DisCoVery

SARS-CoV-2 RT-PCR

Detection Kit Rox

biotech

PRODUCT NAME

Dis**CoV**ery SARS-CoV-2 RT-PCR Detection Kit Rox.

PACKAGING SPECIFICATIONS

200 tests/kit.

INTENDED USE

This kit is intended for in vitro qualitative detection of *ORF1ab and N* genes from the 2019-nCoV in pharyngeal swab or bronchoalveolar lavage specimens collected from Coronavirus disease 2019 (COVID-19) suspected cases, suspected clusters of cases, or other individuals who need 2019-nCoV infection diagnosis or differentiation diagnosis.

The definitions of COVID-19 related groups such as "suspected cases" or "suspected clusters of cases" should be referred to *Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia*, Surveillance Protocol for Novel Coronavirus Pneumonia or other COVID-19 related documents (the latest version) from China CDC.

This kit is only for use in auxiliary diagnosis or storage for emergency use of COVID-19 in vitro diagnosis during COVID-19 outbreak since December of 2019. It cannot be used as a conventional in vitro diagnosis reagent for clinical practice. The use of this kit should be under the requirements of *Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia*, *Protocol for Prevention and Control of COVID-19* and other COVID-19 related documents (the latest version).

The nucleic acid detection of 2019-nCOV should conform to requirements of COVID-19 related documents such as *Laboratory testing for COVID-19* (the latest version) from China CDC. The biosafety requirements should be strictly complied with.

The detection results of this kit should be regarded as a reference for clinical practice, but not as the sole standard for clinical diagnosis. It is suggested to make a comprehensive analysis combined with clinical symptoms and other laboratory testing methods.

The laboratory personnel for 2019-nCOV detection should be professionally trained in gene amplification or molecular biology detection and qualified for related experimental operations. Biosafety protective equipment and programs are required for the laboratories.

PRINCIPLES

The kit is designed for RNA detection of 2019-nCoV in specimens using multiplex real time RT-PCR technology and with the conserved regions of ORF1ab and N genes as target sites of the primers and probes. Simultaneously, this kit contains an endogenous control detection system (the control gene is labeled with Rox) to monitor the process of specimen collection, nucleic acid extraction and PCR, and reduce false negative results.

KIT CONTENTS

Component Name	Main Constituents	Specifications and Quantity (200 test)
PCR Reaction Mix	Reaction buffer, dNTPs, Taq polymerase, uracil-DNA glycosylase (UDG), etc.	1000 μL × 3 tubes
PCR Reverse Transcriptase	Reverse transcriptase, RNase inhibitor	100 μL × 1 tube
PCR Primer/Probe Mix 2	Primers and probes for ORF1ab and N genes; primers and probes for the control-RNase P gene (RP-Rox)	900 μL × 1 tube
Positive Control	*In vitro* transcribed RNAs with ORF1ab, N and control-RP genes sequences	200 μL × 1 tube*
Negative Control	Nuclease-free Water	200 μL* × 1 tube*

Note: Components from different lots should not be mixed for use.

**CDC Guidelines recommend testing subsets of 10 samples with the corresponding positive and negative controls.*

STORAGE CONDITIONS AND SHELF LIFE

Store the kit at -20±5ºC away from light for 6 month.

Ship the kit at low temperature. Dry ice should be used for long-distance shipping; avoid repeated freeze-thaw cycles (freeze-thaw cycles should be fewer than 10).

Manufacture date and expiration date are shown on the label.

SPECIMEN REQUIREMENTS

Suitable specimen types: pharyngeal swab or bronchoalveolar lavage specimens.

Sampling of specimen: Follow the routine specimen sampling method or Laboratory testing for COVID-19 (the latest version) from China CDC.

Specimen storage and shipping: specimens to be used immediately or within 24 hours should be stored at 4ºC. Specimens which cannot be used within 24 hours should be stored at or below 70ºC. If -70ºC is not possible, the specimens to be tested can be stored at -20ºC for 10 days and nucleic acid can be stored at -20ºC ±5ºC for 15 days. Repeated freeze-thaw cycles should be avoided. Specimens should be shipped on ice in sealed foam boxes for transportation or adding ice constantly on the way.

TEST METHOD

1. Specimen preparation (specimen preparation area)

Prepare 200 µL of specimen for nucleic acid extraction. Extracted RNA can be used directly for detection. If the extracted RNA is not for the subsequent detection after extraction immediately, it can be stored at -70ºC, avoiding repeated freeze-thaw cycles.

2. Reagent preparation (reagent preparation area)

Thaw PCR Reaction Mix and PCR Primer/Probe Mix 2 at room temperature. Mix thoroughly to ensure homogeneity, then centrifuge briefly. Briefly spin down PCR Reverse Transcriptase, and put on ice for the next step.

Prepare the reaction mix for the number of reactions based on the table below. It is recommended to set up a negative and positive control for each test. When the number of specimens is **n**, the number of reactions N = the number of specimens (n) + positive control (1) + negative control (1) + 1.

PREPARATION OF THE REACTION

Kit Components	Volume per Reaction
PCR Reaction Mix	15 µL × N
PCR Primer/Probe Mix 2	4.5 µL × N
PCR Reverse Transcriptase	0.5 µL × N

Mix the reagents thoroughly, then dispense equal 20 µL into each microcentrifuge tube and transfer to the specimen handling area.

3. Specimen addition (specimen handling area)

Add 5 µL of extracted Positive Control, Negative Control and specimen nucleic acid to the aliquoted system to reach a total reaction volume of 25 µL. Tightly cap the reaction tube, then centrifuge briefly at low speed and move to the test area.

4. PCR amplification (amplification and analysis area)

Place the PCR tube in sequence into the PCR instrument and set the specimen types of positive control, negative control, and specimen nucleic acid, and the specimen names.

Select the FAM and VIC channels to detect the 2019-nCoV gene ORF1ab and N respectively, and the Rox channel to detect the internal control gene RP. “Quencher dye” and “passive reference” are set to “none”.

STEPS	TEMPERATURE	REACTION TIME	CYCLES
Reverse Transcription	50°C	5 min	1
Pre-denaturation	95°C	30 s	1
Denaturation	95°C	5 s	45
PCR cycling	60°C	34 s	

5. Result analysis

The results are automatically saved after the reaction. Then analyze the amplification curves of the target genes and the internal control gene separately. According to the analysis of the image, adjust Baseline's Start value, End value and Threshold value, click Analyze for analysis and then record the qualitative results under the Plate window. (As for ABI 7500, the user can adjust manually according to the actual conditions to ensure that all the baselines for the curves are flat. For instance, the Start value can be set from 3 to 15, the End value can be set from 5 to 20 and the threshold can be set just above the summit of the negative control amplification curve for the three fluorophore channels).

6. Quality control (evaluation of experiment effectiveness)

Each control in the kit should meet the following requirements, otherwise the experiment is invalid.

	Positive Control	Negative Control
FAM channel *(ORF1ab gene)*	Ct ≤ 34	No Ct value
VIC channel *(N gene)*	Ct ≤ 34	No Ct value
Rox channel (internal standard gene)	Typical S-shaped curve, and Ct ≤ 34	No Ct value

REFERENCE CT VALUE FOR POSITIVE RESULT

The reference Ct value to determine target gene as positive is set at 38. The internal standard for Ct value is 38.

INTERPRETATION FOR TEST RESULTS

- If typical S-shaped curve is observed in Rox channel of the specimen and Ct ≤ 38, the results can be determined as the table below.

VIC channel FAM		FAM channel: ORF1ab gene	
		Ct ≤ 38	Ct > 38
VIC channel: N gene	Ct ≤ 38	Positive	Suspected Positive
	Ct > 38	Suspected Positive	Negative

The suspected positive specimens should all be double checked. If the double-checked result for both Ct values of FAM and VIC channels is higher than 38, the result is negative; otherwise, it is positive.

- If the Ct value of Rox channel is higher than 38 without showing apparent S-shaped amplification curve, the causes can be listed as following:
- PCR inhibitors exist in the specimen. It is suggested to dilute the specimen before test.
- The operation of nucleic acid extraction is flawed. It is suggested to repeat nucleic acid extraction for the test.
- Eligible specimens were not obtained in the processing procedures or specimens have been degraded during transportation and storage. It is suggested to perform sampling again.

ASSAY LIMITS

- The test result is provided for reference only in clinical practice, but it cannot be the sole evidence for diagnosis.
- Negative results can be caused by low quality of RNA extracted from the specimens, improper storage conditions of solution of extracted RNA, inappropriate storage period, inhibitors in the specimen, nucleic acid degradation, etc.
- False negative or false positive results are likely to be caused by inappropriate collecting, transportation and handling of specimens, or unsuitable experiment operation and environment.
- False negative results may occur by changes in the target sequences of 2019-nCoV due to mutations or other reasons.

PRODUCT SPECIFICITIES

- Minimum detection limit：500 copies/mL.
- The negative and positive controls provided with the kit resulted 100% negative and positive respectively.

NOTES

Please read the manual carefully before testing and follow the protocol strictly.

Test analysts should be trained by professionals and must perform operation in labs following safety guidelines and wearing personal protective equipment.

The kit should be stored away from light to protect the fluorophore from decay. All the centrifuge tubes and tips should be autoclaved to guarantee them to be DNase and RNase free.

The test specimens involved in this kit should be considered as infectious substances, and their treatment and handling must meet the relevant regulations of the General Guidelines for Biosafety of Microbiology and Biomedical Laboratories and the Medical Waste Management Regulations issued by of the Ministry of Health.

REFERENCES

Tang X, Wu C, et al. (2020). On the origin and continuing evolution of SARS-CoV-2. National Science Review, nwaa036, https://doi.org/10.1093/nsr/nwaa036.

(2019) COVID-19: Laboratory Testing Guideline (Fifth Edition) [PDF File]. http://www.chinacdc.cn/en/COVID19/202003/P020200308322036088669.pdf.