The instructions of One Step RT-PCR Detection Kit for COVID-19 Coronavirus (100 Tests)

Catalog Number: MBS2614309

Product name

One Step RT-PCR Detection Kit for COVID-19 Coronavirus

Storage conditions and Expiration

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

Expiration: 6 months. Avoid repeated freezing-thawing.

Application

This kit is suitable for the relative quantitative detection of COVID-19 nucleic acid. The results are for reference only and cannot be used for diagnosis.

Principle

In this Kit, fluorescent RT-qPCR is used to design specific primers and probes targeting the N gene and ORF1ab gene in the conserved region on the genome of COVID-19. The 5' end marks FAM (ORF1ab) and HEX (N gene) of the probe are detected and compared to the base group, and the 3' end marks are reported to the quenched base group (FAM quenched by BHQ1 and HEX quenched by BHQ2). Before PCR amplification, the fluorescent from 5' probe emitted by FAM/HEX is absorbed by the quenching group because the quenching group is close to the reporting group. Therefore, no fluorescent signal is obtained. When the primer extended, the fluorescent probe bound to the template is cut off by Taq enzyme (5'→3' exonuclease activity), and the reporter group is separated from the quenched group to generate a fluorescent signal, to realize the relative quantitative detection of the COVID-19 at the nucleic acid level in the sample.

Contents of the Kit

2X Reaction Buffer	1000 µl x 1 tube
Enzyme Mix	80 µl x 1 tube
Primer & Probe	200 µl x 1 tube
Positive control	500 µl x 1 tube
Negative control	500 µl x 1 tube
50X ROX Dye	40 µl x 1 tube
50X ROX Dye II	40 µl x 1 tube
RNase Free dH_2O	1000 µl x 1 tube

Notes

1. ROX Dye is used to correcting fluorescence signal errors between wells. Applied Biosystems 7300 Real-Time PCR System uses 50X ROX Dye, 7500 Real-Time PCR System uses 50X ROX Dye II, Thermal Cycle Dice Real-Time System and Smart Cycler System, LightCycler Real-Time PCR instrument is not necessary to use ROX dye.
2. Materials required but not provided: Viral RNA extraction Kit, disposable gloves, RNase and DNase free PCR tube.

Estimated operating time

45 to 60 min.

Applicable instrument

Bio-Rad CFX96 Touch, ABI7300, ABI7500 and Roche, etc. fluorescent quantitative PCR instruments with FAM and HEX channel.

Sample request

1. Suitable sample types for this kit: upper respiratory tract specimens (including swabs of the pharynx, swabs of the 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.
2. After sample collection, the test shall be completed on the same day. Otherwise, it shall be stored in the following condition: 2-8°C, no more than 24 hours. Store below -20°C for no more than three days. Can be stored for a long time below -70°C. Repeated freezing-thawing should be avoided.
3. Transportation: the foam box is sealed with ice for transportation.

Operation procedure

Sample solution preparation:

Samples are extracted according to the corresponding requirements and steps with the viral RNA extraction kit, and the extracted RNA could be directly used for detection. If the samples are not immediately detected after extraction, they can also be stored at -70°C, and repeated freezing-thawing should be avoided.

Preparation of amplification reagent:

Take out the reagent from the refrigerator, the reaction liquid and the positive control could be placed on ice to thaw, and the negative control could thaw at room temperature. After thawing thoroughly, mix the mixture upside-down and centrifuge briefly for backup.

Sample added:

Add samples according to the following table, first add negative control, then add samples and positive control. Cover tightly, centrifuge 5 sec at 1,800 rpm. After sampling, put the remaining reagent into -20°C refrigerator for storage immediately.

Reagent	Samples	Positive control	Negative control
2X Reaction Buffer	10 µl	10 µl	10 µl
Enzyme Mix	0.8 µl	0.8 µl	0.8 µl
50X ROX Dye	0.4 µl	0.4 µl	0.4 µl
Primer & Probe	2 µl	2 µl	2 µl
Total RNA Sample	10 pg - 100 ng	5 µl	5 µl
RNase Free dH_2O	to 20 µl	to 20 µl	to 20 µl

Notes:

1. No RNA samples are added to the positive and negative controls. Only add positive and negative controls sample into the PCR reaction tubes.
2. After the Master Mix is prepared, mix well and aliquote into the PCR reaction tube as soon as possible. Centrifuge at 1,000 rpm for 15 seconds. Cap the PCR reaction tube tightly and load it in the qPCR instrument as soon as possible.

RT-PCR amplification (detection part):

Put each reaction tube into the PCR instrument in order, and the reaction is carried out according to the following conditions: when testing with this kit, the FAM channel and HEX channel are both selected for the instrument channel. The reaction volume is 20 µl.

Set the parameters as follows:

Step	Cycle	Temperature	Reaction time (min:sec)
1	1	42	5 min
2	1	95	10 sec
3	40	95	5 sec
		60	30 sec (signal collection)

Results

1. Baseline and Threshold adjustment: adjust the Baseline and Threshold according to the image after analysis. The Begin value of the baseline of the Bio-Rad series of instruments can be 3, and the End value can be 15, and the threshold can be adjusted manually to the point where the threshold line exceeds the peak of the regular blank control product's FAM-channel/HEX channel amplification curve (irregular noise line).

2. The experiment is valid if the following two points are satisfied at the same time. Otherwise, the experiment is invalid and needs to be repeated.
(1) Negative control: neither FAM channel nor HEX channel detected CT value or CT value > 40.
(2) Positive control: both FAM channel and HEX channel showed typical S-shaped curves with CT value < 30.

3. When satisfying the abvoe experimental validity conditions, the following analysis is performed (this kit is for FAM and HEX dual-channel detection):
(1) Positive: Ct≤37 (both FAM and HEX channel), it is to be COVID-19 nucleic acid positive.
(2) Negative: Ct >40 or uncountable (both FAM and HEX channel), and the positive control result is positive, the sample result will be negative for the COVID-19 nucleic acid.
(3) Suspicious, suggest re-extracted and test again: 37<Ct≤40. If the Ct value of the retest results is still in the range of 37-40, the amplification curve is S-shaped and there is a significant exponential growth, it is judged as COVID-19 nucleic acid positive, otherwise it is COVID-19 nucleic acid negative.

Limitations

1. The test results of this product are for reference only and cannot be used as the basis for diagnosis. The test results are only for the reference of Scientific Professionals.

2. The test results are related to the collection, storage and transportation conditions of samples, in which any link error can lead to false-negative results: False-positive results may occur if cross contamination occurs during sample processing.

3. The target sequences detected in this kit are the conserved regions of the COVID-19 N and ORFlab genes.

Product performance index

1. Detection limit: the detection limit of this kit is 100 copies/ml.
2. Precision: repeatable reference is tested for ten consecutive times, and its CV of Ct value is less than 10%.
3. The appearance of the kit is good, the liquid components are clear, transparent and non-soluble, and the dosage of each reagent tube is correct.
4. When the kit tested positive control, both the FAM channel and the HEX channel are positive, while when the kit tested negative control, both the FAM channel and the HEX channel are negative.

Caution for handling

1. Before the experiment, please read the kit instructions carefully and strictly follow the operation steps.
2. Specially trained inspectors are required, and the test should be operated in a laboratory with safety protection and protective equipment.
3. The kit should be kept away from light to avoid fluorescence attenuation. The centrifuge tube and tip head used shall be autoclaved and DNase/RNase free. Labelling on the cap of centrifuge tubes should be avoided, which will interfere with the fluorescent signal.
4. The samples to be tested in this kit shall be considered as infectious substances, and the operation and treatment shall meet the requirements of the ministry of health's <<Gereral Guidelines for Biosafety of Microbial Biomedical Laboratories>> and <<Regulations on clinical Waste Management>>.

References

1. Centers for Disease Control and Prevention. Real-Time RT-PCR Panel for Detection 2019-Novel Coronavirus. January 2020.

2 Nao N, *et al*. Detection of second case of 2019-nCoV infection in Japan.
3. Department of Medical Sciences, Ministry of Public Health. Diagnostic detection of Novel coronavirus 2019 by Real time RT-PCR. January 2020.
4. Corman V *et al*. Diagnostic detection of 2019-nCoV by real-time RT-PCR. Berlin, Jan 17th 2020.

Production batch number

See label and packaging.