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COVID-19 OFR1ab / N /E Triple Nucleic Acid Detection Kit (RT-qPCR)

Product name	Catalog #	
	KN003-24	KN003-48
COVID-19 OFR1ab/N/E Triple Nucleic Acid Detection Kit (RT-qPCR)	24 Tests /Kit	48 Tests /Kit

Description:

Novel coronavirus pneumonia (NCP) that was officially named by the WHO as “Corona virus disease 2019” (COVID-19), is a respiratory infection caused by a new virus that was first identified in late 2019.

This kit utilizes fluorescence quantitative probe-based PCR and guarantee a high specificity to ensure accurate one-step identification of ORF1ab ,N and E genes of 2019-nCoV. The kit offers a highly sensitive test with a limit of detection as low as 500 copies/ml. The results are available within 1.5 hour.

Kit Contents:

Product name	(48 Tests /Kit)
Triple Enzyme solution C	50 µl
Triple Ncvo-O / N/E reaction solution C	1 ml
Triple Ncvo- O / N/E positive control C	200 µl
Triple Negative control C	200 µl

Note: The kit components of different lots can not be interchanged.

Storage and Transportation:

Store at -20℃±5℃. Can be stored at 4 ° C for a short period of time. Shelf life 1 year.

Repeated freezing and thawing ≤5 times.

Protect from light.

Applicable equipment:

ABI series, Bio-Rad, Roche series and other fluorescent real-time PCR instruments.

Specimens requirements:

Specimen type: nasopharyngeal swab from suspected infection; virus cell culture fluid, etc.

2. Specimen collection:

Pharyngeal, respiratory samples. Use a cotton swab to take secretions sample. Place the swabs in a centrifuge tube containing 3.0ml Virus preservation fluid and send them for inspection immediately.

Basic Protocol:

1. Sample preparation.

Take the test sample and extract the RNA nucleic acid according to the instructions of the nucleic acid extraction kit. The nucleic acid extraction product should be stored at -20 ° C.

2. Reaction mixture preparation

According to the total number of test samples, the number of PCR reaction tubes needed is "N". N = number of samples + 1 negative control + 1 positive control.

The following protocol is recommended for a 20 µl reaction volume. If the volume of reaction changes, please adjust proportionally.

Components	Volume
Dual Enzyme solution C	1 µl
Triple Ncvo-O / N/E reaction solution C	19µl
Final Volume:	20 µl

3. Sample adding.

Add 5 µl each of the extracted RNA, positive and of negative control to corresponding reaction tubes. After assembling all the components, cover the tube caps and gently mix the contents of the tube, mix well, and centrifuge briefly.

4. Perform quantitative PCR

Place the reaction tube inside a real-time PCR instrument.

Set the channel and sample information, reaction system volume 25 µl.

Select the following channels: FAM channel for nCOV-ORF1ab, VIC channel for nCOV-N, CY5 channel for nCOV-E. Perform quantitative PCR using recommended cycling parameters settings:

Step	Temperature	Time	Number of cycles	Fluorescence Detector
1	50°C	15 min	1	Off
2	95°C	3 min	1	Off
3	95°C	10sec	45	Off
	60°C	30 sec		On

Result analysis:

1) Set the baseline: Generally, it is set to 6-15cycle for ABI 7500, 7700 and other instruments, 3-15cycle for PE5700, and 6-12cycle for MJ Research Option2. Under special circumstances, the baseline can be adjusted accordantly.

2) Set the threshold: The threshold line just exceeds the highest point of the negative control amplification curve (random noise line).

Result determination:

1) Positive: The Ct value of the two channels of the test sample is ≤ 38.0, and the curve has a significant exponential growth period, the result is determined to be positive;

2) Suspicious: Any test sample with a Ct value > 38.0 and < 40.0, repeat the experiment, if the Ct value is still < 40.0, and the curve has a significant exponential growth period, it is positive, otherwise it is negative;

3) Negative: If the Ct value is not detected or the Ct value is >40, the result is negative.

Quality control standards:

1) Negative quality control: no logarithmic growth phase or Ct value display in the amplification curve;

2) Positive quality control: the amplification curve has a significant logarithmic growth phase, and the Ct value is ≤30;

The above conditions should be met at the same time, otherwise the experiment is deemed invalid.

Limitations of detection method:

- The test results depend on the quality of sample collection, processing, transportation and preservation;
- During the sample extraction process, precautionary measures should be taken to avoid cross-contamination, otherwise it may lead to false positive results;
- Leakage of positive controls and amplification products my lead to false positive results;

- Contaminated consumables and equipment used during the experiment may cause false positive results.
- Different extraction methods have different extraction efficiency, which may lead to false negative results;
- Incorrect sample collection, transfer and processing, low pathogen content in the sample or improper reagent preparation may cause false negative results;
- Variations in the target sequence of the pathogen or sequence changes caused by other reasons may lead to false negative results;
- The test results are for reference only. If a diagnosis is required, please combine with clinical symptoms and other testing methods.

Precautions:

- All operations should be performed strictly in accordance with the protocol;
- The components of the kit should be thawed at room temperature before use, thoroughly mixed and centrifuged briefly;
- N-C buffer should be protected from light;
- Use disposable tips, g tubes loves, and work clothes for each area;
- Sample processing, reagent preparation and sample addition should be performed in different areas to avoid cross-contamination;
- The workbench and various experimental items should be disinfected with 10% sodium hypochlorite, 75% alcohol, and UV lamps after each experiment.
- The test samples involved in this kit should be considered as infectious substances, and their handling and handling must meet the relevant requirements of the General Guidelines for Biosafety of Microbial Biomedical Laboratories and the Medical Waste Management Regulations of the Ministry of Health