

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

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.

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)
Virus Preservation Solution	salt ions and buffers for virus preservation and PCR amplification	1 ml/tube×50
SARS-CoV-2 Reaction Mixture	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
Reaction Dissolvent	Divalent cation, buffer and monovalent cation, etc	300 μL/tube×1
SARS-CoV-2 Positive Control	plasmids carrying target gene and internal control	500 μL/tube×1
SARS-CoV-2 Negative Control	plasmids not carrying target genes	500 μL/tube×1

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.

Instrument Compatibility

Such as, ABI7500、Bio-Rad CFX96 ,CFX96 TOUCH、Hongshi slan96p and Roche Z480,etc.

Sample Requirements

The applicable samples are throat swabs.

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 is used to process throat swab samples. The specific operation is: after collecting samples with throat swab, immerse the swab head in the tube of virus preservation solution, shake and mix well, and then reserve.
- 1.2 When other manufacturers' virus preservation solution (mainly Hanks preservation solution or normal saline) is used to process throat 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 SARS-CoV-2 Reaction Mixture and centrifugate for 1 min to ensure that the SARS-CoV-2 Reaction Mixture is separated to the bottom of the tube for standby.
- 2.2 SARS-CoV-2 Reaction Mixture dissolving: add 5 μ l Reaction Dissolvent into each tube of SARS-CoV-2 Reaction Mixture, shake and mix well, and centrifugate for standby.

Note: SARS-CoV-2 Reaction Mixture shall be dissolved according to the total number of reactions.

3. Add Sample

According to different sample types and different sample collection and processing methods, different sample adding operations are carried out as follows:

- 3.1 SARS-CoV-2 Positive Control or SARS-CoV-2 Negative Control: take 20 μ l 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 SARS-CoV-2 Collection Fluid of our company: directly add 20 μ l 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 μ l of processed samples to be tested and 10 μ l unused virus preservation solution provided in this kit and add them into the tube of the reaction solution.
- 3.4 RNA sample: take 20 μ l RNA sample directly into the tube of the reaction solution (if the RNA sample is less than 20 μ l, 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	

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. SARS-CoV-2 Positive Control: 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. SARS-CoV-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. The internal control VIC channel has typical S-type amplification curve and CT value < 35.
3. Experimental sample: the internal control VIC channel has a typical S-type amplification curve.
4. The experiment result is qualified only if the conditions of 1, 2 and 3 above are all satisfied at the same time, otherwise it is invalid.

Evaluation of Test Results

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, set the value of threshold in the log graph pane to make the threshold line is in the exponential phase of the amplification curve, and the amplification curve of the negative control is flat or lower than 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-type 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. 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: 100 copies / ml, test the detection limit standard products of the enterprise(L), positive detection rate $\geq 95\%$.
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%.

Product Performance Index

1. This product is an in vitro diagnostic reagent kit, tester should be trained and experienced. 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.
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.