User Manual

IVD CE

Document number: BT/CE-PCR-06 rev.A/0

Instruction for use

Novel Coronavirus 2019-nCoV Nucleic Acid Detection Kit (Real Time PCR)

Product name

Novel Coronavirus 2019-nCoV Nucleic Acid Detection Kit (Real Time PCR)

Packing specification

50 test/kit.

Intended use

This detection kit is suitable for qualitative detection of Novel coronavirus 2019 nucleic acid from human nasal cavity, pharyngeal secretions swab, sputum, alveolar lavage solution and feces in vitro. This product is used to assist diagnosis and epidemiological surveillance of novel coronavirus 2019 infection.

Test principle

This product adopts the technology of RT-PCR amplification and fluorescence probe and designs specific primers and probes respectively for novel coronavirus 2019 (ORF1ab gene and N gene). Detection of novel coronavirus 2019 nucleic acid was realized by fluorescence quantitative PCR.

Main component

Reagent components and specifications included in the product:

Component	size（50 test/kit）
2019-nCoV qRT-PCR mixed liquid	770μL / unit*1unit
2019-nCoV RTP enzyme	28μL/ unit*1unit
2019-nCoV DNP enzyme	28μL / unit*1unit
2019-nCoV primer probe（ORF1ab and N, internal label）	110μL / unit*1unit
2019-nCoV positive reference	50μL / unit*1unit
2019-nCoV negative reference	400μL / unit*1unit

Note:

- Different batches of kits are not used interchangeably.
- Self-provided reagent: RNase-free H_2O or using the water from the kit, nucleic acid extraction kit.
- Self-provided instruments and consumables: Real-time fluorescence quantitative PCR instrument, centrifuge, pipette, pipette sucker, quantitative PCR reaction tube (96-hole plate or 8-tube).

Storage conditions and expiration date

Storage condition: -20±5℃in dark. After opening the package, it should be kept in -20±5℃ in dark storage after use, and the repeated freeze-thaw times should not exceed 5 times. It is recommended that the kit which is opened and thawed repeatedly be used up as soon as possible.

Validity period: 6 months.

Applicable instrument

The optimal equipment of this product is ABI 7500 real-time fluorescence quantitative PCR instrument. Other equipment such as Agilent Mx3000p/3005, Roche LightCycler 480/cobas z 480, QIAGEN rotor-gene Q, bio-rad CFX96, HONGSHi and BORI can also be used, which need specific verification.

Sample requirement

1. Sample collection:
   (1) Nasopharyngeal swab: swab the secretions of the nasal cavity and pharynx with a sterile swab, place them in a sterile test tube (containing 1mL sterilized normal saline), pack the test tube tightly with a sterile cotton ball, and then hermetically sealed for sample inspection.
   (2) Sputum: before retention, rinse mouth with water for 3 times, forcefully cough up sputum deep in the respiratory tract, spit into the sterile sputum collector. Patients with deep sputum is not easy to cough out, can be in the sputum before beating the back, help to discharge sputum. The amount of sputum collected should not be less than 1mL.Method of liquefication: equal volume of acetylcysteine (10g/L) was added to the sputum sample, and the sample was oscillated at room temperature for 30 minutes.
   (3) Alveolar lavage fluid: collect bronchoalveolar lavage fluid and hermetically sealed for sample inspection.
2. Sample storage: samples can be stored for 24 hours at 2℃ ~ 8℃, and long-term storage at -70℃.
3. Sample transportation: samples shall be transported at low temperature, and the transportation of samples shall comply with the national biosafety regulations on type ii pathogens.

Test method

1. **Nucleic acid extraction**
   Select the appropriate nucleic acid extraction kit to extract the viral nucleic acid, the specific steps should be in accordance with the corresponding kit instructions.
2. **Reagent preparation**
   2.1 Remove the kit and leave at room temperature for at least 1 hour.
   2.2 Calculate the number of reactions (n) required for the current experiment.
   n= Sample amount +1 tube positive control +1 tube negative control +1

Prepare the reaction mixture according to the following table.The PCR reaction mixture was prepared and loaded into the PCR reaction tube/plate at 15μl/ hole, and the centrifuge was centrifuged for 10 seconds.

Reaction mixture system:

Component name	Feeding sample μL/hole
qRT-PCR mixed liquid	10μl
RTP enzyme	0.5μl
DNP enzyme	0.5μl
Primer probe	2μl
RNase-free H_2O	2μl

   2.3 PCR tubes were transferred to the sample preparation area. The remaining reagent is returned to the -20℃ preparation area. Refrigerate in cold storage away from light.
   2.4 Feeding sample: the samples to be tested were taken successively, and 5μl of positive control and 5 μl of negative control were added to the reaction tube/plate of the separated PCR reaction solution, with the final volume of 20μl. Centrifuge for 10 seconds.
   2.5 Seal or cap: When sealing the film, the hand can only touch the edge part, and cannot directly contact with the light absorption of the sealing film or the tube cover. There should be no gaps or bubbles between the film and the 96-hole.
   2.6 If the PCR reaction tube cannot be put on the machine immediately due to temporary conditions after adding the template into the PCR reaction tube, it is recommended that the PCR reaction tube with the added template be placed in 2～8℃ for temporary storage and be put on the machine for detection as soon as possible within 24 hours.
3. **PCR Amplification :(nucleic acid amplification region)**
   3.1 Turn on the machine and check the performance of the instrument.
   3.2 Take PCR reaction tube prepared in the sample preparation area, place in the corresponding position of the instrument sample tank, and record the placement order.
   3.3 According to table: set the parameters of instrument amplification and start PCR amplification. After the reaction, the appropriate fluorescence threshold was determined according to the amplification curve, and the Ct values of different channels were obtained.

Parameters related to instrument amplification:

Step	Temperature	Time	Cycle
Reverse transcription	50℃	10 min	1 cycle
Pre-degeneration	95℃	5 min	1 cycle
Degeneration	95℃	15s	45 cycles
Annealing/elongation/detection of fluorescence	60℃ (Collect fluorescent)	45s	

Explain:

1. Fluorescence detection was performed at 60 ℃, and the detection channels were FAM, HEX/VIC and CY5.
2. ROX correction was not selected for the ABI series fluorescence PCR instrument, and None was selected for the quenched group.

   3.4 Data processing: after the reaction, the threshold value was determined according to the amplification curve, and the result was interpreted according to the Ct value.

Quality control

1. Negative control: FAM channel, HEX/VIC channel and CY5 channel, Ct value > 38 or not detected.
2. Positive control: the amplification curves of FAM channel, HEX/VIC channel and CY5 channel were s-shaped, and the Ct value was ≤30.
3. Internal reference quality control: the amplification curve of CY5 was s-shaped, and the Ct value was ≤32.
4. The above requirements of the same experiment should be met at the same time, otherwise this experiment will be deemed invalid.
5. A negative and positive control is needed in the experiment, and the baseline threshold is adjusted according to the corresponding negative for different targets.

Interpretation of results

In the case of normal quality control, the results are interpreted as follows:

Novel Coronavirus 2019-nCoV Nucleic Acid Detection Kit (Real Time PCR) test mixed liquid	Result interpretation
If the FAM channel Ct≤38 and the amplification curve is of typical s-type, the result is interpreted to be	ORF1ab gene（+）
If FAM channel Ct > 38, or no value	ORF1ab gene（-）
If the HEX/VIC channel Ct≤38 and the amplification curve is of typical s-type, the result is interpreted to be	N gene（+）
If HEX/VIC channel Ct > 38, or no value	N gene（-）

According to the detection results of the above three channels, the judgment results are as follows:

Test result	Result interpretation
ORF1ab gene and N gene are both (+)	2019-nCoV positive
Only ORF1ab gene（+）	The test needs to be repeated. If it is still positive, it will be judged to be positive for 2019-nCoV.
N gene（+）	If it is positive after repeated test, it may be another proximal coronavirus.
ORF1ab gene and N gene are both（-）	2019-nCoV negative

Limitation of test method

1. The test results of this kit are for clinical reference only and should be considered in the light of its symptoms/signs, medical history, other laboratory tests and treatment response.
2. Excessive nucleic acid degradation or the concentration of the target gene in the amplification reaction system can also result in false negative results.
3. Improper sample collection, transfer and handling, as well as improper test operation and experimental environment may lead to false negative or false positive results.
4. Be clear that the test is limited to the specified sample type and test system (including applicable model, nucleic acid separation/purification reagent, test method, etc.).
5. The detection range of the detection reagent only includes the gene range claimed by the detection reagent, excluding the detection of genes not declared by the detection kit.

Physical Performance of Products

1. The package of the detection kit is complete, no contents overflow; The appearance of the label is complete, without falling off, and the label identification content is clear; The composition in the kit is correct and there are no duplicate or missing components.
2. **Accuracy**
   Test three samples of weak positive, medium positive and strong positive references, and the ORF1ab gene and N gene were positive, and the positive coincidence rate was 100%.
3. **Analytical specificity**
   Test two negative references, and the ORF1ab gene and N gene were both negative, and the negative coincidence rate of 100%.
4. **Minimum detectable limit**
   The detection limit of novel coronavirus is 300 copies/mL.
5. **Within-run precision**
   The same batch of kit was used to test one weak positive reference for 10 times, and the results were positive for ORF1ab and N genes, the positive coincidence rate was 100%, and the coefficient of variation (CV) was $<5\%$.
6. **Between-run precision**
   Three batches of kits were used to test one weak positive reference product for 10 times, and the results of 30 experiments were all positive for ORF1ab gene and N gene, the positive coincidence rate was 100%, and the coefficient of variation (CV) was $<5\%$.

Caution

1. The product test sample is human tissue, there is potential biosafety, so the test should be carefully protected, to prevent other potential infections.
2. This product is a PCR kit, and the clinical laboratory should strictly follow the management rules of the molecular biology laboratory and clinical gene amplification laboratory, such as the management rules of the clinical gene amplification laboratory in medical institutions.
3. The disposal of specimens, the containers for testing specimens and the materials used in the inspection process shall comply with the "regulations on the management of medical waste" and "measures on the management of medical waste in medical and health institutions", as well as the relevant national and regional requirements.
4. In the process of use, attention should be paid to the misuse and cross-contamination of various raw materials.
5. In the process of using the reagent, the operation should be carried out in strict accordance with the IFU requirements of the instrument and reagent instructions.
6. The kit should be transported and stored in strict accordance with the instructions.
7. The test result provided by this product is not the only diagnostic basis, the doctor should combine with other diagnostic methods for comprehensive diagnosis.
8. When using this kit, it should be used in strict accordance with the sample type and sample requirements stipulated in the kit. Using samples of other types may get wrong results.
9. This product is only suitable for in vitro diagnosis. Reagents contain liquid components, there is a certain irritation or toxicity, do not directly contact the skin, eyes. Once in contact, rinse with plenty of water. No swallowing.
10. The expiry date has been marked on the outer surface of the packaging box of the system. Do not use after the expiry date.
11. When feeding samples, each reagent should be centrifuged to avoid cross contamination.
12. Personnel may not leave until the equipment begins to operate normally.

Cited References

1. Technical guidelines for laboratory testing of novel coronavirus infections (3rd edition);
2. Prevention and control of pneumonia by novel coronavirus (third edition);
3. Diagnosis and treatment, prevention and control, laboratory, monitoring, flow guidelines of novel coronavirus pneumonia
4. Primer probe sequence published by CDC: uscdcrt-pcr-panel-primer-probes;
5. CDC-Biosafety Microbiological Biomedical Laboratories-2009-P
6. WHO Laboratory biosafety handbook 3rd edition
7. WHO Laboratory tests on suspected case of novel coronavirus 2019;
8. American CDC RT-PCR test novel coronavirus 2019 operating instructions(original edition) rt-pcr-panel-for-detection-instructions

Manufacturer

Manufacture enterprise: Shanghai Biotecan Pharmaceuticals Co. , Ltd
Manufacture site: First Shanghai Centre, 180 Zhangheng Rd., Pudong New District, Shanghai, China
Zip Code: 201204
Tel: +86-21-50277725

EU representative

Name: Luxus Lebenswelt GmbH
Business Address Kochstr. 1, 47877, Willich, Germany
DIMDI Code: DE/0000047791
Tax Number DE305829099
Contact Person Lin Sun
Telephone 0049-1715605732
Website http://www.ringbio.com
Email Info.m@luxuslw.de

Explanation of symbols

CE	CE Mark		Keep dry
	Consult instruction for use	LOT	Batch number
	Expire date	IVD	IVD product
-25℃ 15℃	-25℃ to - 15℃ storage		Date of manufacture
	Manufacturer		Do not use if package is damaged
EC REP	European union representative		Avoid light