CE IVD

Ver.A/17

Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit (PCR-Fluorescence Probing)

Product: Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit (PCR-Fluorescence Probing)

Size: 48 tests/Box, 96 tests/Box

Applications

The Novel Coronavirus (SARS-CoV-2) FAST Nucleic Acid Detection Kit is developed for qualitative detection of SARS-CoV-2 that is collected from human oropharyngeal swabs in vitro. The applicable population includes: suspected cases of Novel Coronavirus (SARS-CoV-2) infected pneumonia, suspected aggregated cases, and other patients who need to be diagnosed or differentiated with Novel Coronavirus (SARS-CoV-2) infection.

The novel coronaviruses belong to the β genus. COVID-19 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.

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Principle

Highly conserved region containing ORF1ab and N gene in the novel coronavirus (SARS-CoV-2) 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 (SARS-CoV-2). 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 (48 tests/Box)	Size (96 tests/Box)
Reaction Lyophilized Powder	Reverse transcriptase, hot start DNA polymerase; specific ORF1ab and N gene primers, probes; internal control primers, and probes; reaction buffer, etc.(Lyophilized powder)	8 well tube strip×6	8 well tube strip×12
Reaction Lyophilized Powder Dissolvent	Divalent cation, buffer and monovalent cation, etc	300 μL/tube×1	300 μL/tube×2
SARS-CoV-2 Positive Control	Pseudovirus carrying target gene and plasmids carrying internal control (Lyophilized powder)	tube×1	tube×2
SARS-CoV-2 Negative Control	Plasmids not carrying target genes (Lyophilized powder)	tube×1	tube×2
Positive/Negative Control Dissolvent	Tris, EDTA, NaOH, etc.	1 ml/tube×1	1 ml/tube×2

Storage Condition and Valid Period

The kit can be stored and transported at room temperature for a long time before use. After the reagent box is opened for use, it can be stored at room temperature within one month. If it is stored for a long time, it is recommended to place the reagent box at 2-8°C.

The kit production date and expiration date are shown in the outer packaging box, and the validity period is 12 months.

4. The sample should be completely added into the reaction solution. After adding the sample, cover the tube lid as soon as possible and spin the tube.
5. After amplification, take out the reaction tubes, seal them in a special plastic bag, and discard it in a designated place.
6. Do not loosen the lid after amplification in case of aerosol contamination.
7. The used tips shall be directly put into the waste tank containing 10% sodium hypochlorite and disposed together with other waste stuff.
8. The worktable and various experimental stuff should be regularly disinfected with 75% alcohol and ultraviolet lamp.
9. The reagent should be recovered to room temperature before use and avoid repeated freezing and thawing. Avoid cross contamination between reagents.
10. Avoid reuse.

Limitations of Test Methods

1. The test results are only for clinical reference and shall not be the only standard for diagnosis.
2. The negative results can be caused by improper storage conditions of samples, inhibitions in the samples and 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 instrument model.

Product Performance Index

1. Test the positive standard products of the enterprise(P1~P5), and the compliance rate is 100%.
2. Test the negative standard products of the enterprise(N1~N7), and the compliance rate is 100%.
3. Minimum detection limit: 200 copies/ml, test the detection limit standard products of the enterprise(L), positive detection rate ≥ 95%.
4. Test the precision standard products of the enterprise (S1, S2), the test result of each precision standard products of the enterprise is positive, and CV of CT value is less than 5%.

Precautions

1. This product is an in vitro diagnostic reagent kit, tester should be trained and expe rienced. Please read the manual carefully before the experiment.
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 testers and prevent environmental contamination.

Instrument Compatibility

ABI 7500，Bio-rad CFX 96, Tianlong Gentier 96, Lepgen 96, MA-6000.

Sample Requirements

Saliva, sputum, tracheal, aspirate or bronchoalveolar lavage, nasopharyngeal aspirate, nasopharyngeal and pharyngeal swabs, blood urine and stool.

Detection Methods

1. Sample collection and processing

Any of the following methods can be used to process samples:

1.1 The virus preservation solution of our company (Cat.No.CW3129) is used to process swab samples. The specific operation is: after collecting samples with swab, immerse the swab head in virus preservation solution, shake and mix well, and then reserve.

1.2 When other manufacturers' virus preservation solution is used to process swab samples, the samples shall be processed according to the manufacturer's preservation solution operation instructions for standby.

1.3 RNA samples were obtained by nucleic acid extraction kit.

2. Reagents Preparation

2.1 Determine the total number of reactions: calculate the required number of reactions according to the number of test samples. If the number of samples is n, then the total number of reactions is N = n + 2. Take N tubes Reaction Lyophilized Powder and centrifugate for 1 min to ensure that the Reaction Lyophilized Powder is separated to the bottom of the tube for standby.

2.2 Reaction Lyophilized Powder dissolving: add 5 μl Reaction Lyophilized Powder Dissolvent into each tube of Reaction Lyophilized Powder, shake and mix well, and centrifugate for standby.

2.3 SARS-CoV-2 Positive Control dissolving: add 500 μ l Positive/Negative Control Dissolvent into SARS-CoV-2 Positive Control lyophilized powder, shake and mix well, and centrifugate for standby.

2.4 SARS-CoV-2 Negative Control dissolving: add 500 μ l Positive/Negative Control Dissolvent into SARS-CoV-2 Negative Control lyophilized powder, shake and mix, and centrifugate for standby.

Note: The Positive/Negative Control shall be dissolved before the first use. If it needs to be stored for a long time after dissolution, please place it at 2-8 ℃. The Reaction Lyophilized Powder shall be dissolved according to the total number of reactions.

3. Add Sample

According to different sample types, different sample adding operations are carried out as follows:

3.1 SARS-CoV-2 Positive Control or SARS-CoV-2 Negative Control: take 20 μl SARS-CoV-2 Positive Control or SARS-CoV-2 Negative Control and add it into the tube of the reaction solution.

3.2 Samples to be tested processed with virus preservation solution of our company: directly add 20 μl processed samples to the tube of the reaction solution.

3.3 Samples to be tested treated with virus preservation solution of other manufacturers (main component is Hanks preservation solution or normal saline): take 10 μl of processed samples to be tested and 10 μl unused virus preservation solution provided in this kit and add them into the tube of the reaction solution.

3.4 RNA sample: take 20 μl RNA sample directly into the tube of the reaction solution (if the RNA sample is less than 20 μl, it can be supplemented with RNase-Free water).

3.5 Cap the tubes tightly, then shake and mix thoroughly. Transfer the tubes to nucleic acid amplification region for detection.

4. PCR Amplification

4.1 Place tubes mentioned above in the indicated location of the qPCR instrument, and record the placing sequence.

4.2 Open the parameter window to set the cycle conditions, and the reaction procedure is shown in Table 1.

Table 1 Reaction Procedure

Procedure	Temperature	Time	Cycle
Reverse transcription	55°C	1 min	1
Pre-denaturation	96°C	20 sec	1
Denaturation	96°C	5 sec	45
Annealing, extension and Collect fluorescence signal	58°C	30 sec	

Result Interpretation

Positive control and negative control

CT Value			Control Interpretation
IC (VIC)	ORF1ab (FAM)	N (ROX)	
CT≤35	CT≤35	CT≤35	Positive control
CT≤35	CT>40 or None	CT>40 or None	Negative control

The positive control and negative control are consistent with the above table, which proves that the operation is OK and the kit are effective. After that, real sample interpretation can be carried out.

CT Value			Control Interpretation
IC（VIC）	ORF1ab（ FAM）	N（ROX）	
/	CT≤35	CT≤35	Positive
/	CT≤35	CT>40 or None	Positive
/	CT>40 or None	CT≤35	Positive
/	CT≤35	35<CT≤40	Positive
/	35<CT≤40	CT≤35	Positive
/	CT>40 or None	CT>40 or None	Negative
/	35<CT≤40	CT>40 or None	Retest*
/	CT>40 or None	35<CT≤40	Retest*
/	35<CT≤40	35<CT≤40	Retest*
CT>40 or None	CT>40 or None	CT>40 or None	Invalid sample (Resampling)

/ indicate follow situations：CT≤35 or 35<CT≤40 or CT>40 or None.

*Retest result that is not negative as listed in the table is positive.

Amplification curve with CT value is typical S-type with obvious exponential growth period.

If you encounter some special results that are difficult to interpret, our technicians will guide you to interpret.