TÜSEB DiaKit SARS-CoV-2 V01 RT-qPCR Diagnostic Kit Package Leaflet

1. Product Description

TÜSEB DiaKit SARS-CoV-2 V01 RT-qPCR Diagnostic Kit is a single-step Real Time PCR in-vitro diagnostic kit prepared for the qualitative detection of the genomic RNA of the SARS-CoV-2 coronavirus, the causative agent of COVID-19, using the specific Taqman probe system.

2. Description of the Kit

Coronaviruses (CoV) are viruses belonging to the RNA virus family that can cause disease by infecting animals and birds belonging to the mammalian class.

The SARS-CoV-2 virus, which emerged in Wuhan, China, is a new coronavirus that causes respiratory system infection (COVID-19), in which approximately 2% of cases result in death.

The kit works with lower respiratory tract samples (sputum, bronchoalveolar lavage) and upper respiratory tract samples (nasopharyngeal-oropharyngeal swab) taken from individuals showing clinical symptoms for the disease.

The 'ORF1ab' ve 'N' genes encoding the nucleocapsid protein in the SARS-CoV-2 genome was determined as the target with the kit. The "RNAseP" gene was chosen as the internal control gene for the quality and inhibition control of the nasopharyngeal and oropharyngeal swab samples.

3. Devices and Equipment to be provided by the User

1. Biological Cabinet
2. Viral RNA Extraction Kit
3. Real time PCR Device
4. Real time PCR test tubes/strips/plates
5. Adjustable Micropipettes (0.5µL – 1000µL)
6. Filtered Micropipette Tips
7. 1.5 mL Microcentrifuge Tube
8. Cooler Working Block
9. Powder-free disposable inspection glove
10. Medical Waste Container
11. Cooler Cabinets (+4/-20 °C)
12. Desktop centrifuge (Max 2.000 g)
13. Vortex mixer

4. Storage Conditions

(-15 °C) – (-25 °C), 12 months.

5. Contents of the Kit

Kit contents	Quantity		
	100 reactions	500 reactions	1000 reactions
2X Master Mix	1 x 500 µL	2 x 1250 µL	4 x 1250 µL
Primer Mix	1 x 250 µL	2 x 625 µL	2 x 1250 µL
Negative Control	1 x 500 µL	1 x 500 µL	1 x 1000 µL
Positive Control	1 x 100 µL	1 x 200 µL	1 x 250 µL

Table 1. *Contents of the kit*

The positive control sample supplied with TÜSEB DiaKit SARS-CoV-2 V01 RT-qPCR Diagnostic Kit is a fragment containing synthetically produced target gene regions and was extracted by manual method. Nuclease-free water (NFW), used in routine laboratory work, is used as the negative control.

6. RT-qPCR Application Protocol

Please consider the following information before starting the analysis:

1. The kit has been validated only for the volume of molded nucleic acid, which is 25% of the total qPCR volume.
2. The kit should not be used with real-time PCR devices that do not have periodic maintenance records.
3. Program the qPCR device as indicated below and add the reagents to the qPCR tubes in the following order, close the tubes, place them in the qPCR device and start operation. (Table 2-3)

Reaction Setup		qPCR Programı (BIO-RAD CFX96)*		
Content	Volume	Number of Cycle	Temperature	Time
2 x Master Mix	5 µL	1	42°C	5 min
		1	95°C	1 min
Primer Mix	2,5 µL	39	95°C	1 sec
Sample Nucleic Acid	2,5 µL		60°C	1 sec
Total Reaction Volume	10 µL	FAM / HEX Reading		

Table 2. *Details of reaction setup and qPCR program (Bio-Rad CFX96)*

*The mean trashold value of the FAM and HEX channels was determined as 100 RFU. The RFU value is not a fixed value and may vary depending on the viral load of the sample and the sigmoid structure of the graph.

Reaction Setup		qPCR Programı (ROTOR-GENE Q)**		
Content	Volume	Number of Cycle	Temperature	Time
2 x Master Mix	5 µL	1 (Hold)	42°C	8 min
		1 (Hold-2)	95°C	1 min
Primer Mix	2,5 µL	45 (Cyclig)	95°C	10 sec
Sample Nucleic Acid	2,5 µL		60°C	10 sec
Total Reaction Volume	10 µL	GREEN / YELLOW Reading		

Table 3. *Details of reaction setup and qPCR program (Rotor-Gene Q**)*

** During the analysis, Dynamic Tube and Slope Correct should be activated after selecting the appropriate channel. The threshold value should be set to 0.02.

7. Interpretation of Results

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Result	Expected Ct Values		Evaluation
	FAM (Orf1ab&N)	HEX (RnaseP)	
Negative Control	-	-	Expected NTC
Positive Kontrol	≤38	≤38	Expected PC
1.	≤38	≤38 /-	COVID-19 Positive
2.	-	-	Retest
3.	-	≤38	COVID-19 Negative

Table 4. *Evaluation of reactions results*

***For In-vitro Diagnostic Medical Devices Directive (98/79/EC) compliance; We declare the full compatibility of TÜSEB DiaKit SARS-CoV-2 V01 RT-qPCR Diagnostic Kit for use with NAEEKTS (Nucleic Acid Extractor and Preservative Transport Fluid) branded TÜSEB DiaVnat Extraction and Transfer Tube with reference number SBTvNAT2022-100.**

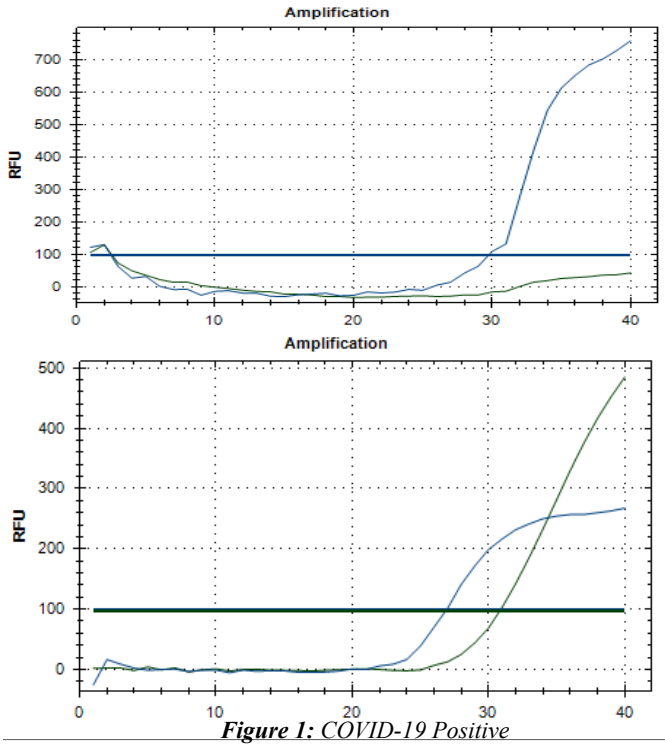

Figure 1: *COVID-19 Positive*

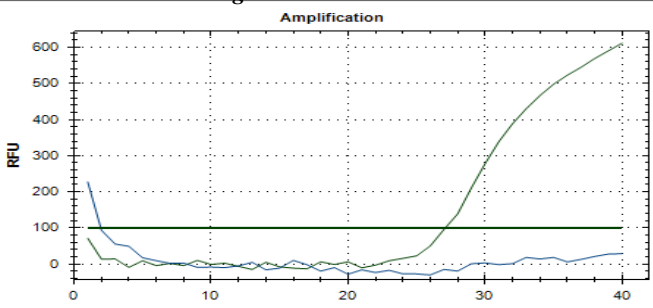

Figure 2: *COVID-19 Negative*

8. Statement of Validation

The validation study of TÜSEB DiaKit SARS-CoV-2 V01 RT-qPCR Diagnostic Kit was performed in triplicate with samples containing synthetic SARS-CoV-2 RNA fragment between 107/ µL copy number and 100/ µL copy number. The reaction results and optimum graphs are given below. TÜSEB DiaKit SARS-CoV-2 V01 RT-qPCR Diagnostic Kit; It can even detect 1 (one) copy number per microliter.

9. Reaction results of solutions prepared with TÜSEB DiaVnat Extraction and Transfer Tube

	Cq Values		
Number of copies/ µL	1	2	3
10^7	14.02	11.31	13.58
10^6	17.18	15.16	17.26
10^5	19.22	19.37	20.22
10^4	21.02	20.38	22.10
10^3	23.83	24.9	24.96
10^2	24.02	24.03	23.24
10^1	-	24.92	24.42
10^0	24.43	24.35	24.23

Table 5. *Reaction results of solutions prepared with TÜSEB DiaVnat Extraction and Transfer Tube*

10. Reaction results of solutions prepared with TÜSEB DiaVnat Extraction and Transfer Tube

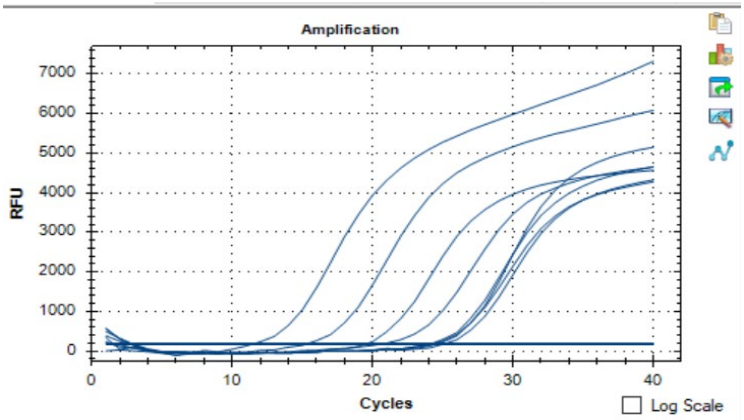

Table 6. *Graphical views of solutions prepared with TÜSEB DiaVnat Extraction and Transfer Tube*