TBG Biotechnology Corp.

ExProbe™ SARS-CoV-2 Testing Kit

Cat. No. 68020

Instructions for Use

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

TBG Biotechnology Corp.
Address: 13F-1, No. 237, Sec. 1, Datong Rd., Xizhi Dist., New Taipei City 221, Taiwan
Tel: +886-2-7741-7777 // Email: COVID19@tbgbio.com // Website: www.tbgbio.com
U.S. Customer and Technical Support: 1-877-822-2461

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Intended Use

ExProbe™ SARS-CoV-2 Testing 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 and mid-turbinate nasal swabs, nasopharyngeal aspirates/washes and nasal aspirate specimens, and bronchoalveolar lavage (BALs) specimens from individuals 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 which is generally detected 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.

The ExProbe™ SARS-CoV-2 Testing 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 ExProbe™ SARS-CoV-2 Testing Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

Principle(s)

The ExProbe™ SARS-CoV-2 Testing Kit uses dual-labeled probes that target a distinct region in the RdRP gene of the SARS-CoV-2 genome and one region each in both the N and E genes of Sarbecovirus (which includes SARS-CoV-2). The detection probe for the RdRP amplicon is labelled with FAM and the probes for the N and E are labelled with Cy5 and Texas Red, respectively. The kit evaluates the presence of one individual amplicon of the SARS-CoV-2 genome, two amplicons of Sarbecovirus/SARS-CoV-2, as well as RNase P as an internal control (IC) that is intended to examine RNA extraction efficiency, the enzyme activity of the kit as well as the assay performance. The RNase P probe is labeled with VIC fluorescent dye which uses an independent fluorescence detection channel from the RdRP, N, and E targets.

Since the probes are labeled with the fluorescent dye reporter and quencher, the fluorescent signals of the intact probes are suppressed by the quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of target DNA amplicon are generated and the signal of the reporter dye increases concomitantly. Each reporter dye is measured at defined wavelengths, which enables simultaneous detection and discrimination of the amplified coronavirus target and the internal control.

Reagents and Equipment

Reagents Included in the Kit

- **SARS-CoV-2 Reaction Mix** (1.5mL/tube) x 1 tube: Main ingredients: dNTPs, $MgCl_2$, Tris-HCl, Primer and Probe.
- **SARS-CoV-2 Enzyme Mix** (500µL/tube) x 1 tube: Main ingredients: RT enzyme, Taq DNA polymerase.
- **SARS-CoV-2 Positive Control** (1.5mL /tube) x 1 tube: Main ingredients: in vitro transcribed RNA containing target genes and internal standard gene fragments (RNase P).
- **SARS-CoV-2 Negative Control** (1.5mL /tube) x 1 tube: Main ingredients: in vitro transcribed RNA containing internal standard gene fragments (RNase P)
- **Instruction For Use** x 1 copy.

Equipment and Materials Required but Not Included

Additional equipment and materials may be required.

- BD UTV 3-mL collection Kit (Cat. No. 220528, BD)
- QIAamp Viral RNA Mini Kit (Cat. No. 52904, Qiagen Germany)
- EZbead Viral Extraction Kit, pre-filled (TBG Biotechnology Corp., Cat. No. 37900a)
- EZbead System-32 with software version v1.2.488.32 (TBG Biotechnology Corp., Cat. No. 37001)
- ABI 7500 Real Time PCR System with Software version 2.3 (ThermoFisher, Cat No. 4351104)
- TBG Q6000 with software version 0.1.0.0102α (TBG Biotechnology Corp., Cat. No. 60001)
- Vortex mixer
- Desktop Centrifuge (for small vials and 96 well plates)
- Ice and/or ice block
- Adjustable pipettes (1000µL, 200µL, 10µL)
- Sterile pipette tips with filters
- 0.2mL PCR Plate (Cat. No. 4346906)
- MicroAMP Optical Adhesive Film (Cat. No. 4311971)
- Bio-safety cabinet
- Power free latex gloves

Warnings and Precautions

- For Prescription Use Only
- 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 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; and,
- 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 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 declaration is terminated or authorization is revoked sooner.
- Do not mix or exchange components from different kit lots. All biological samples in the diagnostic kit have been inactivated.
- Reliability of the results depends on adequate specimen collection, storage, transport, and processing procedure.
- This clinical performance of this test was validated using oropharyngeal (OP) swabs. Other specimen types listed in the intended use are acceptable for testing; however, performance of the ExProbe™ SARS-CoV-2 Testing Kit has not been established.
- If not tested immediately, store extracted RNA at ≤ -70°C until use and keep on ice during testing.
- Wear disposable gloves and change them before entering different areas. Change gloves immediately if contaminated or treat them with nucleic acid decontaminating reagent.
- Supplies and equipment must be dedicated to working areas and should not be moved from one area to another.
- Do not pipette by mouth.
- Do not eat, drink or smoke in laboratory work areas. Wear disposable powder-free gloves, laboratory coats and eye protections when handling specimens and reagents. Wash hands thoroughly after handling specimens and test reagents.
- Avoid contamination of reagents when removing aliquots from reagent tubes.
- Do not pool reagents from different lots or from different tubes of the same lot.
- Do not use the product after its expiry date and do not reuse any disposable items.
- To avoid contaminating working areas with amplified products, open PCR reaction tubes or strips only in designated working areas after amplification.

- Laboratory management shall strictly follow practices of PCR gene amplification laboratory; laboratory personnel must receive professional training; test processes must be performed in separated areas; all consumables should be for single use only after sterilization; special instruments and devices should be used for every process; all lab devices used in different processes and areas should remain in those specific locations.
- All specimens for detection should be handled as if infectious. Wear laboratory coats, protective disposable gloves and change the gloves often to avoid cross-contamination between samples. Handling of specimens and waste must meet relevant requirements outlined in local, state and national regulations.
- Due to the characteristics of swab and other sample collection processes and the viral infection process itself, false negative results may be caused by insufficient sample volume, which should be combined with other clinical diagnosis and treatment information for comprehensive judgment; retest when necessary.

Storage and Handling Conditions

- The diagnostic kit should be stored in a sealed pouch below -20 ± 5°C and protected from light. The kit is provisionally valid for 8 months.
- The number of acceptable freezing and thawing cycles is 4.
- Please refer to the date of manufacture and expiry date on the outer package.
- Do not use reagents past their expiration date.

Specimen Requirements

- Applicable specimen types: Nasal aspirates, nasopharyngeal washes/aspirates, anterior nasal swabs, mid-turbinate nasal swabs, nasopharyngeal swabs, oropharyngeal swabs, and bronchoalveolar lavage (BALs)
- Sample collection devices are not provided with the assay. All testing for COVID-19 should be conducted in consultation with a healthcare provider. Refer to CDC guidelines for sample collection and storage of respiratory specimens:
- https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinicalspecimen s.htmlIt is recommended to use Universal Transport Medium (UTM) for transportation/ temporary storage of collected swabs. Washes/aspirates and BALs can be stored in appropriate sterile containers without preservative matrix.
- After collection and if necessary, specimens can be stored at 2-8°Cfor up to 72 hours. Recommended specimen storage conditions are applicable to both upper and lower respiratory tract samples.

- If shipping and processing are expected to exceed 72 hours, specimens should be stored at -70°C or colder until shipping or processing can occur.

NOTE:

1. Performance may be affected by prolonged storage of specimens.
2. Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulations. Specimens should be collected and handled according to the manufacturer's recommended procedures for the swab collection device that is used.

Protocols

Sample Collection, Transportation and Storage

Validation studies of the ExProbe™ SARS-CoV-2 Testing Kit were completed using oropharyngeal swabs (OP collected with the BD UVT 3-mL Collection Kit (BD, Cat. No. 220528) Nasopharyngeal washes/aspirates or nasal aspirates and BALs can be collected in sterile containers such as the Starplex specimen container (Fisher Scientific, Cat. No. 14-375-459) or other appropriate sterile specimen containers without preservative media.

Viral RNA Extraction

Prepare the QIAamp Viral RNA Mini Kit (Qiagen, Cat. No. 52904, not included in kit) as per the manufacturer's instructions in the "specimen processing area", or prepare the EZbead Viral Extraction Kit, pre-filled (TBG Biotechnology Corp, Cat. No. 37900a, not included in kit) with the EZbead System-32 (TBG Biotechnology Corp, Cat. No. 37001, not included in kit) as per the manufacturer's instructions. 100µL of swab lysate is used as the sample input and elution is completed with RNase free water with volume set to 100µL. If the extracted RNA is not used immediately, please store at -20°C for a maximum of 24 hours.

Preparation of Reagent

1. In the "reagent preparation area", take out each component from the testing kit and thaw on ice. Once the reagents have thawed, vortex and centrifuge the reagents for several seconds and keep on ice for later use.
2. According to the quantity of test specimens (N), pipette appropriate quantity of SARS-CoV-2 Reaction Mix and SARS-CoV-2 Enzyme Mix (SARS-CoV-2 Reaction Mix 15µL*N + SARS-CoV-2 Enzyme Mix 5µL*N). 1.5mL sterile centrifugal tube(s) should be used to prepare the reaction mastermix. After all the reagents are

added, mix them thoroughly and centrifuge for several seconds. 20µL of master mixture is loaded into the 0.2mL PCR reaction plate (ThermoFisher Scientific, Cat. No. 4346906).

Table 1: Mastermix Preparation Table

Reagent Name	1 Sample	24 Samples	96 Samples
SARS-CoV-2 Reaction Mix (µL)	15	360	1440
SARS-CoV-2 Enzyme Mix (µL)	5	120	480

Note: The above configuration is just for your reference and to ensure enough volume of the PCR reaction mixture.

3. Transfer the above-prepared reagents to the "specimen processing area" and keep on ice for later use.

Processing and Loading of Specimens

1. 10µL of the SARS-CoV-2 Positive Control, SARS-CoV-2 Negative Control, or clinical sample is added into the PCR reaction plate containing the mastermix.
2. The plate is tightly sealed (to avoid bubble production) using the MicroAMP Optical Adhesive Film (ThermoFisher Scientific, Cat. No. 4311971). Any liquid on the tube wall is briefly centrifuged to the bottom of the tube.
3. Real-time PCR is performed on the TBG 6000 using the setup protocol below. Or also can performed in the "nucleic acid amplification area" on the ABI7500 Real Time PCR instrument with software version 2.3 using the setup protocol below.

Setting Up TBG Q6000 and Performing the PCR Amplification

1. A maintained instrument will be calibrated for many dyes.
2. A template (.q6t) file contains the settings for the instrument run and will be provided by your local support team and can be transferred via a USB drive or other method to the computer on which TBG Q6000 Software v0.1.0 is installed.
3. In the TBG Q6000 Software v0.1.0 home page, click default experiment template.
4. Open the experiment template (.q6t) file for the TBG Q6000 Real-Time PCR Instrument.
5. In the Experiment Properties window, enter or confirm the following information:
    - Reagents: ExProbe SARS-CoV-2 Testing Kit
    - Fluorophores: FAM, HEX, TEXAS RED, CY5
    - Reaction Volume: 30µL
    - Heated Lid: On
    - Ramp Rate: High 2.5℃
6. In the Plate Setup window, in the Define Targets and Samples tab and the Define Targets pane, confirm that the targets.
    - FAM / RdRP Gene

- HEX / RNase P
- Texas Red / E Gene
- CY5 / N Gene

7. In the Plate Setup window, in the Define Targets and Samples tab and the Define Samples pane, confirm the labeling of the controls. The template has one positive control (PC) and one negative control (NC) assigned to the wells for reference.
8. Select Add Sample to assign a unique sample name to each well that has a patient sample in the physical plate.
9. In the Plate Setup window, in the Assign Targets and Samples tab, confirm that four targets are assigned to each well in the plate layout. To designate a target or sample to a well, select the well, then check the Assign to Wells check box.
10. For wells with a positive control, confirm that Task is set to S for Standard.
11. For wells with a negative control, confirm that Task is set to N for Negative.
12. For wells with a patient sample, confirm that Task is set to U for Unknown.
13. In the Run Method window, confirm that Reaction Volume per well is 30µL, and then confirm the thermal cycling protocol.

Table 2: Thermal cycling Protocol

Segment	Cycle Number	Temp.	Time
1	1	42℃	10 min.
2	1	95℃	10 min.
3	45	95℃	10 sec.
		60℃	45 sec.
		Collect data	-

14. Select Run, enter a file name, then click Save.
15. After the instrument run is complete, click Analysis to “Ct(1)” page.
16. Set Baseline range from 10 to 20, and then Submit.
17. Check Ct Result and Export results.

Setting Up ABI 7500 Real Time PCR System and Performing the PCR Amplification

1. A maintained instrument will be calibrated for many dyes.
2. A template (EDT) file contains the settings for the instrument run and will be provided by your local support team and can be transferred via a USB drive or other method to the computer on which 7500 Software v2.3 is installed.
3. In the 7500 Software v2.3 home page, click Template.
4. Browse to, then open the template file that you transferred in “Transfer the template (EDT) file for the 7500 Real‑Time PCR Instrument”
5. In the Experiment Properties window, enter or confirm the following information:

- Experiment name: Enter a name
- Instrument type: 7500 (96 wells)

- Type of experiment: Quantitation - Standard Curve
- Reagents: ExProbe™ SARS-CoV-2 Testing Kit
- Ramp Speed: Standard

6. In the Plate Setup window, in the Define Targets and Samples tab and the Define Targets pane, confirm that the targets, reporter dyes, and quenchers are listed correctly.
    - RdRP Gene / FAM / BHQ1
    - E Gene / TexasRed / BHQ1
    - N Gene Cy5 / BHQ1
    - RNase P / VIC / BHQ1
7. In the Plate Setup window, in the Define Targets and Samples tab and the Define Samples pane, confirm the labeling of the controls. The template has one Positive Control (PC) and one negative control (NC) assigned to the wells for reference.
8. Select Add New Sample to assign a unique sample name to each well that has a patient sample in the physical plate.
9. In the Plate Setup window, in the Assign Targets and Samples tab, confirm that four targets are assigned to each well in the plate layout. To designate a target or sample to a well, select the well, then check the Assign checkbox. Note: Wells that do not have a sample name will not be analyzed by the software.
10. For wells with a positive control, confirm that Task is set to S for Standard.
11. For wells with a negative control, confirm that Task is set to N for Negative.
12. For wells with a patient sample, confirm that Task is set to U for Unknown.
13. Confirm that Passive Reference is set to None.
14. In the Run Method window, confirm that Reaction Volume Per Well is 30µL, then confirm the thermal cycling protocol.

Table 3: Thermal cycling Protocol

Segment	Cycle Number	Temp.	Time
1	1	42℃	10 min.
2	1	95℃	10 min.
3	45	95℃	10 sec.
		60℃	45 sec.

15. Select Start Run, enter a file name, then click Save.
16. After the instrument run is complete, click Analyze, then save the file.

Exporting Data for Analysis

1. After the run has completed, select the Results tab at the upper left corner of the software.
2. Select the Amplification Plot tab to view the raw data.
3. Start by highlighting all the samples from the run; to do this, click on the upper left-hand box of the sample wells. All the growth curves should appear on the graph.
4. On the right-hand side of the window, the Data drop down selection should be set to Delta Rn vs. Cycle.
5. Select N1 from, the Detector drop down menu, using the downward arrow. Please note that each detector is analyzed individually to reflect different performance profiles of each primer and probe set.
6. In the Line Color drop down, Detector Color should be selected.
7. Under Analysis Settings select Manual Ct. Make sure that the Manual Baseline are set to 3 and 15.
8. Using the mouse, click and drag the red threshold line until it lies within the exponential phase of the fluorescence curves and above any background signal.
9. Click the Analyze button in the lower right corner of the window. The red threshold line will turn to green, indicating the data has been analyzed.
10. Repeat steps 5-9 to analyze results generated for each set of markers (RdRP, E, N).
11. Save analysis file by selecting File then Save As from the main menu.
12. After completing analysis for each of the markers, select the Report tab above the graph to display the Ct values. Record the Ct values and use them for data interpretation.

Reference Range

Assay Controls Materials

- **The negative control (NC)** is needed to ensure that the reagents, equipment and environment involved in the process are not contaminated with the SARS-CoV-2 viral RNA. This is done by adding 10µL from the "Negative control" vial that contains only the in vitro transcriptional RNA containing the internal standard gene fragment (RNaseP) in DNase-RNase free water to 20µL of the qPCR mastermix. A minimum of one negative control must be performed for each run.

- **The positive control (PC)** is needed to ensure that the reagents and equipment is functioning properly when a sample with SARS-CoV-2 RNA is added to the reaction

mix. This is done by adding 10µL from the "Positive control" vial, that contains in vitro transcriptional RNA of all target genes and the internal standard gene fragment (RNase P) at a concentration of (100 copies/µL) to 20µL of the qPCR mastermix. A minimum of one positive control should be performed for each run.

- **The internal control (IC)** is needed to ensure that the sample is added to each qPCR reaction and that the reaction is functioning properly. The IC is present in every reaction. The internal standard gene fragment (RNase P) should result in positive RNase P detection and will act to ensure each reaction is functioning properly.

- **The extraction control** is not included within the ExProbe™ SARS-CoV-2 Testing Kit. Recommended material that can be used include SARS-CoV-2 negative human sera or a human lymphoblastoid cell line CRL-9009 (ATCC, Cat. No. CRL-9009) to verify that adequate sample lysis and efficient nucleic acid extraction have occurred.

Control Acceptance Criteria

Table 4: Valid Controls Acceptance Criteria

Positive Control Well	All Channels should have Ct < 34
Negative Control Well	FAM/Texas Red/Cy5 should have undetectable Ct; VIC (RNase P) should have Ct < 32

Note: All Ct values less than the indicated cutoff should be calculated from a normal S curve associated with a typical real time PCR reaction. In the absence of a normal S curve, the Ct value should be disregarded and the test should be repeated starting from the residual extracted nucleic acid.

In the case that the above criteria have not been met, please disregard any sample test result from that run and repeat the test. If the issue persists, please contact your local representative for troubleshooting.

Explanation of Detection 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. Assuming the controls are acceptable, here is the guideline to interpretation of patient specimen results:

Table 5: Ct Cutoff for Each Channel

Gene (Reporter Dye)	Ct Cutoff for Positive Reaction
RdRP (FAM)	Ct < 40
E (Texas Red)	Ct < 40
N (Cy5)	Ct < 40
RNase P (VIC)	If any other channels are positive, Ct can be any value If all other channels are negative, Ct < 32

Table 6: Interpretation of Patient Results

RdRP (FAM)	E (Texas Red)	N (Cy5)	RNAse P (VIC)	Assay Interpretation	Final Result	Follow-Up Actions
+	+/-			SARS-CoV-2 Positive	Detected	Report results to sender and appropriate public health authorities.
-	Either one of both are positive		+/-	Presumptive Positive	Presumptive Positive	Sample is repeated once. If the repeated result remains the same, 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 Negative	Not Detected	Sample is negative for SARS-CoV-2 virus. Report results to sender and appropriate public health authorities.
-	-		-	Invalid	Invalid	Sample is repeated once. If a second failure occurs, it is reported to sender as invalid and recommend recollection if patient is still clinically indicated.

Limitations

- The use of this test as an in vitro diagnostic under the FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, that meet requirements to perform high complexity tests.
- Use of this test is limited to personnel who are trained in the procedure.
- The laboratory should be equipped with instruments and operators in strict accordance with relevant requirements outlined in local, state and national regulations. Operate in strict accordance with the product manual. Failure to follow these instructions may result in erroneous results.
- The ExProbe™ SARS-CoV-2 Testing Kit performance was established using oropharyngeal swab samples only. While other specimen types listed in the IU are acceptable specimens (i.e., nasopharyngeal swabs, nasopharyngeal washes/aspirates or nasal aspirate, and BALs) for testing, performance with the ExProbe™ SARS-CoV-2 Testing Kit has not been established.
- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.
- Extraction and amplification of nucleic acid from clinical samples must be performed according the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.
- False-negative results may arise from:
    - Improper sample collection
    - Degradation of the SARS-CoV-2 RNA during shipping/storage
    - Specimen collection after SARS-CoV-2 RNA can no longer be found in the specimen matrix
    - Using unauthorized extraction or assay reagents
    - The presence of RT-PCR inhibitors
    - Mutation in the SARS-CoV-2 virus
    - Failure to follow instructions for use
- False-positive results may arise from:
    - Cross contamination during specimen handling or preparation
    - Cross contamination between patient samples
    - Specimen mix-up
    - RNA contamination during product handling.
- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or

immunosuppressant drugs have not been evaluated.

- The ExProbe™ SARS-CoV-2 Testing Kit cannot rule out diseases caused by other bacterial or viral pathogens.
- Negative results do not preclude infection with SARS-CoV-2 virus, and should not be the sole basis of a patient management decision.
- A positive result indicates the detection of nucleic acid from the relevant virus. Nucleic acid may persist even after the virus is no longer viable.

Conditions of Authorization for the Laboratory

The ExProbe™ SARS-CoV-2 Testing 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 ExProbe™ SARS-CoV-2 Testing Kit, the relevant Conditions of Authorization are listed below.

- Authorized laboratories using the ExProbe™ SARS-CoV-2 Testing Kit will include with result reports of the ExProbe™ SARS-CoV-2 Testing Kit, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories* using the ExProbe™ SARS-CoV-2 Testing Kit will perform the ExProbe™ SARS-CoV-2 Testing Kit as outlined in the ExProbe™ SARS-CoV-2 Testing Kit Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to perform the ExProbe™ SARS-CoV-2 Testing Kit are not permitted.
- Authorized laboratories that receive the ExProbe™ SARS-CoV-2 Testing Kit must notify the relevant public health authorities of their intent to run the test prior to initiating testing.
- Authorized laboratories using the ExProbe™ SARS-CoV-2 Testing Kit will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

- Authorized laboratories will collect information on the performance of ExProbe™ SARS-CoV-2 Testing Kit and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and TBG Biotechnology Corp. (Covid19@tbgbio.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.
- All laboratory personnel using The ExProbe™ SARS-CoV-2 Testing Kit must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the test in accordance with the authorized labeling.
- TBG Biotechnology Corp., its authorized distributor(s) and authorized laboratories using the ExProbe™ SARS-CoV-2 Testing 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.

*The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests" as "authorized laboratories."

Performance Characteristics

Limits of Detection (LoD) - Analytical Sensitivity

The LoD study established the lowest SARS-CoV-2 viral RNA concentration (genomic copies/mL) that consistently yielded a 95% positivity rate with the ExProbe™ SARS-CoV-2 Testing Kit.

Preliminary LoD studies for each SARS-CoV-2 target in the assay were performed using synthetic SARS-CoV-2 RNA obtained from Twist Biosciences (Cat # MN9089478.3 containing 1 million copies of SARS-CoV-2 per µL). The Twist reference material includes 6 non-overlapping 5kb fragments that cover over 99.9% of the viral genome. The synthetic RNA was spiked into pooled negative clinical oropharyngeal swab matrix and tested at three different concentrations in triplicate using the following extraction kit and RT-PCR instrument combinations:

- QIAamp Viral RNA Mini Kit/ABI7500 (Table 7)
- EZbead Viral Extraction Kit on the EZbead System-32/ABI7500 (Table 8)
- QIAamp Viral RNA Mini Kit/TBG Q6000 Real-Time PCR System (Table 9)
- EZBead Viral Extraction Kit on the EZbead System-32/TBG Q6000 Real-Time PCR

System (Table 10)

The preliminary LoD was confirmed by testing 20 independent extraction replicates for all extraction kit and instrument combinations and was determined to be 10 copies/µL (See Tables 11-14).

Table 7: Preliminary Range Finding Study Results Using Replicates Extracted with the QIAamp Viral RNA Mini Kit Performed on the ABI7500 Platform

Copies/ µL	RdRP Gene			E Gene			N Gene		
	Mean Ct	SD	Detection Rate	Mean Ct	SD	Detection Rate	Mean Ct	SD	Detection Rate
100	29.70	0.84	(3/3) 100%	29.37	0.55	(3/3) 100%	31.17	0.31	(3/3) 100%
10	32.80	1.10	(3/3) 100%	32.57	0.50	(3/3) 100%	34.70	0.56	(3/3) 100%
2*	ND	ND	(2/3) 66%	34.80	0.85	(3/3) 100%	ND	ND	(1/3) 33%

*At 2 copies/µL, some of the reactions had not detectable (ND) results.
SD; standard deviation

Table 8: Preliminary LoD Range Finding Study Data Using the Replicates Extracted with the EZbead Viral Extraction Kit Performed on the ABI7500 Platform

Copies/ µL	RdRP Gene (FAM)			E Gene (Texas Red)			N Gene (Cy5)		
	Mean Ct	SD	Detection Rate	Mean Ct	SD	Detection Rate	Mean Ct	SD	Detection Rate
100	29.99	0.78	(3/3) 100%	29.89	0.80	(3/3) 100%	32.77	0.50	(3/3) 100%
10	33.50	0.52	(3/3) 100%	34.62	0.49	(3/3) 100%	35.77	0.38	(3/3) 100%
2*	37.1	ND	(1/3) 33%	35.98	0.28	(2/3) 66%	40.04	0.09	(2/3) 66%

*At 2 copies/µL, some of the reactions had not detectable (ND) results.
SD; standard deviation

Table 9. Preliminary LoD Range Finding Study Data Using the Replicates Extracted with the Qiagen QIAamp Viral RNA Mini Kit Performed on the TBG Q6000 Real-Time PCR System

Copies/ µL	RdRP Gene (FAM)			E Gene (Texas Red)			N Gene (Cy5)		
	Mean Ct	SD	Detection Rate	Mean Ct	SD	Detection Rate	Mean Ct	SD	Detection Rate
100	30.86	0.76	(3/3) 100%	31.07	0.33	(3/3) 100%	33.64	0.69	(3/3) 100%
10	34.12	0.78	(3/3) 100%	35.20	0.37	(3/3) 100%	36.95	0.61	(3/3) 100%
2*	ND	ND	(0/3) 0%	35.78	ND	(1/3) 33%	38.89	ND	(1/3) 33%

*At 2 copies/µL, some of the reactions had not detectable (ND) results.
SD; standard deviation

Table 10. Preliminary LoD Range Finding Study Data Using the Replicates Extracted with the EZbead Automated Workflow Performed on the TBG Q6000 Real-Time PCR System

Copies/ µL	RdRP Gene (FAM)			E Gene (Texas Red)			N Gene (Cy5)		
	Mean Ct	SD	Detection Rate	Mean Ct	SD	Detection Rate	Mean Ct	SD	Detection Rate
100	31.28	0.43	(3/3) 100%	31.79	0.66	(3/3) 100%	33.78	1.05	(3/3) 100%
10	34.90	1.22	(3/3) 100%	35.73	0.35	(3/3) 100%	37.74	0.52	(3/3) 100%
2*	ND	ND	(0/3) 0%	ND	ND	(0/3) 0%	ND	ND	(0/3) 0%

*At 2 copies/µL, none of the reactions had detectable (ND) results.
SD; standard deviation

Table 11: LoD Confirmation Study with 20 Extraction Replicates with the Qiagen QIAamp Viral RNA Mini Kit Performed on the ABI7500 Platform

Copies/ µL	Specimen	Positive Rate			Mean Ct			
		RdRP	E	N	RdRP	E	N	RNase P
10	Oropharyngeal Swab	100%	100%	100%	31.91	32.34	34.96	23.05

Table 12: LoD Confirmation Study with 20 Extraction Replicates with the EZbead Viral Extraction Kit Performed on the ABI7500 Platform

Copies/ µL	Specimen	Positive Rate			Mean Ct			
		RdRP	E	N	RdRP	E	N	RNase P
10	Oropharyngeal Swab	100%	100%	100%	30.45	35.29	34.65	24.01

Table 13: LoD Confirmation Study with 20 Extraction Replicates with the Qiagen QIAamp Viral RNA Mini Kit Performed on the TBG Q6000 Real-Time PCR System

Copies/ µL	Specimen	Positive Rate			Mean Ct			
		RdRP	E	N	RdRP	E	N	RNase P
10	Oropharyngeal Swab	100%	100%	100%	34.06	34.37	37.61	24.32

Table 14: LoD Confirmation Study with 20 Extraction Replicates with the EZBead Viral Extraction Kit Performed on the TBG Q6000 Real-Time PCR System

Copies/ µL	Specimen	Positive Rate			Mean Ct			
		RdRP	E	N	RdRP	E	N	RNase P
10	Oropharyngeal Swab	100%	100%	100%	34.55	35.31	38.11	24.77

Matrix Equivalency

A matrix equivalency study was completed to demonstrate that oropharyngeal (OP) and nasopharyngeal (NP) swab matrix performed similarly with the ExProbe SARS-CoV-2 Testing Kit. Ten replicates were prepared at 1X, 2X, and 5X LoD (10 copies/µL) using negative NP and OP swab matrix spiked with Twist Biosciences viral RNA material. Ten negative NP and OP swab matrices were also included in this study. All negative and spiked positive samples were blinded and randomized for testing. Samples were extracted with the EZBead System and tested on the ABI 7500 RT-PCR platform. The evaluation of NP and OP swab matrices showed that both clinical matrices performed similarly with the ExProbe SARS-CoV-2 Testing Kit.

Inclusivity - Analytical Reactivity

To assess the inclusivity of the ExProbe SARS-CoV-2 Testing Kit, the RdRP, E, and N gene specific primer and probe sets were queried against the SARS-CoV-2 genomes (TaxID: 2697049) on the NCBI BLAST website in April 2020. The database consists of

GenBank+EMBL+DDBJ+PDB+RefSeq sequences, but excludes EST (Expressed Sequence Tags), STS (Sequence Tagged Sites), GSS (Genome Survey Sequence), WGS (Whole Genome Shotgun Projects), TSA (Transcriptome Shotgun Assembly), patent sequences as well as phase 0, 1, and 2 HTGS (High Throughout Genomic Sequences) sequences and sequences longer than 100Mb.

Table 15: *In Silico* Inclusivity Blast Results

Primer and Probe ID	Blast Results
RdRP SARSr-F2 Primer	Out of 523 sequences, primer/probe sequence demonstrates over 95% match.
RdRP SARSs-R1 Primer	Out of 523 sequences, primer/probe sequence demonstrates over 95% match.
RdRP SARSr-P2 Probe	Out of 523 sequences, primer/probe sequence demonstrates over 95% match.
E Sarbeco-F1 Primer	Out of 523 sequences, primer/probe sequence demonstrates over 95% match.
E Sarbeco-R2 Primer	Out of 523 sequences, primer/probe sequence demonstrates over 95% match.
E Sarbeco-P1 Probe	Out of 523 sequences, primer/probe sequence demonstrates over 95% match.
N Sarbeco-F1 Primer	Out of 523 sequences, primer/probe sequence demonstrates over 95% match.
N Sarbeco-R1 Primer	Out of 523 sequences, primer/probe sequence demonstrates over 95% match.
N Sarbeco-P1 Probe	Out of 523 sequences, primer/probe sequence demonstrates over 95% match.

The RdRP Forward Primer (5'-GTGA**R**ATGGTCATGTGTGGCGG-3') has a degenerate base with "R" at position 5 and the RdRP Reverse Primer (5'-CA**R**ATGTTAAA**S**ACACTATTAGCA TA-3') also has a degenerate base designated as "R" at position 3 and "S" at position 12. Due to these degenerate bases in the RdRP primer set, 95% homologies to SARS-CoV-2 sequences were shown. A single base mismatch of one NCBI sequence was located in the probe sequence of the RdRP gene. This mismatch is not predicted to impact assay performance. The primers and probe targeting the N and E genes of Sarbecovirus and SARS-CoV-2 had no mismatches against any of the published 523 sequences used in the inclusivity *in silico* evaluation.

Cross-Reactivity - Analytical Specificity

The potential cross-reactivity of the assay primers and probes were evaluated using both *in silico* and wet testing approaches with other potential respiratory pathogens.

Cross-reactivity *in silico* testing with other organisms was completed using the entire Nucleotide Collection (nt/nr) at NCBI. The same default algorithm parameters applied in the inclusivity study were applied to the exclusivity study with the exception being the Max Target Sequence. For the *in silico* cross-reactivity study, the maximum target sequence was defined at 5,000 bases instead of 100 bases used in the inclusivity analysis.

For each virus, only the GenBank ID with the highest % coverage is listed in the table below. The analysis showed that some microorganism sequences have >80% homology to one or more of the ExProbe's primers or probes; however, none of the organisms are expected to be amplified by the test due to one of the following reasons:

- □ Only one primer showed >80% homology to a sequence, while the other primer showed less homology to the same sequence. □
- □ While the probe showed >80% homology to a sequence, neither of the primers showed significant homology to the same sequence. □
- □ While one or both primers showed >80% homology to a sequence, there are >3 mismatches at the 3' end of the primers. □

Table 16: *In Silico* Cross-Reactivity Analysis

	Percent Homology of ExProbe™ SARS-CoV-2 Primers/Probes								
	RdRP P2	**RdRP F2**	**RdRP R1**	**N P1**	**N F1**	**N R1**	**E P1**	**E F1**	**E R2**
Coronavirus 229E (11137)	0%	0%	0%	52%	50%	68%	0%	40%	0%
Coronavirus OC43 (31631)	95%	0%	56%	47%	70%	40%	46%	95%	0%
Coronavirus HKU1 (290028)	100%	0%	56%	57%	90%	64%	69%	63%	44%
Coronavirus NL63 (277944)	81%	0%	0%	47%	70%	48%	53%	50%	44%
MERS (1335626)	86%	96%	52%	100%	80%	40%	65%	100%	48%
Bordetella pertussis	0%	0%	0%	52%	68%	0%	58%	0%	63%
Candida albicans	72%	50%	0%	84%[1]	89%[1]	65%	54%	69%	59%
Chlamydia pneumoniae	52%	0%	0%	56%	0%	0%	54%	0%	77%
Enterovirus EV68	0%	0%	59%	52%	68%	0%	0%	0%	0%
Haemophilus influenzae	52%	57%	0%	100%[2]	68%	0%	58%	0%	86%[1]
hMPV	0%	0%	0%	0%	0%	0%	0%	0%	0%

	Percent Homology of ExProbe™ SARS-CoV-2 Primers/Probes								
	RdRP P2	**RdRP F2**	**RdRP R1**	**N P1**	**N F1**	**N R1**	**E P1**	**E F1**	**E R2**
Human Rhinovirus	52%	0%	0%	0%	0%	0%	4%	0%	0%
Influenza A	56%	50%	0%	60%	0%	70%	0%	0%	86%[1]
Influenza B	0%	0%	0%	0%	0%	0%	0%	0%	0%
Legionella pneumophila	88%[2]	61%	59%	92%[1]	89%[1]	65%[2]	100%	69%	59%
Mycobacterium tuberculosis	0%	0%	0%	0%	0%	0%	0%	0%	0%
Parainfluenza virus 1-4	0%	0%	0%	0%	0%	0%	0%	0%	0%
Pneumocystis jirovecii (PJP)	52%	73%	59%	88%[2]	0%	0%	66%	0%	59%
Pseudomonas aeruginosa	68%	0%	77%	92%[3]	84%[3]	90%[3]	0%	0%	90%[1]
Mycoplasma pneumoniae	0%	0%	0%	0%	0%	0%	0%	0%	81%[1]
Respiratory Syncytial Virus	0%	0%	0%	0%	0%	0%	0%	0%	0%
Staphylococcus aureus	0%	0%	0%	0%	0%	0%	0%	0%	0%
Staphylococcus epidermidis	0%	0%	0%	56%	0%	65%	70%	0%	0%
Streptococcus pneumoniae	80%[2]	0%	0%	88%[1]	68%	95%[1]	70%	0%	81%[1]
Streptococcus pyogenes	96%[2]	0%	0%	80%[2]	0%	0%	54%	53%	59%
Streptococcus salivarius	68%	61%	72%	80%[1]	89%[1]	65%	54%	60%	81%[1]

For most organisms that demonstrated > 80% homology to any primer or probe of the ExProbe™ Kit wet testing was performed (i.e., *H. influenzae*, *L. pneumophilia*, *P. aeruginosa*, and *S. pyogenes*). Each organism was tested in triplicate at the following concentrations, respectively; 2.5 x 10^3 genome copies/mL, 1.6 x 10^3 genome copies/mL, 7.2 x 10^3 genome copies/mL, and 2.6 x 10^3 genome copies/mL concentration. All results were negative indicating that the ExProbe™ SARS-CoV-2 Testing Kit is not expected to cross-react with these organisms and cause potential false positive RT-PCR results.

In addition, wet testing was performed against a variety of viral strains using the Non WHO Reference Material-Viral Multiplex Organisms (TWBIOsciences, Cat # NIB-11-242-1EA, UK) to assess the potential cross-reactivity of the assay's primers and probes. Each organism identified in the table below (Table 17) was tested in triplicate with the ExProbe™ SARS-CoV-2 Testing Kit at the concentrations indicated if known. Either RNA or DNA of each organism was spiked into negative oropharyngeal matrix and tested on the ABI 7500 Real-Time instrument for SARS-CoV-2 (RdRP gene) and pan-Sarbecovirus (N and

E genes). All results were negative.

Table 17. Non WHO Reference Material Viral Multiplex Organisms Used to Evaluate Assay Cross-Reactivity

Pathogen	Replicate	Concentration	Cross-Reactivity
Human Herpesvirus 1	3	Unknown	Negative
Human Herpesvirus 2	3	Unknown	Negative
Human Herpesvirus 3 (VZV)	3	Unknown	Negative
Human Herpesvirus 4 (EBV)	3	7.6×10^3 genome copies/mL	Negative
Human Herpesvirus 5 (CMV)	3	4.6×10^4 genome copies/mL	Negative
Rotavirus A	3	5.8×10^6 genome copies/mL	Negative
Astrovirus	3	Unknown	Negative
Norovirus GI	3	Unknown	Negative
Norovirus GII	3	Unknown	Negative
Sapovirus C12	3	Unknown	Negative
Coronavirus 229E	3	Unknown	Negative
Coxsackievirus B4	3	Unknown	Negative
Rhinovirus A39	3	Unknown	Negative
Parechovirus 3	3	1.2×10^7 genome copies/mL	Negative
Influenza A Virus H1N1	3	Unknown	Negative
Influenza A virus H3N2	3	Unknown	Negative
Influenza B virus	3	Unknown	Negative
Metapneumovirus A	3	Unknown	Negative
Parainfluenzavirus 1	3	Unknown	Negative
Parainfluenzavirus 2	3	Unknown	Negative
Parainfluenzavirus 3	3	Unknown	Negative
Parainfluenzavirus 4	3	Unknown	Negative
Respiratory Syncytial Virus A2	3	5.6×10^3 genome copies/mL	Negative

Clinical Evaluation

To evaluate the clinical performance of the ExProbe™ SARS-CoV-2 Testing Kit, oropharyngeal swabs that were previously tested by two separate comparator EUA authorized molecular RT-PCR assays were used. A total of 200 negatives and 43 positive oropharyngeal swabs were tested retrospectively. There was one false positive result for which further discordant analysis was not conducted. Results of the study are displayed in Table 18.

Table 18. Results from Preliminary Clinical Study Using an EUA Authorized Molecular Assay as a Comparator

Oropharyngeal Swabs		Comparators – EUA Authorized Molecular Assays		
		Positive	Negative	Total
ExProbe™ SARS-CoV-2 Testing Kit	Positive	43	1	44
	Negative	0	199	199
	Total	43	200	243
Positive Percent Agreement		43/43; 100.00% (91.80% - 100.00%)[1]		
Negative Percent Agreement		199/200; 99.50% (97.22% - 99.91%)[1]		

[1]Two-sided 95% score confidence intervals

Contrived Clinical Performance Study

The contrived clinical performance of the ExProbe™ SARS-CoV-2 Testing Kit was also evaluated using SARS-CoV-2 synthetic RNA (Twist Biosciences Cat # MN9089478.3) spiked into negative oropharyngeal swab matrix. Positive specimens were prepared by spiking known concentrations of SARS-CoV-2 synthetic RNA into individual, unique oropharyngeal swab matrices that were determined to be negative prior to spiking the RNA. Spiking concentrations included low positive (1X LoD; 10 copies/µL), moderate positive (5X LoD; 50 copies/µL), and high positive concentrations (10X LoD; 100 copies/µL). In addition to the 30 contrived positive samples, 30 negative samples were obtained by spiking with water instead of synthetic viral genome copies.

Prepared samples were blinded and randomized for testing. RNA was extracted using the Qiagen QIAamp Viral RNA Mini Kit and testing was performed on the ABI 7500 PCR system in one RT-PCR run that included five positive control reactions and five negative control reactions along with the 30 positive and negative contrived samples. Results of the study are summarized below in Table 19.

Table 19: Clinical Evaluation Using Contrived Oropharyngeal Swabs

Concen.	Replicates	RdRP Gene			E Gene			N Gene		
		Detection Rate	Mean Ct	%CV	Detection Rate	Mean Ct	% CV	Detection Rate	Mean Ct	% CV
unspiked	30/30	0%	ND	N/A	0%	ND	N/A	0%	ND	N/A
1x LoD	20/20	100%	31.9	2.3	100%	32.3	1.3	100%	35.0	1.3
5x LoD	5/5	100%	30.7	0.7	100%	30.8	0.6	100%	33.0	0.4
10x LoD	5/5	100%	29.5	2.6	100%	29.3	1.4	100%	31.1	0.8

Note: ND = Not Detected, N/A = Not Applicable.

All 30 negative specimens were non-reactive while all 30 positive specimens had positive results. All results had proper controls and each positive sample had a proper S-curve as

part of the Ct value quality control.

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TBG Biotechnology Corp.,
13F-1, No.237, Sec. 1, Datong Rd, Xizhi Dist., New Taipei City 221, Taiwan

For Product Support, please contact TBG Biotechnology Corp. at Tel: +886-2-7741-7777 or email:

COVID19@tbgbio.com.
For U.S. Customer and Technical Support: 1-877-822-2461