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

Cat.#DA-930

Version 4, June, 2020

CE

Table of Contents

1. Name

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

2. Serial Number of Kit

Cat.#DA-930

3. Specification

Large package, 24 tests/kit.

Large package, 48 tests/kit.

Large package, 96 tests/kit.

4. Shelf life

Reagent kits stored at -20°C±5°C, and the validity period is temporarily 6 months.

5. Intended Use

This kit is used for the in vitro qualitative detection of the ORF1ab and N genes of novel coronavirus (2019-nCoV) in pneumonia suspected cases of novel coronavirus infection, patients with suspected cluster cases, and other patients requiring diagnosis or differential diagnosis of novel coronavirus infection throat swabs and sputum samples. The test results of this kit are for clinical reference only and should not be used as the sole criterion for clinical diagnosis. It is recommended to conduct a comprehensive analysis of the condition in combination with the clinical manifestations of the patient and other laboratory tests.

6. Principle

PCR technique for diagnosis of pathogen is based on the amplification of special fragment of genome from the pathogen. Different from classical PCR techniques, fluorescent PCR uses fluorochrome to directly reflect the quantity change of PCR amplified products via changes of fluorescence energy released by excitation light stimulation. Variant of fluorescent signal is directly proportional to that of the amplified product. Collection and analysis of fluorescence is realized by highly sensitive automatic instrument so that original template quantity can be quantified.

This kit is based on one-step RT-PCR technology. The novel coronavirus (2019-nCoV) ORF1ab and N genes were selected as amplification target regions, and specific primers and fluorescent probes (N-gene probes were labeled with FAM, ORF1ab probes were labeled with Yellow) were designed to detect the RNA of novel coronavirus (2019-nCoV) sample. This kit is also includes an endogenous internal control detection system (the internal control probe is labeled with Cy5) for monitoring the sample collection,

nucleic acid extraction process and PCR amplification process which can reduce the occurrence of false negative results. The common experimental instruments are real-time fluorescent detection instruments such as *ABI 7500,* or Roche *LightCycler ® Instrument*. The quantification result can be gotten directly by computer analysis when the reaction is over.

7. Product Description

Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing) applies to qualitative in vitro diagnosis of 2019 Novel Coronavirus in the samples. This diagnostic method is based on PCR technique, combined with fluorescent probe technique, which can realize rapid auxiliary diagnosis of 2019 Novel Coronavirus infection.

Detailed clinical study validates high specificity, sensitivity, and reproducibility of the kit, which can be used for early diagnosis of clinical infection of 2019 Novel Coronavirus. See relevant information in 17. Technological Specification.

8. Contents

Name		Content
PCR detection reagent (large package, 24 tests/kit)	NC (ORF1ab/N) PCR reaction solution A	● 1×450μl ● Specific primers, probes, Tris-HCl, KCl, $(NH_4)_2SO_4$, $MgCl_2$
	NC (ORF1ab/N) PCR reaction solution B	● 1×100μl ● Hot start Taq DNA polymerase, c-MMLV enzyme, Rnasin, dNTPs, etc.
PCR detection reagent (large package, 48 tests/kit)	NC (ORF1ab/N) PCR reaction solution A	● 1×900μl ● Specific primers, probes, Tris-HCl, KCl, $(NH_4)_2SO_4$, $MgCl_2$
	NC (ORF1ab/N) PCR reaction solution B	● 1×200μl ● Hot start Taq DNA polymerase, c-MMLV enzyme, Rnasin, dNTPs, etc.
PCR detection reagent (large package, 96 tests/kit)	NC (ORF1ab/N) PCR reaction solution A	● 2×900μl ● Specific primers, probes, Tris-HCl, KCl, $(NH_4)_2SO_4$, $MgCl_2$
	NC (ORF1ab/N) PCR reaction solution B	● 2×200μl ● Hot start Taq DNA polymerase, c-MMLV enzyme, Rnasin, dNTPs, etc.
Quality Control (large package, 24 tests/kit, large package, 48	NC (ORF1ab/N) negative control	● 1×400μl ● Pseudoviruses with internal control segments
	NC (ORF1ab/N) positive	● 1×400μl

tests/kit, large package, 96 tests/kit)	control	● Pseudoviruses with target segments and internal control segments
	Instructions for Use	● 1 copy

9. Applicable Instruments

ABI PRISM® 7500 SDS and LightCycler480.

10. Warnings and Precautions

10.1 Users should read the instruction carefully.

10.2 For in vitro diagnostic use only.

10.3 Do not pipet by mouth.

10.4 Do not eat, drink, or smoke in laboratory working area. Wear protective disposable glove, laboratory coats, and eye protection when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and reagents.

10.5 Avoid microbial contamination of reagents when removing aliquots from reagent bottles. The use of disposable pipet tips is recommended.

10.6 Do not reverse the reagent.

10.7 Dispose of unused reagents and waste in accordance with country, federal, state, and local regulations.

10.8 Do not use a kit after its expiration date.

10.9 Workflow in the laboratory must proceed in a unidirectional manner, beginning in the Pre-Amplification area and moving to the Post-Amplification (Amplification/Detection) area. Pre-amplification activities must begin with reagent preparation and proceed to specimen preparation. Supplies and equipment must be dedicated to each pre-amplification activity and not used for other activities or moved between areas. Gloves must be worn in each area and must be changed before leaving that area. Equipment and supplies used for reagent preparation must not be used for specimen preparation activities or for pipetting or processing amplified DNA or DNA extraction buffer. Post-amplification supplies and equipment must remain to the Post-amplification area at all times.

10.10 Specimens should be regarded as infectious and processed in accordance with safe laboratory procedures required in country, federal, state, and local regulations. Thoroughly clean and disinfect all work surfaces with 10% bleaching solution. Supplies and equipment that have contacted specimens must be

processed with high pressure before being discarded.

10.11 This detection only applies to throat swab and sputum samples.

11. Stability and Storage

11.1 Properties of all the components of the kit are stable during transport under low temperature with dry ice.

11.2 After receiving the kits, open the outer packaging carton to validate there is residual dry ice, which can demonstrate that the quality of the reagents is not influenced during transport. Otherwise, please contact the authorized representative of our company in European Union to change the kits.

11.3 When getting the new kits, please immediately store them at -20°C±5°C, avoiding repeated freeze thawing.

11.4 The kit is stable until expiry date when operating according to the Instruction for Use.

11.5 The rest kit after opened should be stored at -20°C±5°C, and it is stable until the expiry date.

12. Materials and instruments required but not provided

- Nucleic acid extraction or purification reagents
- Stroke-physiological saline solution
- Disposable gloves and masque, powderless
- 1.5ml centrifuge tube and 0.5ml centrifuge tube
- Vortex mixer
- Adjustable pipettes and pipette tips with filters
- Table model high speed centrifuge with rotor for 1.5ml reaction tube
- Thermostat-controlled waterbath or other thermostatic equipment
- Real-time PCR amplification apparatus
- Specific reaction tube for PCR amplification apparatus (0.2ml light reaction tube and glass capillary)
- Ice

13. Pathogen Information

Coronaviruses are an unsegmented single-stranded positive-strand RNA virus, belonging to the Orthocorona mirinae subfamily of the Corona viridae family of the Nidovirales. Based on different serotypes and genomic characteristics, Coronaviruses can be divided into four generas-α, β, γ, δ. There are six

known coronaviruses that can infect human, including 229E and NL63 of the genus α, 0C43 and HPU of the genus β, Middle East Respiratory Syndrome Coronavirus (MERST-CoV), and Severe Acute Respiratory Syndrome Coronavirus (SARSR-CoV). Coronavirus isolated from the lower respiratory tract of patients with pneumonia is a new type of coronavirus belonging to the genus β. Coronaviruses have an envelope, and the particles are round or oval, usually in polymorphism, with a diameter of 50 to 200 nm. S protein is located on the surface of the virus to form a rod-like structure. As one of the virus's major antigen proteins, S protein is the main gene used for typing. The N protein encapsulates the virus genome and can be used as a diagnostic antigen. Most of the knowledge about the physicochemical properties of coronavirus comes from the research of SARS-CoV and MERS-COV. The virus is sensitive to heat. For 30 minutes at 56°C, diethyl ether, 75% ethanol, chlorine-containing disinfectant, peracetic acid, and chloroform can effectively inactivate the virus. Chlorhexidine can not effective in inactivating the virus.

The main clinical symptoms of 2019 novel coronavirus (2019-nCoV) infection were fever, fatigue and dry cough. Nasal congestion, runny nose and other symptoms of the upper respiratory tract are rare. About half of the patients develop dyspnea after one week. In severe cases, they progress rapidly to acute respiratory distress syndrome, septic shock, hard-to-correct metabolic acidosis, and coagulation dysfunction. It is worth noting that in the course of severe and critically ill patients, there can be moderate to low fever, even without obvious fever. Some patients have mild onset symptoms and no fever. Most patients who recover after 1 week have a good prognosis, and a few patients are critically ill and even die. Therefore, the early diagnosis and early treatment of 2019 novel coronavirus (2019-nCoV) infection has great significance to the prognosis and control of the disease, especially to control its large-scale spread.

At present, real-time PCR is the fastest and most reliable method for screening 2019 novel coronavirus (2019-nCoV).

14. Specimen Type, Collection, Pre-treatment, Transport, and Storage

14.1 Applicable specimens: throat swab and sputum.

14.2 Specimen Collection

⚠ ***Note***: All specimens have to be treated as potentially infectious material.

14.2.1 Collection of throat swab

14.2.1.1 Wipe the tonsils and pharyngeal wall with two swabs at the same time.

14.2.1.2 Put the swab head in the tube containing the sampling solution and seal up.

14.2.1.3 Label the tube with patient information and date/time collected.

14.2.2 Collection of sputum

14.2.2.1 Collect the deep coughed sputum to the spiral tube containing the sampling solution and seal up.

14.2.2.2 Label the tube with patient information and date/time collected.

14.3 Specimen transport

In order to ensure high quality of the specimens for laboratory use, specimens should be transported to laboratory as soon as possible. Specimens should be transported at controlled temperature.

14.3.1 Ice, dry ice or liquid nitrogen is needed to transport to the laboratory as quick as possible.

14.3.2 Transportation of specimens must comply with country and local regulations for the transport of etiologic agents.

14.4 Pretreatment of specimen

14.4.1 Pretreatment of throat swab

14.4.1.1 Add 1ml stroke-physiological saline solution to sterile glass tube, shake thoroughly and mix evenly. Squeeze the swab.

14.4.1.2 Pipette all the supernatant to 1.5ml sterile centrifuge tube for further use.

14.4.2 Pretreatment of sputum

14.4.2.1 Take the sputum and add stroke-physiological saline solution in volume of four times. Shake gently and put in the refrigerator at 4°C overnight to make sputum liquefied completely.

14.4.2.2 Mix with tip or pipette and suck 1ml to the 1.5ml-centrifuge tube for further use.

14.5 Specimen storage

Note: Routine freezing or prolonged storage of specimens may affect performance.

Store specimens that are not tested upon receipt at liquid nitrogen or below -70ºC for later detection.

15. Test Procedure

15.1 Sample processing and nucleic acid extraction

It is recommended to take 200μl of liquid specimen for nucleic acid extraction. The RNA extraction kit or purification kit produced by Da An Gene Co., Ltd. of Sun Yat-sen University (e.g. RNA extraction or purification reagent (immunomagnetic beads): YSXB No. 20170583, and RNA extraction or purification

reagent (immunomagnetic beads): YSXB No. 20150302). For specific steps, please follow the Instruction for Use of the kit.

Both negative and positive controls in this kit are involved in the extraction.

⚠ For specimen handling and RNA extraction, please reference to either the methods issued by Ministry of Health or the relevant health associations, or other methods of equivalent to manipulate the nucleic acid extraction kit.

15.2 PCR amplification

15.2.1 Preparation of PCR mixture

When calculating the total of the reaction, negative control and positive control should be calculated. Please prepare in reference to Table 1.

Table 1 Example for preparation of system

	Numbers of sample	1	3	24
PCR mixture	NC (ORF1ab/N) PCR reaction solution A	17.0μl	51.0μl	408.0μl
	NC (ORF1ab/N) PCR reaction solution B	3.0μl	9.0μl	72.0μl
PCR detection preparation	PCR mixture	20.0μl	20.0μl	20.0μl
	Processed sample	5.0μl	5.0μl	5.0μl
	Total volume	25.0μl	25.0μl	25.0μl

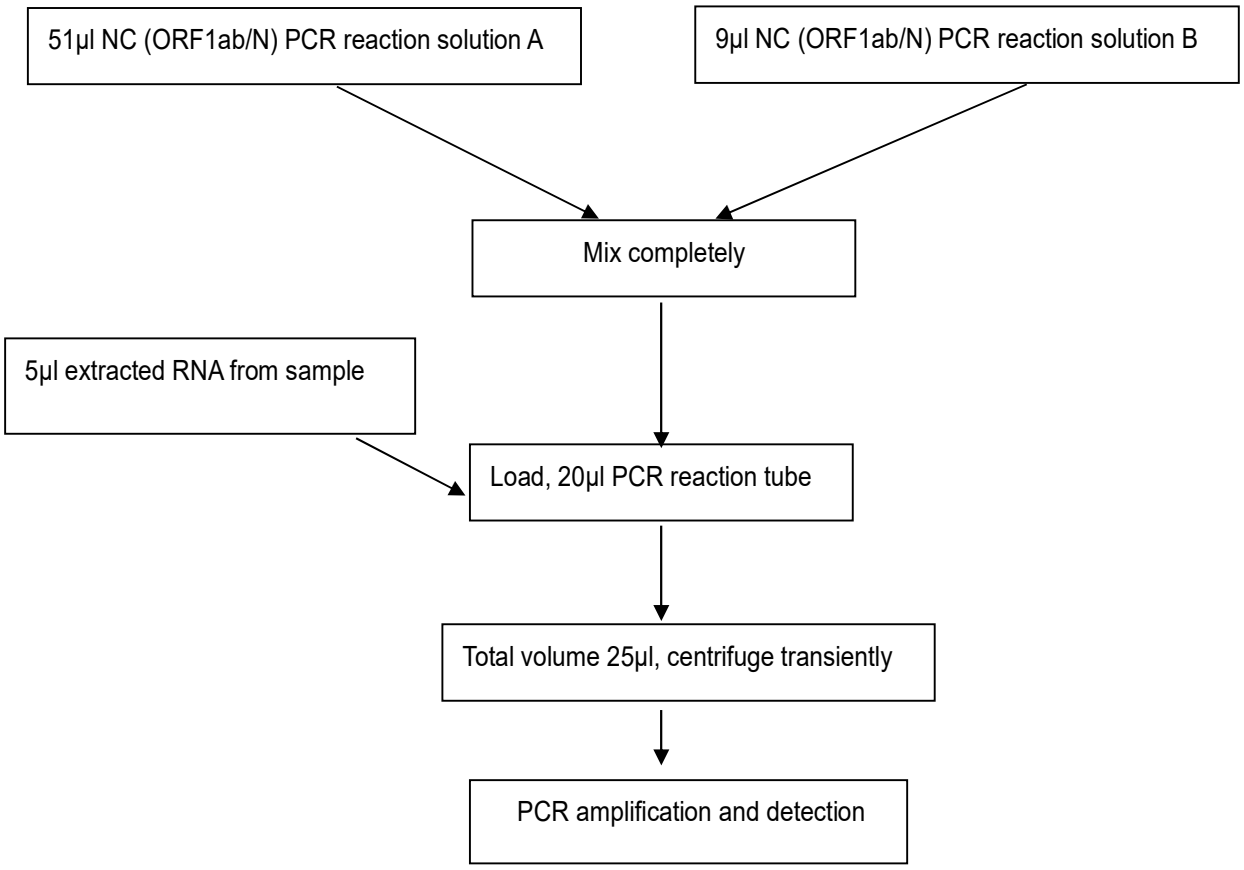
⚠ ***Note***: In reagent preparation area, take out kits from refrigerator, and make sure the components of the kit are complete, then completely thaw reagents to be used, swirl to ensure homogeneity, and centrifuge transiently. Pipette the components according to requirements into 1.5ml centrifuge tube, and swirl gently. Load to reagent tube with a specification of 20μl/test. Place the loaded PCR system on ice. PCR amplification should be performed in 45 minutes. All operations should be performed on ice. The final mixed solution should be blended completely.

15.2.2 Application of sample

Add 5μl extracted RNA from samples to each reaction tube (including negative control, sample, and positive control). During the whole process of application of sample, the samples should be applied in the

following order: first negative control, second detection sample, last positive control. After application of sample, all PCR reaction tubes should be enclosed, avoiding cross contamination.

Figure 1: A diagram for reagent preparation and application of sample (3 tests)



15.2.3 Programming ABI PRIM® 7500 Sequence Detection Systems

15.2.3.1 Procedure setup (See detailed information in operation manual of each instrument.)

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: Reporter 1: FAM, Quencher 1: NONE, Reporter 2: VIC, Quencher 2: NONE, Reporter 3: Cy5, Quencher 3: NONE, Passive Reference: NONE.

15.2.3.2 Set up the cycle conditions

Set up the detection time and temperature, according to the following three stages (See Table 2). If further information is needed, please look up in Chapter 4 of Applied Biosystems 7500 Real-Time PCR System Absolute Quantification Getting Started Guide.

Stage 1: Reverse transcription; Stage 2: Pre-denaturation; Stage 3: Nucleic acid amplification and

fluorescence detection.

Table 2 Setup of detection time and temperature

Stage	Reps	Target (°C)	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.

15.2.4 Programming LightCycler480 Instrument

15.2.4.1 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.

15.2.4.2 Set cycling conditions:

Program name	Cycles	Target（°C）	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

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

15.2.5 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.

15.2.6 Result determination

15.2.6.1 If the test sample has no amplification curve or Ct value > 40 in the FAM and VIC channels, and

has amplification curve in Cy5 channel, the sample can be judged as negative for 2019 Novel Coronavirus (2019-nCoV).

15.2.6.2 If the Ct value of the test sample in the FAM and VIC channels is NMT (i.e., not more than) 40, and there is obvious amplification curve, the sample can be judged as positive for 2019 Novel Coronavirus (2019-nCoV).

15.2.6.3 If the Ct value of the test sample is NMT 40 only in one channel between FAM and VIC, and no amplification curve in the other channel, it is recommended to repeat the test. If the result of the retest consist with the original, it can be determined as positive for 2019 Novel Coronavirus; if the result of the retest is negative, it can be determined as negative for 2019 Novel Coronavirus.

16. Quality Control

16.1 NC (ORF1ab/N) negative control: FAM and VIC detection channels have no obvious amplification curve, Cy5 detection channel has obvious amplification curve.

16.2 NC (ORF1ab/N) positive control: FAM and VIC detection channels have obvious amplification curves, and the Ct value is ≤ 32, Cy5 detection channel has or has no amplification curve.

⚠ ***Note***: The above requirements should be met in the same experiment, otherwise, the experiment is invalid and it should be re-performed.

⚠ ***Note***: Perform quality control for each experiment.

17. Technological specification

17.1 Sensitivity: the analytical sensitivity of all instruments is 5.0×10^2 copies/ml by laboratory evaluation; diagnostic sensitivity is 100% for use with *ABI PRISM® 7500 SDS* and *LightCycler480 Instrument* by clinical study evaluation.

17.2 Specificity: the analytical specificity of all instruments is 100% by laboratory evaluation; diagnostic specificity is 100% for use with *ABI PRISM® 7500 SDS* and *LightCycler480 Instrument* by clinical investigation evaluation.

17.3 Precision: within run/ between run precision, within day/ between day precision, precision variation coefficient between different operators is not more than 5%.

17.4 Stability: Result of experimental study on stability shows that the Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing) can be stored for 3 days at 4°C. Perform acceleration testing at 37°C for 3 days, and does not affect the performance of the kit. Long-term stability

under storage conditions of -20°C±5°C is still in progress.

18. Product Use Limitations

18.1 Polymerase repression may result in fault negative result.

18.2 Reliable results depend on correct specimen collection and transport.

18.3 Detection of novel Coronavirus depends on quantity of microorganism contained in the specimen, and is influenced by collection methods, patient factors (for example, age and forthcoming symptoms), and infection.

18.4 The kit only applies to specified specimen types. Detection of other types may result in false positive or false negative results.

18.5 The kit can not be used to assess a treatment.

18.6 Like other diagnostic experiments, all clinical and laboratory results should be considered and then an interpretation can be made.

18.7 The product is to be used by personnel specially trained on PCR technique only.

19. Troubleshooting

19.1 No fluorescent increase signal in reaction tube of positive control

●Procedure setup error

Check according to 15.2.3.1or 15.2.4.1 in the Instructions for Use.

●Preparation error of PCR reaction system

Check one by one according to preparation table, when necessary, repeat PCR reaction.

●Use kit after its expiration date or use deteriorated reagent

Check the storage condition of the kit prior to use and use the reagents during its shelf life. Store reagents according to the storage condition specified in the Instructions for Use.

19.2 Fluorescent increase signal emerges in reaction tube of negative control

19.2.1 Contamination occurs in experiment

◆ Laboratory management should be strictly performed according to management specification for PCR gene amplification laboratory. The experimenters should receive special training. The experiment should be strictly processed in separate areas (reagent preparation area, specimen preparation area, amplification area and amplicon analysis area). All consumables used should be single-use. Each stage of the experiment should apply special apparatus and equipment, and cross-use should be

avoided between different areas and different stages.

- Repeat the experiment using new reagents.
- During application of samples, keep to the following order: first negative control, second detection sample, last positive control. After application of sample, all PCR reaction tubes should be enclosed.

If there are other problems, please contact our technique supporters. Email service@daangene.com, toll-free hot line: +86-8008304008

20. Manufacturer

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Tel: +86-20-32290789, Fax: +86-20-32068126

Website：http://www.daangene.com

21. Reference standard

ISO 15223-1:2016 Medical devices -- Symbols to be used with medical device labels, labelling and information to be supplied -- Part 1: General requirements

BS EN ISO 18113-3:2011 In vitro diagnostic medical devices - Information supplied by the manufacturer (labelling) - Part 3: In vitro diagnostic instruments for professional use

BS EN ISO 18113-2:2011 In vitro diagnostic medical devices - Information supplied by the manufacturer (labelling) - Part 2: In vitro diagnostic reagents for professional use

22. European Authorised Representative

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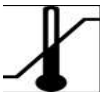
23. Explanation of Symbols

	Use by
	Batch code
	Date of manufacture
	Manufacturer
	Catalogue number
	Temperature limitation
	Contains sufficient for <n> tests
	In vitro diagnostic medical device
	For single use
	CE marking
	Warning sign
	European Authorised Representative
	Consult Instructions For Use