Colorimetric And Isothermal Detection Kit For COVID-19 Coronavirus

【Product name】

Colorimetric And Isothermal Detection Kit For COVID-19 Coronavirus

【Unit】

50 tests

【Intended use】

This kit is suitable for the relative quantitative detection of new coronavirus (SARS-CoV-2) nucleic acid. The test results are for clinical reference only and cannot be used as the basis of diagnosis and treatment alone.

【Detection principle】

This product is based on isothemal amplification technology at constant temperature. Under the condition of constant temperature (60-65℃), using reverse transcriptase and DNA polymerase with strand replacement activity, amplify the target sequence with specific primers. RNA or DNA is amplified in efficient, rapid and accurate manner, so as to achieve the purpose of rapid detection. In this kit, COVID-19 virus is converted into cDNA by reverse transcriptase, and then nucleic acid amplification is performed by modified Bst3 DNA polymerase, SARS-CoV-2 ORF1ab gene is used to design specific primers. The detection process does not need electrophoresis or fluorescent detection system. The whole process from amplification to detection is completed in a reaction tube, and the target sample can be detected simply and quickly.

【Contents of the kit】

Component name	Size	Number
Reaction solution	500μL	1 tube
Primer mix	100μL	1 tube
Positive control	50μL	1 tube

DEPC water could be used as the negative control

【Estimated operating time】

This kit uses reverse transcriptase and Bst3 enzyme for RNA-cDNA conversion and amplification. Mix sample, primer mix and reaction solution, when primer can identify SARS-CoV-2 to exist, its signal could be amplified with Bst DNA polymerase activity. In the reaction, DNA polymerase plays the role of polymerization to produce protons, and the accumulation of protons in the presence of a large amount of DNA polymerase activity leads to the reduction of pH value, which facilitates the real-time and simple detection of amplions. The presence of SARS-CoV-2 can be determined if the reaction solution changes from pink to yellow, the whole reaction time is 40 minutes.

【How to use】

1. Materials required but not provided: Disposable gloves; PCR tube/fluorescent quantitative eight-line reaction tube, ice and ice box.
2. Preparation of reagents: remove all reagents from -20 °C, melt them at room temperature, and place them on ice.

【Operation procedure】

1. Sample added: take out the reagent and add samples according to the following table. First add negative control, then add samples and positive control. Cover tightly, centrifuge 5 sec at 1800 rpm. After sampling, put the remaining reagent into -20°C refrigerator for storage immediately . (ice operation).

Reagent	Negative control	RNA Samples	Positive control
Reaction solution	**10**μL	**10**μL	**10**μL
Primer mixture	**2**μL	**2**μL	**2**μL
Samples	**-**	**1**μL*	**1**μL
DEPC water	**8**μL	**7**μL	**7**μL
Total volume	**20**μL	**20**μL	**20**μL

*The sample size under test should not exceed 10% of the total volume. If there are many samples, the total mixture of reaction liquid, primer mixture and DEPC water can be prepared according to the number of test samples (one more experimental quantity than the actual test sample), and then the total mixture of

19μL can be added to 1μL sample for the experiment.

2. Amplification reaction and real-time monitoring:

2.1 Check whether the reaction mixture after adding the sample is bright pink, indicating that the pH value meets the experimental requirements.

2.2 The mixture is covered and placed into the PCR instrument or a constant temperature water bath/metal bath, and incubated at 65°C for 20-30 minutes "note 1" (40 minutes is approximately equivalent to 40 cycles detected by the probe method), then take out the PCR tube and observed with the naked eye.

The positive reaction changes from pink to yellow, while the negative control remains pink. If the color change is not obvious, such as orange, the reaction will continue at 65℃ for another 10-15 minutes.

2.3 The results can be photographed or scanned to record the colorimetric results.

【Experimental result diagram】

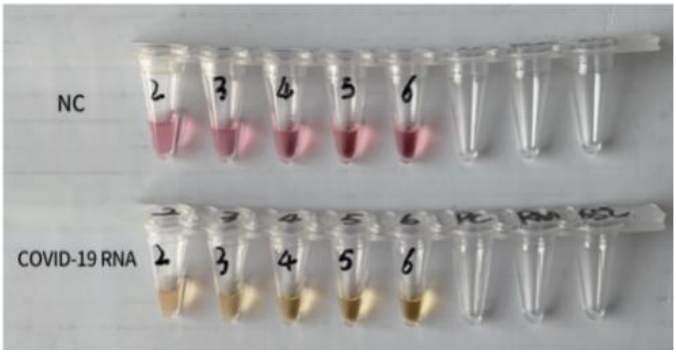

Figure 1: RNA amplification results of five primers against COVID-19 (20 minutes amplification time)

【Caution for handling】

1. Sample requirements:

1.1 Suitable sample types for this kit: upper respiratory tract specimens (including swabs of pharynx, swabs of nose, nasopharynx extracts and deep cough sputum) collected freshly. Lower respiratory tract specimens (including respiratory tract extracts, bronchial lavage fluid, alveolar lavage fluid, lung biopsy specimens) and other samples.

1.2 After sample collection, the test shall be completed on the same day. Otherwise, it shall be stored in the following condition: 2~8℃, no more than 24 hours. Store Below -20℃ for no more than 3 days. Can be stored for a long time

below -70℃. Repeated freezing-thawing should be avoided

(the sample detection should ensure the sample RNA extraction procedure, and verify the RNA recovery and purity before the sample test).

1.3 Transportation: the foam box is sealed with ice for transportation.

2. Reagent treatment:

2.1 The kit should be stored at -20℃. To prevent degradation of the reagent, remove the required test dose from the refrigerator before use (in order to maintain reagent performance and avoid unnecessary freeze-thaw).

2.2 The reagent should be thawed at room temperature and placed on ice for operation. Before use, reverse the reagent 2-3 times, after the reagent is fully mixed, instantaneous centrifugal reserve. Move gently to avoid inactivation of enzyme mix.

2.3 Be careful not to contaminate other samples or reagents with positive controls. Open the lid of the positive control tube as soon as possible and make sure that all other tubes are closed when adding the positive control.

2.4 Keep positive control and suspected specimens away from reagents during treatment.

3. Reaction tube treatment after experiment:

3.1 Do not open the cap of the reaction tube after the experiment to avoid contamination of other samples or test area.

【Applicable instrument】

It is suitable for PCR instruments such as bio-rad c1000 touch; thermostatic water bath; constant temperature metal bath.

【Caution for operation】

1. Metal bath, water bath or PCR instrument without cover, need to add a drop of mineral oil after adding the sample to prevent the reagent or sample from evaporating, resulting in an error in the test results.

2. The reaction is very sensitive, even a small amount of amplification products may contaminate other reactions or reagents, leading to wrong results. Therefore, it is recommended that samples and reagents could be added at different workstations. Avoid electrophoresis or manipulation of amplification products.

3. This kit is used for the detection of human COVID-19 new coronavirus.

4. If the operator has no experience or knowledge in the field of nucleic acid testing, it is possible to make wrong judgments. Therefore, ensure that the kit is used under the supervision of experienced and knowledgeable technicians.

【Storage conditions and Expiration】

Storage conditions: -15~-30℃, avoid light.

Expiration: 6 months. Avoid repeated freezing-thawing after opening.

【Date of approval and modification】

February 15, 2020

【Production batch number】

See label and packaging.