SARS-CoV-2 RT-PCR Test kit

【Catalog Number】

REF CoV19-PCR

【Package Size】

☐ 48 Tests/box ☐ 96 Tests/box

【Intended Use】

This kit is recommended for detection of the novel coronavirus (2019-nCoV) in biological samples using multiplex direct RT-PCR method without the need of the RNA extraction.

Since its outbreak in December 2019, the COVID 19 became a significant factor in human health. The Novel Coronavirus "2019-nCoV or SARS-CoV-2" virus was identified as the cause of the disease and its detection became one of the most important factors in coping with the pandemic.

The nucleic acid detection location of 2019-nCoV genes is placed in the open reading frame of ORF1ab gene and in the nucleocapsid protein N genes. This kit is designed to enable the high-speed amplification with the test run being possibly completed within 1 hour.

The usage of this kit is limited to medical institutions (not for the home use).

【Test Principle】

This kit uses only highly resistant and stable reverse transcription enzyme, activators and lysing agents in the reaction process. The multiplied segments are located in the open frame of the viral ORF1ab gene, viral nucleocapsid protein N genes and human housekeeping genes. All of the genes are being detected simultaneously, in a single tube, using multiplex real-time RT-PCR technology, facilitating fluorescent probe in the process. Human housekeeping gene is used as the internal quality control feature to monitor the sample quality.

【Content of the Kit】

1. Reaction Mix tube containing PCR primers for virus detection.
2. Enzyme Mix tube containing Hi-Taq Reverse Transcription enzyme mix.
3. Negative Control (NC) tube containing distilled water.
4. Positive Control (PC) tube containing fragments of the target viral sequence and internal quality components.

【Storage and Handling】

1. Store at -20°C for up to 6 months, avoid repeated freezing-thawing.
2. For repeated use, the storage at 4°C in the dark is possible, however only for maximum of 7 days
3. The kit should be transported refrigerated (2-8°C); the transport should not exceed 7 days.

【Applicable Instruments】

ABI 7500、Roche LightCycler480、Bio-Rad CFX96 and other real-time quantitative PCR instruments that are able to meet the detection channel settings for this kit.

【Sample Requirements】

1. The samples for testing are biological material obtained from the individuals with suspected coronaviral infection (nasal / pharyngeal swabs, sputum, lung lavage fluid / fecal material / anal swabs, etc.). The collected samples should be kept on ice in the case, they cannot be processed immediately.
2. The obtained specimens could be used for the RT-PCR testing directly, there is no need of nucleic acid

extraction. The reagents of the kit are highly resistant to potential inhibitors present in the samples, but too voluminous specimens, or samples contaminated with blood may lead to erroneous results. In order to control the false negativity caused by the inhibition of the reaction, the sample volume should not exceed <2μL/reaction. Samples could be eventually diluted as described below.

3. This kit cannot facilitate the samples collected by the inactivated sampling tube, due to the presence of the enzyme inactivators. Samples collected to such tubes must have their nucleic acids extracted and purified before detection. It is also recommended that the samples with very low viral load should be tested after concentration of the viral nucleic acids. This kit can be used also for samples with extracted nucleic acids.

【Testing】

1. Sampling

① **Nasal / throat swabs*:** After the sample is collected to the viral sampling tube and resuspended, transfer 200μl of the collected solution to the centrifuge tube. Centrifuge briefly at the high speed (see below). Discard the supernatant, but leave 10-20μL of the sample at the bottom of the tube. Gently resuspend the pellet (if present) and take 2μL for the test.

② **Deep expectoration, lung lavage / aspirate:** Samples collected to non-viral collection tubes should be transferred to 3ml viral sampling solution NS or PBS, or to the distilled water. Centrifuge briefly. Remove the majority of the supernatant and resuspend the rest. Take 2μL for detection avoiding any floating particles.

③ **Anal swabs:** The samples collected to 3ml virus sampling tubes shall be directly processed after full resuspension (anal swabs containing larger amount of feces shall be treated as feces; see below); take 2μL for detection.

④ **Feces:** Take a proper amount of the material (about the size of the swab head) and transfer it to 3ml viral sampling solution or NS, PBS or distilled water. Resuspend and centrifuge at low speed. Take 2μL of the supernatant for detection, avoid the sediment.

⑤ **Serum, Plasma:** 1:1 dilution and resuspension with NS, PBS or distilled water is recommended, take 2μL for detection.

⑥ **Whole blood** (collection to a non-heparin tube): Dilute and resuspend with NS or PBS solution 1:5. Centrifuge at low speed, take 2μL for detection.

Notes: The sample type marked with * is preferred for this kit. High speed centrifugation refers to 10000rpm for 2min; low speed centrifugation refers to 3000rpm for 30sec. When the specimens are not being used for other diagnostic purposes (culture), it is recommended to inactivate them at 56°c for 30min before processing.

2. System preparation

After removal from the storage, let all the reagents thaw thoroughly. Centrifuge the content of the vials to concentrate it to the bottom of the tubes before each use. For the “n” number of the test reactions prepare the reagent mixture by mixing the “n” times together the following components:

Reaction Composition	Dosage (μl/reaction)
Reaction Mix	18
Enzyme Mix	2
Distilled water	3
Sample/PC/NC	2

Take the 25μl aliquot to each test vial. Add 2μL of the specimen per vial.

3. Amplification

This kit is designed for high-speed PCR with high amplification efficiency (instrument support is required). The conventional, standard PCR amplification is possible as well.

Amplification parameters:

① **Standard amplification**

50°C 10min;

95°C 10sec;

45 cycles:

95°C 10sec, 55°C 30sec (read)

② **High-speed amplification**

50°C 10min;

95°C 10sec;

5 cycles:

95°C 5sec, 55°C30sec

40 cycles:

95°C 3sec, 55°C10sec (read)

③ **Detection channels**

ORF1ab：FAM, 520nm

N：HEX/VIC, 555nm

IC：CY5, 668nm

Note: Please set the corresponding wavelength for the Roche device. Set the reference fluorescence of ABI device to “None” and the quenching genes parameter to “None”.

【Interpretation of the Results】

1. The typical "S" shaped amplification curve of the target gene indicates that if Ct value is ≤38, the sample is positive, if Ct is more than 40 or no amplification is detected, the sample is negative, Ct values between 38-40 are inconclusive and the sample has to be retested.
2. If the two target genes are detected in the sample, the results of the test can be declared as positive for COVID-19 nucleic acid. If only one of the target genes is detected and the internal reference value (IC) is less than 30, the sample should be retested.
3. If no target genes are detected, the test results could be declared as negative.

【Quality Control】

1. For the test results being valid, the negative control (NC) should not contain any amplified DNA. Positive control (PC) and internal control samples (IC) are amplified. The Ct value <30 indicates that the experiment ran properly. If these criteria are not met, results are invalid and the test needs to be repeated.
2. If the IC parameter is <30, the sample quality is acceptable. If IC parameter is >30 the results are not acceptable and resampling is warranted.
3. Internal control (IC) uses human housekeeping genes for amplification (using CY5 detection channel). Human samples (anal swabs, pharyngeal swab, etc.) should be positive for housekeeping genes. Using the IC parameter, the sample collection quality and presence of the PCR inhibitors in the sample could be assessed.

【Performance Characteristics】

1. Specificity of this test is: 96.15%, Sensitivity: 95.65%.
2. Limits of the Detection (LOD): LOD values are: 500, 700 and 400 copies/ml for ORF1ab, N genes and IC, respectively.
3. The linear detection range is 500-2 × 10 ^ 7 copies / ml.
4. No cross reactions with following coronaviruses (or other viruses) has been found: Human coronaviruses (hku1, OC43, nl63 and 229E), SARS coronavirus and MERS coronavirus; H1N1 (new H1N1 influenza virus (2009), seasonal H1N1 influenza virus), H3N2, H5N1, H7N9, influenza B Yamagata, Victoria, respiratory syncytial virus A, B, parainfluenza virus 1, 2, 3, rhinovirus a, B, C,

adenovirus 1, 2, 3, 4, 5, 7, 55, enterovirus a, B, C, D, human lung virus, human partial lung virus, Epstein Bar Virus, measles virus, human cytomegalovirus, rotavirus, norovirus, mumps virus, and varicella zoster virus. Also, no cross reaction with bacterial cells such as Mycoplasma pneumoniae and Chlamydia pneumonia was detected.

【Test Limitations】

The test results could be affected by the viral load in the sample (too high or too low), the sample collection quality, its storage and transportation. Other, potentially limiting factors are: the environmental conditions during the test (possible contamination), insufficient experience of the personnel, and unsatisfactory status of the instrument. The quality control measures of this kit are conducive to the detection of false negatives, but cannot completely rule out false negative or false positive results. When the test results are used for clinical diagnosis, we strongly recommend to use them in conjunction with the clinical data. The kit results alone should not be used for the diagnosis confirmation or exclusion.

【Warnings and Precautions】

1. Testing is recommended to be performed in a biosafety cabinet to avoid possible contamination.
2. Ware protective cloths, gloves, masks and goggles.
3. The kit should be stored in dark, at -20°C, if storage for a prolonged time is required.
4. Each kit component has been performance optimized, do not mix it with any other reagents.
5. Standard amplification and high-speed amplification process might produce slightly different Ct values due to different reaction conditions. This should not have a significant impact on quality of the results;
6. For the production and expiration dates see the kit's outer package.

【Kit and Samples Disposal】

The test samples shall be considered as infectious material. Reagent and sample handling shall be in accordance with the local requirements, rules and regulations with the special attention to the biosafety regulations. All the used samples and reagents shall be disposed according to the local biohazardous waste regulations.

【Contact Information】

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	Manufacturer
EC REP	Authorized EU Representative
	Expiration Date
LOT	Batch Code
REF	Catalog Number
	Consult Instructions
IVD	In Vitro Diagnostic Medical Device