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# SARS-CoV-2 Detection Kit (qPCR Probe Assays)

**Product:** SARS-CoV-2 Detection Kit (qPCR Probe Assays)

**Size:** 25 rxns, 50 rxns

## Applications

The SARS-CoV-2 Detection Kit is developed for qualitative detection of SARS-CoV-2 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 SARS-CoV-2 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 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 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 new coronavirus (SARS-CoV-2).

PCR detection system uses Human Actin 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 rxn) | Size and Volume (50 rxn) |
|---|---|---|---|
| SARS-CoV-2 Enzyme | reverse transcriptase, hot start DNA polymerase, etc | 25 μL/tube×1 | 50 μL/tube×1 |
| SARS-CoV-2 Reaction Mixture | specific ORF1ab and N gene primers, probes, internal control primers, and probes, reaction buffer, etc | 475 μL/tube×1 | 950 μL/tube×1 |
| SARS-CoV-2 Positive Control | plasmid carrying target genes | 100 μL/tube×1 | 200 μL/tube×1 |
| SARS-CoV-2 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.

The kit is light-sensitive, it can be stored at -20°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.

## Applicable Equipment

ABI 7500 Real-Time PCR, ABI ViiA7500 Real-Time PCR, Light Cycler 480, Bio-Rad CFX96 Real-Time PCR, SLAN Real Time PCR.

## 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 SARS-CoV-2 reaction mixture out of the kit, melt and mix at room temperature, and centrifugate at low speed for 10 seconds. Take out the SARS-CoV-2 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 |
|---|---|
| SARS-CoV-2 Reaction Mixture | 19 μl × N |
| SARS-CoV-2 Enzyme | 1 μl × 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 20 μL / 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 5 μl of sample to be tested, SARS-CoV-2 positive control and SARS-CoV-2 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 work place for amplification 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 standard gene reported fluorescence is VIC in this product.**

1. Positive control: FAM and ROX channels have typical S-type amplification curve or CT value < 35. The internal standard VIC channel has typical S-type amplification curve.
2. Negative control: FAM and ROX channel value CT > 40 or no CT value, linear 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

Adjust the start and end values of the baseline according to the image after analysis (the user can adjust them according to the actual situation, start value can be 3-7, and the end value can be 10-20).

<table>
<tr><th>Target</th><th>Channel</th><th>Results explanation</th></tr>
<tr><td>Opening reading frame (ORF1ab)</td><td>FAM</td><td rowspan="2">Positive: the test sample CT is less than 37, the curve is S-shaped with an obvious exponential growth period, and the two channels meet the above conditions at the same time;<br>Negative: all channels CT value is more than 40 or no CT value, the result is negative;<br>Suspicious: one channel results CT value is less than 37, the other channel test result is 37 ≤ CT value < 40, the sample need to be tested again, If the value is still in the range of 37-40, the curve is typical s type with exponential growth period, then it is positive, otherwise it is negative.</td></tr>
<tr><td>Nucleo -capsid (N)</td><td>ROX</td></tr>
</table>

## 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.

## Product Performance Index

1. Check the positive and negative standard products of the enterprise, and the compliance rate is 100%.
2. Minimum detection limit: $1 \times 10^3$ copies / ml.

## 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 tube lid or sealing film shall be closed as soon as possible, and centrifugate immediately.
5. Avoid bubbles as much as possible once reaction liquid is divided. 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.

## Reference


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2. Drosten C, Gunther S, Preiser W, van der Werf S, Brodt HR, Becker S, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med. 2003;348(20):1967-76.
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