# TÜSEB DiaKit Quad RT-qPCR Diagnostic Kit (RSV, SARS-CoV-2, Influenza A&B) Package Leaflet

## 1. Intended Use of the Kit and Test Principle

TÜSEB DiaKit Quad RT-qPCR Diagnostic Kit (RSV, SARS-CoV-2, Influenza A&B) is used for the rapid and accurate diagnosis of SARS-CoV-2, Influenza A, Influenza B, Respiratory Syncytial Virus A/B agents in clinical samples. The kit is applied to nucleic acid isolates obtained from nasopharyngeal swab, oropharyngeal swab, bronchoalveolar lavage, nasopharyngeal aspirate and sputum samples. Rapid diagnosis with the kit is performed by one-step reverse transcription (RT) and real-time PCR (qPCR) (qRT-PCR) targeting genomic RNA and DNA regions specific to the target agent. Diagnosis with the kit can be performed ***in less than 45 minutes****. ***Bio-Rad CFX96 Touch** TÜSEB DiaKit Quad RT-qPCR Diagnostic Kit (RSV, SARS-CoV-2, Influenza A&B) was validated with "TÜSEB DiaVnat Extraction and Transfer Tube **(SBTVNAT2022-100)**". The kit is applied to nasopharyngeal swab, oropharyngeal swab, bronchoalveolar lavage, nasopharyngeal aspirate and sputum samples taken by health care providers from individuals with suspected disease.

## 2. Shelf Life

12 months; look at the expiration date on the box. Each reagent stored at storage temperature can be used until the expiration date indicated on the tube. The expiration date of the kit is determined according to the expiration date of the reagents.

## 3. Warnings

1. Keep the kit away from nucleic acid sources and qPCR amplicons.
2. Do not mix kit components with different lot numbers or chemicals of the same name but belonging to different manufacturers.
3. Keep the main stock reagents in the cold block during PCR setup.
4. If possible, set up the PCR in the cold block.
5. Mix the kit components slowly before use.
6. Use separate micropipettes to pipette qPCR mixtures and template nucleic acids.
7. Keep the template nucleic acid and positive control tubes closed at all times, except for liquid transfers.
8. Clean the erasable surfaces of the rooms, benches and devices where the test is performed regularly with 10% NaClO.
9. Remove the qPCR before opening the completed reaction tubes in the laboratory.

## 4. Storage Conditions

As long as the storage temperature is maintained (between -15 °C and -25 °C), the kit can remain stable until the expiration date written on the packaging.

## 5. Contents of the Kit

| **Storage Temperature: -20 °C; Transfer Conditions: 2-8 °C** | | | | |
|---|---|---|---|---|
| **Content/Intended Use** | **Channel** | **Content** | **Quantity (10 µL Reaction)** | **Unit Reaction Consumption** |
| DNA polymerase, dNTP mix, reaction buffer, reverse transcriptase and ribonuclease inhibitor | - | 2X Master Mix | 6 x 1670 µL | 10 µL |
| SARS CoV-2 | FAM | Primer Mix | 4 x 1250 µL | 5 µL |
| IC; Internal Control, (Human RNase P gene) | HEX | | | |
| Respiratory Syncytial Virus A/B | ROX | | | |
| Influenza A&B | Cy5 | | | |

**Table 1.** Contents of the kit

| **Storage Temperature: 2-8 °C; Transfer Temperature: 2-8 °C**<br>**If components are frozen, store at -20 °C. Store at 2-8 °C after the first thawing.** | | | |
|---|---|---|---|
| Negative Control (Nuclease-Free Water)<br>For contamination control, test at each operation. | NTC | 1 x 1000 µL | 5 µL |
| Positive Control: Plasmid containing target gene regions<br>For reagent stability control, test at each operation. | PC | 1 x 500 µL | 5 µL |

***Table 2.*** *Contents of the kit – Controls*

## 6. Devices and Equipment to be provided by the User

| Devices and Equipment to be provided by the User | |
|---|---|
| **1.** Real-Time PCR Device: 4-channel, Ramp rate ≥3 °C/sec.<br>**2.** 1-10 µL micropipette and compatible pipette tip (DNase and RNase-free)<br>**3.** Spin Centrifuge: min.3000 rpm (compatible with 8 strips and 2 mL microcentrifuge tube)<br>**4.** Vortex mixer | **5.**UV cabinet for PCR setup<br>**6.**Cold Tube stand (for microcentrifuge tubes and PCR tube/strips)<br>**7.**Powder-free, disposable, nitrile gloves |

***Table 3.*** *Devices and equipment to be provided by the user*

## 7. Validated qPCR Devices

TÜSEB DiaKit Quad RT-qPCR Diagnostic Kit (RSV, SARS-CoV-2, Influenza A&B) was validated with the devices given in Table 4.

| CFX96 Touch* | Rotor-Gene® Q | MIC-4 qPCR | QuantStudio |
|---|---|---|---|
| Cat#: 1845098; CFX Qualification plate and film (96 wells) | Cat#: 981103; Qiagen Strip Tube and Cap 0.1 mL (4 wells) | Cat#: 71-107C; BMSMIC qPCR Tube and Cap 0.1 mL (4 wells) | Cat#: AB-2800/W; ABgene 96 white plate |
| Cat#: BS2001 Ultra optical half qPCR plate and film (96 wells) | | | Cat#: AB-1170 ABsolute qPCR seal |
| Cat#: TLS0851; White Strip (8 wells) | | | Cat#: AB-1502/W; white PCR tube |

*** Use only white plates/white strips with the Bio-Rad CFX96 qPCR!**

***Table 4.*** *Plastic consumables specific to the validated qPCR device*

## 8. Collection, Storage and Transfer of Nucleic Acid Extracts

Swab samples should be collected using Dacron or Polyester swabs. Other types of samples should be transferred in sterile containers. During the transport phase, Viral transport media (VTM) (Viral transport media preparation, Centers for Disease Control and Prevention, SOP #: DSR-052-01) or TÜSEB DiaVnat Extraction and Transfer Tube (SBTvNAT2022-100) should be used. 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 types of samples within 2 days. If a delay in shipment is expected, the samples should be frozen at -70 °C and shipped with dry ice. It is important that the samples are not subjected to repeated freezing-thawing. The samples that brought to the laboratory in the VTM must be subjected to the Nucleic Acid Purification process. Samples from inside the TÜSEB DiaVnat Extraction and Transfer Tube can be directly included in the PCR reaction.

## 9. Analytical Performance

The analytical performance studies of the mentioned pathogens were carried out with synthetic pUC57 plasmid containing the target gene regions of the pathogens. (Figure 1.)

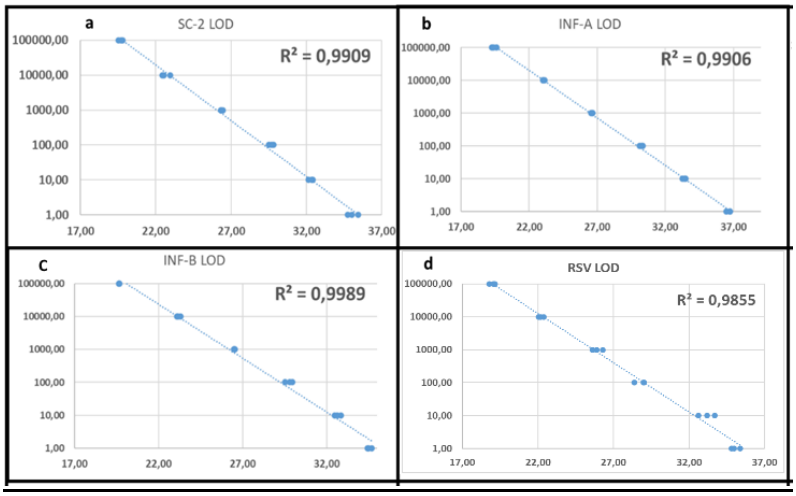


***Figure 1.*** *Limit of Observability (LOD) a.SC-2, b. INF-A, c. INF-B, d. RSV, the horizontal axis represents CT, the vertical axis represents Concentration Gc/µl.Relevant pathogens were studied in triplicate at concentrations ranging from 10^5 Gc/µl to 10^0 Gc/µl.*

## 10. RT-qPCR Protocol

Before starting the analysis, please consider the following information:

1.The kit is validated only for the molded nucleic acid volume, 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.Do not use qPCR plates/strips that are not validated with the kit!
4.Program the qPCR device as indicated below and add the reagents to the qPCR tubes in the order indicated below, close the tubes, place them in the qPCR device and start operation (Table 5-6)

| Reaction Setup | | qPCR Program (BIO-RAD CFX96) | | |
|---|---|---|---|---|
| **Content** | **Volume** | **Number of Cycles** | **Temperature** | **Time** |
| 2 x Master Mix | 10 µL | 1 | 50°C | 5 min. |
| | | 1 | 95°C | 3 min. |
| Primer Mix | 5 µL | 40 | 95°C | 5 sec. |
| Template Nucleic Acid | 5 µL | | 60°C | 10 sec. |
| Total Reaction Volume | 20 µL | FAM / HEX / ROX / CY5 Reading | | |

***Table 5.*** *Reaction setup and qPCR program details (Bio-Rad CCFX96)*

| Reaction Setup | | qPCR Program (ROTOR-GENE Q) | | |
|---|---|---|---|---|
| **Content** | **Volume** | **Number of Cycles** | **Temperature** | **Time** |
| 2 x Master Mix | 10 µL | 1 (Hold) | 50°C | 8 min. |
| | | 1 (Hold-2) | 95°C | 3 min. |
| Primer Mix | 5 µL | 40 (Cycling) | 95°C | 15 sec. |
| Template Nucleic Acid | 5 µL | | 60°C | 15 sec. |
| Total Reaction Volume | 20 µL | FAM / HEX / ROX / CY5 Reading | | |

***Table 6.*** *Reaction setup and qPCR program details (Rotor-Gene Q**)*

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

## 11. Interpretation of Test Results

The shape of the amplification curves should be examined. If a Cq value has been assigned to a sample by the device's software and the curve is sigmoidal, the Cq value can be used in the final evaluation. Non-sigmoidal curves should be recorded as negative. If a sample is assigned a Cq value, but the curve is not sigmoidal, the result should be recorded as negative.

The Cq value of the IC should be examined for samples with a suspicious sigmoidal curve pattern below the threshold in the channel of the targets. If the IC Cq is≤34, (Ct ≤ 40.0 for Rotor-Gene) the sample should be reported as negative. If Cq>34, (Ct ≤ 40.0 for Rotor-Gene) the test should be repeated after the sample is frozen and thawed. If the problem persists after freeze-thaw, a new sample should be requested.

| Control Type | Name | Control Purpose | Expected Result | |
|---|---|---|---|---|
| **Adding NTC** | NTC | Contamination control | No Cq = Valid | |
| **No adding template** | NRC | Reactive contamination control | No Cq = Valid | |
| **Adding PC** | PC | Positive reactive control | Cq ≤ 38.0 = Valid | |
| **Human RNase P*** | IC | Sampling, RNA integrity, nucleic acid extraction and control of reverse transcription and qPCR inhibition | Cq≤34.0 = Valid (Ct ≤ 40.0=Valid for Rotor-Gene) | If IC Cq ≥34.0, but target Cq≤38.0, the IC is valid (Ct ≤ 43.0 for Rotor-Gene) |
| * If any factor is positive in a multiplex reaction with internal control, the target is interpreted as positive even if the internal control (IC) is negative. If any factor is not positive in the multiplex reaction with internal control, the internal control (IC) should give a positive result. | | | | |

***Table 7.*** *Expected performance of kit controls*

If any control does not work as described in Table 7, the work is considered invalid and the test is repeated.

1. Invalid PC: Contact the manufacturer, renew the reagents and repeat the reaction.
2. Invalid NRC: Contact the manufacturer, renew the reagents and repeat the reaction.
3. Invalid NTC: Repeat the analysis, paying attention to the “Warnings” section.
4.

If all controls are valid, proceed to the interpretation of the results.

- If the Cq value of the gene targets is **≤ 38**, conclude **positively.**
- If the Cq value of the gene targets is **> 38**, conclude **negatively.**

**12. Limitations**

- The performance of TÜSEB DiaKit Quad RT-qPCR Diagnostic Kit (RSV, SARS-CoV-2, Influenza A&B) was determined in nasopharyngeal aspirate, bronchoalveolar lavage, nasopharyngeal swab, oropharyngeal swab and sputum samples.
- Mutations in the target regions of the kit can affect primary and/or probe binding and cause the presence of the virus to go undetected.
- Errors made during sample collection, transportation or processing can lead to false negative results.
- Inhibitors or other interfering factors may cause a false negative result. False negative results can also occur in cases where there is an insufficient number of target organisms in the sample.