ExProbe™ SARS-CoV-2 Testing Kit (96 Tests)

REF 68020

1 Intended Use

ExProbe™ SARS-CoV-2 Testing Kit is used for qualitative detection of the RdRP, N and E genes of SARS-CoV-2 in pharyngeal swabs and sputum samples. The test results of this kit can qualitatively determine whether the suspected patient is infected with SARS-CoV-2 virus.

2 Summary And Explanation

The Corona Virus Disease in 2019 (COVID-19) is known to be caused by the SARS-CoV-2 virus. Symptoms include severe viral pneumonia, respiratory failure, and can quickly progress to life threatening conditions. Transmission of the Corona Virus primarily occurs through aerosols and droplets. Once infected, most patients will experience symptoms within 2 weeks.

3 Principle(s)

By applying Real-time PCR (RT-PCR) technology contain a panel of primer mixes and fluorescent probes. This method is based on sequence specific primers that completely match the target sequences resulting in cDNA amplification. This test utilizes the SARS-CoV-2 RdRP and the specific conserved sequence of coding nucleocapsid protein N and E genes as the target regions which are designed for the conserved sequence of the triple-target genes, to achieve detection of sample RNA through fluorescent signal changes. The PCR detection system uses the positive internal control, which monitors the presence of PCR inhibitors in test specimens by detecting whether the internal control signal is normal, to avoid a false negative result.

4 Reagents And Equipment

4.1 Contents of the ExProbe™ SARS-CoV-2 Testing Kit

Each Kit provides sufficient reagents for 96 tests.

4.1.1 **SARS-CoV-2 Assay (500μL/tube) x 1 tubes:** Main ingredients: Primer and Probe.

4.1.2 **SARS-CoV-2 Master Mix (1.5mL /tube) x 1 tube:** Main ingredients: dNTPs, MgCl2, Tris-HCl, Reverse Transcriptase, Taq DNA polymerase.

4.1.3 **SARS-CoV-2 Positive Control (1.5mL/tube) x 1 tube:** Main ingredients: Virus-like Particles(VLPs) containing target sequence for each target gene (RdRP, E, N) and internal standard gene fragments (RNase P).

4.1.4 **SARS-CoV-2 Negative Control (1.5mL/tube) x 1 tube:** Main ingredients: Virus-like Particles(VLPs) containing internal standard gene fragments (RNase P).

4.2 Equipment

4.2.1 Self-prepared reagent and instrument: RNA extraction system. Extraction of RNA is recommended to use the EZbead Virus Extraction Kit, pre-filled (Cat. No. 37900a) and EZbead System-32 (Cat. No. 37001) from TBG Biotechnology Corp.

4.2.2 Materials required but not provided: fluorescence quantitative PCR instrument, bio-safety cabinet, PCR reaction tubes, pipette tips (10μL, 200μL and 1000μL tips with filters are preferred), dry type constant temperature meter, desktop centrifuge, desktop vortex mixer various models of pipette guns, and powder free latex gloves.

4.3 Storage and Stability

4.3.1 The diagnostic kit should be stored in a sealed pouch below -20 ± 5℃ and protected from light. The kit is provisionally valid for 6 months. The number of repeated freezing and thawing shall not be more than 4 times. It can be transported under dark conditions below -20 ± 5℃ and can be kept stable for 5 days.

4.3.2 Please refer to the date of manufacture and expiry date on the outer package.

4.3.3 The reagents keep valid and stable within the expiry date if not used. As long as the container of the reagent is opened, the freeze/thaw cycles should not exceed three.

5 Specimen Requirements

5.1 Applicable specimen type: Sputum, and pharyngeal swab.

5.2 Storage and delivery of specimens: Specimens to be tested can be immediately processed, specimens to be tested within 24 hours can be stored at 4℃. Specimens that cannot be detected within 24 hours should be stored at -70℃ or below for 6 months (in the absence of -70℃ storage conditions, specimens to be tested can be stored at -20 ± 5℃ for 7 days). Multiple freeze/thaw cycles should be avoided. Specimens should be transported in a sealed frozen pitcher with ice or in a sealed foam box with ice.

6 Protocol

6.1 Preparation of reagent

6.1.1 Take out each component from the testing kit and thaw them on ice. Once the reagents have thawed, vortex and centrifuged the reagents for several seconds and keep on ice for later use.

6.1.2 According to the quantity of test specimens (N), pipette appropriate quantity of SARS-CoV-2 Assay and SARS-CoV-2 Master Mix (SARS-CoV-2 Assay 5 μL*N + SARS-CoV-2 Master Mix 15 μL*N). 1.5ml sterile centrifugal tube was used to prepare the reaction system, after all the reagents were added, mix them thoroughly and centrifuged for several seconds. Then the above mixture 20 μL/tube was loaded into the PCR reaction tube.

Note: The above configuration is just for your reference and to ensure enough volume of the PCR reaction mixture, more volume of the actual pipetting may be required.

6.1.3 Transfer the above-prepared reagents to the “specimen processing region” for later use.

6.2 Processing and loading of specimens

6.2.1 Use Sample Release Reagent: EZbead Virus Extraction Kit, pre-filled (Cat. No. 37900a) and EZbead System-32 (Cat. No. 37001) manufactured by TBG Biotechnology Corp. is recommended to extract the nucleic acid as per the product manual.

6.2.2 Extracted SARS-CoV-2 Positive Control, extracted SARS-CoV-2 Negative Control, and clinical sample RNA were added 10 μL into the PCR reaction tube, and the tube cover was tightly sealed (to avoid bubble production). Any liquid on the tube wall is briefly centrifuged to the bottom of the tube.

6.2.3 Fluorescence quantitative PCR was performed on Applied Biosystems® 7500 Real-Time PCR System or TBG Q6000.

6.3 PCR Amplification

(Refer to user manual of each instrument to adjust the settings.)

6.3.1 Place PCR reaction tubes into the specimen wells of the amplification equipment. Set up the SARS-CoV-2 Positive Control, SARS-CoV-2 Negative Control and specimens to be tested in the corresponding sequence and input specimen name.

6.3.2 Select PCR test channel and sample volume:

(a) Select FAM/Texas Red/Cy5 channel (Reporter: FAM/ Texas Red/ Cy5, Quencher: None) to test RdRP gene、E gene and N gene of SARS-CoV-2 respectively.

(b) Select VIC/HEX channel (Reporter: VIC/HEX, Quencher: None) to test internal control.

(c) Sample Volume: 30μL.

(d) Reference Dye: None (for Applied Biosystems series instruments only).

6.3.3 Set cycle parameters:

Table 1 Thermal Protocol

Segment	Cycle Number	Temperature	Time
1	1	42℃	30 min.
2	1	95℃	10 min.
3	45	95℃	15 sec.
		60℃	45 sec.

6.3.4 When the settings are completed, save the settings and carry out the reaction procedure.

6.4 Result Analysis

(Refer to user manual of instrument to adjust the settings.)

Results will be saved automatically when reactions are completed. Analyze amplification curve of target of detection and internal control. Adjust baseline and threshold values according to analysis result (Users can adjust the values according to the actual situation. Start value can be set to 10 to 20 for Applied Biosystems® 7500 Real-Time PCR System or TBG Q6000, respectively. Adjust the amplification curve of negative control to be flat or below threshold). Click “Analyze” to implement the analysis, make sure each parameter satisfies the requirements given in “6.5 Quality Control”. Go to “Plate” window to record qualitative results.

6.5 Quality Control

Table 2 Valid Controls Acceptance Criteria

Channel / Control	C_T Value			
	FAM (Gene RdRP)	Texas RED (Gene E)	Cy5 (Gene N)	HEX/VIC (RNase P)
Negative Control	Undetermined	Undetermined	Undetermined	≤ 30
Positive Control	≤ 32	≤ 32	≤ 32	≤ 30

The test result is treated as valid if all the conditions in the above-mentioned are met for the same test. Otherwise the test result is treated as invalid and needs to be re-tested.

7 Explanation of Detection Result

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 3 C_T cutoff for each channel

Gene (Reporter Dye)	C_T Cutoff for Positive Reaction
RdRP (FAM)	$C_T < 40$
E (Texas Red)	$C_T < 40$
N (Cy5)	$C_T < 40$
RNase P (VIC/HEX)	If any other channels are positive, C_T can be any value If all other channels are negative, $C_T < 34$

Table 4 Data Analysis Table

RdRP	E	N	RNase P	Assay Interpret	Final Result	Follow-Up Actions
+	+/-			Positive	Virus Detected	Report results to sender and appropriate public health authorities
-	Either one or both are positive		+/-	Presumptive Positive	Presumptive Positive	Sample is repeated once. If the repeated result remains the same, additional confirmatory testing may be conducted.
-	-		+	Negative	No virus detected	Sample is negative for SARS-CoV-2 virus.
-	-		-	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.

8 Limitations of Detection Method

8.1 The symptoms and physical signs, disease history, other laboratory examinations and therapeutic reactions of the patients should be comprehensively considered during their clinical diagnosis and treatment.

8.2 The possibility analysis of false negative/positive results:

8.2.1 The unreasonable of specimen collection, delivery, processing and specimen in low concentrations may lead to false negative results.

8.2.2 The mutation in the target sequence of SARS-CoV-2 to be measured or the change in the sequence due to other causes may lead to false negative results.

8.2.3 The unreasonable of reagent storage may lead to false negative results.

8.2.4 Unverified interferences or PCR inhibitors may lead to false negative results.

8.2.5 Cross-contamination occurring in the specimen processing may lead to false positive results.

8.2.6 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.

9 Product Performance Index

9.1 **Limit of detection:** The limit of detection of this kit is 50 copies/reaction.

10 Waring and Precautions

For In Vitro Diagnostic Use only. For professional use only.

10.1 Do not mix or exchange components from different kit lots.

10.2 All biological samples in the diagnostic kit have been inactivated.

10.3 Please read the product manual carefully before operation.

10.4 Please learn and be familiar with the operation procedures and precautions for each instrument before test.

10.5 Laboratory management shall strictly follow practices of PCR gene amplification laboratory, laboratory personnel must receive professional training, test processes must be performed in separated regions, 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 regions should not be cross-used.

10.6 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.

10.7 Note: Improper operation during the storage, transportation and use of the reagent may affect the test results. For example, improper storage and transportation, sample collection, sample processing and test process are not standardized, please strictly follow the instructions.

10.8 Due to the characteristics of swab and other sample collection process and 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.

11 Bibliography

11.1 Clinical management of severe acute respiratory infection when novel coronavirus (nCoV) infection is suspected, WHO, 2020.

11.2 Diagnostic Detection of 2019-nCoV by Real-Time RT-PCR, WHO, 2020.

11.3 Real-Time RT-PCR Panel for Detection 2019-Novel Coronavirus Instructions for Use, US FDA, 2020.

11.4 2019-Novel Coronavirus (2019-nCoV) Real-Time rRT-PCR Panel Primers and Probes, US FDA, 2020.

11.5 2019-nCoV Virus Nucleic Acid Test, Taiwan Centers for Disease Control, 2020.

12 Trademark Used in This Document

12.1 TBG Biotechnology Corp.

12.2 Applied Biosystems

13 Patents Used in This Document

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