Bio-Speedy®

SARS CoV-2 N RT-qPCR Kit

Cat. No: BS-SY-WCOR-306-100
BS-SY-WCOR-306-250
BS-SY-WCOR-306-500
BS-SY-WCOR-306-1000

Package Insert

Manual Version: 201031-1
Approval Date for Use: 31.10.2020

For in vitro diagnostic use only.
For laboratory and professional use only.

Table 1. Kit content [**Shelf Life:** 18 months; refer to the expiration date on the box. Each reagent stored at storage temperature, may be used until the expiration date indicated on the tube. The expiration date of the kit is determined by the expiration date of the reagents.]

Storage Temperature: -20°C, Transport Temperature: +2°C- +8°C						
Intended Use	**Content**	**Quantity (10 µL Reactions)**				**Consumption / Reaction**
		100 Rxns	**250 Rxns**	**500 Rxns**	**1000 Rxns**	
SARS-CoV-2 Detection Nucleocapsid gene (N) (**FAM**)	Oligo Mix	1 x 250 µL	1 x 625 µL	1 x 1250 µL	2 x 1250 µL	2.5 µL
Internal Control (IC) (RNase P) (**HEX**)						
DNA polymerase, dNTP mix, reaction buffer, reverse transcriptase and ribonuclease inhibitor	2X Prime Script Mix	1 x 500 µL	1 x 1250 µL	2 x 1250 µL	4 x 1250 µL	5 µL
Storage Temperature: +2-8°C/-20°C; Transport Temperature +2-8°C/-20°C **If the components are frozen store at -20°C, it should be stored at 2-8 °C without being frozen again after thawing.**						
Negative Control Template **Test it in each run for contamination control**	NTC	1 x 1000 µL	1 x 1000 µL	1 x 1000 µL	1 x 1000 µL	2.5 µL
Positive Control Template: Synthetic SARS-CoV-2 genom fragment **Test it in each run for reactive stability control**	PC	1 x 250 µL	1 x 250 µL	1 x 500 µL	2 x 500 µL	2.5 µL

Instruments and equipments supplied by the user	
1) Real-Time PCR Instrument: FAM / HEX (Green/Yellow) channel, Ramp rate ≥3 °C/sec. **2)** 1-10 µL, 10-100 µL and 100-1000 µL micropipettes and the compatible filtered tips (DNase and RNase free) **3)** Quick Spin Centrifuge: min. 3000 rpm **4)** Vortex **5)** Nuclease-free water / Viral Transport Medium / Serum physiologic	**6)** 1.5 or 2 mL microcentrifuge tubes **7)** Reaction tubes and their caps/seals compatible with the qPCR instrument and the reaction volume, nuclease-free **Extra components recommended to use:** **8)** UV Cabinet for PCR Setup **9)** Cold Tube Rack (for microcentrifuge tubes and PCR tubes/strips) **10)** Disposable powder-free nitrile gloves

Intended Use and Test Principle

Kit is used for detecting the epidemic virus SARS-CoV-2 causing Coronavirus Disease 2019 (COVID-19). ***The kit allows to achieve RT-qPCR result in less than 50 minutes***. The kit is applied to *nasopharyngeal aspirate/lavage, bronchoalveolar lavage, nasopharyngeal swab, oropharyngeal swab and sputum samples*. Rapid diagnosis with the kit is achieved via one-step reverse transcription (RT) and real-time PCR (qPCR) (RT-qPCR) targeting SARS-CoV-2 specific *N* gene fragment. The oligonucleotide set targeting human *RNase P* gene functions as a control of the sampling, nucleic acid extraction and inhibition.

A positive RT-qPCR result is recommended for *at least two different targets on the COVID-19 virus genome in an area with no COVID-19 virus circulation. In areas where COVID-19 virus is widely spread, RT-qPCR of a single discriminatory target is considered sufficient* (Laboratory testing for 2019 novel coronavirus 2019-nCoV in suspected human cases, World Health Organization, published on March 2, 2020).

Analytical Specifications

The kit is validated with *vNAT™* buffer that extracts and preserves viral nucleic acids from respiratory tract samples. *vNAT™* allows going from sample to qPCR within 5 minutes. If the samples are transferred to the laboratory in *vNAT™,* there is no need for further processing; they can directly be added to the RT-qPCR. Limit of detection (LoD) of the kit using *vNAT™* is 200 genomes/mL

for nasopharyngeal aspirate/lavage and sputum, 281 genomes/mL for bronchoalveolar lavage, 562 genomes/mL for oropharyngeal swab, 89 genomes/mL (polyester flocked) – 200 genomes/mL (dacron) for nasopharyngeal swab. Kit is also validated with *RINA™ M14 Nucleic Acid Extraction Robot (Cat No: RINA-M14-01)* and its consumables *(Cat No: RN-NA-14-111-100)*. LoD of the kit using the robotic extraction is 20 genomes/mL for all the respiratory tract sample types.

The kit is validated for 10 and 20 µL qPCR volumes using Roche LightCycler® 96, Bio-Rad CFX96 Touch™, Qiagen Rotor-Gene® 5 Plex Real-Time PCR Systems. Analytical and clinical performance of the kit was determined by the "Turkish Ministry of Health, General Directorate of Public Health, Department of Microbiology Reference Laboratories and Biological Products (HSGM)". The inclusivity was tested wet with 38 different clinical samples confirmed SARS-CoV-2 positive by DNA sequence analysis, and tested in silico with SARS-CoV-2 whole genomes from 42 different geo locations.

The exclusivity was tested wet and in silico with Coronavirus 229E/OC43/NL63/HKU1, MERS, SARS CoV strain Frankfurt 1, Influenza A H1/H3, Influenza B, Parainfluenza 1/2/3/4, Metapneumovirus, Rhinovirus, Respiratory syncytial virus (RSV) A/B, Bocavirus (BoV), Enterovirus, Adenovirus, *Legionella pneumophila*, *Chlamydia pneumoniae*, *Mycobacterium tuberculosis*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, *Streptococcus pyogenes*, *Bordetella pertussis*, *Pneumocystis jirovecii*, *Candida albicans*, *Legionella bozemanii*, *Legionella micdadei*, *Corynebacterium diphtheriae*, *Bacillus anthracis*, *Moraxella catarrhalis*, *Neisseria meningitidis*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Coxiella burneti*, *Staphylococcus aureus, Streptococcus salivarius, Leptospira interrogans, Chlamydia psittaci* and a pooled nasal wash from 20 different people. 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.

Repeatability of the kit is 100%. The reproducibility combined with the robotic extraction is 100% at concentrations over the LoD, and 83% at concentrations below the LoD. The reproducibility combined with the manual extraction is 100% at concentrations over the LoD, and 67-83% at concentrations below the LoD. Mucin, blood and nasal sprays at >10% w/v, nasal corticosteroids and gels at >1% v/v, throat lozenges and anti-virals at >0.1% w/v, antibiotics at >0.01% w/v may interfere with the kit.

The kit was applied to 500 clinical samples concurrently with concurrently with another RT-qPCR kit authorized by the FDA. DNA sequence analysis was applied when the assays were not in agreement. The overall tests resulted in 383 true positives and 117 true negatives. Sensitivity and specificity of the *Bio-Speedy®* kit are 98.7%-100% respectively.

Collection, Storage and Shipment of Clinical Specimens

Swab samples should be collected using Dacron or Polyester swabs. Other specimen types should be transferred in sterile containers. In the transport phase, Viral Transport Medium (VTM) (Preperation of viral transport medium, Center for Disease Control and Prevention, SOP#: DSR-052-01) or *Bio-Speedy® vNAT Viral Transfer Tubes (Cat No:BS-NA-513-100).* Samples should be stored and transported at 2-8 °C until they arrive at the laboratory. Swab samples should be transferred within 5 days, other sample types should be transferred within 2 days. If a delay in shipment is expected, samples should be frozen at -20 or -70°C and shipped with dry ice. It is important that samples are not exposed to continuous freeze-thaw exposure.

Warnings

1. The kit should be stored away from nucleic acid sources and qPCR amplicons.
2. 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.
3. 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.
4. Kit components should be mixed by gently shaking before use.
5. The micropipettes used for pipetting qPCR mixes and template nucleic acids should be separate.
6. Template nucleic acid and positive control tubes should always be kept closed, except for fluid transfers.
7. The wipeable surfaces of the rooms, benches and devices where the test is performed should be cleaned regularly with 10% NaClO.
8. The qPCR completed reaction tubes should be disposed of before opening in the laboratory.

RT-qPCR Application Protocol

Before starting the assay, please consider the following:

- The kit was validated only for the template nucleic acid volume that is 25% of the total qPCR volume.
- The kit can not be used with real-time PCR instruments without the periodic maintenance records.
- Both white and clear 0.1 mL qPCR plates can be used for the assay, while slightly better performance can be obtained using the white plates for Bio-Rad CFX 96 and Roche Light Cycler 96 instruments.
- 0.1 ml and 0.2 ml clear qPCR tubes can be used for the assay, while slightly better performance can be obtained using the 0.1 ml tubes for Rotor Gene Q instrument.
- Both 10 µL and 20 µL qPCR volumes can be used for the assay on Bio-Rad CFX 96 and Roche Light Cycler 96 instruments.
- 10 µL qPCR volume can be used on Rotor Gene Q instruments (72-well rotor) with 0.1 ml clear qPCR tubes.
- 20 µL qPCR volume can be used on Rotor Gene Q instruments (both 36- and 72-well rotor) with both 0.1 and 0.2 ml clear qPCR tubes.

Program the qPCR device as follows and add the reagents to the qPCR tubes in the order specified below, close the tubes, place them into the qPCR device and start the run (Table 2).

Table 2. Reaction set-up and qPCR program details

Reaction setup			**qPCR Program**		
Component	**Reaction**		**Cycle Number**	**Temperature**	**Duration**
2X Prime Script Mix	5 µL	10 µL	1	52 °C	5 min
Oligo Mix	2.5 µL	5 µL	1	95 °C	10 sec
			40	95 °C	1 sec
Template Nucleic Acid	2.5 µL	5 µL		55 °C	1 sec
TOTAL REACTION VOLUME	10 µL	20 µL		FAM/HEX Read* (Green/Yellow Read)	
*If your qPCR devices have FAM / HEX reading only selection option, using this option will shorten the protocol by 4 minutes.					

Interpretation of The Assay Results

The recommended threshold levels to calculate the number of threshold cycles (Cq) for both 10 µL and 20 µL reactions are 0.05 and 200 RFU for Roche LightCycler® 96, Bio-Rad CFX96 Touch™ respectively.
In Rotor-Gene® instruments, the sigmoidality of the amplification curves should be evaluated from the "Raw Data" screen. To see the Ct values of sigmoidal curves in Rotor-Gene® devices; on the analysis screen, "Dynamic Tube" should be active, "Slope Correct" options should be passive, "Outlier Removal" option should be "0", the threshold level should be set to 0.02. Shape of the amplification curves obtained in the FAM/HEX channels are examined and non-sigmoidal curves are recorded as negative. The result is recorded as negative if there is no sigmoidal curve. The result is recorded as positive if Cq<38. The analysis is repeated with the same nucleic acid extract if Cq≥38, if the result is Cq≥38 again, a new sample from the patient is taken. If there is no sigmoidal curve in the negative control and the sample is Cq≥38, the nucleic acid extract removed to -20 °C should be analyzed again, if it is Cq≥38, a new sample should be requested from this person again and the analysis should be repeated.

Table 3. Interpretation of Patient Samples

Cases	**NA Isolate**		**Positive Control**		**Negative Control**		**Comment**
	Target	**IC**	**Target**	**IC**	**Target**	**IC**	
Case 1	Pos	Pos	Pos	Pos	Neg	Neg	Target Positive
Case 2	Pos	Pos	Neg	Neg	Neg	Neg	Target Positive: Since the sample gives a positive result, it is concluded that the kit works, the patient result is reported.If the problem persists in the positive control, contact the manufacturer and request a new positive control.

Case 3	Pos	Neg	Pos	Pos	Neg	Neg	Target Positive
Case 4	Neg	Pos	Pos	Pos	Neg	Neg	Target Negative
Case 5	Pos	Pos	Pos	Pos	Pos	Neg	Contamination Problem: The experiment is repeated by paying attention to the issues in the warnings section.
Case 6	Neg	Pos	Pos	Pos	Pos	Neg	Target Negative: Since the target is negative, there is no contamination problem. NTC supplied with the kit contents may be contaminated. If the problem persists, the manufacturer is contacted and a new negative control is requested.
Case 7	Neg	Neg	Pos	Pos	Neg	Neg	Sampling / Extraction / Inhibition Problem: Nucleic acid isolate is diluted 1/10 and the experiment is repeated. If the diluted sample does not produce positive result in the HEX channel, a new sample is requested.
Case 8	Neg	Neg	Neg	Neg	Neg	Neg	Reagent Problem: Reagents are renewed by contacting the manufacturer and the reaction is repeated.
Case 9	Neg	Pos	Pos	Pos	Neg	Pos	If the IC Cq value in the negative control reaction is 3 cycles or greater than the IC Cq value obtained from the NA isolate, the result is evaluated and reported as negative. Otherwise, a sampling / extraction / inhibition problem is concluded.
Case 10	Pos	Pos	Pos	Pos	Neg	Pos	Target Positive: Since the target is negative in the negative c ontrol and the target is positive in the NA isolate, the sample is reported as positive regardless of the contamination in the IC.

Limitations

- Performance of the *Bio-Speedy® SARS CoV-2 N RT-qPCR Kit* has only been established in nasopharyngeal swab, oropharyngeal (throat) swab, nasopharyngeal aspirate or lavage, bronchoalveolar lavage and sputum samples.
- Mutations within the target regions of the *Bio-Speedy® SARS CoV-2 N RT-qPCR Kit* could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- A false negative result may occur if a specimen is improperly collected, transported or handled.
- Inhibitors or other types of interference may produce a false negative result. 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 kit. 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.

WARNINGS: On the web page linked with the QR code, examples of sigmoidal and non-sigmoidal curves are given for different device types. The results obtained with this kit should NOT be interpreted without examining these samples.