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SARS-CoV-2 Nucleic Acid Detection Kit (Multiplex Real Time RT-PCR) (48 tests/kit)

Revision: A/0

【Product Name】

DV101-01: SARS-CoV-2 Nucleic Acid Detection Kit (Multiplex Real Time RT-PCR) (48 tests/kit)

【Packaging Specifications】

48 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 with gene amplification or molecular biology detection and qualified for related experimental operations. Biosafety protective equipment and programs are required for the laboratories.

【Principles】

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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 by Cy5) 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 (48 tests)
2019-nCoV PCR Reaction Mix	Reaction buffer, dNTPs, Taq polymerase, uracil-DNA glycosylase (UDG), etc.	720 μL×1tube
2019-nCoV PCR Reverse Transcriptase	Reverse transcriptase, RNase inhibitor	24 μL×1 tube
2019-nCoV PCR Primes / Probes	Primes and probes for *ORF1ab* and *N* genes; primes and probes for the control-RNase P gene (RP)	216 μL×1 tube
2019-nCoV Positive Control	*in vitro* transcribed RNA with *ORF1ab* and *N* gene sequences; *in vitro* transcribed RNA with the control-RP gene sequence	50 μL×1 tube
2019-nCoV Negative Control	RNase-free Water	50 μL×1 tube

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

【Storage Conditions and Shelf Life】

- Store the kit at -20±5°C away from light for 12 months.
- 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.

【Instrument】

ABI7500 Real-Time PCR instrument.

【Specimen requirements】

1. Suitable specimen types: pharyngeal swab or bronchoalveolar lavage specimens

CE

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

3. Specimen storage and shipping: specimens to be used immediately or within 24 hours should be stored at 4°C. Specimens which cannot be used within 24 hours should be stored at or below 70°C. If -70°C is not possible, the specimens to be tested can be stored at -20°C for 10 days and nucleic acid can be stored at -20°C ±5°C 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 and avoid repeated freeze-thaw cycles. The positive and negative controls in this kit are involved in the extraction process.

2. Reagent preparation: (Reagent preparation area)

Thaw 2019-nCoV PCR reaction mix and 2019-nCoV PCR combined primer/probe mix at room temperature. Mix thoroughly to ensure homogeneity, then centrifuge briefly. Briefly spin down 2019-nCoV PCR reverse transcriptase, and put on the 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 table

Kit Components	Volume per reaction (μL)
2019-nCoV PCR reaction mix	15 μL × N
2019-nCoV PCR combined primer/probe mix	4.5 μL × N
2019-nCoV 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)

CE

Add 5 μL of extracted 2019-nCoV positive control, 2019-nCoV 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 Cy5 channel to detect the internal control gene RP. For the ABI 7500 real-time PCR instrument, “Quencher dye” and “passive reference” are set to “none”.

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

5. Result analysis

The results are automatically saved after the reaction. Then analyze the amplification curve of the target gene and 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, End value can be set at 5 to 20. Threshold value should be set right above the summit of the amplification curve of negative control.)

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 ≤ 32	No Ct value or Ct>40
VIC channel (*N* gene)	Ct ≤ 32	No Ct value or Ct>40
Cy5 channel (internal standard gene)	Typical S-shaped curve, and Ct ≤ 32	No Ct value or Ct>40

【Reference Ct value for positive result】

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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】

1. If typical S-shaped curve is observed in Cy5 channel of the specimen and Ct<=38, the results can be determined as the table below.

VIC channel FAM	FAM channel		
	Ct<=38	38<Ct<=40 (in a sigmoidal shape)	Ct>40
Ct<=38	Positive	Positive	Suspected positive
38<Ct<=40 (in a sigmoidal shape)	Positive	Suspected positive	Suspected positive
Ct>40	Suspected positive	Suspected positive	Negative

For specimens tested as positive, when the Ct values of a target gene are between 38-40, it is necessary to observe if the amplification curve of the target gene is in sigmoidal shape. If not, the specimen should be regarded as suspected positive.

The suspected positive specimens should all be double checked. If the double-checked result shows both amplification curves of FAM and VIC channels are in a sigmoidal shape with Ct vales no higher than 40, it is positive, If the Ct values of both channels are higher than 40, it is negative. Other results are suspected positive and it is suggested to conduct the detection again.

2. If the Ct value of Cy5 channel is higher than 38 without showing apparent S-shaped amplification curve, the causes can be listed as following:

1) PCR inhibitors exist in the specimen. It is suggested to dilute the specimen before test.
2) The operation of nucleic acid extraction is flawed. It is suggested to repeat nucleic acid extraction for the test.
3) 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.

【Limitations of detection method】

1. The test result is provided for reference only in clinical practice, but it cannot be the sole evidence for diagnosis.

2. 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.

3. 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.

CE

Other clinical observations and relative information should be combined for determination. Conduct the detection again when necessary.

4. False negative results may occur by sequence changes of target sequence of 2019-nCoV due to mutations or other reasons.

【Product performances】

1. Minimum detection limit: 500 copies/mL.

2. The negative and positive reference products of the testing enterprises were 100%.

3. Linearity: the linear range for ORF1ab is 1.24×103 copies/μL- 1.24 copies/μL; the standard curve is Y=-3.263X+34.223; the linear correlation coefficient is no lower than 0.997.The linear range for N is 9.8×102 copies/μL- 0.98 copies/μL; The standard curve is Y=-3.358X+34.542; the linear correlation coefficient is no lower than 0.996.

4. Accuracy

The positive detection rate should be 100%. The negative detection rate for negative control should be 100%.

5. Analytical Specificity

The SARS-CoV-2 was compared with four human coronaviruses of HCoV-HKU1, HCoV-229E, HCoV-OC43 and HCoV-NL63. The results indicate that the SARS-CoV-2 is tested as positive and the other four coronaviruses are tested as negative. The reference standards with and without mucin both should be tested as positive. The negative samples should be tested as negative.

6. Precision

Quantitative fluorescence PCR is used with negative samples, limited positive samples, and strong positive RNA samples. The results indicate that the negative detection rate of the negative samples is 100%; the positive detection rate of the strong positive samples and the limited positive samples are 100% and ≥95% respectively.

【Precaution】

1. Please read the manual carefully before test and follow the protocol strictly.

2. Set both positive and negative controls for each test.

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

4. The kits should avoid light for storage to protect the fluorophore from decay. All the centrifuge tubes, tips should be autoclaved to ensure DNase and RNase free.

5. Separate laboratory areas rigorously and perform the procedures in the predefined areas. To avoid cross contamination, all materials used in their designated area should not be moved or used in other areas. False positive results can be caused when cross contamination is not controlled during the sample treatment process.

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6. All lab workbench and supplies, such as pipettes, centrifuges, PCR cyclers should be disinfected using hypochlorous acid 1% or UV light for 25-30 mins.

7. After amplification, take out the reaction tubes and seal in a specially designed plastic bag to dispose in a designated area.

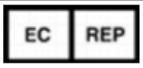
8. The test specimens involved in this kit should be considered as infectious substances, and their treatment and handling must meet the relevant regulations of the General Guidelines for Biosafety of Microbiology and Biomedical Laboratories and the Medical Waste Management Regulations issued by of the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

【References】

1. X Tang, C Wu, et al. On the origin and continuing evolution of SARS-CoV-2. National Science Review. 2020

2. Guidelines for Laboratory testing for COVID-19 (the fifth edition)

【Symbols and Interpretations】

				
For in vitro diagnostic use only	Attention, see instruction for use	Do not use if package is damaged	Limiting temperature	Do not reuse
				
Afraid of the sun	Manufacturer	Date of production	Validity	Batch code
				
Conformity of European	Authorized Representative	Tests per kit	Keep dry	Catalog

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