# Novel Coronavirus (2019-nCoV) RT-PCR Detection Kit
# Instruction for Use

| Label | Description |
|---|---|
|  | Contains sufficient for <N> tests |
|  | Batch number |
|  | Date of manufacturing |
|  | Date by which the device should be used |
|  | Name and address of Manufacturer |
|  | In vitro diagnostic |
|  | Consult instruction before use |
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|  | Temperature limitation |

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**【PRODUCT NAME】**

Novel Coronavirus (2019-nCoV) RT-PCR Detection Kit (Commercial names: Fosun 2019-nCoV qPCR)

**【SIZE】**

32 tests/kit, 48 tests/kit, 96 tests/kit

**【INTENDED USE】**

This product is intended for the rapid detection of 2019-nCOV in human throat swab or sputum samples.

Coronavirus is a +ssRNA virus with envelope. Its diameter is about 80-120 nm. Its genetic material is the largest of all RNA viruses. It is an important pathogen of many livestock, pets, including human diseases, and can cause many kinds of acute and chronic diseases. According to *Virus Taxonomy-- Ninth Report of the International Committee on Taxonomy of Viruses*, the Coronaviridae is divided into three genera: α, β and γ. Among them, α Coronavirus (such as human Coronavirus 229E, human Coronavirus NL63), β Coronavirus (such as human Coronavirus HKU1, human Coronavirus OC43 and SARS) can cause disease of varying degree to human.

The common signs of people infected with coronavirus are respiratory symptoms, fever, cough, shortness of breath and dyspnea. In more serious cases, infection can lead to pneumonia, severe acute respiratory syndrome, renal failure, and even death. The "2019-nCoV" was named by WHO in January 12, 2020.

According to the confirmed case request in *New Coronavirus Pneumonia Prevention and Control Program (6$^{th}$ ed)*, clinical diagnosis case or suspected case has the following pathogenic evidences: RT-PCR detection result of 2019-nCOV in respiratory tract or blood sample is positive. This kit is intended for the detection of 2019-nCOV and is helpful for clinical diagnosis of 2019-nCOV infection.

**【PRINCIPLE OF DETECTION】**

This product is a fluorescent probe-based Taqman RT-PCR assay system. Firstly, the RNA of 2019-nCov will be reverse transcribed into cDNA by reverse transcriptase, and then PCR amplification will be performed with cDNA as template. During amplification of the template, the Taqman probe will be degraded due to the 5'-3' polymerase activity and exonuclease activity of Taq DNA polymerase, then the separation of fluorescent reporter and quencher enables the fluorescent signal to be detected by instrument. The ORF1ab gene of 2019-nCoV will be detected qualitatively by FAM channel, the N gene of 2019-nCoV will be detected qualitatively by JOE channel, the E gene of 2019-nCoV will be detected qualitatively by ROX channel, and the internal reference will be detected by CY5 channel.

dUTP and UNG enzyme are used in the kit to prevent contamination of the amplified products.

Internal reference is used in the kit for quality control starting from sample collection.

## 【PRODUCT CONTENTS】

| Components | Amount | | | Amount per reaction | Ingredient |
|---|---|---|---|---|---|
| | 32 Tests/kit | 48 Tests/kit | 96 Tests/kit | | |
| 2019-nCoV Reaction Reagent | 448 μL | 672 μL | 672 μL*2 | 14 μL | dNTPs, $MgCl_2$, Primers (ORF1ab gene, E gene and N gene of 2019-nCOV), Probes |
| RT-PCR Enzyme | 192 μL | 288 μL | 288 μL*2 | 6 μL | Taq DNA polymerase, Reverse Transcriptase, UNG enzyme |
| Positive Control of 2019-nCoV | 200 μL | 200 μL | 200 μL*2 | - | RNA template |
| Negative Control | 200 μL | 200 μL | 200 μL*2 | - | NaCl |
| Internal Reference A | 160 μL | 240 μL | 240 μL*2 | 5 μL | RNA template |

Note: Do not mix the components from different batches for detection. The positive control of 2019-nCOV and internal reference were constructed artificially, and they were not infectious.

## 【STORAGE & SHELF LIFE】

All reagents should be stored at -15℃~-25℃ with protection from light, and the reagents are stable for 12 months (to be determined) when stored at the recommended condition. See label for production date and expiration date.

The kit should be transported by cold chain transport or sealed foam box with ice. The temperature should be controlled below -8℃ and the transportation time should not exceed 4 days. Repeated freeze-thaw should be less than 5 times.

## 【INSTRUMENTS】

Our recommendation for platform to use Novel Coronavirus (2019-nCoV) RT-PCR Detection Kit: Real-time PCR instrument-- Roche LightCycler 480、Life Technologies 7500、SLAN-96P.

## 【SAMPLING & HANDING】

(1) Throat Swab: Use the plastic rod swab with polypropylene fiber head to wipe the bilateral pharyngeal tonsils and the posterior pharyngeal wall at the same time, immerse the swab head into the tube containing physiological saline, discard the tail, and tighten the tube cover.

(2) Sputum: Cough up the sputum in the deep part of the respiratory tract and collect it in the container. Liquefying method: add equal volume of acetylcysteine (10 g/L) into the sputum sample, shake at room temperature for 30 minutes, and then carry out RNA extraction after sufficient liquefying.

(3) Bronchoalveolar Lavage: Collect bronchoalveolar lavage for test.

The collected sample should be used for detection as soon as possible. If the sample need to be transferred cannot be detected immediately, please store it at low temperature.

The sample can be stored for 24 hours at 2~8℃ and for a long time below -70℃. It can also be stored in refrigerator at -20℃ temporarily.

Samples shall be transported at low temperature in accordance with biosafety regulations.

## 【PROTOCOL】

1. Reagent Preparation

Prepare reagent with ice box, and prepare reaction reagent according to the number of reaction samples (number of reaction samples, n = number of samples to be tested + 2 control samples + 1):

Add n × 6 μL of RT-PCR enzyme and n × 14 μL of 2019-nCOV reaction reagent into the centrifuge tube, mix by shaking, and centrifugate at low speed for a few seconds, then make aliquots of 20 μL into different PCR reaction tubes. The reaction tubes can be placed at 2~8℃ for 3 hours after separation.

2. RNA Extraction

It is recommended to use the RNA extraction and purification reagent (general type) produced by our company, QIAamp Viral RNA Mini Kit (Qiagen) and NX-48 Viral RNA Kit (Genolution) to extract RNA from sample and reference sample.

The volume of sample to be extracted is 200 μL, and 5 μl of internal reference A will be added to each sample (including the reference); after RNA extraction, the extracted RNA shall be added to the reaction tubes within 10 minutes, or transferred to the centrifuge tubes and stored at -15 ℃~-25 ℃.

3. Template Addition

Add 10 μL of extracted Negative Control, 10 μL of extracted Positive Control, and 10 μL of extracted RNA from sample to different PCR reaction tubes. Centrifuge them at low speed. Then, move them to the Real-time PCR instrument.

4. PCR Amplification

Step1: 50℃ for 15 minutes, 1 cycle;

Step2: 95℃ for 3 minutes, 1 cycle;

Step3: 95℃ for 5 seconds to 60℃ for 40 seconds, 5 cycles;

Step4: 95℃ for 5 seconds to 60℃ for 40 seconds, 40 cycles. The signals of FAM, JOE, ROX and CY5 fluorescence channels will be collected at 60℃.

Note: Select "None" from the pull-down menu of the passive reference on operation interface of ABI7500 RT-PCR software.

5. Data Analysis (ABI7500)

Test data file need to be saved after PCR reaction. Please set the parameters and analysis the results of FAM, JOE, ROX and CY5 channels respectively.

(1) Baseline setting: the baseline can be set automatically or adjusted according to the shape of amplification curve.
(2) Threshold setting: the threshold value should be higher than the highest fluorescence value of negative control in this kit.

6. Quality Control

Negative control and positive control provide the calibration for the kit, and shall be set for each test. The result is valid if ALL the below criteria is met. Otherwise, the test is invalid. In this case, the errors of instruments, reagents, amplification conditions, etc. shall be checked, and the experiment shall be repeated.

| Products of Quality Control | Requirements of Quality Control | | | |
|---|---|---|---|---|
| | FAM Channel | JOE Channel | ROX Channel | CY5 Channel |
| Positive Control of 2019-nCoV | Ct ≤ 30 | Ct ≤ 30 | Ct ≤ 30 | No requirement |
| Negative Control | Undet | Undet | Undet | Ct ≤ 32 |

7. Interpreting Test Results

| Interpreting Test Results of each channels |
|---|
| FAM has amplification signal, Ct ≤ 36, and amplification curve is typical S shape, then ORF1ab gene (+); otherwise, ORF1ab gene (-). |
| JOE has amplification signal, Ct ≤ 36, and amplification curve is typical S shape, then N gene (+); otherwise, N gene (-). |
| ROX has amplification signal, Ct ≤ 36, and amplification curve is typical S shape, then E gene (+); otherwise, E gene (-). |
| If the Ct of FAM, JOE and ROX is more than 36 or no value; and the Ct of CY5 is more than 32 or no value, then there is a problem with the sample or operation, which needs to be retested. |

According to the above channel detection results, the judgment results are as follows:

| Test Results | Interpreting Test Results |
|---|---|
| ORF1ab gene (+), N gene (+), E gene (+);<br>OR ORF1ab gene (+), N gene (+), E gene (-);<br>OR ORF1ab gene (+), N gene (-), E gene (+);<br>OR ORF1ab gene (-), N gene (+), E gene (+). | 2019-nCoV (+) |
| Only ORF1ab gene (+) | Test again, and if repeated: 2019-nCoV (+) |
| Only N gene (+) or E gene (+) | 2019-nCoV (-) |
| ORF1ab gene (-), N gene (-), E gene (-) | 2019-nCoV (-) |

**【CUT-OFF VALUE OR REFERENCE INTERVAL】**

The cut-off value of 2019-nCoV is Ct ≤ 36.

**【ASSAY EXPLAINATION】**

1. The contamination of laboratory environment and reagent, or cross contamination during specimen treatment may lead to false positive result.

2. The decrease of detection effect even the false negative result may occur if there is any mistakes in the transportation, storage and operation of reagents.

3. 2019-nCOV early infection or other respiratory virus infection can't be excluded in patients with negative results. If conditions permit, it is recommended to collect more sensitive samples such as sputum or bronchoalveolar lavage for retest.

**【ASSAY LIMITATIONS】**

1. The positive result detected by this kit can't indicate whether there is virus in vivo. It is suggested to use other methods for confirmation at the same time.

2. This kit is intended for classification and detection of 2019-nCoV. The result is only for clinical reference, and the clinical management of patients should be considered in combination with their symptoms/signs, history, other laboratory tests and treatment responses.

3. Although the detected target sequences of this kit are the conservative region of 2019-nCoV's gene, the missed detection of coronavirus types with rare mutations in the conservative region can't be completely avoided in theory.

**【PERFORMANCE SPECIFICATIONS】**

Conformity rate of Negative Control: detection results of 2019-nCoV were negative in 15 enterprise reference samples (T1-T15), and the conformity rate of negative control (-/-) should be 15/15.

Conformity rate of Positive Control: detection results of 2019-nCoV were positive in 5 enterprise reference samples (Y1-Y5).

Detection limitation: 300 copies /mL.

Repeatability: The test results of enterprise reference samples (J1-J2) were all positive after 10 repetitions, and the coefficient of variation (CV) of J1's Ct value is less than 5.0%.

Precision: 5 days of continuous testing, 2 times a day for each person, 4 repetitions for each sample, and the coefficient of variation (CV) of their Ct value is less than 5.0%.

Specificity: non-specific interference of other related pathogens (Coronavirus (229E, HKU1, OC43, NL63), Influenza A Virus (H1N1, H3N2), Influenza B Virus, Respiratory Syncytial Virus (type A, B), Parainfluenza virus (type 1, 2, 3, 4a, 4b), Rhinovirus (type 1A, 1B, 14, 57), Adenovirus (type 3, 7), Enterovirus (type 71); Mycoplasma pneumoniae, Chlamydia pneumoniae; Legionella pneumophila (type 1, 2, 4, 6, 14), Legionella feeleii (type 1), Legionella birminghamensis, Legionella micdadei, Legionella sainthelensi, Bordetella pertussis, Haemophilus influenzae (type B, C), Staphylococcus aureus, methicillin-resistant Staphylococcus aureus, methicillin-sensitive Staphylococcus aureus, Streptococcus

pneumoniae (type 1, 3, 5, 6, 14, 19), Streptococcus pyogenes, Klebsiella pneumoniae, Candida albicans, Moraxella catarrhali, Pseudomonas aeruginosa, Staphylococcus epidermidis, Shigella dysenteriae, Campylobacter jejuni, Bacillus cereus, Enterococcus faecium, Enterococcus faecalis, Vibrio parahaemolyticus, Salmonella, Listeria monocytogenes, Enterobacter sakazakii). Normal concentrations of hemoglobin, albumin, mucus, antifungal drugs and other interfering substances were added to the simulated samples, which did not interfere with the test results.

The preliminary results of clinical trial: Conformity rate of Positive Control=100.0%; Conformity rate of Negative Control=97.8%; Overall coincidence rate = 98.4%.

**【ATTENTIONS】**

1. The kit is only used for in vitro diagnosis.
2. Please read this manual carefully before beginning the experiment.
3. All equipment used in the experiment shall be sterilized.
4. Unreasonable sample collection, transfer, storage and operation may lead to wrong test results.
5. RNA extraction shall be carried out as soon as possible after sample collection to avoid degradation. If it cannot be carried out immediately, it shall be stored in accordance with **[SAMPLING & HANDING]**.
6. After the operation of the nucleic acid extractor, the used consumables shall be sealed. After the instrument is cleaned, turn on the ultraviolet lamp for 30 minutes.
7. As this test involves the extraction of viral RNA and PCR amplification, please take care to avoid contamination of the amplification reaction mixture. Regular monitoring of laboratory contamination is recommended.
8. When using this kit, please strictly follow the instructions. The collection, storage and transfer of samples, the extraction and detection of RNA, and the interpretation of results must be carried out in strict accordance with the requirements of the kit instructions. The processes of sample preparation and addition must be carried out in the biosafety cabinet or other basic protective facilities according to the technical requirements of the clinical gene amplification laboratory.
9. 2019-nCOV has strong transmission ability and high-risk coefficient. Personal protection should be a three-level laboratory level of biosafety. The operator must have professional skills and PCR inspection qualification. During the whole operation process, it is necessary to prevent the infection risk of aerosol pollution, and the operator must add samples and use reagents and consumables accurately.
10. To prevent virus spreading, the 2019-nCOV must be detected in a biosafety level 2 (P2) or above laboratory. Laboratory management should strictly follow the management standard of PCR gene amplification laboratory, and the experimental operation must be strictly partitioned. The instruments, equipment, consumables, work clothes used in each region must be distinguished strictly and can't be used intercross to avoid contamination.
11. All test samples shall be regarded as infectious substances. During the experiment, work clothes shall be worn, disposable gloves shall be worn and replaced frequently to avoid cross contamination between samples. The operation of sample and waste shall meet the requirements of relevant laws and regulations such as *The general guidelines for biosafety of microbiological biomedical laboratories* and *The regulations on the management of medical wastes* issued by the Ministry of Health.

**【REFERENCE】**

[1] 《新型冠状病毒感染的肺炎实验室检测技术指南（第四版）》Guidelines for laboratory testing of new coronavirus pneumonia prevention. 2020.
[2] 《新型冠状病毒感染的肺炎诊疗方案（试行第六版）》 New coronavirus pneumonia prevention and control program (6th ed) (in Chinese). 2020.
[3] Clinical management of severe acute respiratory infection when novel coronavirus (nCoV) infection is suspected. WHO. 2020.

**【General Information】**

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**【PRODUCT REGISTRATION No. / TECHNICAL REQUIPMENT No.】**

**【DATE OF APPROVAL AND MODIFICATION OF INSTRUCTION】**

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