REF **BS-SY-SC2-100**

Bio-Speedy® Direct RT-qPCR SARS-CoV-2

Instructions For Use

For *In vitro* Diagnostic Use
Rx Only
For use under Emergency Use Authorization (EUA) only

CE IVD

Bioeksen R&D Technologies Ltd.

Istanbul Technical University, Teknokent ARI 3, No: 4/B 105
Sariyer, Istanbul, TURKEY

Phone: +90 (212) 285 10 17, Fax: +90 (212) 285 10 18
E-mail: info@bioeksen.com.tr, Web: www.bioeksen.com.tr

Table of Contents

INTENDED USE

Bio-Speedy® Direct RT-qPCR SARS-CoV-2 nucleic acid detection kit is a one-step reverse transcription and real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swabs, oropharyngeal (throat) swabs, combined nasopharyngeal/oropharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal or nasopharyngeal aspirates, nasal washes and bronchoalveolar lavage samples 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. 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.

The *Bio-Speedy® Direct RT-qPCR SARS-CoV-2* is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The *Bio-Speedy® Direct RT-qPCR SARS-CoV-2* is only for use under the Food and Drug Administration's Emergency Use Authorization.

TEST PRINCIPLE

The *Bio-Speedy® Direct RT-qPCR SARS-CoV-2* nucleic acid detection kit is a real-time reverse transcription polymerase chain reaction (rRT -PCR) test. The SARS-CoV-2 primer and probe set(s) is designed to detect nucleic acid from the SARS-CoV-2 in nasopharyngeal swabs, oropharyngeal (throat) swabs, combined nasopharyngeal/oropharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal or nasopharyngeal aspirates, nasal washes and bronchoalveolar lavage samples from patients suspected of COVID-19 by their healthcare provider.

Detection with the kit is achieved via rapid nucleic acid extraction from respiratory tract samples followed by multiplex real-time RT-PCR targeting the SARS-CoV-2 specific *ORF1ab* gene and human *RNase P* gene and mRNA in real-time PCR instruments that are equipped with FAM and HEX detection channels.

The oligonucleotide set targeting human *RNase P* gene and mRNA functions as a control of the sampling, nucleic acid extraction and inhibition. The kit also contains negative and positive control templates.

The kit contains vNAT™ buffer that extracts and preserves viral nucleic acids in respiratory tract samples. The vNAT™ component enables the initiation of the real-time RT-PCR within 5 minutes of introduction of the sample. Polyethyleneimine coated tetradecyl dimethyl benzyl ammonium chloride-based nanoparticles (NP) and tween-20 in vNAT™ lyse envelope and nucleocapsid of SARS-CoV-2 and release the genome. NP, guanidinium thiocyanate and NaN_3 in vNAT™ preserve the integrity of the released genomes. BSA in vNAT™ is used as a PCR facilitator to compensate negative effects of PCR inhibitors.

INSTRUMENTS AND SOFTWARE

The *Bio-Speedy® Direct RT-qPCR SARS-CoV-2* test is to be used with the *Roche LightCycler® 96, Bio-Rad CFX96 Touch™, Qiagen Rotor-Gene® 5 Plex* Real-Time PCR Systems and the accompanying software (Table 1).

Table 1. *Instruments and their software validated with the kit*

Manufacturer	Instrument	Instrument Catalog No	Software	Software Catalog No
Roche	LightCycler® 96	5815916001	LightCycler® 96 Application and Instrument Software	Included in 5815916001
Bio-Rad	CFX96 Touch™	1855195	CFX Maestro™ Software	12004110
Qiagen	Rotor-Gene® 5 Plex Platform	9001570	Q-Rex Software v1.0	Included in 9001570

REAGENTS AND MATERIALS PROVIDED

Reagent Names and Concentrations

The kit contents and their formulation are provided in Table 2.

Table 2. *Kit contents*

Component	Amount [1]	Content	Intended Use	Box [2]
Oligo Mix	1 x 250 µL	*ORF1ab* Primer-1; 500 nM	SARS-CoV-2 Detection (*ORF1ab* gene)	Box 1/2
		ORF1ab Primer-2; 500 nM		
		ORF1ab Probe; 150 nM (**FAM**)		
		RNase P Primer-1; 500 nM	Internal Control (IC) (*RNase P* gene and mRNA)	
		RNase P Primer-2; 500 nM		
		RNase P Probe; 150 nM (**HEX**)		

2X Prime Script Mix	1 x 500 µL	DNA polymerase, dNTP mix, reaction buffer, reverse transcriptase and ribonuclease inhibitor	One Step Real-Time RT-PCR Mix	Box 1/2
NTC	2 x 1000 µL	Water, DEPC-Treated, Molecular Biology Grade (CAS 7732-18-5)	No Template (Negative) Control for PCR and Extraction Negative Control	Box 1/2
PC	1 x 250 µL	10^3 copies/mL synthetic RNA for *ORF1ab* oligonucleotide set and 10 ng/µL total nucleic acid extract from human blood for *RNase P* oligonucleotide set	Positive Control Template: Synthetic SARS-CoV-2 genome fragment	Box 1/2
vNAT™	5 x 2 mL	Viral nucleic acid extraction buffer	Extraction and preservation of viral nucleic acids	Box 2/2

[1] for 100-reaction kit.

[2] Components with different storage and transport requirements are packaged in separate boxes. Please refer to the "LABELS" section.

Kit Storage Requirements and Shelf Life of the Kit

- Based on individual component shelf life, the approximate shelf life of the kit is estimated to be 12 months at the recommended storage temperature for each component.
- The “Box 1/2" (PCR components: Oligo Mix, 2X Prime Script Mix, NTC and PC) can be stored at -20°C and can be transported at +2°C - +8°C.
- The “Box 2/2” (Extraction component: vNAT™) can be stored and transported at room temperature (25°C ± 2°C). The temperature for long-term storage is +2°C- +8°C. However, it is recommended to be stored at room temperature because of the risk of precipitation occurring due to cold storage.
- Each reagent (stored at the recommended storage temperature) may be used until the expiration date indicated on the tube.
- The shelf life and safe storage conditions of the kit components were determined by the application of the Arrhenius Equation based on accelerated study data. During accelerated stability studies, all components were stored at different temperatures and tested at weekly intervals throughout the study period. More than 2 cycles deterioration of Cq values for *ORF1ab* compared to the values obtained on day 0 was accepted as a threshold for the shelf life of the “2X Prime Script Mix”, “Oligo Mix” and “vNAT™" components. More than 5 cycles deterioration of Cq values for *ORF1ab* compared to the values obtained on day 0 was accepted as a threshold for the shelf life of the “Positive Control (PC)” component.
- Real-Time stability testing is on-going and the final shelf life will be established after the stability studies have been completed.
- The expiration date of the kit is determined by the expiration date of the included reagents

and is indicated on the label of the kit box. In addition, the expiration date of each reagent is stated on the label of each reagent. Please refer to the expiration date on the box.

- Do not use reagents beyond their expiration date.

MATERIALS REQUIRED BUT NOT PROVIDED

Table 3. *Components required but not included with the kit*

Component	Using for	Specifications
Swabs for nasopharyngeal and oropharyngeal swab samples	Sampling and Transportation	Dacron or polyester, breakable shaft, sterile
Containers for other samples	Sampling and Transportation	Sterile, capped sample container
Viral Transport Medium	Sample transport	Preparation of VTM: Centers for Disease Control and Prevention, SOP#: DSR-052-01
Vortex mixer	NA extraction	Speed up to 3000 rpm
Microcentrifuge tubes	NA extraction	1.5 mL or 2 mL, nuclease-free
	real-time RT-PCR	
Micropipettes	NA extraction	Adjustable volume; 1-10 µL, 10-100 µL, 100-1000 µL
	real-time RT-PCR	
Micropipettes tips	NA extraction	Compatible with the micropipettes (1-10 µL, 10-100 µL and 100-1000 µL), filtered, nuclease-free
	real-time RT-PCR	
LightCycler® 96 with Application and Instrument Software [1]	real-time RT-PCR	Manufacturer: Roche / Catalogue No: 5815916001
CFX96 Touch™ and CFX Maestro™ Software [1]	real-time RT-PCR	Manufacturer: Bio-Rad / Catalogue No: 1855195 (instrument) and 12004110 (software)
Rotor-Gene® 5 Plex Platform with Q-Rex Software v1.0 [1]	real-time RT-PCR	Manufacturer: Qiagen / Catalogue No: 9001570
Reaction tubes and their caps/ seals	real-time RT-PCR	Compatible with the Real-Time PCR instrument and the reaction volume
Quick-spin centrifuge	real-time RT-PCR	Speed up to 3000 rpm, with adaptors for PCR plates and tubes
PCR cabinet	real-time RT-PCR	UV cabinet for PCR Setup
Cold tube rack	real-time RT-PCR	For microfuge tubes (1.5/ 2 mL)

		and for PCR tubes (0.1 / 0.2 mL)
Gloves	All processes	Disposable, powder-free, nitrile
Laboratory coat	All processes	Disposable (preferably)

[1] Any of these instruments may be used.

WARNINGS AND PRECAUTIONS

Use Statements

- For In Vitro Diagnostic (IVD) Use only
- For prescription use (Rx) only
- For use under Emergency Use Authorization (EUA) only
- The *Bio-Speedy® Direct RT-qPCR SARS-CoV-2* assay has not been FDA cleared or approved; the test has been authorized by FDA under an Emergency Use Authorization (EUA) for use in laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.
- The *Bio-Speedy® Direct RT-qPCR SARS-CoV-2* assay has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The *Bio-Speedy® Direct RT-qPCR SARS-CoV-2* assay 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 authorization is terminated or revoked sooner.
- Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

Safety & Hazards

- *Positive Control:* The SARS-CoV-2 *ORF1ab* gene, used as a control material, was obtained synthetically. Infective virus was not used in the production process.
- *Bovine Serum Albumin:* All bovine serum albumin used in the reagents, originate from herds in countries declared free of Transmissible Spongiform Encephalopathies and are obtained from Transmissible Spongiform Encephalopathies-free certified manufacturers.
- Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.
- Proper personal protective equipment including lab coats, gowns, gloves, eye protection, and a biological safety cabinet are recommended for manipulation of clinical specimens. Refer to Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition -

CDC and World Health Organization, Laboratory Biosafety Manual, 3rd Edition, WHO/CDS/CSR/LYO/2004.11.
www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2 https://www.cdc.gov/coronavirus/2019-nCoV/labbiosafety-guidelines.html.
- Specimen processing should be performed in accordance with national biological safety recommendations.
- If infection with SARS-CoV-2 is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions.
- Perform all manipulations of human clinical specimens within a Class II (or higher) biological safety cabinet (BSC).
- The vNAT™ buffer in the kit contains guanidinium thiocyanate. To avoid the danger of cyanide gas production, bleach or acidic solutions should not be added to sample collection tubes or containers.
- Immediately clean up any spill containing potentially infectious material with 0.5-1% (w/v) sodium hypochlorite (10-20% v/v bleach). Dispose of cleaning materials in a biohazard waste stockpot. If the spill contains vNAT™ buffer, do not use bleach or acidic solutions. Due to the danger of cyanide gas formation, clean with a suitable laboratory detergent and water.
- Report incident to supervisor, fill in the Accident Report and consult a physician immediately in the event that infectious materials are ingested or come into contact with mucus membranes, open lacerations, lesions or other breaks in the skin.

Waste Management

Medical Waste

Appropriate waste management and decontamination procedures should be used.

Medical wastes are collected in sealed biohazard bags / containers that are resistant to rupture, puncture, explosion and transportation in accordance with the regulations on medical wastes. The contents of medical waste bags/containers should be never compressed, removed from the bag/container, emptied and transferred to another container.

Dispose of waste in a designated matter in accordance with local, regional and federal regulations.

Molecular Waste

Nucleic acid contamination from molecular waste can be caused by dust and spreading aerosols. PCR products can be destroyed using a 3 % (mass fraction) hypochlorite solution (refer to ISO 22174:2005).

General Requirements for Good Practices on PCR and RT-PCR

Laboratory Setup

To prevent contamination of the reaction mixture by previously amplified target sequences, maintain separate work areas, dedicated equipment, and supplies for:

- Sample preparation
- PCR setup
- PCR amplification
- Analysis of PCR products

Rooms can be simulated using a clean bench or the UV cabinet for PCR setup. Physical separation through the use of different rooms is the most effective and preferable way of ensuring separate work areas and working facilities.

For additional information, refer to ISO 22174 (2005).

Personnel

- Different sets of laboratory coats should be worn pre- and post-PCR.
- Disposable gloves should be worn at sample preparation and when setting up PCR.
- Laboratory coats and gloves should be changed at appropriate frequencies (when suspected that they are contaminated) and before leaving the work area (in passing from one work area to another).
- All personnel who perform aspects of the testing procedures should be trained to work with PCR and microbiology as appropriate.

Protection of Product Performance and Analysis Efficiency

- The components in the kit should not be mixed with components with different lot numbers or chemicals of the same name but from different manufacturers.
- Master stock reagents should be kept on the cold block during the PCR setup; if possible,

the PCR setup should be performed on the cold block.

- Kit components should be mixed by gently shaking before use.
- Maintenance/ calibration interval should be determined for all instruments and equipment used with the kit.
- Sampling should be carried out by personnel with sufficient knowledge and experience.
- For collection of nasopharyngeal/ oropharyngeal swabs, polyester flocked swabs are preferred. Sterile dacron swabs with plastic or flexible metal handles may also be used. Cotton or calcium alginate swabs or swabs with wooden sticks should not be used since they may contain substances that inactivate some viruses and inhibit PCR.
- The kit is intended for use in a laboratory environment by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

Preventing Contamination

- The kit should be stored away from nucleic acid sources and PCR amplicons.
- The micropipettes used for pipetting PCR mixes and template nucleic acids should be separate. Filtered and nuclease-free pipette tips should be used.
- All sample tubes should be opened and closed carefully to avoid contamination.
- Template nucleic acid and positive control tubes should always be kept closed, except for fluid transfers; tube caps should not be interchanged.
- Amplified products should not be brought into the reaction setup area. To avoid false positives due to amplified material, the PCR completed reaction tubes should be disposed of before opening in the laboratory (PCR products can be destroyed using a 3% -mass fraction- hypochlorite solution; refer to ISO 22174:2005).
- To avoid false positives due to cross contamination, all unknown sample tubes should be closed before pipetting the Positive Control.
- It is recommended to use swabs with breakable shaft to prevent contamination during sampling.
- The wipeable surfaces of the rooms, benches and devices where the analysis is performed should be cleaned regularly with freshly diluted 10% bleach solution (0.5% sodium hypochlorite).

Plate Layout Suggestions

- In multi-targeted PCR runs, separate different targets by a row or by a column if enough space is available.

- If possible, put at least one well between unknown samples and controls.
- Separate negative and positive controls by one well if possible.
- Place replicates of one sample for the same target next to each other.
- Start with the unknown samples and put controls at the end of the row or column.
- If possible, put positive controls in one of the outer rows or columns.
- Consider that caps for PCR tubes come in strips of 8 or 12.

SPECIMEN HANDLING AND STORAGE

Collecting the Specimen

Nasopharyngeal swabs, oropharyngeal (throat) swabs, combined nasopharyngeal/ oropharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal or nasopharyngeal aspirates, nasal washes and bronchoalveolar lavage samples shall be collected by a healthcare provider in accordance with the updated version of CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19 (https://www.cdc.gov/coronavirus/2019-ncov/lab /guidelines-clinical-specimens.html).

Swabs (dacron or polyester flocked) should be placed immediately into a sterile transport tube containing 2-3 mL of viral transport medium (VTM) (Preparation of viral transport medium, Centers for Disease Control and Prevention, SOP#: DSR-052-01).

Nasopharyngeal (NP) or nasal aspirate and nasal wash samples should be transferred into sterile containers containing 2-3 mL of VTM (in case of immediate analysis, these samples can be taken into sterile containers by healthcare providers).

Bronchoalveolar lavage (BAL) samples should be collected 2-3 mL into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container.

Transporting Specimens

Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential SARS-CoV-2 specimens. Store specimens at 2-8°C and ship overnight to the laboratory on ice pack. If a specimen is frozen at -70°C or lower, ship overnight to the laboratory on dry ice.

Storing Specimens

Specimens can be stored at 2-8ºC for up to 72 hours after collection. If a delay in extraction is expected, store specimens at -70ºC or lower in accordance with the CDC Interim Guidelines

for Collecting, Handling, and Testing Clinical Specimens for COVID-19. Extracted nucleic acid should be stored at -70ºC or lower. It is important to avoid repeated freezing and thawing of specimens.

INSTRUCTION FOR USE

Preparation of Nucleic Acid Samples

The following procedures are used to extract nucleic acid from nasopharyngeal swabs, oropharyngeal (throat) swabs, combined nasopharyngeal/ oropharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasopharyngeal or nasal aspirates, nasal washes in the VTM and from bronchoalveolar lavage samples.

For the negative extraction control, the same procedures are applied using 100 µL of NTC in the kit instead of the respiratory sample.

- Vortex the sample tube at the highest speed for 15 seconds.
- Transfer 100 µL of vNAT™ into a clean microcentrifuge tube.
- Add 100 µL respiratory sample to the tube containing 100 µL vNAT™.
- Vortex the tube at the highest speed for 15 seconds.
- Incubate the tube for 5 minutes at room temperature.
- The 200 µL mixture is ready to use in Real-Time RT-PCR.
- Store the sample at -70°C.

Planning of the PCR plate & PCR Setup

- Determine the number of reactions and create the PCR plate plan.
- Plan to include the following reactions:
    - Duplicate reactions for each test sample and extraction negative control.
    - Duplicate PCR control reactions;
        - Positive Control (included in the kit)
        - No Template (Negative) Control (included in the kit)
- Thaw all reagents.
- Vortex all reagents to mix thoroughly.
- Centrifuge the reagent tubes briefly to bring the contents to the bottom and place on cold rack / ice.
- Combine the following components for the number of reactions required plus 10% overage to compensate for pipetting errors:

Table 4. *Reaction set-up*

Component	Volume per reaction [1]	Volume for 96 reactions [2]
2X Prime Script Mix	5 µL	528 µL
Oligo Mix	2.5 µL	264 µL

[1] These amounts are valid for a reaction volume of 10 µL. For 20 µL reaction volume, these values should be multiplied by 2.

[2] Includes 10% overage to compensate for pipetting errors.

- Mix the solution thoroughly by vortexing, centrifuge briefly and distribute 7.5 µL (distribute 15 µL for the reaction volume of 20 µL) to each reaction well or tube.
- Add 2.5 µL (add 5 µL for the reaction volume of 20 µL) of Nucleic Acid sample, Extraction Negative Control, Negative (No Template) Control and Positive Control to the appropriate wells.
- Seal the plate or close the tubes, centrifuge briefly to bring the contents to the bottom and place into the Real-Time PCR instrument.

Programing and Running the Real-Time PCR Instrument

- The kit is validated for 10 µL and 20 µL Real-Time PCR volumes using *Roche LightCycler® 96, Bio-Rad CFX96 Touch™,* and *Qiagen Rotor-Gene® 5 Plex* Real-Time PCR Systems. It is recommended to use one of these instruments.
- Program the Real-Time PCR instrument as follows:

Table 5. *Real-Time PCR program details*

Reaction Volume [1]	Step	Cycle Number	Temperature	Duration
10 µL or 20 µL	Reverse Transcription	1	52 °C	5 min
	Hold	1	95 °C	10 sec
	Denature	40	95 °C	1 sec
	Anneal / Extend		55 °C	30 sec
	Detection (Reading)		FAM *(ORF1ab)* / HEX *(RNase P)*	

[1] Both 10 µL and 20 µL PCR volumes can be used for the assay on Bio-Rad CFX96 Touch™, Roche LightCycler® 96, and Qiagen Rotor-Gene® 5 Plex (72-well rotor) instruments. 10 µL PCR volume is recommended for high test capacity on these instruments. But, 20 µL PCR volume should be used on Qiagen Rotor-Gene® 5 Plex instruments with 36-well rotor.

- Define the plate set-up.
- Start the run.

QUALITY CONTROL

- Quality control requirements must be performed in conformance with local, state, and federal regulations or accreditation requirements and the user's laboratory's standard quality control procedures. For further guidance on appropriate quality control practices, refer to 42 CFR 493.1256.
- Quality control procedures are intended to monitor reagent and assay performance.
- Test all positive controls prior to running diagnostic samples with each new kit lot to ensure all reagents and kit components are working properly.
- Good laboratory practice (cGLP) recommends including a positive extraction control in each nucleic acid isolation batch.
- The negative extraction control must proceed through nucleic acid isolation per batch of specimens to be tested.
- Always include a negative control (NTC), and the appropriate positive control (PC) in each amplification and detection run. All clinical samples should be tested for human *RNase P (RP)* gene to control for specimen quality and extraction.

Description of Controls

Controls that will be provided with the test kit are provided in **Table 6**.

Table 6. *Controls to be used with Bio-Speedy® Direct RT-qPCR SARS-CoV-2*

Control Type	Description	Purpose	Frequency of Testing
No Template (Negative) Control / Extraction Negative Control	Molecular grade, DNase and RNase-free water	Contamination control during RNA extraction and RT-PCR	Each extraction batch (100 µL) and each PCR run (2.5 µL or 5 µL per reaction)
Positive Control	10^3 copies/mL (=2~10x LoD) synthetic SARS-CoV-2 RNA and 10 ng/µL total nucleic acid extract from human blood which contains *RNase P* gene	To monitor the integrity of the RT-PCR reagents and process.	Each PCR run (2.5 µL or 5 µL per reaction)
Internal/ Extraction Control	Human *RNase P* gene and mRNA transcript (*RNase P* gene and mRNA targeted primers and probes are included	To monitor the integrity of specimen adequacy and the integrity of nucleic acid extraction and RT-PCR from each	Every collected human respiratory tract specimen

	in Oligo-Mix).	human respiratory tract specimen	

RESULT INTERPRETATION

Interpretation of Control Results

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

No Template (Negative) Control/ Extraction Negative Control (NTC)

The NTC is molecular grade, DNase and RNase-free water, used in place of sample nucleic acid as a no template (negative) control in each PCR run. NTC also serves as an extraction negative control and it is used in place of respiratory sample in each extraction batch/run. No Template (Negative) Control/ Extraction Negative Control should give a negative result (Cq not detected) for both the oligo mixes targeting *ORF1ab* (SARS-CoV-2, FAM channel) and *RNase P* (IC, HEX channel). Otherwise, it shows that there is a contamination problem. In this case, it is recommended to repeat the analysis by paying attention to the "Warnings and Precautions" section.

Positive Control

The Positive Control (PC) includes synthetic SARS-CoV-2 RNA that contains *ORF1ab* sequence and total nucleic acid extract from human blood which contains *RNase P* gene and mRNA. The positive control should give positive results (Cq<38.0) for both the oligo mixes targeting *ORF1ab* (SARS-CoV-2, FAM channel) and *RNase P* (IC, HEX channel). Otherwise, it indicates that there is a reagent stability problem. In this case, it is recommended to contact the manufacturer and renew the reagents and repeat the analysis.

Internal/ Extraction Control

Detection of *RNase P* in extracted nucleic acid serves as an extraction, inhibition and sampling control for each sample. Nucleic acid extracted from each specimen, should yield a positive result in the HEX channel, with a Cq value < 38.0. If the *RNase P* assay is negative on a clinical sample, it is interpreted as follows:

- If the *ORF1ab* assay is positive with a negative *RNase P* result, it is considered that there is no inhibition, extraction or sampling problem and the run is valid. In this case, the result is interpreted as “SARS-CoV-2 Positive” as long as there is no sigmoidal amplification curve in the No Template (Negative) Control and the Positive Control is valid.
- If the *ORF1ab* assay is negative along with a negative *RNase P*, the specimen result is

considered invalid and should be repeated. If residual specimen is available, nucleic acid is re-extracted from the specimen and test is performed again. If the re-tested sample does not give a positive result in the HEX channel, a new specimen should be collected from the patient.

Table 7. *Expected Performance of Bio-Speedy® Direct RT-qPCR SARS-CoV-2 Controls*

Control Type	**Control Name**	**Purpose**	**Expected Results and Cq Values**	
			ORF1ab	***RNase P***
No Template (Negative) Control / Extraction Negative Control	NTC	Contamination control during RNA extraction and RT-PCR	Negative (No Cq)	Negative (No Cq)
Positive Control	PC	Reagent integrity	Positive (Cq<38.0)	Positive (Cq<38.0)
Internal / Extraction Control	IC	To monitor the integrity of nucleic acid extraction and RT-PCR from each human respiratory tract specimen	Not applicable	Positive (Cq<38.0)

If any control does not perform as described above, run is considered invalid and all specimens are repeated from extraction step. The positive control and negative control are interpreted as described in Table 8 below.

Table 8. *Positive and Negative Control Interpretation*

Positive Control		**Negative Control**		**Results**	**Action**
***ORF1ab* (FAM)**	***RNase P* (HEX)**	***ORF1ab* (FAM)**	***RNase P* (HEX)**		
Positive (Cq<38.0)	Positive (Cq<38.0)	Negative (No Cq)	Negative (No Cq)	VALID	Continue to result interpretation of patient specimens
Any of them is Negative (Cq not detected)		Not considered		INVALID (Reagent integrity problem)	Contact the manufacturer, renew the reagents, and repeat the reaction
Not considered		Any of them is Positive (Cq<38.0)		INVALID (Contamination problem)	Repeat the analysis by paying attention to the "Warnings and Precautions" section.

Examination and Interpretation of Patient Specimen Results

Assessment of clinical specimen test results must 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.

The assay results are interpreted as given in Table 9. The results can be interpreted as "SARS-CoV-2 Positive" as long as there is no sigmoidal amplification curve in the negative control. The results can be interpreted as "SARS-CoV-2 Negative" as long as there is a sigmoidal amplification curve with a Cq<38.0 in the internal and positive controls.

Table 9. *Interpretation of Bio-Speedy® Direct RT-qPCR SARS-CoV-2 Patient Samples*

***ORF1ab* / FAM (positive for Cq < 38.0)**	***RNase P* / HEX (positive for Cq < 38.0)**	**Results Interpretation**	**Action**
Positive (+)	Positive (+)	Results are VALID, SARS-CoV-2 RNA is Detected	Report as POSITIVE
Positive (+)	Negative (-)	Results are VALID, SARS-CoV-2 RNA is Detected	Report as POSITIVE
Negative (-)	Positive (+)	Results are VALID, SARS-CoV-2 RNA is Not Detected	Report as NEGATIVE
Negative (-)	Negative (-)	Results are INVALID (Sampling / Extraction / Inhibition problem)	Re-extract the specimen and perform testing again. If the result is still invalid, a new specimen should be obtained. If additional clinical sample is unavailable, report as INVALID

PERFORMANCE CHARACTERISTICS

Limit of detection (LoD)

Limit of detection (LoD) studies determine the lowest detectable concentration of SARS-CoV-2 at which greater or equal to 95% of all (true positive) replicates test positive.

In LoD studies, natural respiratory tract specimens that were RT-PCR negative for SARS-CoV-2 collected from healthy donors and patients were used.

A cultured SARS-CoV-2 virus of an isolate from a patient (provided by Republic of Turkey, Ministry of Health, General Directorate of Public Health) was used for spiking. The cultured

virus was quantified in copies/mL by a molecular assay using dilutions of synthetic SARS-CoV-2 ORF1ab gene partial RNA.

The samples for the LoD study were obtained by spiking natural respiratory samples, which were RT-PCR negative for SARS-CoV-2, with the cultured SARS-CoV-2 virus that was quantified previously. For each sample type, a total of 4 concentration levels with 1.5, 2 and 3-fold dilutions between the levels, were tested with a total of 120 replicates (20 replicates in 3 different instruments; *Roche LightCycler® 96, Bio-Rad CFX96 Touch™, Qiagen Rotor-Gene® 5 Plex* and at 2 different reaction volumes; 10 µL and 20 µL) per concentration, with an additional 60 replicates of blank samples (negative clinical specimens for SARS-CoV-2). The results of the LoD study for Nasopharyngeal Dacron Swab (NPDS) samples are summarized in Table 10. Similar results were obtained in the LoD study performed at both 10 µL and 20 µL reaction volumes using 3 different instruments.

Table 10. *Results of the LoD study for the Nasopharyngeal Dacron Swab (NPDS) samples*

Instrument	Reaction Volume	Concentr. of Viral RNA (copies/mL)	Number Tested	SARS-CoV-2 *(ORF1ab)*			Internal Control *(RNase P)*		
				Positive	Cq (Avg)	SD	Positive	Cq (Avg)	SD
Bio-Rad CFX96 Touch™	10 µL	**200**	**20**	**20 (100.0%)**	**36.53**	**0.03**	20 (100.0%)	33.78	0.08
		133	20	18 (90.0%)	37.05	0.24	20 (100.0%)	33.49	0.04
		100	20	11 (55.0%)	37.54	0.04	20 (100.0%)	34.07	0.04
		66	20	4 (20.0%)	37.91	0.03	20 (100.0%)	33.66	0.04
		0	10	0 (0.0%)	-	-	10 (100.0%)	33.73	0.03
	20 µL	**200**	**20**	**20 (100.0%)**	**36.52**	**0.04**	20 (100.0%)	33.71	0.09
		133	20	18 (90.0%)	37.06	0.23	20 (100.0%)	33.51	0.05
		100	20	10 (50.0%)	37.55	0.03	20 (100.0%)	34.02	0.03
		66	20	4 (20.0%)	37.88	0.03	20 (100.0%)	33.67	0.06
		0	10	0 (0.0%)	-	-	10 (100.0%)	33.63	0.05
Roche LightCycler® 96	10 µL	**200**	**20**	**20 (100.0%)**	**36.54**	**0.04**	20 (100.0%)	33.84	0.15
		133	20	17 (85.0%)	37.04	0.23	20 (100.0%)	33.55	0.11
		100	20	12 (60.0%)	37.55	0.04	20 (100.0%)	34.08	0.02
		66	20	5 (25.0%)	37.94	0.03	20 (100.0%)	33.69	0.03
		0	10	0 (0.0%)	-	-	10 (100.0%)	33.75	0.08
	20 µL	**200**	**20**	**20 (100.0%)**	**36.53**	**0.04**	20 (100.0%)	33.75	0.07
		133	20	17 (85.0%)	37.07	0.24	20 (100.0%)	33.53	0.08
		100	20	11 (55.0%)	37.54	0.03	20 (100.0%)	34.09	0.02
		66	20	4 (20.0%)	37.94	0.03	20 (100.0%)	33.59	0.02
		0	10	0 (0.0%)	-	-	10 (100.0%)	33.66	0.07

Instrument	Reaction Volume	Concentr. of Viral RNA (copies/mL)	Number Tested	SARS-CoV-2 *(ORF1ab)*			Internal Control *(RNase P)*		
				Positive	Cq (Avg)	SD	Positive	Cq (Avg)	SD
Qiagen Rotor-Gene® 5 Plex	10 µL	**200**	**20**	**20 (100.0%)**	**36.55**	**0.03**	20 (100.0%)	34.05	0.16
		133	20	17 (85.0%)	37.00	0.07	20 (100.0%)	33.68	0.12
		100	20	11 (55.0%)	37.56	0.03	20 (100.0%)	34.13	0.06
		66	20	4 (20.0%)	37.96	0.02	20 (100.0%)	33.72	0.08
		0	10	0 (0.0%)	-	-	10 (100.0%)	33.80	0.06
	20 µL	**200**	**20**	**20 (100.0%)**	**36.54**	**0.04**	20 (100.0%)	33.92	0.20
		133	20	17 (85.0%)	37.07	0.24	20 (100.0%)	33.65	0.07
		100	20	11 (55.0%)	37.55	0.03	20 (100.0%)	34.11	0.05
		66	20	4 (20.0%)	37.96	0.02	20 (100.0%)	33.66	0.03
		0	10	0 (0.0%)	-	-	10 (100.0%)	33.69	0.08

LoD of the *Bio-Speedy® Direct RT-qPCR SARS-CoV-2* is 200 copies/mL for nasopharyngeal aspirate; 281 copies/mL for bronchoalveolar lavage; 562 copies/mL for oropharyngeal swab; 89 copies/mL for nasopharyngeal swab samples collected with polyester flocked swabs; 200 copies/mL for nasopharyngeal swab samples collected with dacron swabs. The summary of the LoD study stratified per specimen type is provided in Table 11.

Table 11. *The summary of LoD study results for each specimen type*

Sample Type	Concentration of Viral RNA (copies/mL)	Number Tested	SARS-CoV-2 *(ORF1ab)*			Internal Control *(RNase P)*		
			Positive	Cq (Avg)	SD	Positive	Cq (Avg)	SD
Nasopharyngeal Aspirate (NPA)	**200**	**120**	**120 (100.0%)**	**36.44**	**0.08**	120 (100.0%)	34.28	0.04
	133	120	107 (89.2%)	36.85	0.18	120 (100.0%)	34.42	0.07
	100	120	52 (43.3%)	37.46	0.12	120 (100.0%)	33.95	0.05
	66	120	25 (20.8%)	37.93	0.09	120 (100.0%)	34.83	0.05
	0	60	0 (0.0%)	-	-	60 (100.0%)	33.67	0.04
Bronchoalveolar Lavage (BAL)	562	120	120 (100.0%)	36.27	0.05	120 (100.0%)	29.32	0.12
	374	120	120 (100.0%)	36.71	0.04	120 (100.0%)	29.41	0.09
	281	**120**	**116 (96.7%)**	**37.30**	**0.05**	120 (100.0%)	29.07	0.10
	187	120	79 (65.8%)	37.71	0.11	120 (100.0%)	29.19	0.08
	0	60	0 (0.0%)	-	-	60 (100.0%)	29.05	0.04
Naso	**200**	**120**	**120 (100.0%)**	**36.53**	**0.04**	120 (100.0%)	33.84	0.12

Sample Type	Concentration of Viral RNA (copies/mL)	Number Tested	SARS-CoV-2 *(ORF1ab)*			Internal Control *(RNase P)*		
			Positive	Cq (Avg)	SD	Positive	Cq (Avg)	SD
	133	120	104 (86.7%)	37.05	0.21	120 (100.0%)	33.57	0.08
	100	120	66 (55.0%)	37.55	0.03	120 (100.0%)	34.08	0.04
	66	120	25 (20.8%)	37.93	0.04	120 (100.0%)	33.67	0.04
	0	60	0 (0.0%)	-	-	60 (100.0%)	33.71	0.06
Nasopharyngeal Polyester Flocked Swab (NPFS)	**89**	**120**	**120 (100.0%)**	**36.58**	**0.07**	120 (100.0%)	30.27	0.05
	59	120	104 (86.7%)	37.17	0.05	120 (100.0%)	30.19	0.08
	44	120	57 (47.5%)	37.53	0.11	120 (100.0%)	30.43	0.09
	29	120	33 (27.5%)	37.92	0.04	120 (100.0%)	30.10	0.04
	0	60	0 (0.0%)	-	-	60 (100.0%)	29.86	0.07
Oropharyngeal Dacron Swab (OPDS)	**562**	**120**	**120 (100.0%)**	**36.72**	**0.04**	120 (100.0%)	34.72	0.06
	374	120	110 (91.7%)	37.22	0.03	120 (100.0%)	34.86	0.02
	281	120	50 (41.7%)	37.66	0.04	120 (100.0%)	34.57	0.04
	187	120	28 (23.3%)	37.95	0.03	120 (100.0%)	34.63	0.04
	0	60	0 (0.0%)	-	-	60 (100.0%)	34.60	0.05
Oropharyngeal Polyester Flocked Swab (OPFS)	**562**	**120**	**120 (100.0%)**	**36.71**	**0.04**	120 (100.0%)	35.16	0.05
	374	120	104 (86.7%)	37.23	0.03	120 (100.0%)	34.92	0.04
	281	120	55 (45.8%)	37.65	0.03	120 (100.0%)	35.07	0.06
	187	120	29 (24.2%)	37.95	0.03	120 (100.0%)	34.88	0.04
	0	60	0 (0.0%)	-	-	60 (100.0%)	34.73	0.04

The effect of Real-Time PCR volume on test performance was also examined during LoD studies. Average Cq values of 10 µL and 20 µL Real-Time PCRs in LoD concentrations for each sample type were given in Table 12. These results showed that both 10 µL and 20 µL PCR volumes can be used for the assay.

Table 12. *The effect of Real-Time PCR volume on test performance*

Sample Type	Viral RNA copies/µL (LoD)	SARS-CoV-2 *(ORF1ab)*				Internal Control *(RNase P)*			
		20 µL		10 µL		20 µL		10 µL	
		Cq (Avg)	SD	Cq (Avg)	SD	Cq (Avg)	SD	Cq (Avg)	SD
NPA	200	36.46	0.09	36.42	0.06	34.35	0.03	34.21	0.05
BAL	281	37.29	0.05	37.31	0.05	29.13	0.09	29.01	0.10
NPDS	200	36.53	0.04	36.54	0.03	33.79	0.11	33.89	0.14

Sample Type	Viral RNA copies/µL (LoD)	SARS-CoV-2 *(ORF1ab)*				Internal Control *(RNase P)*			
		20 µL		10 µL		20 µL		10 µL	
		Cq (Avg)	SD	Cq (Avg)	SD	Cq (Avg)	SD	Cq (Avg)	SD
NPFS	89	36.62	0.06	36.54	0.06	30.25	0.06	30.29	0.05
OPDS	562	36.72	0.04	36.72	0.04	34.80	0.06	34.64	0.06
OPFS	562	36.72	0.04	36.71	0.04	35.35	0.07	34.97	0.03

Inclusivity

All SARS-CoV-2 nucleotide sequences in available nucleotide databases *(NCBI; https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome)* were searched against the SARS-CoV-2 targeted oligonucleotide sequences. The results are summarized in Table 13. 99.7% and 99.9% of the available 15324 SARS-CoV-2 nucleotide sequences resulted in 100% identity for the forward and reverse primers respectively; 99.8% of the available 15324 SARS-CoV-2 nucleotide sequences resulted in 100% identity for the probe sequence. The maximum number of mismatches is 2 for the 25-base long probe, 1 for the 22-base long forward primer, and 1 for the 28-base long reverse primer.

Table 13. *Inclusivity test results (in-silico tests were carried out on August 27, 2020.)*

Oligo Name	Minimum Identity[1]	Maximum Mismatches[2]	Number of Total Alignments	Number of Alignments with Mismatches	Number of Alignments with 100% Identity
ORF1ab Primer-1	95.5%	1	15324	41	15283 (99.7%)
ORF1ab Primer-2	96.4%	1	15324	13	15311 (99.9%)
ORF1ab Probe	92.0%	2	15324	23	15301 (99.8%)

[1] Minimum identity%= [(base long of the oligonucleotide - number of maximum mismatches) / base long of the oligonucleotide] x 100.

[2] The decrease in Tm values when there are 1 base mismatch in the primer sequences or 2 base mismatches in the probe sequence does not prevent the primers and probe from remaining attached during the amplification step.

Exclusivity/ Cross-Reactivity

Wet Testing

The exclusivity was wet tested with a total of 44 samples, consisting of 43 respiratory pathogens at clinically relevant concentrations (10^5 genome copies/mL) and a pooled, SARS-CoV-2 nasal wash from 20 different people, following extraction by using vNAT™. All samples were tested in duplicate and none produced any detectable reactivity with the *Bio-Speedy® Direct RT-qPCR*

SARS-CoV-2.

The wet tests showed that the kit does not cross-react with the other respiratory pathogens or the microbial flora in the human respiratory tract (Table 14).

In-Silico Tests

In silico tests were carried out using Primer Blast tool of NCBI (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) by entering the tested cross reacting organism/strain and oligonucleotide sequences into the relevant fields. BLAST search

(https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome) was also used for comparing the whole target region on SARS-CoV-2 Ref Seq Genome (NC_045512, 15431-15532) with the genomes of cross reacting organisms/strains (Table 14 and Table 15). SARS-CoV-2 taxon was excluded in the BLAST searches while the cross-reacting organisms/strains were included.

The in-silico tests showed that the primers and probe were not homologous to any organism/strain except some SARS-CoV strains other than SARS-CoV-2 (Table 14). The blast search showed that the target region on SARS-CoV-2 genome resembles more than 90% to some SARS-CoV strains, however, detection is not expected as there are no known SARS-CoV strains circulating in the population (Table 15).

Table 14. *The exclusivity (cross-reactivity) test results of 43 respiratory pathogens and a pooled nasal wash sample from 20 different people*

Organism	**In-Silico Analysis**	**Wet Testing (10^5 copies/mL)**			
		ORF1ab		***RNase P***	
		Cq (Avg)[1]	**SD**	**Cq (Avg) [1]**	**SD**
Human Coronavirus 229E	Not detected	No Cq	-	31.49	0.02
Human Coronavirus OC43	Not detected	No Cq	-	31.51	0.05
Human Coronavirus NL63	Not detected	No Cq	-	31.50	0.06
Human Coronavirus HKU1	Not detected	No Cq	-	31.46	0.03
MERS-coronavirus	Not detected	No Cq	-	31.51	0.01
SARS CoV strain Frankfurt 1	Not detected	No Cq	-	31.45	0.04
Influenza A H1	Not detected	No Cq	-	31.44	0.06
Influenza A H3	Not detected	No Cq	-	31.53	0.10
Influenza B	Not detected	No Cq	-	31.56	0.07
Parainfluenza 1	Not detected	No Cq	-	31.51	0.03
Parainfluenza 2	Not detected	No Cq	-	31.44	0.01
Parainfluenza 3	Not detected	No Cq	-	31.39	0.06
Parainfluenza 4	Not detected	No Cq	-	31.44	0.05
Human Metapneumovirus (hMPV)	Not detected	No Cq	-	31.40	0.06

Organism	In-Silico Analysis	Wet Testing (10^5 copies/mL)			
		ORF1ab		*RNase P*	
		Cq (Avg)[1]	SD	Cq (Avg) [1]	SD
Rhinovirus	Not detected	No Cq	-	31.42	0.01
Respiratory syncytial virus (RSV) A	Not detected	No Cq	-	31.54	0.07
Respiratory syncytial virus (RSV) B	Not detected	No Cq	-	31.50	0
Bocavirus (BoV)	Not detected	No Cq	-	31.52	0.04
Enterovirus	Not detected	No Cq	-	31.53	0.06
Adenovirus	Not detected	No Cq	-	31.48	0.06
Legionella pneumophila	Not detected	No Cq	-	31.49	0.05
Chlamydia pneumoniae	Not detected	No Cq	-	31.44	0.09
Mycobacterium tuberculosis	Not detected	No Cq	-	31.46	0.07
Haemophilus influenzae	Not detected	No Cq	-	31.48	0.07
Streptococcus pneumoniae	Not detected	No Cq	-	31.55	0.05
Mycoplasma pneumoniae	Not detected	No Cq	-	31.47	0.04
Streptococcus pyogenes	Not detected	No Cq	-	31.49	0.04
Bordetella pertussis	Not detected	No Cq	-	31.52	0.02
Pneumocystis jirovecii	Not detected	No Cq	-	31.49	0.11
Candida albicans	Not detected	No Cq	-	31.50	0.04
Legionella bozemanii	Not detected	No Cq	-	31.51	0.01
Legionella micdadei	Not detected	No Cq	-	31.52	0.05
Corynebacterium diphtheriae	Not detected	No Cq	-	31.49	0.05
Bacillus anthracis	Not detected	No Cq	-	31.54	0.07
Moraxella catarrhalis	Not detected	No Cq	-	31.49	0.06
Neisseria meningitidis	Not detected	No Cq	-	31.53	0.08
Pseudomonas aeruginosa	Not detected	No Cq	-	31.48	0.01
Staphylococcus epidermidis	Not detected	No Cq	-	31.49	0.08
Coxiella burneti	Not detected	No Cq	-	31.53	0.06
Staphylococcus aureus	Not detected	No Cq	-	31.55	0.03
Streptococcus salivarius	Not detected	No Cq	-	31.48	0.08
Leptospira interrogans	Not detected	No Cq	-	31.47	0.04
Chlamydia psittaci	Not detected	No Cq	-	31.63	0.07
Pooled human nasal wash - *to represent diverse microbial flora in the human respiratory tract*	-	No Cq	-	28.32	0.08

[1] Average Cq values of duplicate PCRs in 10^5 copies/mL concentrations for each organism in VTM:Sample (1:1) mixtures.

Table 15. *In-silico test results of the potential cross-reacting SARS-CoV strains*

Description	Accession	Identity %		
		Forward	Probe	Reverse
Rhinolophus bat coronavirus BtCoV/4991 RNA-dependent RNA polymerase (RdRp) gene, partial cds	KP876546.1	100.0%	96.4%	100.0%
Bat coronavirus RaTG13, complete genome	MN996532.1	100.0%	96.4%	100.0%
Bat SARS-like coronavirus isolate 151569 RNA-dependent RNA polymerase (RdRp) gene, partial cds	MN312711.1	95.5%	100.0%	92.0%
Bat SARS-like coronavirus isolate 8548 RNA-dependent RNA polymerase (RdRp) gene, partial cds	MN312738.1	95.5%	100.0%	92.0%
Bat SARS-like coronavirus isolate 8561 RNA-dependent RNA polymerase (RdRp) gene, partial cds	MN312739.1	95.5%	100.0%	92.0%
Bat SARS-like coronavirus isolate 8572 RNA-dependent RNA polymerase (RdRp) gene, partial cds	MN312740.1	95.5%	100.0%	92.0%
Bat SARS-like coronavirus isolate 8586 RNA-dependent RNA polymerase (RdRp) gene, partial cds	MN312741.1	95.5%	100.0%	92.0%
Bat coronavirus Rc_CoV-3 RdRp gene for RNA dependent RNA polymerase, partial cds	LC469044.1	100.0%	96.4%	92.0%
SARS bat coronavirus RdRp gene for RNA dependent RNA polymerase, partial cds, strain: Is68	AB889999.1	95.5%	96.4%	92.0%
Bat SARS coronavirus Rp3, complete genome	DQ071615.1	95.5%	96.4%	92.0%
Bat SARS CoV Rs672/2006, complete genome	FJ588686.1	95.5%	96.4%	92.0%
SARS-related bat coronavirus isolate Anlong-29 RNA-dependent RNA polymerase gene, partial cds	KF294439.1	95.5%	96.4%	92.0%
SARS-related bat coronavirus isolate Anlong-97 RNA-dependent RNA polymerase gene, partial cds	KF294440.1	95.5%	96.4%	92.0%
SARS-related bat coronavirus isolate Anlong-111 orf1ab polyprotein and orf1a polyprotein genes, complete cds	KF294455.1	95.5%	96.4%	92.0%
Rhinolophus affinis coronavirus isolate LYRa3 RNA-dependent RNA polymerase gene, partial cds	KF569973.1	95.5%	96.4%	92.0%
Rhinolophus affinis coronavirus isolate LYRa11, complete genome	KF569996.1	95.5%	96.4%	92.0%
BtRs-BetaCoV/GX2013, complete genome	KJ473815.1	95.5%	96.4%	92.0%
SARS-related coronavirus isolate F21 RdRP mRNA, partial cds	KU973686.1	95.5%	96.4%	92.0%
SARS-related betacoronavirus Rp3/2004 PREDICT-EHA-156-12-HZ13479 RNA-dependent RNA polymerase mRNA, partial cds	KX285180.1	95.5%	96.4%	92.0%
SARS-related betacoronavirus Rp3/2004 PREDICT-EHA-156-12-HZ13484 RNA-dependent RNA polymerase mRNA, partial cds	KX285181.1	95.5%	96.4%	92.0%

Description	Accession	Identity %		
		Forward	Probe	Reverse
Bat SARS-like coronavirus isolate Rf4092, complete genome	KY417145.1	95.5%	96.4%	92.0%
Bat SARS-like coronavirus isolate Rs4231, complete genome	KY417146.1	95.5%	96.4%	92.0%
Bat coronavirus isolate Anlong-103, complete genome	KY770858.1	95.5%	96.4%	92.0%
Bat coronavirus isolate Anlong-112, complete genome	KY770859.1	95.5%	96.4%	92.0%
Bat coronavirus Rc_CoV-4 RdRp gene for RNA dependent RNA polymerase, partial cds	LC469045.1	95.5%	96.4%	92.0%
Coronavirus BtRs-BetaCoV/YN2018A, complete genome	MK211375.1	95.5%	96.4%	92.0%
Bat SARS-like coronavirus isolate 8794 RNA-dependent RNA polymerase (RdRp) gene, partial cds	MN312742.1	95.5%	96.4%	92.0%
Bat SARS-like coronavirus isolate HZ13479 RNA-dependent RNA polymerase (RdRp) gene, partial cds	MN312829.1	95.5%	96.4%	92.0%
Bat SARS-like coronavirus isolate HZ13484 RNA-dependent RNA polymerase (RdRp) gene, partial cds	MN312830.1	95.5%	96.4%	92.0%
Bat SARS-like coronavirus isolate HZ13488 RNA-dependent RNA polymerase (RdRp) gene, partial cds	MN312831.1	95.5%	96.4%	92.0%
Bat SARS-like coronavirus isolate NL140346 RNA-dependent RNA polymerase (RdRp) gene, partial cds	MN312843.1	95.5%	96.4%	92.0%
Bat SARS-like coronavirus isolate NL140352 RNA-dependent RNA polymerase (RdRp) gene, partial cds	MN312844.1	95.5%	96.4%	92.0%
Bat SARS-like coronavirus isolate NL140494 RNA-dependent RNA polymerase (RdRp) gene, partial cds	MN312856.1	95.5%	96.4%	92.0%

Endogenous Interference Substances Studies

Nasopharyngeal dacron swab samples that were qPCR negative for SARS-CoV-2 collected from healthy donors and patients were used in the interference substances studies. Eight (8) NPDS samples, each in 2 mL of VTM, were pooled to obtain a total of 16 mL of negative clinical nasopharyngeal dacron swab sample. For each interfering substance listed in Table 16, 1 mL of negative NPDS sample was aliquoted into microcentrifuge tubes. 1 mL of sample in each tube was spiked with 50% of the relevant interfering substance. From each SARS-CoV-2 negative NPDS samples containing 50% interfering substance, 200 µL dilutions were prepared with final interfering substance concentrations of 10%, 1%, 0.1%, and 0.01%, as shown in Table 16. All dilutions were spiked with synthetic SARS-CoV-2 RNA to a final concentration of 1000 copies/mL. Nucleic acid was extracted from all samples using the vNAT™ buffer in *Bio-Speedy® Direct RT-PCR SARS-CoV-2* and then real-time RT-PCR was performed with the kit.

According to the results of the interfering substance inhibition tests, mucin at 50% (w/v), blood

at 50% (v/v), nasal spray (Nasonex) at 10% (v/v), nasal corticosteroids and gels at 10% (w/v), throat lozenges at 10% (w/v), anti-viral at 1% (v/v), antibiotics at 0.1% (w/v) may interfere with the *Bio-Speedy® Direct RT-qPCR SARS-CoV-2*.

Table 16. *Interfering substance inhibition of the Real-Time RT-PCR assay on the triplicate samples containing 1000 SARS-CoV-2 copies/mL*

Interfering substances	Concentration		*ORF1ab*		*RNase P*		Results (positive for Cq < 38.0)
			Cq[1]	SD[2]	Cq[1]	SD[2]	
No interfering substances	-	0	35.07	0.03	32.93	0.05	Positive (+)
Mucin from bovine submaxillary glands (CAS Number 84195-52-8)	w/v %	0.1	35.01	0.06	32.86	0.03	Positive (+)
		1	35.04	0.04	32.91	0.02	Positive (+)
		10	36.48	0.11	34.32	0.04	Positive (+)
		50	**39.07**	**0.08**	**36.95**	**0.13**	**Negative (-)**
Blood	v/v %	0.1	35.05	0.06	32.9	0.05	Positive (+)
		1	34.97	0.03	32.88	0.08	Positive (+)
		10	36.08	0.05	33.92	0.06	Positive (+)
		50	**39.05**	**0.05**	**36.94**	**0.07**	**Negative (-)**
OTRIVINE Adult Nasal Spray	v/v %	0.1	35.03	0.08	32.89	0.07	Positive (+)
		1	35.07	0.09	32.91	0.09	Positive (+)
		10	36.02	0.05	33.87	0.09	Positive (+)
		50	37.06	0.10	34.93	0.10	Positive (+)
Nasonex Nasal Spray	v/v %	0.1	35.04	0.02	32.92	0.10	Positive (+)
		1	36.04	0.06	33.91	0.05	Positive (+)
		10	**39.99**	**0.06**	**37.87**	**0.09**	**Negative (-)**
		50	**N/A[3]**	-	**N/A[3]**	-	**Negative (-)**
Ayr Saline Nasal Gel, With Soothing Aloe	w/v %	0.1	34.96	0.08	32.88	0.06	Positive (+)
		1	35.05	0.06	32.94	0.06	Positive (+)
		10	**39.12**	**0.07**	**36.99**	**0.08**	**Negative (-)**
		50	**N/A[3]**	-	**N/A[3]**	-	**Negative (-)**
Strepsils Sore Throat & Blocked Nose Lozenges	w/v %	0.01	35.08	0.04	32.95	0.04	Positive (+)
		0.1	35.29	0.02	33.14	0.08	Positive (+)
		1	37.03	0.04	34.85	0.07	Positive (+)
		10	**39.98**	**0.07**	**37.88**	**0.14**	**Negative (-)**
Rapivab (peramivir)	v/v %	0.01	35.19	0.03	33.04	0.09	Positive (+)
		0.1	36.07	0.03	33.94	0.06	Positive (+)
		1	**38.96**	**0.07**	**36.8**	**0.12**	**Negative (-)**
		10	**N/A[3]**	-	**N/A[3]**	-	**Negative (-)**

Interfering substances	Concentration		*ORF1ab*		*RNase P*		Results (positive for Cq < 38.0)
			Cq[1]	SD[2]	Cq[1]	SD[2]	
Amoxicillin + Penicillin + Cefadroxil + Erythromycin mixture	w/v %	0.01	36.11	0.12	33.94	0.05	Positive (+)
		0.1	**39.97**	**0.09**	**37.85**	**0.09**	**Negative (-)**
		1	**N/A[3]**	**-**	**N/A[3]**	**-**	**Negative (-)**
		10	**N/A[3]**	**-**	**N/A[3]**	**-**	**Negative (-)**

[1] The average Cq value for triplicate reactions.

[2] "SD" is the standard deviation of results for triplicate samples.

[3] N/A: Not available; not detected any Cq value for all triplicate reactions.

Clinical Evaluation

Bio-Speedy® Direct RT-qPCR SARS-CoV-2 was used for testing 451 clinical samples *(nasopharyngeal swabs, oropharyngeal swabs, nasopharyngeal aspirates and bronchoalveolar lavage samples)* in VTM concurrently with another Real-Time RT-PCR kit authorized by the FDA in a blinded fashion. Samples were obtained from individuals suspected of COVID-19 (47%) or from individuals having COVID-19 (53%). No restrictions were placed on age, gender, medications or known pharmaceutical therapies. 451 individuals in the intensive care unit (ICU) (46%) and non-ICU settings (54%) were enrolled in this study.

Nasopharyngeal (NP) or oropharyngeal (OP) swab samples, NP aspirate and bronchoalveolar lavage (BAL) samples were collected by a healthcare provider in accordance with the updated version of CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19 (https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html). Swabs (dacron or polyester flocked) were placed immediately into sterile transport tubes containing 2-3 mL of viral transport medium (VTM). Nasopharyngeal (NP) aspirate and nasal wash samples were transferred into sterile containers containing 2-3 mL of VTM. Bronchoalveolar lavage (BAL) samples were collected 2-3 mL into sterile, leak-proof, screw-cap sterile dry containers.

The samples were packaged and transferred to "Turkish Ministry of Health, General Directorate of Public Health, Department of Microbiology Reference Laboratories and Biological Products, National Virology Reference Laboratory" in VTM at 2-8 °C within 24 hours for the SARS-CoV-2 real-time RT-PCR tests.

As the samples arrived in the laboratory, they were processed immediately without being stored. The homogenized samples were divided into two separate tubes equally to be tested by two different methods.

Nucleic acid extraction was carried out using vNAT™ buffer for the samples to be tested with *Bio-Speedy® Direct RT-qPCR SARS-CoV-2*. The reactions were set up as described in Table 4 for the reaction volume of 10 µL. Real-Time RT-PCR tests were performed on Bio-Rad CFX96 Touch™ according to the PCR protocol as described in Table 5. Extracted nucleic acids were stored at -80ºC. To observe the effect of reaction volume in clinical samples, 451 samples were

retested at 20 µL reaction volume, in accordance with Table 4 and Table 5, using frozen nucleic acid samples. Similar results were obtained at both 10 µL and 20 µL reaction volumes.

The samples to be tested with the comparator method were treated as specified in the IFU of the relevant kit.

The overall *Bio-Speedy®* tests resulted in 349 true positives, 94 true negatives and 8 false negatives. Sensitivity and specificity of the *Bio-Speedy® Direct RT-qPCR SARS-CoV-2* are 97.8% and100% respectively.

Table 17. *Performance of the Bio-Speedy® Direct RT-qPCR SARS-CoV-2 vs FDA authorized test*

Patient Specimens (for all sample types)		*FDA authorized test*		
		Positive	Negative	Total
Bio-Speedy® Direct RT-qPCR SARS-CoV-2	Positive	349	0	349
	Negative	8 [1]	94	102
	Total	357	94	451
Positive Percent Agreement		349/357 = 97.8% (95% CI: 92.6% - 100%)		
Negative Percent Agreement		94/94 = 100% (95% CI: 86.2% - 98.1%)		

[1] Positive by the comparator for the *ORF1ab* target only:

4 of the 8 samples negative by the *Bio-Speedy®* and positive by the comparator were negative by the DNA sequencing. The sequencing-based screening was performed by amplifying a 251 bp fragment of *Orf 1ab* using the following primer set: Cor-FW (5'-ACTCAAATGAATCTTAAGTATGC-3') and Cor-RV (5'-TCACATTTTGGATAATCCCA-3'). The samples that were negative by DNA sequencing were a BAL sample and three NP / OP swab samples, the Cq values of which were 27.07, 28.56, 26.86 and 32.94, respectively, by comparative testing.

4 of the 8 samples negative by the *Bio-Speedy®* and positive by the comparator were positive by the DNA sequencing. Four of the sequenced 251 bp amplicons were 100% similar to the reference SARS-CoV-2 (NC_045512) genome position between 15058 and 15308. The samples positive by the DNA sequencing were all NP / OP swab samples whose Cq values were determined as 23.55, 24.79, 28.96 and 30.61 by the comparative test.

ASSAY LIMITATIONS

- *Bio-Speedy® Direct RT-qPCR SARS-CoV-2* is intended for use in a laboratory environment by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.
- The clinical specimens shall be collected by a healthcare provider in accordance with the updated version of CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19 (https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html).
- A false negative result may occur if a specimen is improperly collected, transported or handled.

- Performance of the *Bio-Speedy® Direct RT-qPCR SARS-CoV-2* has only been established in nasopharyngeal swab, oropharyngeal (throat) swab, nasopharyngeal aspirate and bronchoalveolar lavage samples. Combined nasopharyngeal/oropharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal aspirates and nasal washes are also considered acceptable specimen types but performance has not been established.
- The use of cotton or calcium alginate swabs or swabs with wooden sticks can lead to false negative results since they may contain substances that inactivate some viruses and inhibit PCR. Flocked (polyester) or dacron swabs are recommended for collection of nasopharyngeal/ oropharyngeal swab samples. Performance of the *Bio-Speedy® Direct RT-qPCR SARS-CoV-2* has only been evaluated using dacron and polyester flocked swabs.
- Mutations within the target regions of the *Bio-Speedy® Direct RT-qPCR SARS-CoV-2* could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- Inhibitors or other types of interference may produce a false negative result. Mucin at 50% (w/v), blood at 50% (v/v), nasal spray (Nasonex) at 10% (v/v), nasal corticosteroids and gels at 10% (w/v), throat lozenges at 10% (w/v), anti-viral at 1% (v/v), antibiotics at 0.1% (w/v) may interfere with the *Bio-Speedy® Direct RT-qPCR SARS-CoV-2*. False negative results may also occur if inadequate numbers of organisms are present in the specimen.
- Detection of SARS-CoV-2 RNA may be affected by patient factors (e.g., presence of symptoms), and/or stage of infection.
- Based on the in-silico analysis, other SARS-like coronaviruses in the same subgenus (Sarbecovirus) as SARS-CoV-2 may cross-react with the *Bio-Speedy® Direct RT-qPCR SARS-CoV-2*. Other SARS-like coronaviruses in the same subgenus (Sarbecovirus) as SARS-CoV-2 are not known to be currently circulating in the human population, therefore are highly unlikely to be present in patient specimens.

CONDITIONS OF AUTHORIZATION FOR THE LABORATORY

The *Bio-Speedy® Direct RT-qPCR SARS-CoV-2* 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/medicaldevices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medicaldevices/vitro-diagnostics-euas

However, to assist clinical laboratories using the *Bio-Speedy® Direct RT-qPCR SARS-CoV-2*, the relevant Conditions of Authorization are listed below:

A. Authorized laboratories[1] using the *Bio-Speedy® Direct RT-qPCR SARS-CoV-2* 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 *Bio-Speedy® Direct RT-qPCR SARS-CoV-2* will use the *Bio-Speedy® Direct RT-qPCR SARS-CoV-2* as outlined in the 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 use the *Bio-Speedy® Direct RT-qPCR SARS-CoV-2* are not permitted.

C. Authorized laboratories that receive the *Bio-Speedy® Direct RT-qPCR SARS-CoV-2* will notify the relevant public health authorities of their intent to run the *Bio-Speedy® Direct RT-qPCR SARS-CoV-2* prior to initiating testing.

D. Authorized laboratories using the *Bio-Speedy® Direct RT-qPCR SARS-CoV-2* will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E. Authorized laboratories will collect information on the performance of the *Bio-Speedy® Direct RT-qPCR SARS-CoV-2* and report to Division of Microbiology (DMD)/Office of Health Technology 7 (OHT7)-Office of In Vitro Diagnostics and Radiological Health (OIR)/Office of Product Evaluation and Quality (OPEQ)/Center for Devices and Radiological Health (CDRH) (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Bioeksen R&D Technologies Ltd (via e-mail: info@bioeksen.com.tr or via phone: +90 212 285 10 17) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the *Bio-Speedy® Direct RT-qPCR SARS-CoV-2* of which they become aware.

F. All laboratory personnel using the *Bio-Speedy® Direct RT-qPCR SARS-CoV-2* must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and use the *Bio-Speedy® Direct RT-qPCR SARS-CoV-2* in accordance with the authorized labeling.

G. Bioeksen R&D Technologies Ltd, its authorized distributor(s), and authorized laboratories using the *Bio-Speedy® Direct RT-qPCR SARS-CoV-2* will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

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

EXPLANATION OF SYMBOLS

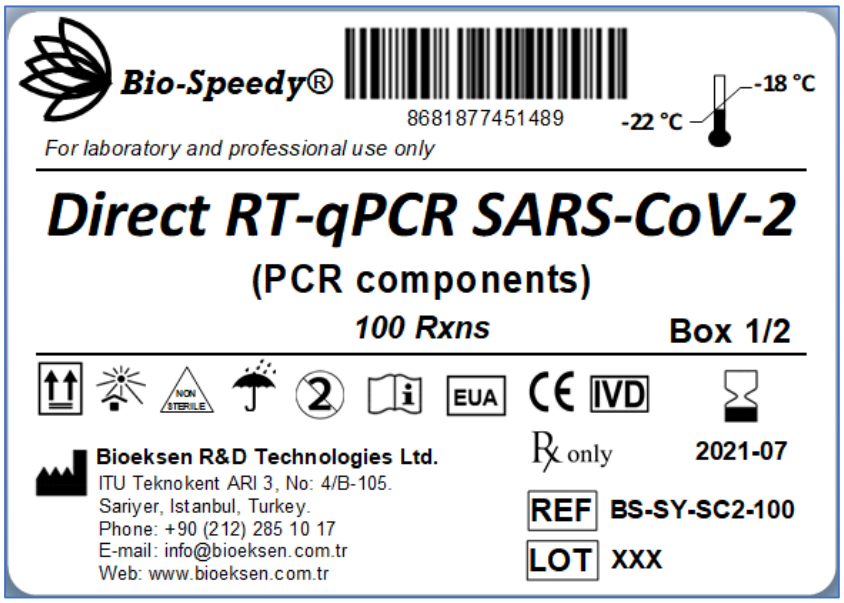
Table 18. *Symbols used in labeling for Bio-Speedy® Direct RT-qPCR SARS-CoV-2*

Symbol	Meaning	Symbol	Meaning
CE	European Conformity		Temperature limit (Store temperature)
IVD	For In Vitro Diagnostic Use		Keep away from light
REF	Catalog Number		Keep away from water/moisture
LOT	Lot Number (Batch Code)		Non-Sterile

Rx only	Prescription Use only (CAUTION! US Federal Law restricts this device to sale by or on the order of a licensed healthcare practitioner.)		Do not reuse
		EUA	For use under Emergency Use Authorization (EUA) only
	Manufacturer		Keep it upright
	Use-by Date (Expiration Date)		Consult Instructions for Use

LABELS

PCR Components Box (Box 1/2) Label of the *Bio-Speedy® Direct RT-qPCR SARS-CoV-2*:



Vial Labels of PCR Components

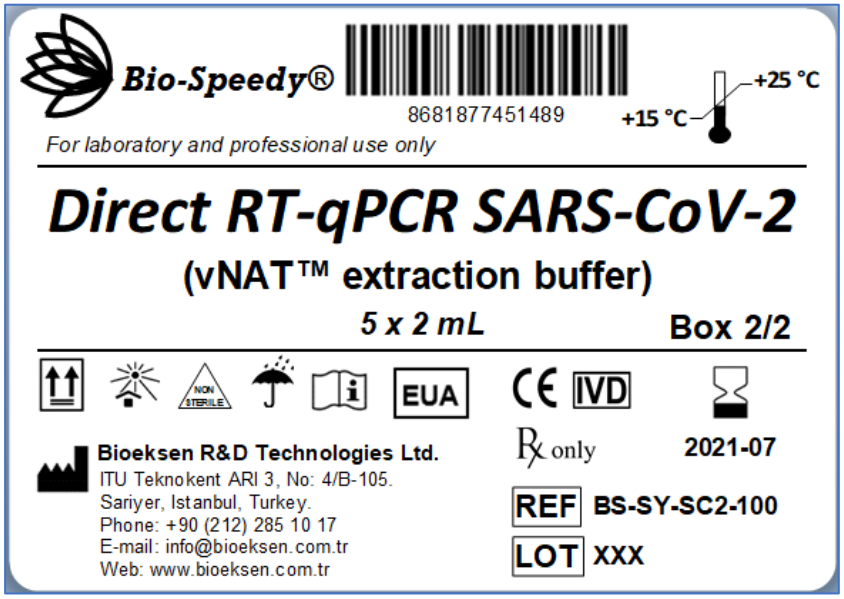
<u>Vial Label of "2X Prime Script Mix"</u>:

Vial Label of "Oligo Mix":

Vial Label of "NTC":

Vial Label of "PC":

vNAT™ Box (Box 2/2) Label of the *Bio-Speedy® Direct RT-qPCR SARS-CoV-2*:



Vial Label of "vNAT™":

MANUFACTURER and TECHNICAL SUPPORT

Bioeksen R&D Technologies Limited Company

Address: Istanbul Technical University Teknokent ARI 3, No: 4/B 105
Sariyer, Istanbul, TURKEY
Phone: +90 (212) 285 10 17
Fax: +90 (212) 285 10 18
Web: www.bioeksen.com.tr
E-mail: info@bioeksen.com.tr