Instructions for Use of Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing)

DA0930~DA0932

[Product Name]

Generic name: Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing)

[Package Specifications]

Large package, 24 tests/kit; large package, 48 tests/kit;

[Intended Use]

This kit is used for the in vitro qualitative detection of novel coronavirus (2019-nCoV) ORFlab and N gene in the throat swabs, sputum specimens of suspected pneumonia patients infected by novel coronavirus, suspected clustering cases and others needing diagnosis or differential diagnosis for novel coronavirus.

Detection of novel coronavirus RNA shall meet the requirements of related guideline,clinical diagnostic/disease control and other documents, so as to carry out the regulations on biosafety. The detection results of this kit are for clinical reference only and should not be used as the sole criteria for clinical diagnosis. It is recommended to conduct a comprehensive analysis on the condition in combination with the clinical manifestations of the patient and other laboratory tests.

[Test Principle]

This kit is based on one-step RT-PCR technique. In practice, 2019 Novel Coronavirus (2019-nCoV) ORF1ab and N genes are selected as amplification target regions. Specific primers and fluorescent probes are designed for the detection of 2019 Novel Coronavirus RNA in the specimens. This kit also includes an endogenous internal standard detection system, which is used for monitoring over the processes of specimen collection, RNA and PCR amplification, thereby reducing false negative results.

[Main Components]

Component name		Specification	Quantity	Main constituents
PCR detection reagents (large package, 24 tests/kit)	NC(ORF1ab/N) PCR reaction liquid A	450 μl/ tube	1	Specific primers, probes, tris(hydroxymethyl)aminomethane-hydrochloric acid buffer
	NC(ORF1ab/N) PCR reaction liquid B	100 μl/ tube	1	Hot start Taq DNA polymerase, c-MMLV reverse transcriptase, etc.
PCR detection reagents (large package, 48 tests/kit)	NC(ORF1ab/N) PCR reaction liquid A	900 μl/ tube	1	Specific primers, probes, tris(hydroxymethyl)aminomethane-hydrochloric acid buffer
	NC(ORF1ab/N) PCR reaction liquid B	200 μl/ tube	1	Hot start Taq DNA polymerase, c-MMLV reverse transcriptase
Control material (large package, 24 tests/kit; large package, 48 tests/kit)	NC (ORF1ab/N) negative control material	400 μl/ tube	1	Pseudovirus with internal standard fragment
	NC (ORF1ab/N) positive control material	400 μl/ tube	1	Pseudovirus containing target fragments, pseudovirus containing internal standard fragments

The above components in different batches of kits cannot be interchangeable.

Reagents to be self-prepared: RNA extraction or purification reagents. It is recommended to use YHXB No. 20170583 and YHXB No. 20150302 products manufactured by DAAN.

Description of negative/positive control materials: the positive control material is pseudovirus containing the target fragments and pseudovirus containing internal standard fragments, while the negative control material is pseudovirus containing an internal standard fragments. During use, they should be involved in extraction and should be considered as an infectious substance. They shall be handled and disposed in accordance with relevant regulations.

[Storage Conditions and Validity Date]

The kit is stored at -20±5℃, and the validity period is 6 months.

The reagent can be stored at 4°C for 3 days; the kit can be stored at 37°C for 3 days; repeated freezing and thawing should be avoided; repeated freezing and thawing cycles should not exceed 7 times and the times of decapping the reagent should not exceed 7.

See the product label for the date of manufacture and validity of the kit.

[Applicable Instruments]

ABI 7500, LightCycler480, AGS4800, Bio-Rad CFX96.

[Specimen Requirements]

1. Applicable sample types: throat swabs, sputum, bronchoalveolar lavage fluid, anus swab, blood.

2. Specimen collection (aseptic technique)

Throat swab: Wipe the tonsil and posterior pharyngeal wall with two swabs at the same time, and immerse the swab heads into the tube containing the sampling liquid;

Sputum: After the patient has a deep cough, collect the coughed sputum in the screw-cap test tube containing the sampling liquid;

Bronchoalveolar lavage fluid(BALF):After local anesthesia, insert the bronchoscope through pharyngeal into bronchus of middle lobe of right lung or lingual segment of left lung via mouth or nose, place the top into the branch opening of bronchus, add sterile saline slowly via trachea biopsy hole, 30-50ml per time, volume 100-250ml, not exceed 300ml.

Anus swab: Insert disinfect cotton swab into anus 3-5cm, pull out after rotation, put into virus preservation solution collection tube immediately, discard the end of swab, screw the collection tube.

Blood sample: Collect blood sample 5ml recommended using EDTA vacuum tube, confirm using whole blood or plasma to perform nucleic acid extraction according to the requirement of nucleic acid kit. If separating plasma is needed, centrifuge the whole blood at 1500-2000rpm for 10 minutes, collect supernatant into sterile collection tube.

3. Sample storage and transportation

The specimens for virus isolation and RNA detection should be tested as soon as possible. The specimens that can be detected within 24 hours can be stored at 4℃; those that cannot be detected within 24 hours should be stored at -70℃ or below (if no -70℃ storage conditions, then temporarily stored in -20℃ refrigerator). The specimens should avoid repeated freezing and thawing during transport. Specimens should be sent to the laboratory as soon as possible after collection. If the specimens need to be transported over long distances, it is recommended to use dry ice for storage.

[Test Method]

1. Specimen processing and RNA extraction (specimen processing area)

It is recommended to take 200 μl of liquid specimen for RNA extraction. The RNA extraction kit or purification kit (magnetic beads) (YHXB No. 20170583 and YHXB No. 20150302) produced by DAAN Gene Co., Ltd. Of Sun Yat-sen University are applicable. Other RNA extraction kits are needed validation by the end user. For specific steps, please follow the Instruction for Use of the kit.

Both negative and negative control materials in this kit are involved in the extraction.

2. PCR reagent preparation (reagent preparation area)

Take out the NC (ORF1ab/N) PCR reaction liquid A and NC (ORF1ab/N) PCR reaction liquid B from the kit. After thawing at room temperature, shake and mix. Centrifuge at 8,000 rpm for a few seconds before use.

Take N (N = number of specimens to be tested + NC (ORF1ab/N) negative control material + NC (ORF1ab/N) positive control material) PCR reaction tube. A NC single-reaction amplification system is prepared as follows:

NC(ORF1ab/N) PCR reaction liquid A	NC(ORF1ab/N) PCR reaction liquid B	Amplification system
17μl	3μl	20μl

After thoroughly mixing the components, centrifuge for a short time to cause all the liquid on the tube wall to fall to the bottom of the tube, and then aliquot 20 μl of the amplification system into the PCR tubes.

3. Sampling (specimen preparation area)

Add 5 μl each of the negative control material, the RNA of specimens to be tested, and the positive control material processed into the PCR reaction tubes, cover the tubes tightly, and transfer them to the amplification detection area after centrifugation at 8,000 rpm for several seconds.

4. PCR amplification (amplification detection area)

4.1 Place the reaction tube in the sample sink of the instrument.

4.2 Setting of ABI Prism 7500 Instrument (take ABI Prism 7500 instrument as an example)

4.2.1 Open the "Setup" window, set the negative control (NTC), positive control and Unknown specimen in the corresponding order, and set the specimen name in the column of "Sample Name"; the probe detection modes are set as: Repoiter1: FAM, Quencher 1: NONE; Reporter2: VIC, Quencher2: NONE; Reporter3: Cy5, Quencher3: NONE; Passive Reference: NONE。

4.2.2 Open the "Instrument" window and set the cycle conditions as follows:

Stage	Reps	Target (℃)	Running Time	Data Collection
1	1	50	00:15:00	
2	1	95	00:15:00	
3	45	94	00:00:15	
		55	00:00:45	√

After setting, save the file and run the program.

4.3 Setting of LightCycler480 Instrument

4.3.1 After opening the software, select "New Experiment", set the detection mode to "Multi Color Hydrolysis Probe/UPL Probe", and the detection channel to FAM, VIC and Cy5.

4.3.2 Setting of cycling conditions:

Program name	Cycles	Target (℃)	Running Time	Analysis Mode	Acquisition Mode
1	1	50	00:15:00	None	None
2	1	95	00:15:00	None	None
3	45	94	00:00:15	None	None
		55	00:00:45	Quantification	Single

4.3.3 Select "Sample Editor" to set the sample name, save the file and run the program after setting.

4.4 Setting of AGS4800 Instrument

4.4.1 Start AGS4800 software, select corresponding layer of machine(upper,middle,bottom)

4.4.2 Click ”New”, edit experiment name, file route, save and click OK button to open a new file.

4.4.3 Program setting: The amplification parameters are as following:

Program name	Cycles	Target (℃)	Running Time	Data Collection
1	1	50	00:15:00	
2	1	95	00:15:00	
3	45	94	00:00:15	
		55	00:00:45	√

4.4.4 Sample setting: click sample setting page, select the needed fluorescence channel in the upper menu(channel 1 FAM, channel 2 VIC, channel 3 Cy5, others blank), select the needed sample pore in the 48 sample position table, click FAM, VIC and Cy5 as fluorescence type at the right side, select the corresponding sample type at the bottom button: negative control, unknown, etc.

4.4.5 Start amplification: Confirm the sample tubes are well placed, click start button to start PCR cycling.

4.4.6 Save the result: After the experiment finished, save automatically to the file route.

4.5 Setting of Bio-Rad CFX96 Instrument

4.5.1 Double click Bio-Rad CFX manager software, guidance interface will appear, select ‘create a new experiment’, click OK to confirm.

4.5.2 Program setting: in the interface of experiment setup, click ‘protocol’, choose’create new’ to set the program. ‘Protocol editor’ interface appear, modify time or temperature is allowed. ‘Insert step’: to increase a step. ‘Insert gradient’: to increase a temperature gradient. ‘Insert goto’: to increase a cycle.

‘Add plate read to step’: Add plate read to this step. Setting as below:

Program name	Target (℃)	Running Time
1	50	00:15:00
2	95	00:15:00
3	94	00:00:15
4	55	00:00:45
5	+Plate Read	
	GOTO 3, 44 more times	

4.5.3 Plate setting: in the experiment setup interface, click ‘plate’ to set up the plate, choose ‘create new’. ‘Plate editor’ interface appear, choose the needed sample area. Click ‘select fluorophores’ to choose fluorescence type, then click OK. Click ‘sample type’ to choose sample type. Click ‘Replicate series’ to give number to the sample. Plate parameter as follow: fluorescence: FAM,VIC,CY5; sample:unknown; Click OK to save.

4.5.4 Start detection: in the experiment setup interface, click ‘open lid’ then put in the samples, click’close lid’ and ‘start run’, confirm the result saving route, click ‘start run’ to start the experiment.

5. Analysis of results (please refer to the instruction for use of each instrument for setting, taking ABI7500 Instrument as an example)

After reaction, save the results. Adjust the Start value, End value and Threshold value of Baseline according to the image after analysis (the user can adjust them according to the actual conditions, the Start value can be set at 3~15 and the End value at 5~20, adjust the Threshold value at the Log chart window, enabling the Threshold value line to be at the log phase, the amplification curve of the negative control to be straight or lower than the threshold line), click Analysis to obtain the analysis result automatically, and read the test result in the "Report" window.

6. Quality control

NC (ORF1ab/N) negative control material: no obvious amplification curve for FAM and VIC detection channels, and obvious amplification curve for Cy5 channel;

NC (ORF1ab/N) positive control material: obvious amplification curves for FAM and VIC detection channels and Ct value ≤ 32, and amplification curve or no amplification curve for Cy5 channel;

The above requirements must be met at the same time in the same experiment; otherwise, the experiment is invalid and needs to be carried out again.

P.S., FAM channel for N gene detection; VIC channel for ORF1ab gene detection; Cy5 channel for internal standard.

7. Determination of results

7.1 If the test sample has no amplification curve or Ct value > 40 in the FAM and VIC channels, there is amplification curve in the Cy5 channel, it can be judged that there is no 2019 Novel Coronavirus (2019-nCoV) RNA in the sample;

7.2 If the test sample has obvious amplification curve in the FAM and VIC channels and Ct value ≤ 40, it can be judged that the sample is positive for 2019 Novel Coronavirus (2019-nCoV);

7.3 If the test sample only has the Ct value of ≤ 40 in a single channel of FAM or VIC, and there is no amplification curve in the other channel, the results need to be re-tested. If the re-test results are consistent, the sample can be judged to be positive for 2019 Novel Coronavirus (2019-nCoV). If the re-test results are negative, it can be judged that no 2019 Novel Coronavirus (2019-nCoV) RNA has been detected.

[Positive Judgment Value]

The ROC curve method is used to determine both the reference CT value of the kit and the internal standard reference value are 40.

[Interpretation of Test Results]

1. Negative and positive control materials should be tested in each experiment. Only when the control materials meet the quality control requirements can the test results be determined;

2. When the FAM and VC detection channels are positive, the result from the Cy5 channel (internal standard channel) may be negative due to the competition of the system;

3. When the internal standard result is negative, if the FAM and VC detection channels of the test tube are also negative, it indicates that the system is inhibited or the operation is wrong, the test is invalid. Therefore, the sample needs to be re-tested;

4. The report is recommended to be in the following format:

The format of negative result report: no 2019 Novel Coronavirus (2019-nCoV) RNA was detected in the specimens, and the concentration was lower than the sensitivity of the kit;

The format of the positive result report: 2019 Novel Coronavirus (2019-nCoV) RNA was detected in the specimens.

[Limitations of Test Method]

1. The specimen test results are related to the quality of specimen collection, processing, transportation and storage. Unreasonable sample collection, transfer, storage and processing may lead to incorrect test results;

2. Cross contamination is not well controlled during specimen processing, and a false positive result may appear;

3. The genetic mutation of the virus during the epidemic period may also lead to false negative results;

4. The test results of this kit are for clinical reference only. The clinical diagnosis and treatment of patients should be considered in combination with their symptoms/signs, medical history, other laboratory tests and treatment response.

[Product Performance Indicators]

Product analysis performance evaluation results:

1. The analytical sensitivity of this kit is 500 copies/ml.

2. Cross-reaction: no cross-reaction with other pathogens such as seasonal influenza A (H1N1) virus, novel influenza A (H1N1-2009) virus, influenza A H3N2, H5N1, H7N9, influenza B Yamagata, influenza B Victoria, RSV A, RSV B, parainfluenza I, parainfluenza II, parainfluenza III, adenovirus types 1, 2, 3, 4, 5, 7 & 55, enterovirus types A, B, C and D, hMPV (human metapneumovirus), EB virus, measles virus, human cytomegalovirus, rotavirus, norovirus, mumps virus, varicella zoster virus, mycoplasma pneumoniae, chlamydia pneumoniae, legionella, bordetella pertussis, haemophilus influenzae, staphylococcus aureus, streptococcus pneumoniae, streptococcus pyogenes, klebsiella pneumoniae, mycobacterium tuberculosis, aspergillus fumigatus, candida albicans, candida glabrata, cryptococcus neoformans, coronavirus (HKU1, OC43, NL63, 229E), SARS coronavirus, MERS coronavirus, and human genomic DNA that are similar to 2019 Novel Coronavirus or cause similar symptoms.

3. Exogenous interfering substances: the therapeutic drugs against 2019 Novel Coronavirus in the specimens, such as benzylline, oxymetazoline, sodium chloride, beclomethasone, dexamethasone, flunisolide, triamcinolone, budesonide, mometasone, fluticasone, histamine hydrochloride, interferon, zanamivir, ribavirin, oseltamivir, peramivir, lopinavir, mupirocin, levofloxacin, azithromycin, tobramycin, ritonavir, meropenem, arbidol, and ceftriaxone will not interfere with the test results of the kit.

4. Endogenous substances such as whole blood and mucus that may be present in sputum and throat swab specimens have no interference with the test results of the kit.

5. Precision: within-run/between-run precision, within-day /day-to-day precision, and the coefficient of variation (CV) of precision between different operators are not more than 5%.

6. Coincidence rate of negative/positive reference materials: The coincidence rates of 3 positive reference materials and 10 negative reference materials all are 100%.

7. The clinical evaluation result is compared with the confirmed/exclusion results obtained by the recommended methods in the *Diagnosis and Treatment Scheme for Pneumonia Patients Infected by Novel Coronavirus* and *Monitoring Scheme for Pneumonia Patients Infected by Novel Coronavirus* (Second Edition). The actual usage data collected from 8 medical institutions such as Hainan Hospital of PLA General Hospital and Guangdong Center for Disease Control and Prevention was analyzed. Through initial analysis, it was basically confirmed that the clinical performance of the product could meet the emergency needs of the epidemic situation. Sample types for clinical evaluation include throat swabs and sputum. Further clinical data will be collected after its marketing to confirm its clinical performance.

[Precautions]

1. This product is used for in vitro test only. Please read the Instruction carefully before experiment;

2. In order to avoid any potential biological hazards in the specimens, the test specimens should be regarded as infectious and avoid contact with human skin and mucosa; the specimens should be handled in a biosafety cabinet that prevents aerosol outflow. The test tubes and tips used in the sample preparation area should be poured into a container containing disinfectant and sterilized with the medical wastes before discarding; sample handling and processing must comply with relevant regulations: including the General Biosafety Standard for Microbiological and Biomedical Laboratories and Regulations on the Administration of Medical Wastes issued by the Ministry of Health;

3. Product processing: After PCR, the product is likely to cause pollution. All reaction tubes should be put into a biosafety garbage disposal bag or other container by the person who is no longer involved in the experiment that day, and then discarded after these reaction tubes are completely sealed;

4. Avoid RNase contamination during the whole process. Wear work clothes, disposable gloves and masks during the experiment. Complete the operation in a well-ventilated chemical hood or biosafety cabinet that is clean, disinfected and sterilized by ultraviolet light to prevent any harmful substances from entering the respiratory tract;

5. Use autoclaved disposable centrifuge tubes and tips or purchase DNase-free and RNase-free centrifuge tubes and tips;

6. Thaw PCR detection reagents completely before use, and use after centrifugation at 8000 rpm for several seconds, but avoid repeated freezing and thawing;

7. False positive result may appear if cross contamination is not well controlled during specimen processing;

8. The laboratory management shall be in strict accordance with the management practice of PCR gene amplification laboratory. The laboratory personnel must receive professional training. The experimental process shall be strictly divided into different areas (reagent preparation area, specimen preparation area, amplification test area). All consumables shall be sterilized for single use. Special instruments and equipment shall be used in each stage of experiment. No cross-utilization of the supplies of each area in each stage shall be allowed;

9. After the experiment, 10% hypochlorite or 75% alcohol should be used to disinfect the worktable and pipette, followed by exposing them in ultraviolet light for 20-30 minutes;

10. After the RNA sample extraction is completed, it is recommended to proceed to the next experiment immediately; otherwise, please store the extract at -20℃ for use (within 24h);

11. Quality control must be performed over each experiment.

12. A variety of factors may cause performance changes during the storage, transportation, and use of reagents, such as improper storage and transportation, non-standard sample collection, sample processing and testing. Please strictly follow the Instruction. Due to the characteristics of the sample collection process such as sampling with swabs and the virus infection process itself, there may be false negative results caused by insufficient samples collected. Therefore, the test results should be comprehensively judged in combination with other clinical diagnosis and treatment information and re-testing should be carried out if necessary.

[References]

1. Clinical management of severe acute respiratory infection when novel coronavirus (nCoV) infection is suspected — Interim guidance. 2020.

[Basic Information]

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Production License number: YSYJXSCX No. 20040999

[Medical Device Registration Certificate No./Product Technical Requirements No.] GXZZ No. 20203400063

[Date of approval and amendment of the Instruction for Use] January 28, 2020