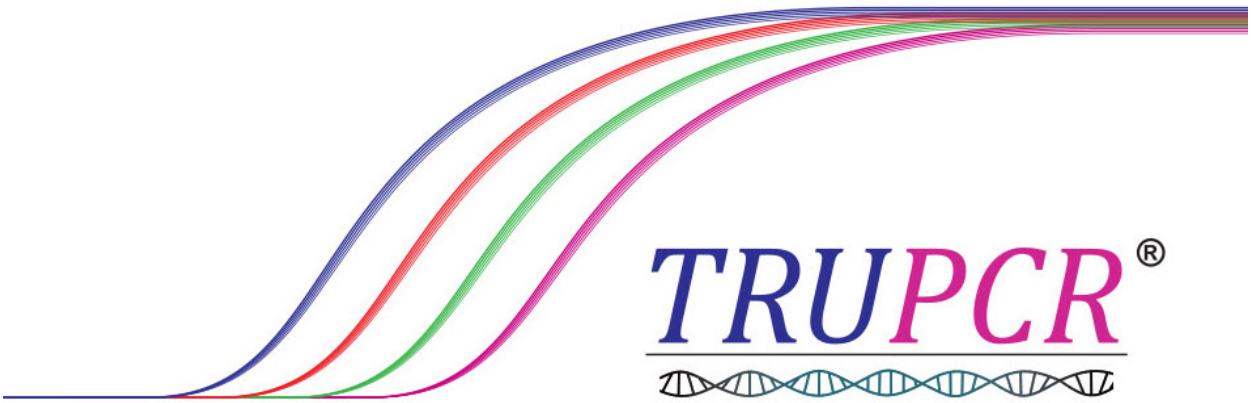

TRUPCR® SARS-CoV-2 KIT

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

Version 2.0

Instructions for Use

FOR IN VITRO DIAGNOSTIC USE
FOR PRESCRIPTION USE ONLY
FOR USE UNDER EMERGENCY USE AUTHORIZATION ONLY

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REF Product No.: 3B304

Σ 100 tests

Temperature limitation

June 2021

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

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INTENDED USE

The 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 swabs 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 (HCP).

The TRUPCR SARS-CoV-2 Kit is also for use with saliva specimens that are collected with the assistance of a HCP in a healthcare setting using the OMNIgene·ORAL OM-505/OME-505 saliva collection device by individuals suspected of COVID-19 by their HCP.

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 RNA; 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 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. Negative results for SARS-CoV-2 RNA from saliva should be confirmed by testing of an alternative specimen type if clinically indicated.

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, bronchoalveolar lavage (BAL) specimens and saliva samples 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 Use Under Emergency Use Authorization (EUA) Only
- For In Vitro Diagnostic Use Only
- For Prescription Use (Rx)
- This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an Emergency Use Authorization (EUA) for use by authorized laboratories.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests 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 declaration is terminated or authorization is 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) • $MgCl_2$ 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 • The TRUPCR SARS-CoV-2 kit may be used without this tube in order to follow Approach 3	233 µL X 2
Primer Probe Mix-2	• Primer Probe mix for RdRP gene and N gene and human RNaseP gene detection	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

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 with software version 1.4.3

- Applied Biosystems QuantStudio 5 with software version 1.5.0
- Qiagen Rotor-Gene Q 5Plex HRM Platform with 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:

- Respiratory Specimens

  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.

- Saliva Specimens Collected with the Assistance of a Healthcare Provider
  Saliva specimens must only be collected using the OMNIgene·ORAL collection device from DNA Genotek (OMNIgene·ORAL OM-505 or OMNIgene·ORAL OME-505) using the manufacturer's instructions Saliva specimens must be couriered to the testing location at ambient temperature and tested within 72 hours of collection.

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 specimen 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 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 gene assay, SARS-CoV-2 detection) to confirm whether SARS-CoV-2 RNA is present in the patient sample.
- Approach #3: Use ONLY the Tube 2 (RdRP gene assay, SARS-CoV-2 detection). This approach can be used for the remaining tubes if using the approach detailed in #2 above.

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 Biosystems QuantStudio 3 (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 directed, 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 Biosystems QuantStudio 3 (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 Biosystems QuantStudio 3 (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

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

Approach #2 (E Assay for Screening)

		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:

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.

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 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. Please work with your local public health authority to resolve this testing scenario.
+	+/-	-	-	**SARS-CoV-2 Presumptive Positive**	

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 Negative**	Report results to healthcare provider and appropriate public health authorities.
-	-	-	+	**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 RdRP/N gene) RNA load in the sample can lead to reduced or absent Internal Control signals.

Approach #2:

Patient Results Interpretation (E Assay for Screening, RdRP/N Assay for SARS-CoV-2 Confirmation)

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

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)		
						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. Please work with your local public health authority to resolve this testing scenario.
+	+/-		-	-	**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 Tube 2 markers remain negative after re-test, 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. Please work with your local public health authority to resolve this testing scenario.
-	+	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 and appropriate public health authorities.
-	-	**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 Running Only 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 and appropriate public health authorities.
-	-	**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

- Testing of saliva specimens is limited to patients with symptoms of COVID-19.
- Negative results for SARS-CoV-2 RNA from saliva should be confirmed by testing of an alternative specimen type if clinically indicated.
- Detection of RNase P indicates that human nucleic acid is present and implies that human biological material was collected and successfully extracted and amplified. It does not necessarily indicate that the specimen is of appropriate quality to enable detection of SARS-CoV-2.
- 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, Applied Biosystems QuantStudio 5, 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 clinical performance of TRUPCR® SARS-CoV-2 Kit was established using oropharyngeal and nasopharyngeal swab samples and saliva specimens collected in the OM-505/OME-505 device 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, 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/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas.

However, 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.us 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] For ease of reference, this 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 respiratory sample matrices (nasopharyngeal swab, oropharyngeal swab, and bronchoalveolar lavage (BAL)) and saliva samples collected with the assistance of a healthcare provider using the OMNIgene·ORAL OM-505/OME-505 collection device 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 experiments were performed on the Applied Biosystems QuantStudio 3, Applied Biosystems QuantStudio 5, 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 for respiratory specimens was established at 10 copies/µL with the QuantStudio 3, QuantStudio 5, and Rotor-Gene Q 5Plex HRM platforms. The LoD of the TRUPCR® SARS-CoV-2 Kit for saliva was also established at 10 copies/µL using the QuantStudio 3 instrument.

Applied Biosystems 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

SD; Standard deviation

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

SD; Standard deviation

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

SD; Standard deviation

Saliva Samples Collected in the OM-505/OME-505						
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	31.21	0.32	31.62	0.425
50	20/20	20/20	31.86	0.722	32.02	0.562
25	20/20	20/20	32.77	0.651	32.76	0.611
10	20/20	20/20	34.06	0.656	33.92	0.754
5	10/20	10/20	36.62	0.812	35.95	0.925

SD; Standard deviation

Applied Biosystems QuantStudio 5 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	29.71	0.5121	29.24	0.4987
100	20/20	20/20	31.64	0.5184	31.32	0.5871
50	20/20	20/20	32.71	0.6380	32.26	0.5447
10	20/20	20/20	33.55	0.6971	33.47	0.6985
5	13/20	14/20	35.65	0.7584	35.26	0.8105

SD; Standard deviation

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	29.75	0.7789	29.62	0.6412
100	20/20	20/20	31.50	0.7503	31.48	0.7288
50	20/20	20/20	33.09	0.6889	32.72	0.6659
10	20/20	20/20	33.84	0.7498	34.62	0.9287
5	15/20	15/20	35.42	0.8555	35.49	0.8822

SD; Standard deviation

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
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

SD; Standard deviation

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

SD; Standard deviation

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

SD; Standard deviation

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, RNA extraction components, and common substances that could be found in respiratory samples 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 and Endogenous 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
Phenol Red	Transport Media	0.10%
BSA	Transport Media	1%

Component	Found In	Concentration Tested
GuSCN	Extraction Reagent	2M
GUHCl	Extraction Reagent	1.5M
Mucin from bovine submaxillary glands	Clinical Sample	50% (w/v)
Blood	Clinical Sample	50% (v/v)
Adult Nasal Spray	Clinical Sample	10% (v/v)

E. Clinical Performance Evaluation – Respiratory Samples (NP and OP Swabs)

Testing of Real Clinical Samples:

Performance of the TRUPCR® SARS-CoV-2 Kit was evaluated using 61 clinical samples that were previously tested using an FDA authorized molecular assay. 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 EUA authorized molecular 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 an EUA Authorized Molecular Assay

		EUA Authorized Molecular Assay - Comparator		
		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

F. Clinical Performance Evaluation – Saliva Specimens Collected in the OM-505/OME-505

Prospective Paired Nasopharyngeal Swab and Saliva Clinical Study:

A study was performed to evaluate the use of saliva as a specimen type for detection of SARS-CoV-2 in patients who were suspected of COVID-19 by their healthcare provider (HCP). The study was conducted prospectively with patients presenting with signs and symptoms of COVID-19 in a healthcare facility. Nasopharyngeal swab specimens and their corresponding paired saliva samples were collected from each patient by an HCP; the saliva was collected with the assistance of an HCP in the FDA authorized collection device following the manufacturer's instructions (OMNIgene-ORAL (DNA Genotek, Canada). The HCP gave the patient the OM-505/OME-505 device and instructed the patient to spit until the fill-line was reached on the collection tube. The HCP observed the collection process. The HCP prepared the specimen for transport to the on-site testing laboratory by inserting the collected specimen into a biohazard bag and by placing it into an insulated foam box without cold chain (i.e., ambient temperature).

RNA was extracted from the paired nasopharyngeal swab and saliva samples using the TRUPCR Viral RNA Extraction Kit. Both the saliva and swabs were transported at ambient temperature and tested using the TRUPCR SARS-CoV-2 Test on the QuantStudio 3 instrument within 48 hours of collection. A total of 60 paired study samples (30 NP positive and 30 NP negative) were evaluated using Approach #1 to establish the clinical performance of the assay when using HCP-collected saliva. A summary of the results of the paired study is presented below.

There was 96.67% positive percent agreement (PPA) and 96.67% negative percent agreement (NPA), respectively between the results obtained from testing nasopharyngeal swabs and those obtained from testing the corresponding saliva samples. Of the 30 confirmed positive NP swab samples, 29 paired saliva specimens produced positive results for the E and N+RdRp genes (29/30; 96.67%); however, there was one false negative where the NP swab showed positive amplification (Ct < 35) but the saliva sample was negative. For the 30 confirmed NP swab negative patients, the TRUPCR Test generated one false positive result for saliva (both the E and N+RdRp targets had Ct values below the cutoff of 35).

		TRUPCR® SARS-CoV-2 Kit (Nasopharyngeal Swab)		
		Positive	**Negative**	**Total**
TRUPCR® SARS-CoV-2 Kit (Saliva)	**Positive**	29	1	30
	Negative	1	29	30
	Total	30	30	60
Positive Percent Agreement		29/30; 96.67% (83.33% to 99.41%) [1]		
Negative Percent Agreement		29/30; 96.67% (83.33% - 99.41%)[1]		

[1] 95% Confidence Interval

G. **FDA SARS-CoV-2 Reference Panel Testing for Nasopharyngeal Swabs and Saliva**

FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to corroborate the LoD. The extraction method and instrument used were the TRUPCR® Viral RNA Extraction Kit (3B213V/3B214V) and the Applied Biosystem QuantStudio 3 respectively.

Summary of LoD Confirmation Result Using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal Swab	$1.8x10^4$ NDU/mL	N/A
MERS-CoV		N/A	ND
SARS-CoV-2	Saliva	$1.8x10^4$ NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL: RNA NAAT detectable units/mL

N/A: Not Applicable

ND: Not Detected

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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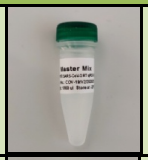
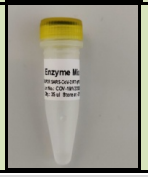
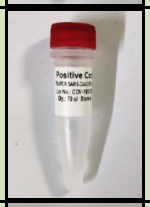
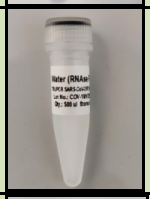
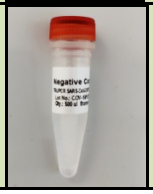
PHOTOS OF KIT COMPONENTS

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

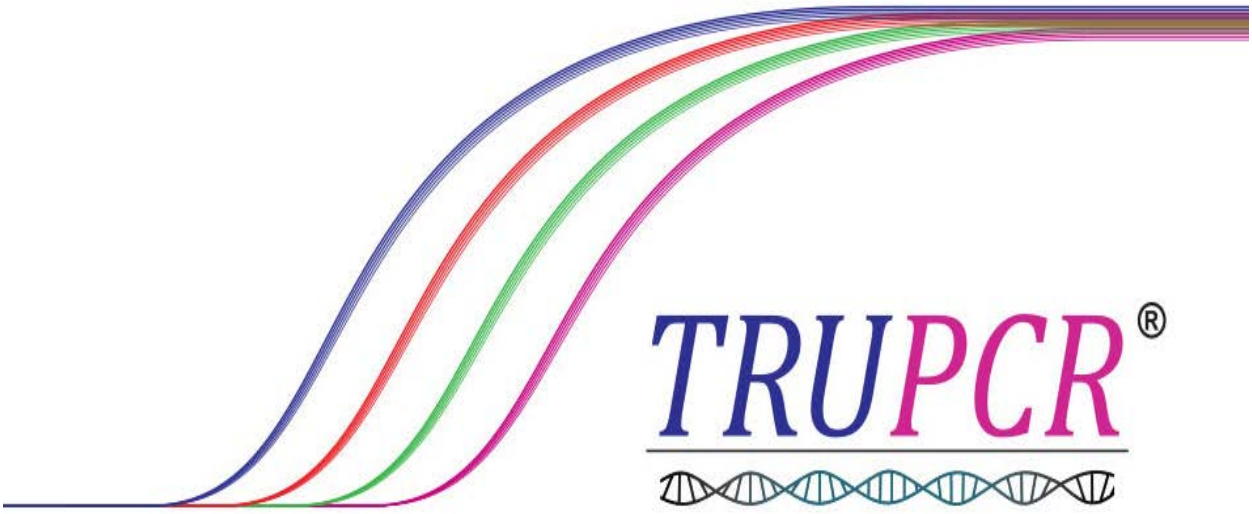
100 Davidson Avenue

Suite 109

Somerset, NJ, 08873

(732) 945-0190

mail@genophyll.us



TRUPCR® VIRAL RNA EXTRACTION KIT

Designed for rapid isolation of highly pure viral RNA

Version 1.0

Instruction for Use

For Prescription Use
For In Vitro Diagnostic Use Only
For Use Under Emergency Use Authorization Only

- This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an Emergency Use Authorization (EUA) for use by authorized laboratories.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests 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 declaration is terminated or authorization is revoked sooner.

3B BlackBio Biotech India Ltd
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TRUPCR® Viral RNA Extraction Kit

Version 1.0

Designed for rapid isolation of highly pure viral RNA

REF Product No.: 3B213V

100 tests

Temperature limitation

December 2020

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

CONTENT	PAGE NO.

KIT CONTENTS

TRUPCR® Viral RNA Extraction Kit	100 Rxns
TRUPCR® Silica Membrane Binding Columns	100
Collection Tubes	100
Buffer BAVL1* (Lysis Buffer)	60 ml
BAW1* (Concentrate) (Wash Buffer-I)	38 ml
BAW2 (Concentrate) (Wash Buffer-II)	26 ml
Buffer BRE (Elution Buffer)	8 ml
Carrier RNA (Poly A)‡	300 µl x 2
Protocol	1

*Contains chaotropic salt which is an irritant. Not compatible with disinfecting reagents that contain bleach.

‡ Stored in dry ice in a separate box.

STORAGE

1. Kit components except Carrier RNA should be stored at room temperature (15 - 25 °C).

2. Carrier RNA should be stored at -20 °C.

3. TRUPCR® Silica Membrane Binding Columns should be stored dry at room temperature (15–25°C); storage at higher temperatures should be avoided.

BASIC PRINCIPLE

TRUPCR® Viral RNA Extraction Kit is designed for the rapid preparation of highly pure viral RNA using silica membrane column manual centrifugation method from biological samples:

- Respiratory specimens (bronchoalveolar lavage, nasopharyngeal aspirates/washes or nasal aspirates, nasopharyngeal and oropharyngeal swabs, and anterior nasal and mid-turbinate nasal swabs)
- Saliva

In a first step the sample containing virus RNA is lysed by incubation in a lysis solution (Buffer BAVL1) containing guanidine thiocyanate (RNase inhibitor). Lysis buffer and ethanol creates the appropriate conditions for binding of nucleic acid to the silica membrane of the column. This binding process is reversible and specific to nucleic acids. Carrier RNA improves binding and recovery of the low concentrated viral RNA. Contaminations (potential PCR inhibitors) like salts, metabolites and soluble macromolecular cellular components are removed in simple washing steps with ethanolic buffers BAW1 and BAW2. In a final elution step the nucleic acids can be eluted in low salt buffer or water and are ready for use in subsequent reactions.

INTENDED USE

With the **TRUPCR® Viral RNA Extraction Kit** method, viral RNA is isolated and purified from biological samples e.g. respiratory specimens and saliva. The prepared nucleic acids are suitable for applications like automated fluorescent nucleic acids sequencing, RT-PCR, or any kind of enzymatic manipulation.

The detection limit for certain viruses depends on individual detection procedures e.g. in-house nested (RT) PCR. We highly recommend the use of internal (low-copy) standards as well as positive and negative controls in order to monitor the purification, amplification and detection processes.

General Characteristics of the Kit	
Sample Volume	**25 µl-300 µl (Ideal 200 µl)**
Typical Recovery Rates	**>90%**
Elution Volume	**30 µl-100 µl (Ideal 40 µl)**
Binding Capacity	**40 µg**
Time for batch of 24 samples	**≈ 50 min**
Spin Column	**Mini**

CARRIER RNA

Carrier RNA serves two purposes. Firstly, it enhances binding of viral nucleic acids to the Spin column, especially if there are very few target molecules in the sample. Secondly, the addition of large amounts of carrier RNA reduces the chance of viral RNA degradation in the rare event that RNase molecules escape denaturation by the chaotropic salts and detergent in Buffer BAVL1. If carrier RNA is not added to Buffer BAVL1 this may lead to reduced viral RNA or DNA recovery. The amount of lyophilized carrier RNA (provided) is sufficient for the volume of Buffer BAVL1 supplied with the kit. The concentration of carrier RNA has been adjusted so that the protocol can be used as a generic purification system compatible with many different amplification systems and is suitable for a wide range of RNA and DNA viruses.

Different amplification systems vary in efficiency depending on the total amount of nucleic acid present in the reaction. Elutes from this kit contain both viral nucleic acids and carrier RNA, and amounts of carrier RNA will greatly exceed amounts of viral nucleic acids. Calculations of how much elute to add to downstream amplifications should therefore be based on the amount of carrier RNA added. To obtain the highest levels of sensitivity in amplification reactions, it may be necessary to adjust the amount of carrier RNA added to Buffer BAVL1.

PREPARATION OF REAGENTS

Addition of carrier RNA to Buffer BAVL1

Check Buffer BAVL1 for any precipitate, and if necessary, incubate at 50°C until the precipitate is dissolved. Calculate the volume of Buffer BAVL1–carrier RNA mix needed per batch of samples by selecting the number of samples to be simultaneously processed from below table.

Table 1: Volumes of Buffer BAVL1 and carrier RNA mix required for the TRUPCR® Viral RNA extraction spin procedure:

No. of Sample	Buffer BAVL1 Volume (ml)	Carrier RNA volume (µl)	No. of Sample	Buffer BAVL1 Volume (ml)	Carrier RNA volume (µl)
1	0.56	5.6	**11**	6.16	61.6
2	1.12	11.2	**12**	6.72	67.2
3	1.68	16.8	**13**	7.28	72.8
4	2.24	22.4	**14**	7.84	78.4
5	2.80	28.0	**15**	8.40	84.0
6	3.36	33.6	**16**	8.96	89.6
7	3.92	39.2	**17**	9.52	95.2
8	4.48	44.8	**18**	10.08	100.8
9	5.04	50.4	**19**	10.64	106.4
10	5.60	56.0	**20**	11.20	112.0

Note: The sample preparation procedure is optimized for 5.6 µg of carrier RNA per sample. If less carrier RNA has been shown to be better for your amplification system, transfer only the required amount of dissolved carrier RNA to the tubes containing Buffer BAVL1.

BUFFER BAW1

Buffer BAW1 is supplied as a concentrate. Before using for the first time, add the appropriate amount of molecular biology grade ethanol (96–100%) as indicated on the bottle and in Table 2. Tick on the label to indicate that ethanol has been added. Store reconstituted Buffer BAW1 at room temperature (15–25°C). Reconstituted Buffer BAW1 is stable for up to 1 year when stored at room temperature.
Note: Always mix reconstituted Buffer BAW1 by shaking before starting the procedure.

Table 2: Preparation of Wash Buffer BAW1

No. of Reactions	Wash Buffer BAW1 (Concentrate)	Ethanol	Final Volume
50	19 ml	25 ml	44 ml

100	38 ml	50 ml	88 ml

Buffer BAW2

Buffer BAW2 is supplied as a concentrate. Before using for the first time, add the appropriate amount of molecular biology grade ethanol (96–100%) as indicated on the bottle and in Table 3. Tick on the label to indicate that ethanol has been added. Store reconstituted Buffer BAW2 at room temperature (15–25°C). Reconstituted Buffer BAW2 is stable for up to 1 year when stored at room temperature.

Note: Always mix reconstituted Buffer BAW2 by shaking before starting the procedure.

Table 3: Preparation of Wash Buffer BAW2

No. of Reactions	Wash Buffer BAW2 (Concentrate)	Ethanol	Final Volume
50	13 ml	30 ml	43 ml
100	26 ml	60 ml	86 ml

ELUTION OF NUCLEIC ACIDS

Elution buffer should be equilibrated to room temperature before it is applied to the column. Yields will be increased if the Spin column is incubated with the elution buffer at room temperature for 5 minutes before centrifugation.

PROTOCOL: PURIFICATION OF VIRAL RNA

This protocol is for purification of viral RNA from 200 µl of sample using the TRUPCR® VIRAL RNA EXTRACTION KIT.

Important point before starting

- All centrifugation steps are carried out at room temperature (15–25°C).

Things to do before starting

- Equilibrate samples to room temperature.
- Equilibrate Buffer BRE to room temperature for elution in step 12.
- Add carrier RNA to Buffer BAVL1 according to the instruction (Page No. 6)
- Ensure that Buffer BAW1 and Buffer BAW2 have been prepared according to the instructions (Page No. 6 & 7)

PROCEDURE

1. Pipette 560 µl of prepared Buffer BAVL1 containing carrier RNA into a 1.5 ml micro-centrifuge tube.
   (Please refer to the preparation of Buffer BAVL1 and carrier RNA – Page No. 6)

2. Add 200 µl of sample into the buffer BAVL1-carrier RNA containing micro centrifuge tube.
   Note: *(It is essential that the sample is mixed thoroughly with the Lysis Buffer BAVL1 to yield a homogenous solution for efficient lysis of the sample)*

3. Incubate the mixture at room temperature (15-25°C) for 10 minutes.

4. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.

5. Add 560 µl of molecular biology grade ethanol (96–100%) to the sample, close the cap and mix thoroughly by pulse-vortexing for 15 s. Incubate the lysate with the ethanol for 5 minutes at room temperature (15–25°C).

   Note: *If ambient temperature exceeds 25°C, ethanol should be cooled on ice before adding to the lysate.*

6. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.

7. Carefully apply 630 µl of the lysate from step 5 onto the TRUPCR® silica membrane binding column without wetting the rim. Close the cap and centrifuge at 10,000 rpm for 1 min. Place the column in collection tube, if the lysate has not completely passed through the column after centrifugation, centrifuge again at higher speed until the column is empty.

8. Discard the lysate from the collection tube. Carefully open the Spin column and repeat step 7.

9. Place the column into same collection tube carefully, open the column, and add 500 µl of Buffer BAW1 without wetting the rim. Close the cap and centrifuge at 10,000 rpm for 1 min and discard the filtrate.

10. Place the column into same collection tube carefully, open the column, and add 500 µl of Buffer BAW2 without wetting the rim. Close the cap and centrifuge at full speed (≈20,000 x g; 14,000 rpm) for 3 min and discard the filtrate with collection tube.

11. **Recommended:** Place the column in a new 2 ml collection tube. Centrifuge at full speed (≈20,000 x g; 14,000 rpm) for 1 min to dry the membrane completely.

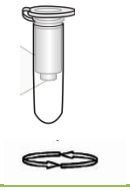
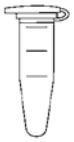
12. Place the column in a clean 1.5 ml micro-centrifuge tube (not provided) and discard the collection tube. Carefully open the lid of the column and apply 30-40 µl of Buffer BRE or RNase-free water directly to the centre of the membrane. Close the lid and incubate at room temperature for 1 min. Centrifuge at 10,000 rpm for 1 min.

Important: Ensure that the elution buffer is equilibrated to room temperature. If elution is done in small volumes (<30 µl), the elution buffer must be dispensed onto the centre of the membrane for complete elution of bound RNA.

Elution volume is flexible and can be adapted according to the requirements of the downstream application. Remember that the recovered elute volume will be approximately 5 µl less than the elution buffer volume applied onto the column. Incubating the column loaded with Buffer BRE or water for 5 min at room temperature before centrifugation generally increases RNA yield.

BRIEF PROCEDURE: TRUPCR® VIRAL RNA EXTRACTION KIT

Step	Description	Approximate Time* Required for Batch of 24 Samples
1. Sample Lysis 	560 µl Lysis Buffer BAVL1 (containing carrier RNA) + 200 µl Sample + Mix (Pulse Vortexing) Incubate at RT for 10 min	15 minutes
2. Optimize Binding Conditions 	+ Add 560 µl molecular biology grade ethanol (96-100%) + Mix (Pulse Vortexing) Incubate at RT for 5 min	10 minutes
3. Bind Viral Nucleic Acid 	Load lysate into the TRUPCR® Binding column *Centrifuge at 10,000* rpm, 1 min	6 minutes
4. Wash silica membrane 	+ 500 µl Wash Buffer- I BAW1 *1 min, 10,000 rpm* + 500 µl Wash Buffer- II BAW2 *3 min, full speed (≈20,000 x g; 14,000 rpm)*	10 minutes

5. Dry silica membrane 	*Centrifuge 1 min, full speed (≈20,000 x g; 14,000 rpm)*	3 minutes
6. Elute Pure Viral Nucleic Acid 	30-40 µl BUFFER BRE Incubate for 1 min at RT *1 min, 10,000rpm*	6 minutes
Total Time		**≈50 minutes**

*****Considering centrifuge with minimum 24 well rotor.

Note: Briefly centrifuge the 1.5 ml tubes to remove drops from the inside of the lid after each incubation and vortexing step.

TROUBLESHOOTING

Problem	Possible cause and suggestions
Small amounts or no viral nucleic acids in the elute	**Problem with carrier RNA** • Carrier RNA not added. • See remarks concerning storage of Buffer BAVL1 with carrier RNA. **Viral nucleic acids degraded** • Samples should be processed immediately. If necessary, add RNase inhibitor to the sample and ensure appropriate storage conditions up to the processing. • Check that all buffers have been prepared and stored correctly. If in doubt, use new aliquots of Buffer BAVL1, carrier RNA and Elution Buffer BRE.
Problems with subsequent detection	**Reduced sensitivity** • Change the volume of elute added to the PCR/RT-PCR. • Incubation time and temperature are critical for lysis as well as RNA stability. For sensitive RNA preparations, incubation at room temperature is sufficient without significant loss of sensitivity. For parallel isolation of viral RNA and DNA, incubation time (5-15 min) and temperature (RT/ 56°C/ 72°C) may be adapted in order to get optimal recovery rates for both species. **Ethanol carryover** • Prolong centrifugation steps in order to remove Buffer BAW2 completely.
General problems	**Clogged membrane** • Centrifuge sample lysate before the addition of ethanol and subsequent loading onto the corresponding RNA Virus Binding Column.

For technical support contact at info@3bblackbio.com.

SAFETY INSTRUCTIONS

Component	Hazard Contents	GSH Symbol	Hazard Phrases	Precaution Phrases
BAVL1	Guanidine thiocyanate 30-60%	 Warning	H 302: Harmful if Swallowed H 412: Harmful to aquatic life with long lasting effects EUH031: Contact with acids liberates toxic gas	P 260: Do not breathe vapours P 273: Avoid release to the environment P301+312: If swallowed call a poison centre of doctor/ physician if you feel unwell P 330: Rinse mouth
BAW1	Guanidine hydrochloride	 Warning	H 226: Flammable liquid and vapour H 302: Harmful if Swallowed	P 210: Keep away from heat/sparks /open flames/hot surfaces-No smoking P 233: Keep container tightly closed P 301+312: If swallowed call a poison centre of doctor/ physician if you feel unwell P 330: Rinse mouth P 403+235: Store in a well-ventilated place. Keep cool

MATERIAL AND DEVICES REQUIRED BUT NOT PROVIDED

- Adjustable pipettes with sterile filter or positive displacement tips
- Disposable powder-free gloves
- Sterile bidistilled water
- Sterile 1.5 ml and 2 ml microcentrifuge tubes
- 15 ml conical tubes

- Vortex mixer
- Desktop centrifuge
- Laminar airflow cabinet
- 96 – 100% ethanol
- Personal protection equipment (lab coat, gloves, goggles)

SYMBOLS

Catalog number


Temperature limitation


Caution; consult accompanying documents


Manufacturer

In Vitro Diagnostic Medical Device


Consult instructions for use


Contains sufficient for (n) amount tests