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

1. Product Description

TÜSEB DiaKit SARS-CoV-2 RT-qPCR Diagnostic Kit is a one step in-vitro Diagnostic Kit using the specific Taqman probe system, prepared for qualitative detection of genomic RNA of the COVID-19 coronavirus SARS–CoV–2.

2. Description of the kit

Coronaviruses (CoV) are viruses belonging to the RNA virus family that can cause disease by infecting mammalian animals and birds. 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 determined for the disease. With the kit, the "N" gene encoding the nucleocapsid protein found in the SARS-CoV-2 genome was identified as the target. The "RNaseP" gene was selected as the internal control gene for the quality and inhibition control of samples taken by nasopharygeal or oropharyngeal swab.

3. Devices and Equipment to be provided by the User

1. Real-Time PCR Device Minimum 2-Channel (FAM/HEX)
2. 1-10 µL, 10-100 µL, and 100-1000 µL micropipette and micropipette tips (sterile, RNase-Dnase-free)
3. Spin Centrifuge - min. 3000rpm
4. Vortex mixer
5. 0.5, 1.5, or 2 mL Eppendorf type microcentrifuge tube
6. Sterile laminar flow cabinet for qPCR reaction setup
7. Disposable nitrile gloves
8. Reaction strip or plate compatible with the qPCR instrument
9. Plate sealing film

4. WARNING AND PRECAUTIONS

- For in vitro diagnostic use only.
- For professional use in laboratories.
- Micropipettes used in the preparation of reaction mixtures should be sterile and pipettes providing sample transfer should be separate.
- Micropipette tips used in procedures should not contain "Rnase-Dnase".
- Reference materials of all samples and kits to be tested must be considered infectious agents and handled strictly by laboratory biosafety requirements.
- Sterile centrifuge tubes and filter tips should be used. Tips should be discarded after use in a bin containing 10% sodium hypochlorite solution. After the procedure, the work area surface and the instrument surface should be disinfected with a freshly prepared 10% sodium hypochlorite solution and then cleaned with 75% ethanol or distilled water.
- Use only the reagents provided in the kit and recommended by the manufacturer. Do not mix reagents from different batches.
- Repetitive freeze-thaw processes should be avoided.
- Freezing is recommended after small portions.
- Ice or cooler must be used during the reaction setup. It is not used during sample preparation.
- Measurements should be made by instructions to obtain accurate and reliable results.

5. Storage Conditions

All reactants should be stored at -20 °C. Reactants can be stored for 12 months under recommended conditions. Expired kits should not be used. Extreme temperature and light may damage product performance. Kits stored in inappropriate conditions should not be used.

6. Contents of the kit

Kit contents	Cap	Quantity	
		500 reactions	1000 reactions
Prime Script	Green	2 x 1000 µL	4 x 1000 µL
Oligo Mix	Blue	2 x 1000 µL	4 x 1000 µL
Positive Control	Red	1 x 300 µL	2 x 300 µL
Negative Control	White	1 x 500 µL	2 x 500 µL

Table 1. *Contents of the kit*

Oligo Mix contains primer and fluorescent labeled probes of viral target gene and internal control gene.

Prime Script contains Taq polymerase and reverse transcriptase enzymes, which are the basic components of RT-PCR reaction, as well as DNA nucleotide bases (dNTPs) and buffers necessary for the reaction.

The positive control contains synthetically synthesized target viral and human gene oligonucleotides.

The negative control contains "Rnase-Dnase Free PCR grade" water.

7. Preparation of the Reaction Mixture

Before preparing the reaction mixture, attention should be paid to the sterility of the working cabinet. Before and after the procedures, the work area surface and the instrument surface should be disinfected with a freshly prepared 10% sodium hypochlorite solution and then cleaned with 75% ethanol or distilled water. After this process, the reaction should be established according to the amounts specified in Table 2.

Component	Reaction (µL) (per sample)
Prime Script	4
Oligo Mix	4
Sample	2
Total Volume	10

Table 2*. Preparation of the reaction*

After the tubes are taken out of the -20ºC conditions, they should be waited to dissolve in the tube stand in the cabinet. After the dissolution process,

the tubes should be centrifuged for 3-5 seconds in the spin centrifuge device. After this process, the amounts specified for a sample in Table 2 should be considered in the empty microcentrifuge tubes of 1.5 – 2 mL and Prime Script, and then Oligo Mix should be added according to the number of samples. Do not vortex the mixture. Mix by gently pipetting. After, the prepared mixture should be transferred to plates or strips with a micropipette, 8 µL for each sample.

Before the sample transfer process, the tubes containing the samples should be vortexed for 3-5 seconds. After the process, 2 µL of the samples should be transferred to the plate or strip with the help of a micropipette. For each sample a new pipette tip should be used. After sample transfer is complete, use plate-centrifuge device for plate and strip-centrifuge device for strips. It should be centrifuged for 5 seconds to remove any bubbles present in the plate or strip.

8. Protocol

After the reaction preparation process, the plate or strip is placed in the device, the steps in Table 3 should be set up in accordance with the device.

Cycle	Temperature	Time
1	50°C	3 min
1	95°C	10 sec
40	95°C	1 sec
	60°C	1 sec

Table 3. *Protocol*

If the device has a ramp rate option, it can be used optionally according to table 4.

Cycle	Temperature	Time
1	50°C	3 min
Ramp rate 5ºC sec		
1	95°C	10 sec
Ramp rate 5ºC sec		
40	95°C	1 sec
	60°C	1 sec
Ramp rate 5ºC sec		

Table 4*. Protocol (Ramp Rate added)*

After the protocol setup, the target fluorophore dye and the channels should be selected as in Table 5

Fluorophore	Target
HEX	N gene
FAM	RNASE-P gene

Table 5. *Fluorescence detectors profile (dyes)*

TÜSEB DiaKit SARS-CoV-2 RT-qPCR Diagnostic Kit detects the viral gene in the HEX channel and the internal control RNASEP gene in the FAM channel.

9. Interpretation of Results

After the run in the Real-Time PCR device is completed, it should be interpreted according to the amplification values and curves.

The threshold value for the Bio-Rad CFX96 Touch™ device is 200 RFU.

Before the analysis in Rotor Gene Q devices:

- Dynamic Tube option enabled,
- Slope Correct option disabled,
- Outlier Removal option should be 0,
- The threshold level should be set to 0.02.

N Gene	RnaseP gene	Assessment
+	+	The patient is SARS-CoV-2 positive.
-	+	The patient is SARS-CoV-2 negative
+	-	Inconclusive result. The test should be repeated.
-	-	

Table 6. *Evaluation of reaction results*

- ✓ When interpreting for the FAM and HEX channels Cq < 37 should be considered valid.
- ✓ Those above Cq > 37 should be considered invalid.