-2 as a SARS-CoV-2 confirmatory assay if applicable

STORAGE AND HANDLING

All the components of TRUPCR® SARS-CoV-2 RT qPCR kit should be stored at -20˚C and are stable until the date of expiry stated. Do not use reagents past their expiration date. The reagents can be aliquoted and stored at -20˚C in order to maintain the stability and sensitivity.

MATERIAL AND DEVICES REQUIRED BUT NOT PROVIDED

- TRUPCR® Viral RNA Extraction Kit (3B213V/3B214V)
- Applied Biosystem QuantStudio 3 software version 1.4.3
- Qiagen Rotor-Gene Q 5Plex HRM Platform software version 2.3.1
- Adjustable pipettes with sterile filter or positive displacement tips
- Disposable powder-free gloves
- Sterile bi-distilled water
- Sterile 1.5 ml and 2 ml microcentrifuge tubes
- 50 ml conical tubes
- Vortex mixer
- Real time PCR
- Laminar airflow cabinet
- PCR vials (0.2 ml, thin-walled)
- 96 – 100% ethanol
- Personal protection equipment (lab coat, gloves, goggles)

INSTRUCTIONS FOR USE FOR APPLIED BIOSYSTEM AND ROTOR-GENE Q INSTRUMENTS

Description of Test Steps

Specimen Collection and Transport:

The TRUPCR® SARS-CoV-2 Kit is a qualitative test based on real-time reverse transcription polymerase chain reaction (RT-PCR) for the detection of the SARS-CoV-2 RNA extracted from nasopharyngeal, oropharyngeal, anterior nasal and mid-turbinate nasal swabs, nasopharyngeal wash/aspirate or nasal aspirates, and BAL specimens. Swab specimens are collected using sterile flocked nylon swabs (Himedia, Cat # PW1172) placed into TRUPCR® Viral Transport Medium (3B Blackbio Biotech India Ltd, Cat # 3B305). Flocked swabs and viral transport media/universal transport media are also acceptable for processing with the TRUPCR® workflow. BALs and washes/aspirates should be collected in sterile, DNase/RNase free Cryotubes (Cryochill™ Vial Self Standing sterile, Tarsons Products Pvt Ltd., Cat # 523182) or

other appropriate sterile containers without preservative. Specimens must be packaged and shipped in accordance with the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulations and Guidance of the Centers for Disease Control and Prevention (CDC). Briefly and if necessary, the specimens can be stored at 2-8°C for up to 72 hours. If the delay in shipping or extraction is expected to extend beyond 72 hours, storage at -70°C is recommended until the workflow can proceed. The storage conditions are the same for both upper and lower respiratory tract specimens.

RNA Extraction Step:

Performance of RT-PCR amplification-based assay depends on the amount and quality of sample template RNA. RNA extraction procedures should be performed by TRUPCR® Viral RNA Extraction Kit (3B213V/3B214V), by following manufacturer's recommended procedures for sample extraction. Briefly, RNA is extracted from 200 µL (input volumes are same for both upper and lower respiratory tract specimens) of clinical specimens and eluted with 40 µL of elution buffer consisting of RNase-free water supplied with the kit. Following the extraction, RNA should be used immediately or stored at -80°C for a maximum of 1 month.

There are three recommended approaches for using the TRUPCR SARS-CoV-2 Kit:

- Approach #1: Run both Tube 1 (E gene assay, Sarbecovirus detection) and Tube 2 (RdRP/N gene assay, SARS-CoV-2 detection) for every clinical sample and positive/negative control.
- Approach #2: Use Tube 1 (E gene assay, Sarbecovirus detection) as a screening assay. If positive, run Tube 2 (RdRP/N gene assay, SARS-CoV-2 detection) to confirm whether SARS-CoV-2 RNA is present in the patient sample.
- Approach #3: Use ONLY Tube 2 (RdRP/N gene assay, SARS-CoV-2 detection) for every clinical sample and positive/negative control.

Approach #1:

Real Time PCR Protocol:

1. **Reaction Preparation for Samples**

   NOTE: Two separate tubes are run for every single sample in this approach

Tube - 1	Tube - 2
	
E	***RdRP & N***
RNaseP	***RNaseP***

Prepare the PCR Mix as follows:

Name of the Reagent	Tube-1 (SARS-like coronaviruses)	Tube-2 (SARS-CoV-2 specific)
Master Mix	10 µL	10 µL
Enzyme Mix	0.35 µL	0.35 µL
Primer Probe Mix-1	4.65 µL	-
Primer Probe Mix-2	-	4.65 µL
RNase free water	5 µL	5 µL
Total reaction volume	**20 µL**	**20 µL**

a) Transfer 20 µL of the above prepared Reaction mixes into separate 0.2 mL PCR tubes and close the tubes.

b) In duplicate (one for each tube), add 5 µL of RNA sample, positive control, or negative control to make a final volume of 25 µL.

2. **Program Set Up**

   Define the following settings for the Temperature Profile and Dye Acquisition on the PCR instruments. These settings apply to both the Qiagen Rotor-Gene Q 5Plex HRM (software 2.3.1) and Applied Biosystem QuantStudio3 (software 1.4.3) platforms that were validated for use with the assay.

Step	Temperature, °C	Time	Dye Acquisition	Cycles
1	50	15 min	-	1
2	95	05 min	-	1
3	95	05 sec	-	40
4	60	40 sec	Yes	
5	72	15 sec	-	

Passive Reference Dye – ROX

3. **Channel Selection**

   Define the following setting for channel selection

Detection	Detector Name	Reporter	Quencher	Gain Setup
Tube 1	E gene	FAM	BHQ1	Auto
	RNaseP	HEX	BHQ1	Auto
Tube 2	RdRP + N	FAM	BHQ1	Auto
	RNaseP	HEX	BHQ1	Auto

4. **Result Analysis**

   Use the Approach #1 Patient Results Interpretation chart after testing the samples.

Approach #2:

Real Time PCR Protocol:

1. **Reaction Preparation for Samples**

   NOTE: Prepare the Tube 1 assay (Screening).

Tube - 1

E

RNaseP

Prepare the PCR Mix as follows:

Name of the Reagent	Tube-1 (SARS-like coronaviruses)
Master Mix	10 µL
Enzyme Mix	0.35 µL
Primer Probe Mix-1	4.65 µL
Primer Probe Mix-2	-
RNase free water	5 µL
Total reaction volume	**20 µL**

a) Transfer 20 µL of the above prepared Reaction mix into a 0.2 mL PCR tube and close the tube.

b) Add 5 µL of RNA sample, positive control, or negative control to make a final volume of 25 µL.

2. Program Set Up

Define the following settings for the Temperature Profile and Dye Acquisition on the PCR instruments. These settings apply to both the Qiagen Rotor-Gene Q 5Plex HRM (software 2.3.1) and Applied Biosystem QuantStudio3 (software 1.4.3) platforms that were validated for use with the assay.

Step	Temperature, °C	Time	Dye Acquisition	Cycles
1	50	15 min	-	1
2	95	05 min	-	1
3	95	05 sec	-	40
4	60	40 sec	Yes	
5	72	15 sec	-	

Passive Reference Dye – ROX

3. Channel Selection

Define the following setting for channel selection

Detection	Detector Name	Reporter	Quencher	Gain Setup
Tube 1	E gene	FAM	BHQ1	Auto
	RNaseP	HEX	BHQ1	Auto

4. Result Analysis: Use the Approach #2 Patient Results Interpretation chart after testing the samples. If the E-gene assay is positive follow the below steps for the Tube 2 confirmatory assay:

Tube – 2 (Confirmatory)

RdRP* & *N

RNaseP

Prepare the PCR Mix as follows:

Name of the Reagent	Tube-2 (SARS-CoV-2 specific)
Master Mix	10 µL
Enzyme Mix	0.35 µL
Primer Probe Mix-1	-
Primer Probe Mix-2	4.65 µL
RNase free water	5 µL
Total reaction volume	**20 µL**

a) Transfer 20 µL of the above prepared Reaction mix into a 0.2 mL PCR tube and close the tube.

b) Add 5 µL of RNA sample, positive control, or negative control to make a final volume of 25 µL.

5. **Program Set Up**

Define the following settings for the Temperature Profile and Dye Acquisition on the PCR instruments. These settings apply to both the Qiagen Rotor-Gene Q 5Plex HRM (software 2.3.1) and Applied Biosystem QuantStudio3 (software 1.4.3) platforms that were validated for use with the assay.

Step	Temperature, °C	Time	Dye Acquisition	Cycles
1	50	15 min	-	1
2	95	05 min	-	1
3	95	05 sec	-	40
4	60	40 sec	Yes	
5	72	15 sec	-	

Passive Reference Dye – ROX

6. **Channel Selection**

Define the following setting for channel selection

Detection	Detector Name	Reporter	Quencher	Gain Setup
Tube 2	RdRP + N	FAM	BHQ1	Auto
	RNaseP	HEX	BHQ1	Auto

Approach #3:

Real Time PCR Protocol:

1. **Reaction Preparation for Samples**

Tube - 2

RdRP* & *N

RNaseP

Prepare the PCR Mix as follows:

Name of the Reagent	Tube-2 (SARS-CoV-2 specific)
Master Mix	10 µL
Enzyme Mix	0.35 µL
Primer Probe Mix-1	-
Primer Probe Mix-2	4.65 µL
RNase free water	5 µL
Total reaction volume	**20 µL**

a) Transfer 20 µL of the above prepared Reaction mixes into a 0.2 mL PCR tube and close the tube.

b) Add 5 µL of RNA sample, positive control, or negative control to make a final volume of 25 µL.

2. **Program Set Up**

Define the following settings for the Temperature Profile and Dye Acquisition on the PCR instruments. These settings apply to both the Qiagen Rotor-Gene Q 5Plex HRM (software 2.3.1) and Applied Biosystem QuantStudio3 (software 1.4.3) platforms that were validated for use with the assay.

Step	Temperature, °C	Time	Dye Acquisition	Cycles
1	50	15 min	-	1
2	95	05 min	-	1
3	95	05 sec	-	40
4	60	40 sec	Yes	
5	72	15 sec	-	

Passive Reference Dye – ROX

3. **Channel Selection**

Define the following setting for channel selection

Detection	Detector Name	Reporter	Quencher	Gain Setup
Tube 2	RdRP + N	FAM	BHQ1	Auto
	RNaseP	HEX	BHQ1	Auto

4. **Result Analysis**

Use the Approach #3 Patient Results Interpretation chart after testing the samples.

THRESHOLD SETTINGS

*Range of threshold: An absolute value of threshold varies from instrument to instrument depending upon instrument's make, age, model and calibration. Please set threshold above the maximum level of no Template control curve (random noise curve), then analyze the results.

RESULT ANALYSIS

Approach #1 (Both E and RdRP/N Assays)

Control Results Interpretation

	Expected Results and Ct Values	
	Primer Probe Mix 1 SARS-like coronaviruses	Primer Probe Mix 2 SARS-CoV-2

Control Type/Name	Used to Monitor	E (FAM)	RNase P (HEX)	RdRP/N (FAM)	RNase P (HEX)
Negative control (NTC)	Reagent and/or environmental contamination	ND	ND	ND	ND
High Positive Control (PC)	PCR reagent failure including primer and probe integrity	Positive Ct < 27	Positive Ct < 27	Positive Ct < 27	Positive Ct < 27
Low Positive Control (PC)		Positive Ct < 33	Positive Ct < 33	Positive Ct < 33	Positive Ct < 33
RNase P Internal Control (IC)	Failure in lysis and extraction procedure	N/A	Positive ≤ 35/ ND*	N/A	Positive ≤ 35/ ND*

N/A; Not Applicable
ND; Not Detected
* Detection of the internal control is not required for samples that are positive for SARS-CoV-2

1. The negative control reactions for probe/primer sets should not exhibit fluorescence growth curves that cross the threshold line. If a false positive occurs with one or more of the primers and probe sets for the no template control (NTC) reactions, sample contamination may have occurred. Invalidate the run and repeat the assay using the same NTC. If the NTC continues to show a Ct of ≤ 35, stop using the kit and refer to the troubleshooting section in the Instructions for Use or use different molecular biology grade water.
2. There are 2 positive controls included with the TRUPCR® SARS-CoV-2 Kit; a high positive and a low positive that consists of DNA plasmids of RdRP, E, and N genes mixed in equal concentrations. The high positive control (PC) should be considered valid if the Ct values for E, RdRP, N, and RNase P targets are < 27. The low positive control (PC) should be considered valid if the Ct values for E, RdRP, N, and RNase P are < 33.

Serial Number	Control	Expected Ct Values
1	High Positive control Tube 1	<27
2	High Positive control Tube 2	<27
3	Low Positive control Tube 1	<33
4	Low Positive control Tube 2	<33
5	Negative control Tube 1	No amplification
6	Negative control Tube 2	No amplification

3. All clinical samples should exhibit RNaseP reaction curves that cross the threshold line at or before 35 cycles, thus indicating the presence of sufficient RNA from the human RNaseP gene indicating the specimen is of acceptable quality. However, it is possible that some samples may fail to give positive reactions due to low cell numbers in the original clinical sample. Failure to detect RNaseP in any of the clinical samples may indicate:

   (a) Improper extraction of nucleic acid from clinical materials resulting in loss of RNA or carry-over of RT-PCR inhibitors from clinical specimens

   (b) Absence of enough human cellular material in sample to enable detection

   (c) Improper assay set up and execution

   (d) Reagent or equipment malfunction

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.

Approach #2 (E Assay for Screening, followed by RdRP/N Assay if Applicable)

		Expected Results and Ct Values		Next Steps as defined by the result of the Screening Assay	Expected Results and Ct Values	
		Primer Probe Mix 1 SARS-like coronaviruses			Primer Probe Mix 2 SARS-CoV-2	
Control Type/Name	**Used to Monitor**	**E (FAM)**	**RNase P (HEX)**		**RdRP/N (FAM)**	**RNase P (HEX)**
Negative control (NTC)	Reagent and/or environmental contamination	ND	ND	Controls must be valid to proceed to the RdRP/N assay	ND	ND
High Positive Control (PC)	PCR reagent failure including primer and probe integrity	Positive Ct < 27	Positive Ct < 27		Positive Ct < 27	Positive Ct < 27
Low Positive Control (PC)		Positive Ct < 33	Positive Ct < 33		Positive Ct < 33	Positive Ct < 33
RNase P Internal Control (IC)	Failure in lysis and extraction procedure	N/A	Positive ≤ 35/ ND*		N/A	Positive ≤ 35/ ND*

N/A; Not Applicable
ND; Not Detected
* Detection of the internal control is not required for samples that are positive for SARS-CoV-2

Approach #3 (RdRP/N Assay Only)

		Expected Results and Ct Values	
		Primer Probe Mix 2 SARS-CoV-2	
Control Type/Name	**Used to Monitor**	**RdRP/N (FAM)**	**RNase P (HEX)**
Negative control (NTC)	Reagent and/or environmental contamination	ND	ND
High Positive Control (PC)	PCR reagent failure including primer and probe integrity	Positive Ct < 27	Positive Ct < 27
Low Positive Control (PC)		Positive Ct < 33	Positive Ct < 33
RNase P Internal Control (IC)	Failure in lysis and extraction procedure	N/A	Positive ≤ 35/ ND*

N/A; Not Applicable
ND; Not Detected
* Detection of the internal control is not required for samples that are positive for SARS-CoV-2

Examination of Patient Specimen Results:

Cutoff- This assay runs for 40 cycles however for clinical sample interpretation, the threshold cutoff cycle Ct is 35. When all controls meet stated requirements, specimen results are interpreted according to the following:

Approach #1:

Patient Results Interpretation Running Both the E and RdRP/N Assays Simultaneously

Primer Probe Mix 1 SARS-like coronaviruses		Primer Probe Mix 2 SARS-CoV-2		Result Interpretation	Actions
E (FAM)	**RNase P (HEX)**	**RdRP/N (FAM)**	**RNase P (HEX)**		
+	+/-	+	+/-	**SARS-CoV-2 Positive**	Report results to healthcare provider and appropriate public health authorities.
-	+	+	+/-	**SARS-CoV-2 Positive**	
-	-	+	+/-	**SARS-CoV-2 Positive**	As amplification is seen in the SARS-CoV-2 specific master mix (RdRP/N), the sample is considered SARS-CoV-2 positive. Report results to healthcare provider and appropriate

Primer Probe Mix 1 SARS-like coronaviruses		Primer Probe Mix 2 SARS-CoV-2		Result Interpretation	Actions
E (FAM)	RNase P (HEX)	RdRP/N (FAM)	RNase P (HEX)		
					public health authorities.
+	+/-	-	+	**SARS-CoV-2 Presumptive Positive**	Sample is repeated once using residual extracted nucleic acid. If the repeated result remains Presumptive Positive, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and other SARS-like viruses for epidemiological purposes or clinical management.
+	+/-	-	-	**SARS-CoV-2 Presumptive Positive**	
-	+	-	+	**SARS-CoV-2 Negative**	Report results to healthcare provider.
-	-	-	+	**SARS-CoV-2 Negative**	
-	+	-	-	**SARS-CoV-2 Negative**	
-	-	-	-	**Invalid**	Repeat test using new extracted nucleic acid from original patient sample. If all markers remain negative after re-test, report the results as invalid and re-collect patient sample.

NOTE:

1. If needed, manually inspect amplification curves for all the samples. Assign a Ct value to verify the positive amplification.
2. Detection of the Internal Control in the HEX detection channel is not required for positive results in the FAM™ detection channel. A high Sarbecovirus (target E gene) and/or SARS-CoV-2 (target N/RdRP gene) RNA load in the sample can lead to reduced or absent Internal Control signals.

Approach #2:

Patient Results Interpretation Running the E-gene Assay 1st and if directed, the RdRP/N Assay 2nd

Screening Assay Step #1 Primer Probe Mix 1 SARS-like coronaviruses		Next Steps as defined by the result of the Screening Assay	Confirmatory Assay Step #2 Primer Probe Mix 2 SARS-CoV-2		Result Interpretation	Actions
E (FAM)	RNase P (HEX)		RdRP/N (FAM)	RNase P (HEX)		
+	+/-	Sample is repeated using the confirmatory assay (Tube 2) and residual extracted nucleic acid.	+	+/-	**SARS-CoV-2 Positive**	Report results to healthcare provider and appropriate public health authorities.
+	+/-		-	+	**SARS-CoV-2 Presumptive Positive**	Sample is repeated once using residual extracted nucleic acid and the confirmatory assay. If the repeated result remains Presumptive Positive. Additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and other SARS-like viruses for epidemiological purposes or clinical management.
+	+/-		-	-	**SARS-CoV-2 Presumptive Positive**	Tube 2 results are invalid. Repeat the confirmatory assay using newly extracted nucleic acid from the original patient sample. If all Tube 2 targets remain negative after re-test, report the result as Presumptive Positive. Additional confirmato testing may be conducted, if it is necessary t differentiate between SARS-CoV-2 and other SARS-like viruses for epidemiological purposes or clinical management.
-	+	Report results to healthcare provider. Tube 2 is preserved for future testing.	Tube 2 Assay is not performed		**SARS-CoV-2 Negative**	Report results to healthcare provider.
-	-	**Invalid Results**. Repeat Tube 1 using newly extracted nucleic acid from original patient sample.	Tube 2 assay is not performed		**Invalid**	If Tube 1 markers remain negative after re-rest, report the results as invalid and re-collect the patient sample.

NOTE:

1. If needed, manually inspect amplification curves for all the samples. Assign a Ct value to verify the positive amplification.

2. Detection of the Internal Control in the HEX detection channel is not required for positive results in the FAM™ detection channel. A high Sarbecovirus (target E gene) and/or SARS-CoV-2 (target N/RdRP gene) RNA load in the sample can lead to reduced or absent Internal Control signals.

Approach #3:

Patient Results Interpretation for ONLY Running the RdRP/N Assay

Primer Probe Mix 2 SARS-CoV-2		Result Interpretation	Actions
RdRP/N (FAM)	RNase P (HEX)		
+	+/-	**SARS-CoV-2 Positive**	As amplification is seen in the SARS-CoV-2 specific master mix (RdRP/N), the sample is considered SARS-CoV-2 positive. Report results to healthcare provider and appropriate public health authorities.
-	+	**SARS-CoV-2 Negative**	Report results to healthcare provider.
-	-	**Invalid**	Repeat test using new extracted nucleic acid from original patient sample. If markers remain negative after re-test, report the results as invalid and re-collect patient sample.

NOTE:

1. If needed, manually inspect amplification curves for all the samples. Assign a Ct value to verify the positive amplification.
2. Detection of the Internal Control in the HEX detection channel is not required for positive results in the FAM™ detection channel. A high SARS-CoV-2 (target N/RdRP gene) RNA load in the sample can lead to reduced or absent Internal Control signals.

10. Limitations

- This test is a qualitative test and does not provide the quantitative value of viral load in the original specimens.

- The specimens to be tested shall be collected, processed, stored and transported in accordance with the conditions specified in the instructions. Inappropriate specimen preparation and operation may lead to inaccurate results.
- Extraction of nucleic acid from clinical samples must be performed according to the specified methods listed in this procedure. Other extraction approaches have not been evaluated.
- Amplification and detection of SARS-CoV-2 with this kit has only been validated with the Applied Biosystems QuantStudio 3 and the Qiagen Rotor-Gene Q 5Plex HRM. Use of other instrument systems may cause inaccurate results.
- Based on the *in silico* analysis, SARS-CoV and other SARS-like coronaviruses in the same subgenus (Sarbecovirus) as SARS-CoV may cross react with the E, N, and/or RdRP primer sets of the TRUPCR® SARS-CoV-2 Kit. SARS-CoV is not known to be currently circulating in the human population, therefore it is highly unlikely to be present in patient specimens.
- The TRUPCR® SARS-CoV-2 Kit performance was established using oropharyngeal and nasopharyngeal swab samples only. While other specimen types listed in the IU are acceptable specimens for testing (i.e., anterior nasal swabs, mid-turbinate nasal swabs, and nasopharyngeal wash/aspirate or nasal aspirates, and bronchoalveolar lavage (BAL) specimens), clinical performance with the TRUPCR® SARS-CoV-2 Kit has not been established.
- False-negative results may occur if the viruses are present at a level that is below the analytical sensitivity of the assay or if the virus has genomic mutations, insertions, deletions, or rearrangements or if performed very early in the course of illness.
- Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C.§263a, that meet requirements to perform high complexity tests.

11. Conditions of Authorization for the Laboratory

The TRUPCR® SARS-CoV-2 Kit Letter of Authorization, User Manual, and Labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas.

To assist clinical laboratories using the TRUPCR® SARS-CoV-2 Kit, the relevant Conditions of Authorization are listed below.

a) Authorized laboratories[1] using the TRUPCR® SARS-CoV-2 Kit will include with test result reports, 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 the TRUPCR® SARS-CoV-2 Kit will use it as outlined in the authorized labeling. Deviation from the authorized procedures, such as the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use the TRUPCR® SARS-CoV-2 Kit are not permitted.

c) Authorized laboratories that receive the TRUPCR® SARS-CoV-2 Kit will notify the relevant public health authorities of their intent to run the product prior to initiating testing.

d) Authorized laboratories using the TRUPCR® SARS-CoV-2 Kit will have a process in place for reporting test results to healthcare providers and relevant public health authorities.

e) Authorized laboratories will collect information on the performance of the TRUPCR® SARS-CoV-2 Kit and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and 3B Blackbio (via email: mail@genophyll.com and info@3bblackbio.com) if they become aware of any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the TRUPCR® SARS-CoV-2 Kit.

f) All laboratory personnel using the TRUPCR® SARS-CoV-2 Kit must be appropriately trained in molecular techniques and use appropriate laboratory and personal protective

equipment when handling this kit and use the TRUPCR® SARS-CoV-2 Kit in accordance with the authorized labeling.

g) 3B Blackbio, authorized distributors including Genophyll Enterprises, and authorized laboratories using the TRUPCR® SARS-CoV-2 Kit 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, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests" as "authorized laboratories."

PERFORMANCE CHARACTERISTICS

A. Limit of detection (Sensitivity):

LoD studies determine the lowest detectable concentration of SARS-CoV-2 at which approximately 95% of all (true positive) replicates test positive. The LoD was determined using a reference RNA control from ThermoFisher Scientific, USA (AcroMetrix™ Coronavirus 2019 (COVID-19) RNA Control (Cat # 954519)). Different sample matrices (nasopharyngeal swab, oropharyngeal swab, and bronchoalveolar lavage (BAL) were screened negative by an EUA authorized assay and spiked with AcroMetrix™ Coronavirus 2019 (COVID-19) RNA Control at several concentrations. RNA was extracted using the TRUPCR® Viral RNA Extraction Kit and the experiment was performed on the Applied Biosystem QuantStudio 3 and the Qiagen Rotor-Gene Q 5Plex HRM. A two-phase approach was used to determine the LoD for each specimen type. In phase I the preliminary LoD was established using a limited number of replicates and various concentrations of spiked RNA material. In phase II, the LoD was confirmed by testing 20 independent extraction replicates at different concentrations. The LoD of the TRUPCR® SARS-CoV-2 Kit was established at 10 copies/µL with both the QuantStudio and Rotor-Gene Q platforms.

Applied Biosystem QuantStudio 3 LoD confirmatory study results

Nasopharyngeal swab						
Dilution Copies/µl	Detection Rate E gene (FAM)	Detection Rate RdRP/N (FAM)	Average Ct E gene (FAM)	SD E gene	Average Ct RdRP/N genes (FAM)	SD RdRP/N genes
500	20/20	20/20	29.50	0.5332	29.75	0.4257
100	20/20	20/20	31.40	0.5722	31.22	0.5266
50	20/20	20/20	32.47	0.6225	32.61	0.6121
10	20/20	20/20	33.86	0.6485	33.90	0.7227
5	12/20	10/20	35.17	0.8349	35.67	0.8925

Oropharyngeal swab						
Dilution Copies/µl	Detection Rate E gene (FAM)	Detection Rate RdRP/N (FAM)	Average Ct E gene (FAM)	SD E gene	Average Ct RdRP/N genes (FAM)	SD RdRP/N genes
500	20/20	20/20	29.48	0.7643	29.88	0.6699
100	20/20	20/20	31.34	0.7307	31.63	0.7451
50	20/20	20/20	32.59	0.7261	32.35	0.5693
10	20/20	20/20	34.14	0.6931	34.32	0.7932
5	13/20	12/20	35.22	0.8345	35.62	0.8753

BAL						
Dilution Copies/µl	Detection Rate E gene (FAM)	Detection Rate RdRP/N (FAM)	Average Ct E gene (FAM)	SD E gene	Average Ct RdRP/N genes (FAM)	SD RdRP/N genes
500	20/20	20/20	29.62	0.7572	29.22	0.6324
100	20/20	20/20	31.55	0.7751	31.63	0.6862
50	20/20	20/20	32.68	0.7907	32.67	0.7758
10	19/20	19/20	34.03	0.7833	34.12	0.8125
5	12/20	11/20	35.31	0.8756	35.62	0.8360

Qiagen Rotor-Gene Q 5Plex HRM LoD confirmatory study results

Nasopharyngeal swab						
Dilution Copies/µl	Detection Rate E gene (FAM)	Detection Rate RdRP/N (FAM)	Average Ct E gene (FAM)	SD E gene	Average Ct RdRP/N genes (FAM)	SD RdRP/N genes
500	20/20	20/20	28.52	0.4262	28.85	0.4741

Nasopharyngeal swab						
Dilution Copies/µl	Detection Rate E gene (FAM)	Detection Rate RdRP/N (FAM)	Average Ct E gene (FAM)	SD E gene	Average Ct RdRP/N genes (FAM)	SD RdRP/N genes
100	20/20	20/20	30.70	0.4216	30.82	0.5987
50	20/20	20/20	32.11	0.5474	32.24	0.6454
10	20/20	20/20	33.05	0.5971	33.24	0.6985
5	17/20	15/20	34.87	0.7441	35.95	0.9310

Oropharyngeal swab						
Dilution Copies/µl	Detection Rate E gene (FAM)	Detection Rate RdRP/N (FAM)	Average Ct E gene (FAM)	SD E gene	Average Ct RdRP/N genes (FAM)	SD RdRP/N genes
500	20/20	20/20	28.25	0.6891	28.98	0.6120
100	20/20	20/20	30.95	0.7010	30.66	0.6428
50	20/20	20/20	32.22	0.7122	31.99	0.6894
10	20/20	20/20	33.94	0.6998	34.44	0.7820
5	16/20	14/20	35.68	0.8889	36.12	0.9482

BAL						
Dilution Copies/µl	Detection Rate E gene (FAM)	Detection Rate RdRP/N (FAM)	Average Ct E gene (FAM)	SD E gene	Average Ct RdRP/N genes (FAM)	SD RdRP/N genes
500	20/20	20/20	28.25	0.7379	28.62	0.6626
100	20/20	20/20	30.89	0.6329	30.85	0.6325
50	20/20	20/20	32.23	0.7115	32.13	0.6759
10	20/20	19/20	33.95	0.6719	34.03	0.7485
5	14/20	13/20	35.66	0.8360	35.22	0.8684

B. Inclusivity

Inclusivity of this kit was assessed via *in silico* analysis against 16237 whole genome sequences of SARS-CoV-2 of which 14197 were published via GISAID (www.gisaid.org) and 2040 were published via the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov). The BLASTn module of Legacy BLAST software (blast-2.7.1+) was used for the analysis. The database of genomes from NCBI and GISAID were created using the blastdb module. The p Value was kept as 0.0001. Word size and mismatch scores were kept at default settings.

GENBANK					
Name Oligo	Total_Sequences genebank	100%_aligned sequences	sequences with atleast_1 mismatch	Mismatch at 5' end	Mmismatch at 3' end
N-F	2040	1975	65	65	0
N-R	2040	2039	1	1	0
N-Prob	2040	2040	0	0	0
RdRP_SARSr-F2	2040	2040	0	0	0
RdRP_SARSr-R1	2040	2040	0	0	0
RdRP_SARSr-Probe	2040	2035	5	5	0
E_Sarbeco_F1	2040	2039	1	1	0
E_Sarbeco_R2	2040	2040	0	0	0
E_Sarbeco_Probe	2040	2040	0	0	0
GISAID					
Name Oligo	Total_Sequences GISAID	100%_aligned sequences	sequences with atleast_1 mismatch	Mismatch at 5' end	Mmismatch at 3' end
N-F	14197	12277	1920	1920	0
N-R	14197	14162	35	35	0
N-Prob	14197	14197	0	0	0
RdRP_SARSr-F2	14197	14197	5	5	0
RdRP_SARSr-R1	14197	14188	9	9	0
RdRP_SARSr-Probe	14197	14194	3	3	0
E_Sarbeco_F1	14197	14192	5	5	0
E_Sarbeco_R2	14197	14191	6	6	0
E_Sarbeco_Probe	14197	14192	5	5	0

In a single oligonucleotide sequence, mutation events leading to ≤ 2 mismatch/es will not have any significant negative impact on the amplification of the respective target sequence. None of the analyzed sequences showed mismatches in more than one oligonucleotide and none of the mismatching sequences showed mismatches with both SARS-CoV-2 specific genes (N and RdRP); hence reactivity of the specific oligonucleotides included in the TRUPCR® SARS-CoV-2 Kit is not expected to be affected.

C. Cross-Reactivity Wet Testing

The specificity of the assay was evaluated by using wet testing against normal and pathogenic organisms associated with the respiratory tract. Test specimens for laboratory testing were prepared by spiking cultured isolates/inactivated organisms (18 viruses and 11 bacteria according to the table below) into negative matrix (Viral transport medium). Each sample was extracted using the TRUPCR® Viral RNA extraction Kit and tested with the TRUPCR® assay in duplicate or up to ten replicates.

Cross-Reactivity Wet Testing Results

Organism	Tested concentration	Number of samples tested	Result
HCoV-HKU1	>1.2×10^5 Copies/mL	2	Negative
HCoV-OC43	>1.1×10^5 Copies/mL	2	Negative
HCoV-NL63	>1.1×10^6 Copies/mL	2	Negative
HCoV-229E	>3.5×10^5 Copies/mL	2	Negative
MERS-CoV	>1.9×10^5 Copies/mL	2	Negative
Influenza A (H1N1/09)	>8.2×10^6 Copies/mL	10	Negative
Influenza A (H3N2)	>1.2×10^6 Copies/mL	6	Negative
Influenza B	>2.3×10^5 Copies/mL	5	Negative
Rhinovirus	>1.3×10^5 Copies/mL	4	Negative
Respiratory Syncytial Virus	>4.3×10^5 Copies/mL	4	Negative
Parainfluenza 1 virus	>5.1×10^5 Copies/mL	6	Negative
Parainfluenza 2 virus	>3.5×10^5 Copies/mL	5	Negative
Parainfluenza 3 virus	>1.8×10^6 Copies/mL	2	Negative
Parainfluenza 4 virus	>3.1×10^5 Copies/mL	2	Negative
Metapneumovirus	>3.5×10^5 Copies/mL	2	Negative
Enterovirus	>2.7×10^5 Copies/mL	2	Negative
Adenovirus	>4.3×10^5 Copies/mL	2	Negative
Legionella spp.	>1.5 X 10^8 CFU/mL	2	Negative
Mycoplasma spp.	>1.5 X 10^8 CFU/mL	10	Negative
Rhinovirus	>1.5 X 10^8 CFU/mL	2	Negative
S. pneumoniae	>1.5 X 10^8 CFU/mL	10	Negative
S. aureus	>1.5 X 10^8 CFU/mL	10	Negative
S. agalactiae	>1.5 X 10^8 CFU/mL	10	Negative
K. pneumoniae	>1.5 X 10^8 CFU/mL	10	Negative
E. coli	>1.5 X 10^8 CFU/mL	10	Negative
E. cloacae	>1.5 X 10^8 CFU/mL	10	Negative
H. influenzae	>1.5 X 10^8 CFU/mL	10	Negative
K. aerogenes	>1.5 X 10^8 CFU/mL	10	Negative
P. aeruginosa	>1.5 X 10^8 CFU/mL	10	Negative

No detectable amplification curve (Ct) was observed for the E, RdRP, or N targets. As expected, the internal control did show 100% detection for all tested replicates for all organisms

evaluated for potential cross-reactivity. Cross-reactivity and/or interference with the assay's performance due to the presence of the organisms displayed above is therefore, unlikely to occur.

D. Interfering Substances Study

Because the TRUPCR® Viral RNA Extraction Kit was an extraction methodology that FDA was unfamiliar with, an interfering substances study was completed. This study evaluated the potential interference of sample collection media and RNA extraction components from the TRUPCR® extraction kit on performance of the TRUPCR® SARS-CoV-2 Kit. Pooled negative nasopharyngeal swab matrix and pooled negative BAL matrix were spiked with each potential interferent at the concentration listed in the table below. Similarly, pooled SARS-CoV-2 positive nasopharyngeal swab matrix and pooled positive clinical BAL specimens were also spiked with each substance at the concentration provided in the table below. SARS-CoV-2 positive samples were prepared by spiking AcroMetrix™ material into each matrix at 2X LoD. RNA was extracted from the positive samples using the TRUPCR® Viral RNA Extraction Kit and each substance was tested with 3 extraction replicates. Results from the study demonstrated that the TRUPCR® Viral RNA Extraction Kit was sufficient for extracting nucleic acid and did not impact the assay's performance. No false positive or false negative results were observed for any of the substances evaluated at the given concentrations.

Evaluation of Exogeneous Interfering Substances

Component	Found In	Concentration Tested
NaCl	Transport media/Extraction Reagent	0.14M
KCl	Transport Media	0.005M
CaCl2	Transport Media	0.001M
MgSO4-7H2O	Transport Media	0.0004M
MgCl2-6H2O	Transport Media	0.0005M
Na2HPO4-2H2O	Transport Media	0.0003M
KH2PO4	Transport Media	0.0004M
D-glucose	Transport Media	0.006M
NaHCO3	Transport Media	0.0004M

Component	Found In	Concentration Tested
Phenol Red	Transport Media	0.10%
BSA	Transport Media	1%
GuSCN	Extraction Reagent	2M
GUHCl	Extraction Reagent	1.5M

E. Clinical Performance Evaluation

Testing of Real Clinical Samples:

Performance of the TRUPCR® SARS-CoV-2 Kit was evaluated using 61 clinical samples that were previously tested using the FDA authorized RealStar SARS-CoV-2 RT-PCR Kit (authorized April 22, 2020). Samples were blinded, randomized, and given to an unbiased operator for evaluation with the TRUPCR® SARS-CoV-2 Kit. 25 oropharyngeal and 36 nasopharyngeal specimens were tested as part of the clinical evaluation.

The TRUPCR® SARS-CoV-2 Kit detected 30/31 positives and 29/30 negative clinical oropharyngeal swab and nasopharyngeal swab specimens. The positive and negative percent agreements between the TRUPCR® SARS-CoV-2 Kit and the comparator method are shown below in the table. No additional discordant analyses were conducted for the one false positive and one false negative result and the data from the clinical evaluation were considered acceptable.

Method Comparison Study results using the Altona EUA Authorized Assay

		Comparator – Altona Real-Star SARS-CoV-2 RT-PCR Kit		
		Positive	**Negative**	**Total**
TRUPCR® SARS-CoV-2 Kit	**Positive**	30	1	31
	Negative	1	29	30
	Total	30	30	61
Positive Percent Agreement		30/31; 96.77% (83.81% - 99.43%)[1]		
Negative Percent Agreement		29/30; 96.67% (83.33% - 99.41%)[1]		

[1] 95% Confidence Interval

TROUBLESHOOTING GUIDE

No.	Observation	Probable causes	Comments
1	Amplification signal in negative control	Cross contamination during handling	Check for contamination of kit's component and contact **info@3bblackbio.com**
2	No amplification signal with positive controls	Incorrect PCR mixture	Check whether all components are added.
		Missing control sample during RNA mixing	Be careful when pipetting
		Changing RNA during RNA mixing	Write down sample number on the 1.5 mL micro centrifuge tube and the PCR tube
		Leaving reagents at room temperature for a long time or incorrect storage condition	Please check the storage condition and the expiration date (see the kit label) of the reagents and use a new kit, if necessary
		The PCR conditions do not comply with the protocol	Repeat the PCR with corrected settings
3	Weak or no signal of the RNaseP gene (Internal control)	Reagent has been thawed and frozen too often or exposed to inappropriate storage conditions	Please mind the storage conditions given in manual
		The PCR was inhibited	RNA of Poor quality may interfere with the PCR reaction, use a recommended isolation method

You can also take the following steps:

- Video conference orientation
- Upon purchase, you will also receive the contact information of a technical expert whom you may text with for any questions.
- US-based helpdesk number: +1-732-945-0190
- Product Support website: www.genophyll.com/contact
- Helpdesk email: info@3bblackbio.com

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PHOTOS OF KIT COMPONENTS

Sl No.	Reagent	Tube Picture
A	Master Mix	
B.	Enzyme Mix	

Sl No.	Reagent	Tube Picture
C.	Primer Probe Mix-1 (not used if using only Approach #3)	
D.	Primer Probe Mix-2	
E.	High Positive Control	
F.	Low Positive control	
G.	Water (RNase free)	
H.	Negative Control	

SYMBOLS

Catalog number

Temperature limitation

Caution; consult accompanying documents

Manufacturer

IVD In Vitro Diagnostic Medical Device


Consult instructions for use


Contains sufficient for (n) amount tests

Ordering Information

For ordering information, please contact Genophyll Enterprises, LLC

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