Real-Time PCR Kit for Rapid Detection of 2019-nCoV *E* gene

【Product name】

Real-Time PCR Kit for Rapid Detection of 2019-nCoV *E* gene

【Cat No.】A7721-50T

【Packing specification】50 tests/kit

【Intended use】

The kit is suitable for qualitative detection of 2019-nCoV *E* gene extracted from clinical samples or virus cultures. The experimental results are for reference of basic research only, not for clinical diagnosis.

【Storage conditions and expiration date】

The kit should be stored in the dark at-20 ℃ ± 5 ℃ and is valid for 12 months.

Avoid repeated freezing and thawing.

Date of manufacture and expiry date see label.

Transportation conditions: cold chain or dry ice transportation below 0℃.

【Applicable instrument】

Fully automatic fluorescent PCR detector with channel-calibrating, and the FAM detection channel is necessary.

【Test Principle】

The kit uses real-time fluorescent PCR technology to design primers and probes to target the conserved and specific regions of the *E gene* of 2019-nCoV. During PCR amplification, the probe binds to the template, and the 5'-end reporter group of the probe is cleaved by the Taq enzyme (5'→3' exonuclease activity), thereby moving away from the quenching group to generate a fluorescent signal. The real-time amplification curve is automatically plotted based on the detected fluorescence signal, and the sample Ct value (the number of cycles experienced when the fluorescent signal in each reaction tube reaches the set domain value) is calculated. FAM fluorophore is used to label *E* gene probe in the reaction solution.

【The main components】

No.	Components	Specifications and quantity
1	Nuclease-free water	1000μL×1 tube
2	2×Nucleic acid amplification reaction solution	500μL×1 tube
3	20×Reverse transcriptase	50μL×1 tube
4	10× Primer and Probe reaction solution(A7721)	100μL×1 tube
5	Positive control(E)	50μL×1 tube

Note: The different lots of components CANNOT be exchanged.

【Sample requirements】

RNA samples extracted from clinical samples or virus cultures.

【Test method】

1. Sample preparation

RNA extraction from clinical samples or virus culture should be according to the corresponding manual in the virus RNA extraction kit. The extracted RNA can be detected as soon as possible after preparation. If the sample is not detected immediately after extraction, it should be stored at -70 ℃ for standby, avoiding repeated freezing and thawing.

2. System preparation:

1).Take out the reagent and let the reagent thaw completely. Invert the mixture and centrifuge immediately. One or N test reactions (N = number of samples to be tested + positive control + negative control) are prepared for reaction systems, respectively, as follows.

Components	1 reaction	N reactions
Nuclease-free water	2μL	2μL×（n+1）
2 × Nucleic acid amplification reaction solution	10μL	10μL×（n+1）
20×Reverse transcriptase	1μL	1μL×（n+1）
10× Primer and Probe reaction solution(A7721)	2μL	2μL×（n+1）

2).Reaction distribution: The reaction solution was mixed and centrifuged, and each tube was dispensed in an amount of 15 μL in a PCR tube suitable for a fluorescence PCR apparatus.

3). Loading: 5 μL of the extracted sample nucleic acid and positive control nucleic acid are added to the reaction system, and the total reaction volume was 20 μL.

3. Cycle parameter setting:

Program	Number of cycles	Temperature	Reaction time	
1	1	45℃	10min	
2	1	95℃	5min	
3	45	95℃	15sec	
		60℃	45sec	Fluorescence Collection

The detection channel is set to FAM, corresponding to *E* genes of the tube. "Quencher Dye" and "Passive Reference" are set to “None” for the ABI 7500 instrument.

4. Threshold setting

The principle of the threshold setting is to choose the highest point of fluorescence signal just exceeding the normal negative control as the threshold line, or adjust it according to the Signal-to-noise ratio of the results.

5. Quality control standard

The experimental results are valid if there is no amplification curve in blank control and negative control, and there is S-type amplification curve in FAM detection channel of the positive control. Otherwise, the experimental results are invalid. .

6. Calculation and Interpretation of test results

A) The 2019-nCoV *E* gene is positive if there is S type amplification curve in the FAM channel and Ct value≤36;

B)The 2019-nCoV *E* gene result is not definite if there is S type amplification curve in the FAM channel, and 36 < Ct value ≤ 40. It is necessary to RE-test the nucleic acid after re-extraction. It is judged that the *E* gene is positive if there is S type amplification curve in the FAM channel and Ct value≤40. Otherwise, the 2019-nCoV *E* gene is negative.

C)The 2019-nCoV *E* gene is negative if there is no significant S-type amplification curve in the FAM channel or Ct value＞40.

Note: It is recommended to re-test the sample of the same patient if the *E* gene of the sample is positive. The 2019-nCoV is positive if the 2019-nCoV *E* gene of the sample is still positive.

【Limitations】

1. The target sequence of 2019-nCoV *E* gene is conserved region and the target gene is highly conserved and stable. But mutations in the target sequences or sequence changes due to other causes may result in false negative results.

2. Unreasonable sample collection, delivery and storage conditions, and low virus content in the sample may all lead to false negative results.

3. Unverified other interference or PCR inhibitors may result in false negative results.

【Product performance】

1. Limit of detection: 200 copies /mL.

2. Fluorescence quantitative linear range：500~2×10^{10}copies/mL.

3. Cross-reactions: there is no cross-reactions with other pathogens (parainfluenza virus ,rhinovirus , respiratory syncytial virus, adenovirus, measles virus, mumps, Human coronavirus NL63/229E/OC43/HKU1, cytomegalovirus, Bordetella pertussis, Human metapneumovirus, Enterovirus Mycoplasma pneumoniae, Pseudomonas aeruginosa, Streptococcus pyogenes, Streptococcus salivarius salivarius).

【Precautions】

1. Please read the instruction carefully before use and operate in strict accordance with the requirements.
2. Each component in the kit should be fully melted and mixed, and centrifuge with high-speed and short-term before use.
3. The kit must be kept in the dark to avoid the decay of fluorescent substances. The centrifugal pipe and tip used should be autoclaved and be free of DNase and RNase.
4. The whole operation process, and the software and hardware facilities of PCR laboratory should meet the requirements of the regulations such as *the Administrative measures for clinical gene amplification Laboratory of medical institutions* and *the Guidelines for clinical gene amplification laboratory in medical institutions* certificated by the NMPA or other authorities. The waste and amplification products produced in the process of experiment should be properly treated to prevent cross contamination.

【Instruction of symbol】

IVD	IVD product		Manufacturer
	Consult instruction for use		Store at RT
	Expire date		Keep dry
	Date of manufacture	REF	Catalogue number
LOT	Batch code		Fragile,handle with care
	Keep away from sunlight	EC REP	European union representative

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