Bio-Speedy®

Kat. No: BS-SY-WCOR-305-100

Kul. Klv. Vrs.: 01312020MK

CE IVD

REPUBLIC OF TURKEY
MINISTRY OF HEALTH
GENERAL DIRECTORATE OF PUBLIC HEALTH

COVID-19 qPCR Detection Kit

100 Tests

Table 1. Kit content (Shelf Life: 12 months) Storage: -20 °C; Transport: +2-8 °C				
Intended Use / Content / Target	**Content**		**Quantity**	**Consumption per reaction**
	Oligo Mix			
	Code	**Dye**		
SARS-CoV-2 (2019-nCoV) (RdRp gene)	Wuhan-RdRp	ROX	1 x 500 µL	5 µL
Internal Control (IC) (RNase P gene)		HEX		
DNA polymerase, dNTP mix, reaction buffer	2X RT-qPCR Mix		1 x 1000 µL	10 µL
Reverse transcriptase and ribonuclease inhibitor	RT-RIN Mix		1 x 100 µL	1 µL
Negative Control Template **Test it in each run for contamination control**	NC		1 x 500 µL	5 µL
Positive Control Template: Synthetic SARS-CoV-2 genom fragment **Test it monthly for reactive stability control**	PC		1 x 50 µL	5 µL

Intended Use and Test Principle: Kit is used for detecting the epidemic virus "SARS-CoV-2 (2019-nCoV)" causing Coronavirus Disease 2019 (COVID-19). The kit is applied to nucleic acid isolates from 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 (2019-nCoV)-specific RdRp (RNA-dependent RNA polymerase) gene fragment. The RdRp gene-targeted Wuhan-RdRp oligonucleotide set gives positive results only with SARS-CoV-2 (2019-nCoV). In the SARS-CoV-2 (2019-nCoV) routine screening, Wuhan-RdRp olionucleotide set is applied; if the result is Wuhan-RdRp positive, SARS-CoV-2 (2019-nCoV) is interpreted as positive; and if the result is Wuhan-RdRp negative SARS-CoV-2 (2019-nCoV) is interpreted as negative.

Analytical Specifications: Kit is validated with Roche LightCycler® 96, Bio-Rad CFX96 Touch™, Qiagen Rotor-Gene® 5 Plex Real-Time PCR Systems. The kit's detection limit (LOD) is the lowest analyte concentration that can be detected with a 95% probability. The LOD of the oligonucleotide sets included in the kit, inclusivity and exclusivity studies were carried out using 35 different genotypes and published by the World Health Organization (WHO Protocol, 13.01.2020, Diagnostic detection of Wuhan coronavirus 2019 by real-time RT-PCR). LOD of the test for the RdRp gene is 3.8 copy-RNA/reaction. The kit is validated with RINA™ M14 Nucleic Acid Extraction Robot (Cat No: RINA-M14-01) and its consumables (Cat No: RN-NA-14-111-100) and the manual Bio-Speedy® Nucleic Acid Isolation Kit (Cat No: BS-NA-102-100).

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 analysis is performed should be cleaned regularly with 10% NaClO. 8) The qPCR completed reaction tubes should be disposed of before opening in the laboratory.

APPLICATION PROTOCOL

Table 2. 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.

Reaction setup			qPCR Program		
Component	**Add order**	**Reaction**	**Cycle Number**	**Temperature**	**Duration**
2X RT-qPCR Mix	1	10 µL	1	45 °C	15 min
RT-RIN Mix	2	1 µL	1	95 °C	3 min
Oligo Mix	3	5 µL	40	95 °C	5 sec
Template Nucleic Acid	4	5 µL		55 °C	35 sec
TOTAL REACTION VOLUME		21 µL		FAM/HEX/ROX read	

Table 3. Interpretation of Results: 1) Examine the shape of the amplification curves obtained in the FAM / HEX / ROX channels and record non-sigmoidal curves as negative. 2) Calculate the number of threshold cycles (Cq). 3) Record the result as negative if 38≤Cq and as positive If Cq <38. 4) Interpret results according to Table 3.

Template →	**NA Isolate**		**Positive Control**		**Negative Control**		**Interpretation**
Target →	**Wuhan-RdRp**	**IC**	**Wuhan-RdRp**	**IC**	**Wuhan-RdRp**	**IC**	
Case 1	Pos	Pos	Pos	Pos	Neg	Neg	SARS-CoV-2 (2019-nCoV) Positive → Consult the reference laboratory
Case 2	Neg	Pos	Pos	Pos	Neg	Neg	SARS-CoV-2 (2019-nCoV) Negative → Report the test result
Case 3	Pos	Pos	Pos	Pos	Pos	Neg	Contamination Problem: The experiment is repeated by paying attention to the issues in the Warnings section.
Case 4	Neg	Neg	Pos	Pos	Neg	Neg	Extraction/Inhibition Problem: 1) Extraction is repeated 2) Nucleic acid isolate is diluted 1/10 and the experiment is repeated
Case 5	Neg	Neg	Neg	Neg	Neg	Neg	Reagent Problem: By contacting the manufacturer, reagents are renewed and the reaction is repeated