

Novel Coronavirus (2019-nCoV) Nucleic Acid Detection Kit (PCR-Fluorescence Probing)

Product: Novel Coronavirus (2019-nCoV) Nucleic Acid Detection Kit
(PCR-Fluorescence Probing)

Size: 25 tests/Box, 50 tests/Box

Applications

The Novel Coronavirus (2019-nCoV) Nucleic Acid Detection Kit is developed for qualitative detection of 2019-nCoV that is collected from human nasopharyngeal swabs, sputum and bronchoalveolar lavage fluid in vitro. It is only used as an in vitro diagnostic emergency response for 2019-nCoV infection happened in December 2019. It is suggested to follow the newest document instructions of "Diagnosis and treatment of novel coronavirus infection pneumonia" and "Strategic Preparation and Response Plan of novel coronavirus infection pneumonia" (current version).

The novel coronaviruses belong to the β genus. 2019-nCoV is an acute respiratory infectious disease. People are generally susceptible. Currently, the patients infected by the novel coronavirus are the main source of infection; asymptomatic infected people can also be an infectious source. Based on the current epidemiological investigation, the incubation period is 1 to 14 days, mostly 3 to 7 days. The main manifestations include fever, fatigue and dry cough. Nasal congestion, runny nose, sore throat, myalgia and diarrhea are found in a few cases.

The novel coronavirus detection should meet the requirements of "new laboratory technology related to new coronavirus pneumonia detection guidelines" and address biosafety issues.

Manufacturer

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Principle

Highly conserved region containing ORF1ab and N gene in the novel coronavirus (2019-nCoV) genome is used to design specific primers and probes in this kit. Based on the fluorescence quantitative PCR platform, the principle of one-step fluorescence quantitative reverse transcription PCR was used to qualitatively detect the novel coronavirus (2019-nCoV).

PCR detection system uses Human ACTB gene to design primers and probes as internal control, which can monitor the quality of respiratory nucleic acid and whole PCR reaction system. It avoids the risk of blank samples and false negatives.

Kit Components

Reagent	Component	Size and Volume (25 tests/Box)	Size and Volume (50 tests/Box)
2019-nCoV Reaction Buffer	reaction buffer, etc	150 µL/tube×1	300 µL/tube×1
2019-nCoV Detection Mixture	specific ORF1ab and N gene primers, probes, internal control primers, and probes, dNTP, etc	50 µL/tube×1	100 µL/tube×1
2019-nCoV Enzyme	reverse transcriptase, hot start DNA polymerase, etc	25 µL/tube×1	50 µL/tube×1
2019-nCoV Positive Control	plasmids carrying target gene and internal control	100 µL/tube×1	200 µL/tube×1
2019-nCoV Negative Control	empty plasmid	100 µL/tube×1	200 µL/tube×1

Storage Condition and Valid Period

See the packing box for the production date and expiration date of the kit.

Reagents in this kit is light-sensitive, it should be stored at -20°C±5°C, and the shelf life is 6 months.

The foam box and dry ice or ice bag is needed for sealed transportation especially for long distance shipping. And avoid repeated freezing and thawing.

Instrument Compatibility

ABI 7500; ABI ViiA7500; Light Cycler 480; Bio-Rad CFX96; SLAN series medical PCR instrument; Analytikjena qTower3.

Product Performance Index

1. Check the positive and negative standard products of the enterprise, and the compliance rate is 100%.
2. Minimum detection limit: 300 copies / ml(7.5 copies / reaction).

Precautions

1. This product is an in vitro diagnostic reagent in urgent need of rare disease. Professional and experienced laboratory personnel is required before the experiment. Please read the manual carefully.
2. The laboratory management shall be in strict accordance with the management specifications of PCR gene amplification laboratory. The laboratory personnel must be trained professionally. The experiment process shall be carried out in strict divisions (reagent preparation area, specimen preparation area, amplification and product analysis area). All consumables are disposable after sterilization. Special instruments and equipment shall be used in each stage of the experiment operation, and the supplies in each zone shall not be used in cross.
3. Sample processing shall be carried out in the biosafety cabinet to protect the safety of operators and prevent environmental contamination.
4. The sample shall be completely added into the reaction liquid. After adding the sample, the pipe cover or sealing film shall be closed as soon as possible, and centrifugate immediately.
5. Avoid bubbles as much as possible when aliquoting reaction mixture. Check carefully whether the reaction tubes are tightly capped or the sealing film is open before run the equipment.
6. After amplification, take out the reaction tube / plate, seal it in a special plastic bag, and discard it in a designated place.
7. Do not loosen the cap or tear the sealing film after amplification in case of aerosol contamination.
8. The used tubes shall be directly put into the waste tank containing 10% sodium hypochlorite and disposed together with other waste stuff.
9. The worktable and various experimental stuff should be regularly disinfected with 75% alcohol and ultraviolet lamp.
10. The reagent should be recovered to room temperature before use and avoid repeated freezing and thawing. Wherever, avoid cross contamination between reagents.
11. Avoid reuse.

Sample Requirements

The applicable sample used for kit: upper respiratory tract samples (including throat swabs, nasal swabs, nasopharynx extracts, expectoration fluid); lower respiratory tract samples (including respiratory tract extracts, bronchoalveolar lavage fluid, alveolar lavage fluid, lung tissue biopsy samples); tissue culture samples, etc.

Detection Methods

1. Reagents Preparation

- 1.1 Take 2019-nCoV Reaction Buffer Detection Mixture and 2019-nCoV Detection Mixture Reaction Buffer out of the kit, melt and mix at room temperature, and centrifugate at low speed for 10 seconds. Take out the 2019-nCoV enzyme solution from the kit, centrifugate for 10 seconds, and put it on ice for use.
- 1.2 Calculate reactions N and prepare reaction mixture: calculate the number of reactions according to the number of test samples. If the number of samples is n, the number of reactions N = (number of samples to be tested n + negative control 1 + positive control 1 + 1). Prepare reaction system according to table 1 below.

Table 1 Reaction System Preparation

Component	Reagents volume needed/ each x N
2019-nCoV Reaction Buffer	5 μ L×N
2019-nCoV Detection Mixture	2 μ L×N
2019-nCoV Enzyme	1 μ L×N

- 1.3 Reaction system preparation: prepare a corresponding number of PCR reaction tubes, shake and mix the above reaction mixture, centrifugate it for 5 seconds, and then divide it into the PCR tube according to 8 μ L / tube. Shift the PCR tube to the sample preparation area.

2. Sample Preparation

- 2.1 The obtained samples can be extracted with commercialized virus RNA extraction kit. It is recommended to use the magnetic bead or column-based virus DNA / RNA extraction kit of CoWin Biosciences Co., Ltd. Please check the corresponding kit instructions for details.

2.2 Take 17 µL of sample to be tested, 2019-nCoV positive control and 2019-nCoV negative control, respectively, and add them to the reaction mixture that has been repacked. Seal with film or cap the PCR reaction tube tightly and centrifugate at low speed. The PCR reaction tube should be transferred to nucleic acid amplification region for detection.

3. PCR Amplification (Nucleic acid amplification region)

- 3.1 Place the above PCR reaction tube in the indicated location of the instrument, and record the placing sequence.
- 3.2 Open the parameter window to set the cycle conditions, and the reaction procedure is shown in Table 2.

Table 2 Reaction Procedure

Procedure	Temperature	Time	Cycle
Reverse transcription	50°C	15 min	1
Pre-denaturation	94°C	1 min	1
Denaturation	94°C	15 sec	45
Annealing, extension and Collect fluorescence signal	58°C	30 sec	

Experimental Validity

Note: ORF1ab gene reported fluorescence is FAM, N gene reported fluorescence is ROX, and internal control gene reported fluorescence is VIC in this product.

1. Positive control: Both FAM and ROX channels have typical S-type amplification curve and CT value < 35. The internal control VIC channel has typical S-type amplification curve and CT value < 35.
2. Negative control: FAM and ROX channel value CT > 40 or no CT value, the amplification curve is straight line or slight oblique line, no exponential growth period.
3. Experimental sample: the internal standard VIC channel has a typical S-type amplification curve.
4. The experiment result is qualified only if the conditions of 1, 2 and 3 are met at the same time, otherwise it is invalid.

Evaluation of Test Results

Take ABI 7500 as an example: automatically save the result after the reaction, adjust the start value, end value and threshold value of the baseline according to the image after analysis (The user can adjust it according to the actual situation, Start value can be 3-15, end value can be 5-20, and the Threshold Value can be set in the Log window, so that the threshold line is in the exponential phase of the amplification curve, the amplification curve of the negative control is straight or below the threshold line), click Analysis to get the analysis results automatically, and read the test results in the report window.

Target	Channel	Results explanation
Opening reading frame (ORF1ab)	FAM	Positive: both FAM and ROX CT value is less than 35, and the curve is S-shaped with an obvious exponential growth period; Negative: one channel (either FAM or ROX) CT value is more than 35, the other CT value is more than 40 or no CT value; Suspicious: when one channel (either FAM or ROX) CT value is less than 35, the other CT value is in the range of 35-40, or both FAM and ROX CT value is in the range of 35-40, the sample needs to be tested again, it is positive if the CT value of repeated result is still in the range of 35-40 and the curve is typical S type with exponential growth period, otherwise it is negative.
Nucleocapsid (N)	ROX	

Limitations of Test Methods

1. The test results are only for clinical reference and shall not be the only standard for diagnosis.
2. Several reasons can cause negative results like poor RNA quality, the improper preservation, the presence of inhibitors in the sample and the degradation of nucleic acids.
3. Inappropriate sample collection, transfer and treatment, improper experimental operation and experimental conditions may lead to false negatives or false positives.
4. This product is only limited to the specified sample type and applicable model.
5. Internal control can monitor the effectiveness of PCR reaction system, but it may not be able to judge the efficiency of nucleic acid extraction.