# BioeXsen Health Technology SARS-CoV-2 RT PCR Kit

CE IVD

## Package Insert

***Rx only***
***For use under Emergency Use Authorization (EUA) only***

### 1. Kit Content

**Shelf life:** 12 months; refer to 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 by the expiration date of the reagents.

**Table 1a.** Kit content

| Intended Use | Content | Quantity | | Storage [1] / Transport |
|---|---|---|---|---|
| | | 100 Rxns | 1000 Rxns | |
| SARS-CoV-2 detection (*ORF1ab gene*) (**FAM**) | Oligo Mix | 1 x 250 µL | 2 x 1250 µL | -20°C / +2°C- +8°C (Box 1/2) |
| Internal Control (IC) (*RNase P gene and mRNA*) (**HEX**) | | | | |
| One step real-time RT-PCR Mix | 2X Prime Script Mix | 1 x 500 µL | 4 x 1250 µL | |
| No Template (Negative) Control for PCR and Extraction Negative Control **Test it in each run for contamination control** | NTC | 2 x 1000 µL | 16 x 1250 µL | |
| Positive Control template (Synthetic SARS-CoV-2 genom fragment) **Test it in each run for reactive stability control** | PC | 1 x 250 µL | 2 x 500 µL | |
| Extraction and preservation of viral nucleic acids | *vNAT*® | 5 x 2 mL | 1 x 100 mL | Room Temperature (Box 2/2) |

**Table 1b.** Instruments and equipment supplied by the user

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

### 2. Intended Use and Test Principle

*BioeXsen SARS-CoV-2 RT PCR* nucleic acid detection kit is a one-step reverse transcription and real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swabs, oropharyngeal (throat) swabs, combined nasopharyngeal/oropharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal or nasopharyngeal aspirates, nasal washes and bronchoalveolar lavage samples from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

Detection with the kit is achieved via rapid nucleic acid extraction from respiratory tract samples followed by multiplex real-time RT-PCR targeting the SARS-CoV-2 specific *ORF1ab* gene and human *RNase P* gene and mRNA in real-time PCR instruments that are equipped with FAM and HEX detection channels.

The oligonucleotide set targeting human *RNase P* gene and mRNA functions as a control of the sampling, nucleic acid extraction and inhibition. The kit also contains negative and positive control templates.

The kit contains *vNAT*® buffer that extracts and preserves viral nucleic acids in respiratory tract samples. The *vNAT*® component enables the initiation of the real-time RT-PCR within 5 minutes of introduction of the sample.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

### 3. Analytical Specifications

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 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.

Limit of detection (LOD) of the kit using *vNAT*® is 200 genomes/mL for nasopharyngeal aspirate/lavage, 281 genomes/mL for bronchoalveolar lavage, 562

genomes/mL for oropharyngeal swab, 89 genomes/mL (polyester flocked) – 200 genomes/mL (dacron) for nasopharyngeal swab.

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. 99.7% and 99.9% of the available 15324 SARS-CoV-2 nucleotide sequences resulted in 100% identity for the forward and reverse primers respectively; 99.8% of the available 15324 SARS-CoV-2 nucleotide sequences resulted in 100% identity for the probe sequence.

The exclusivity was tested with 43 different viral/bacterial/fungal respiratory tract pathogens 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. The in-silico tests showed that the primers and probe were not homologous to any organism/strain except some SARS-CoV strains other than SARS-CoV-2. The blast search showed that the target region on SARS-CoV-2 genome resembles more than 90% to some SARS-CoV strains, however, detection is not expected as there are no known SARS-CoV strains circulating in the population.

The interfering substance inhibition tests showed that the mucin at 50% (w/v), blood at 50% (v/v), nasal spray (Nasonex) at 10% (v/v), nasal corticosteroids and gels at 10% (w/v), throat lozenges at 10% (w/v), anti-viral at 1% (v/v), antibiotics at 0.1% (w/v) may interfere with the *BioeXsen SARS-CoV-2 RT PCR*

The kit was applied to 451 clinical samples concurrently with another Real-Time RT-PCR kit authorized by the FDA. DNA sequence analysis was applied when the assays were not in agreement. The overall tests resulted in 349 true positives, 94 true negatives and 8 false negatives. Sensitivity and specificity of the *BioeXsen SARS-CoV-2 RT PCR* are 97.8% and 100% respectively.

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material, blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to corroborate the LoD. RNA extraction from the samples was performed using the *vNAT*® buffer included in the kit. The results are summarized in Table 2.

**Table 2.** Summary of LoD confirmation result using the FDA SARS-CoV-2 Reference Panel

| Reference Materials Provided by FDA | Specimen Type | Product LoD (NDU/mL) | Cross-Reactivity |
|---|---|---|---|
| SARS-CoV-2 | Nasopharyngeal Dacron Swab | $1.8x10^3$ | N/A |
| MERS-CoV | | N/A | ND |

NDU/mL = RNA NAAT detectable units/mL
N/A: Not Applicable
ND: Not Detected

## 4. Collection, Storage and Shipment of Clinical Specimens

*Collecting the Specimen*

Nasopharyngeal swabs, oropharyngeal (throat) swabs, combined nasopharyngeal/ oropharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal or nasopharyngeal aspirates, nasal washes and bronchoalveolar lavage samples shall be collected by a healthcare provider in accordance with the updated version of CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19 (https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens. html). Swabs (dacron or polyester flocked) should be placed immediately into a sterile transport tube containing 2-3 mL of viral transport medium (VTM) (Preparation of viral transport medium, Centers for Disease Control and Prevention, SOP#: DSR-052-01). Nasopharyngeal (NP) or nasal aspirate and nasal wash samples should be transferred into sterile containers containing 2-3 mL of VTM (in case of immediate analysis, these samples can be taken into sterile containers by healthcare providers). Bronchoalveolar lavage (BAL) samples should be collected 2-3 mL into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container.

*Transporting Specimens*

Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential 2019-nCoV specimens. Store specimens at 2-8°C and ship overnight to the laboratory on ice pack. If a specimen is frozen at -70°C or lower, ship overnight to the laboratory on dry ice.

*Storing Specimens*

Specimens can be stored at 2-8°C for up to 72 hours after collection. If a delay in extraction is expected, store specimens at -70°C or lower in accordance with the CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19. Extracted nucleic acid should be stored at -70°C or lower. It is important to avoid repeated freezing and thawing of specimens.

## 5. Warnings and Precautions

1. Specimen processing should be performed in accordance with national biological safety recommendations.
2. All patient specimens and positive controls should be considered potentially infectious and handled accordingly. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2 (https://www.cdc.gov).
3. Proper personal protective equipment including lab coats, gowns, gloves, eye protection, and a biological safety cabinet are recommended for manipulation of clinical specimens.
4. The *vNAT*® buffer in the kit contains guanidinium thiocyanate. To avoid the danger of cyanide gas production, bleach or acidic solutions should not be added to sample collection tubes or containers.
5. Immediately clean up any spill containing potentially infectious material with 0.5-1% (w/v) sodium hypochlorite (10-20% v/v bleach). Dispose of cleaning materials in a biohazard waste stockpot. If the spill contains *vNAT*® buffer, do not use bleach or acidic solutions. Due to the danger of cyanide gas formation, clean with a suitable laboratory detergent and water.
6. All personnel who perform aspects of the testing procedures should be trained to work with PCR and microbiology as appropriate. Sampling should be carried out by personnel with sufficient knowledge and experience.
7. The kit should be stored away from nucleic acid sources and PCR amplicons.
8. To prevent contamination of the reaction mixture by previously amplified target sequences, maintain separate work areas, dedicated equipment.
9. Different sets of laboratory coats should be worn pre- and post-PCR.

10. The micropipettes used for pipetting PCR mixes and template nucleic acids should be separate. Filtered and nuclease-free pipette tips should be used.
11. Template nucleic acid and positive control tubes should always be kept closed, except for fluid transfers; tube caps should not be interchanged.
12. For collection of nasopharyngeal/ oropharyngeal swabs, polyester flocked swabs are preferred. Sterile dacron swabs with plastic or flexible metal handles may also be used. Cotton or calcium alginate swabs or swabs with wooden sticks should not be used since they may contain substances that inactivate some viruses and inhibit PCR.
13. It is recommended to use swabs with breakable shaft to prevent contamination during sampling.
14. 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.
15. Master stock reagents should be kept on the cold block during the PCR setup.
16. Kit components should be mixed by gently shaking before use.
17. Maintenance/ calibration interval should be determined for all instruments and equipment used with the kit.
18. Amplified products should not be brought into the reaction setup area. To avoid false positives due to amplified material, the PCR completed reaction tubes should be disposed of before opening in the laboratory.
19. The wipeable surfaces of the rooms, benches, and devices where the analysis is performed should be cleaned regularly with freshly diluted 10% bleach solution (0.5% sodium hypochlorite).
20. Dispose of waste in a designated matter in accordance with local, regional, and federal regulations.

## 6. Preparation of Nucleic Acid Samples (*vNAT*® Application Protocol)

1. Vortex the sample tube at the highest speed for 15 seconds.
2. Transfer 100 µL of *vNAT*® into a clean microcentrifuge tube.
3. Add 100 µL liquid respiratory sample to the tube containing 100 µL *vNAT*®.
4. Vortex the tube at the highest speed for 15 seconds.
5. Incubate the tube for 5 minutes at room temperature.
6. The 200 µL mixture is ready to use in real-time RT-PCR.
7. Store the sample at -20 ° C.

Samples that need to be liquefied, such as bronchoalveolar lavage, can be mixed and homogenized with an equal volume of nuclease-free water or viral transport medium or serum physiologic before nucleic acid extraction.

For the negative extraction control, the same procedures are applied using 100 µL of NTC in the kit instead of the respiratory sample.

## 7. 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.
- 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 PCR volumes can be used for the assay on *Bio-Rad CFX96 Touch™, Roche LightCycler® 96,* and *Qiagen Rotor-Gene®* 5 Plex (72-well rotor) instruments. 10 µL PCR volume is recommended for high test capacity on these instruments. But 20 µL PCR volume should be used on *Qiagen Rotor-Gene® 5 Plex* instruments with 36-well rotor.

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 3).

**Table 3.** Reaction set-up and qPCR program details

| **Reaction setup** | | | **qPCR Program (Set reaction volume to 10 µL or 20 µL)** | | |
|---|---|---|---|---|---|
| **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 |
| Template Nucleic Acid | 2.5 µL | 5 µL | 40 | 95 °C | 1 sec |
| TOTAL REACTION VOLUME | 10 µL | 20 µL | | 55 °C | 30 sec |
| | | | | FAM/HEX read | |

## 8. Interpretation of the Assay Results

Assessment of clinical specimen test results must be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.

Shape of the amplification curves obtained in the FAM/HEX channels are examined and non-sigmoidal curves are recorded as negative. The recommended threshold levels to calculate the number of threshold cycles (Cq) for both 10 µL and 20 µL reactions are 0.05, 200 and 0.02 RFU for *Roche LightCycler® 96, Bio-Rad CFX96 Touch™* and *Qiagen Rotor-Gene® 5 Plex*, respectively. The result is recorded as negative if there is no sigmoidal curve. The result is recorded as positive if Cq<38.

**Table 4.** Positive and No Template (Negative) Control Interpretation

| Positive Control | | Negative Control | | Results |
|---|---|---|---|---|
| ***ORF1ab* (FAM)** | ***RNase P* (HEX)** | ***ORF1ab* (FAM)** | ***RNase P* (HEX)** | |
| Positive (Cq<38.0) | Positive (Cq<38.0) | Negative (No Cq) | Negative (No Cq) | VALID (Continue to result interpretation of patient specimens) |
| Any of them is Negative (Cq not detected) | | Not considered | | INVALID (Reagent stability problem) |
| Not considered | | Any of them is Positive (Cq<38.0) | | INVALID (Contamination problem) |

**Table 5.** Interpretation of Patient Samples (**positive for Cq<38**)

| ***ORF1ab* / FAM** | ***RNase P* / HEX** | **Results Interpretation** | **Action** |
|---|---|---|---|
| Positive (+) | Positive (+) | Results are valid, SARS-CoV-2 RNA is Detected | Report as POSITIVE |
| Positive (+) | Negative (-) | Results are valid, SARS-CoV-2 RNA is Detected | Report as POSITIVE |
| Negative (-) | Positive (+) | Results are valid, SARS-CoV-2 RNA is Not Detected | Report as NEGATIVE |
| Negative (-) | Negative (-) | Results are invalid | If residual specimen is available, re-extract nucleic acid from the specimen and perform the test again. If the result is still invalid, a new specimen should be obtained. If additional clinical sample is unavailable, report as INVALID |

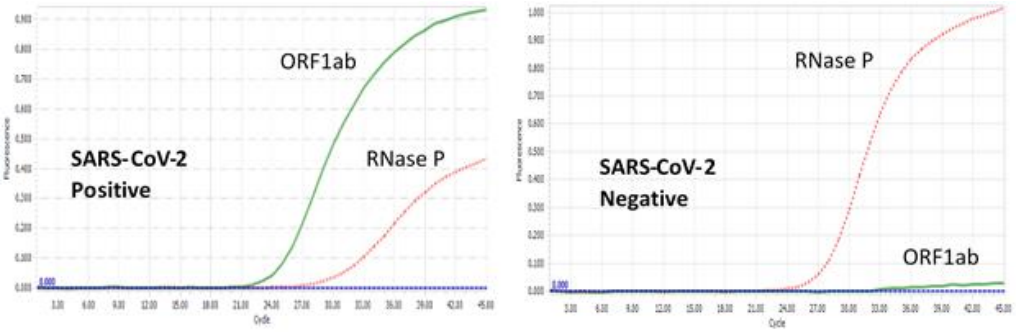


***Figure 1.*** *Examples of the amplification curves.*

## 9. Limitations

- The *BioeXsen SARS-CoV-2 RT PCR* assay has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- Performance of the *BioeXsen SARS-CoV-2 RT PCR* has only been established in nasopharyngeal swab, oropharyngeal (throat) swab, nasopharyngeal aspirate, or lavage and bronchoalveolar lavage samples.
- Mutations within the target regions of the *BioeXsen SARS-CoV-2 RT PCR* 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.
- The in-silico tests showed that the kit may cross-react with some SARS-CoV strains other than SARS-CoV-2.
- The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

## 10. Distributor and Technical Support

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