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2019-Novel Coronavirus (ORF1ab, E &N genes) Nucleic Acid Test Kit

(Fluorescent Probe-Based Real-Time PCR Assay)

Instruction for Use

English VER 1.0

REF **R205T011B0C2 50Tests/Box** **R205T012B0C2 100 Tests/Box** **R205T013B0C2 200Tests/Box**

PRODUCT NAME

2019-Novel Coronavirus (ORF1ab, E &N genes) Nucleic Acid Test Kit (Fluorescent Probe-Based Real-Time PCR Assay)

INTENDED USE

This kit is intended for qualitative detection of *ORF1ab*, *E* & *N* genes of 2019-Novel Coronavirus (2019-nCoV, SARS-COV-2) in the specimens including nasopharyngeal swab, oropharyngeal swab, bronchoalveolar lavage fluid, saliva, sputum and fecal. The *ORF1ab* gene sequences are higher specificity for 2019-nCoV, while the sequences of the *E* and *N* genes are homogenous to the same genes in some virus such as SARS-like coronaviruses and coronaviruses carried by some bats. For professional in-vitro diagnostic use.

SUMMARY AND EXPLANATION

The novel coronaviruses belong to the β genus. COVID-19 is an acute respiratory infectious disease. People are generally susceptible. Currently, the patients infected by the novel coronavirus are the main source of infection; asymptomatic infected people can also be an infectious source. Based on the current epidemiological investigation, the incubation period is 1 to 14 days, mostly 3 to 7 days. The main manifestations include fever, fatigue and dry cough. Nasal congestion, runny nose, sore throat, myalgia and diarrhea are found in a few cases.

TEST PRINCIPLE

This kit uses polymerase chain reaction (PCR) technology based on TaqMan fluorescent probe to detect *ORF1ab*, *E* & *N* genes of 2019-Novel Coronavirus from specimens including nasopharyngeal swab, oropharyngeal swab, bronchoalveolar lavage fluid, saliva, sputum and fecal collected from the patients or suspected patients.

REAGENTS

Seq.	Labels	Main contents	*No.* (50 tests)	*No.* (100 tests)	*No.* (200 tests)
1	Buffer (nCoV3-S)	Each deoxyribonucleotide triphosphates (dNTPs)/ magnesium *etc.*	1 tube (800μL/tube)	1 tube (1600μL/tube)	2 tubes (1600μL/tube)
2	Primers/Probes (nCoV3-S)	Specific Primers and Probes	1 tube (160μL/tube)	1 tube (325μL/tube)	1 tube (650μL/tube)
3	Enzyme Mixture (nCoV3-S)	Mixture of reverse transcriptase and Taq DNA polymerase	1 tube (110μL/tube)	1 tube (225μL/tube)	1 tube (425μL/tube)
4	Positive Control (nCoV3-S)	Armored RNA of nCoV and Internal Control	1 tube (500μL/tube)	1 tube (1000μL/tube)	1 tube (1000μL/tube)
5	Negative Control (nCoV3-S)	TE Buffer	1 tube (500μL/tube)	1 tube (1000μL/tube)	1 tube (1000μL/tube)

OTHER MATERIALS REQUESTED BUT NOT PROVIDED

The following list includes the materials that are required for use but not included in this Kit:

- Nucleic acid extraction kit.
- Nuclease-free consumables: Filter tips, 1.5mL tubes, PCR-well strips or 96-well plate.
- Experimental equipment: Centrifuge for 1.5mL tubes and PCR-well strips or 96-well plate (if available), Vortex. Real Time PCR instrument (thermocycler).
- Others: Micropipettes (0.5-20μL, 10-100μL, 20-200μL,100-1000μL), Powder-free disposable gloves, Microplate sealing film.

STORAGE CONDITIONS AND SHELF LIFE

The shelf life of this kit is 12 months when stored in the freezer at - (20±5) ℃. It is suggested to transport the kits in a sealed foam box with dry ice and/or ice packs. The kit needs to be stored away from light. Never leave the kit for more than 14 days at 2-8℃. Never leave the kit for more than 7 days at 37℃. Never repeat freeze-thaw more than 5 times for this kit (The effects of repeated freeze-thaw more than 5 times for this kit haven't been verified).

APPLICABLE EQUIPMENT

Applicable to ABI 7500 Real-Time PCR thermocycles. For other Real-Time PCR thermocycles, please consult the manufacturer before use.

ACCEPTABLE SPECIMENS

The indications of sample collection are the patients or suspected patients with 2019-Novel Coronavirus infection. The specimens to be collected are the nasopharyngeal swab, oropharyngeal swab, bronchoalveolar lavage fluid, saliva, sputum or fecal of the patients or suspected patients.

SPECIMENS COLLECTION AND STORAGE

Oropharyngeal swab: With a special swab wipe moderately the back wall of the pharynx and the two of the tonsils. After collection, quickly insert the swab into a sample tube containing 3 mL of preservation solution and close the tube cap.

Sputum: The best time to collect a sputum specimen is in the morning. Collect specimens before using antibiotics. Ask the patient to rinse his mouth with clean cold water or clean teeth with toothbrush without a toothpaste, and those with dentures should remove them. Take a deep breath, try to cough up the sputum from the deep respiratory tract and spit into a sputum cup, the volume of specimens should be ≥1mL. Those with difficulty coughing up the sputum, may use atomized inhalation with 45℃, 10% NaCl water, to make sputum easy to cough up. For patients with difficulty in coughing sputum naturally, an aseptic sputum suction catheter can be used to extract deep secretions from the trachea. For collecting the sputum from children, first, press the tongue backward with a bent tongue depressor, then put the swab into the throat. When children are irritated to cough

by pressing their tongues, they can spew out secretions or sputum from the lungs or trachea and that will stick to the swabs. Can also tap with fingers over the manubrium sterni, to induce children to cough up sputum.

Bronchoalveolar lavage fluid: The collection of bronchioalveolar lavage fluid should be with the consent of patients in advance. 3 to 4 hours of fasting before the procedure. Let the patient lie flat after anesthesia. Place the oxygen- nasal catheter into the left nostril and secure it with medical transparent tape. Secure the patient's head and send bronchoscopy into the corresponding location of the airways. With a disposable 10-20 mL syringe, inject the 37℃ saline with the air through a bronchoscope, at 5-20 mL per section. Repeatedly press, release the suction valve button of the bronchoscopy operating parts to suck out bronchoalveolar lavage fluid and secretions. Avoid damage to the mucous membrane and should pay attention to sterile operation through the whole procedure.

Saliva: Before collection, rinse mouth with water before sampling, and do not eat before sampling, relax cheeks, spit into the saliva collector, add the preservation solution prefilled in tube. Invert tube for about 10 times to mix. Fulfill the necessary information on the label provided, and stick it to the tube.

Nasopharyngeal swab: Let the patient relax. Stick the swab to the nostril and slowly rotate it into the nasopalatine area along the nostril wall, then slowly take it out while rotating and wiping. Wipe the other nostril with the same swab in the same way. After collection, quickly insert the swab into a sample tube containing 3mL of preservation solution and close the tube cap.

Fecal: Take 5-8 g of fresh feces into the stool sampling tube and tighten the cap.

The accurate clinical information of the specimens, such as specimen number, date of onset and date of collection, should be attached to the transport and preservation process. Avoid repeated freezing and thawing of the clinical specimens during transporting and storing. The samples should be transported at least below 8℃ if the transportation cannot be guaranteed at - (20±5) ℃.

TEST METHODOLOGY

1. Nucleic Acid Extraction (Pre-PCR)

Extract the nucleic acids from the clinical samples, positive control and negative control according to the instructions of the nucleic acid extraction kit:

1.1 Clinical samples: Clinical specimen to be tested

1.2 Positive Control: Positive Control(nCoV3-S) included in this kit.

1.3 Negative control: Negative Control(nCoV3-S) included in this kit.

* We have validated the following kits for nucleic acid extraction from the nasopharyngeal swab, oropharyngeal swabs, bronchoalveolar lavage fluid, saliva, sputum or fecal specimen:

- Nucleic Acid Extraction Kit, using the 32M, 96M or FAST96 instruments (Mole Bioscience). recommended*.
- Nucleic Acid Extraction Kit (Spin Column) (Mole Bioscience). recommended*

If you use nucleic acid extraction kits from other suppliers, please verify first.

2. Amplification Processes (PCR)

2.1 Preparation of Amplification Reagent (PCR Room I)

To prepare the PCR reaction mixtures, take the Primers/Probes(nCoV3-S) tube and Buffer(nCoV3-S) tube from the kit. Thaw them on ice or at 2-8℃. Take out of Enzyme Mixture(nCoV3-S) tube. Shake well and centrifuge all reagent tubes at low speed shortly. Prepare the PCR reaction mixtures according to the following ratios:

Reagent	Buffer(nCoV3-S)	Primers/Probes(nCoV3-S)	Enzyme Mixture(nCoV3-S)
Volume	15.0 µL	3.0µL	2.0µL

Calculate the amount of each reagent. Add the reagents into an appropriate volume centrifugal tube, mix well and centrifuge shortly. Total number of PCR mixtures = number of clinical samples + 1 positive control +1 negative control.

Add 20.0 µL of the PCR mixture into each of the PCR well/tube, and then transfer the plates/tubes to the PCR room II.

2.2 Add the Templates (PCR Room II)

Add 10.0 µL of nucleic acid extracted from each sample (prepared in the first step: Pre-PCR) into each PCR well/tube which were added with PCR reaction mixtures. Vortex the sealed the plate or tubes to mix well and then centrifuge at 2000-3000rpm for 1min.

2.3 Amplification (Detection Area)

Put the reaction tubes/plates into the fluorescent real-time PCR thermocycler and set the cycle program as follows:

Step	Cycles	Temperature (℃)	Time(min:sec)
1	1	55	05:00
2	1	95	00:10
3	5	95	00:05
		58	00:30
4	40	94	00:05
		58*	00:30

Fluorescent dye signals assigning: FAM (*ORF1ab* gene), HEX/VIC (*N* gene), ROX (*E* gene), Cy5 (internal control). *The signal data is collected at 58℃. When using the ABI7500, select the 'Quencher' and 'Passive reference' columns as "none". Set the reaction volume per tube/well to 30 µL.

CUT-OFF VALUE

The cut-off value of FAM, VIC/HEX and ROX is 38.00, 38.00 and 38.00 respectively, it is determined by ROC curve. The cut-off value of internal control is determined to be 35.00 by limited dilution.

EXPLANATION OF THE TEST RESULTS

After the reaction is completed, the instrument automatically saves the results, adjusts the Start, End, and Threshold values of the Baseline after analyzing the image (It is user self-adjustable: Start values can be between 3 and 15. End values can be between 5 and 20). Adjust the amplification

curve of the PCR-negative control to straight or below the threshold line.

For negative control, the Ct value in FAM, HEX/VIC and ROX channels should be negative, and Ct value in Cy5 channel should be negative in general, but sometimes, it is positive, in this case, the results of the test are still valid. For positive control, the Ct values in FAM, HEX/VIC, ROX and Cy5 channels are ≤ 31.00. The above requirements must be met in the same test at the same time, otherwise the PCR reaction is considered invalid and should be re-performed. When all of the above requirements are met, the interpretation and judgment of test results are as follows:

1. When the Cy5(Internal Control) fluorescent Ct value is ≤ 35.00, FAM (ORF1ab), HEX/VIC (N gene) and ROX (E gene) are showed no amplification or no typical S-type amplification curve, the tested sample is determined as a 2019-novel Coronavirus negative sample.
2. When the FAM (ORF1ab) fluorescent Ct value is ≤ 38.00, with a typical S-type amplification curve, the tested sample is determined as an *ORF1ab* gene positive sample. When FAM (ORF1ab) fluorescent Ct value is > 38.00, the sample is determined as an *ORF1ab* gene negative sample.
3. When the HEX/VIC (N gene) fluorescent Ct value is ≤ 38.00, with a typical S-type amplification curve, the tested sample is determined as a *N* gene positive sample. When HEX/VIC (N gene) fluorescent Ct value is > 38.00, the sample is determined as a *N* gene negative sample.
4. When the ROX (E gene) fluorescent Ct value is ≤ 38.00, with a typical S-type amplification curve, the tested sample is determined as an *E* gene positive sample. When ROX (E gene) fluorescent Ct value is >38.00, the sample is determined as an *E* gene negative sample.
5. When the concentration of *ORF1ab*, *E* & *N* gene is too high, the amplification of internal control will be repressed, in this case, the sample can be determined as a 2019-nCoV positive one directly, or the nucleic acid of the sample can be diluted and re-tested.
6. When the *ORF1ab* gene, *E* and *N* gene are all positive, the internal control is negative, the sample can be determined as a 2019-nCoV positive one directly. When the *ORF1ab* gene, *E* and *N* gene are not all positive, the internal control is negative, re-sampling or re-extraction is needed. When all the three genes and internal control are negative, re-sampling or re-extraction is needed.
7. The sample is 2019-nCoV positive with positive *ORF1ab* gene, positive *E* or positive *N* gene. With a negative *ORF1ab* gene, *E* and *N* gene, the sample cannot be excluded from 2019-nCoV positive.
8. Examples for interpretation are listed in the table below.

Seq.	Internal control	*ORF1ab*	*N*	*E*	Interpretation
1	positive	positive	positive	positive	2019-nCoV positive
2	negative	positive	positive	positive	2019-nCoV positive, or re-test after dilution
3	negative	positive	negative	negative	Re-sampling or re-extraction.
4	negative	negative	positive	negative	Re-sampling or re-extraction.
5	negative	negative	negative	positive	Re-sampling or re-extraction.
6	negative	positive	positive	negative	Re-sampling or re-extraction.
7	negative	positive	negative	positive	Re-sampling or re-extraction.
8	negative	negative	positive	positive	Re-sampling or re-extraction.
9	positive	positive	negative	negative	2019-nCoV positive
10	positive	positive	negative	positive	2019-nCoV positive
11	positive	positive	positive	negative	2019-nCoV positive
12	positive	negative	negative	positive	2019-nCoV positive
13	positive	negative	positive	negative	2019-nCoV positive
14	positive	negative	positive	positive	2019-nCoV positive
15	positive	negative	negative	negative	2019-nCoV nondetectable
16	negative	negative	negative	negative	Re-sampling or re-extraction.

LIMITATIONS OF THE TEST METHOD

The results of this kit are only for aiding in diagnosis and shall not be used as the sole basis for diagnosis or exclusion, and should be analyzed by combination with clinical symptoms. A negative result indicates the viral concentration in the sample is lower than the detection limitation of the kit, in this situation, the infection cannot be excluded.

The optimal sample type and the time to reach maximum titer after infection have not been verified. Therefore, collecting samples in the same patient at different times and multiple locations may help to avoid false negative results.

The following conditions can also cause a false positive or false negative test result:

1. The results can be affected by collecting, disposing, transporting and storage of samples, and any errors of these processes will result in false negative.
2. The mutation of sequence related to primers or probes used in this kit may cause false negative results.
3. Cross-contamination during sample processing may lead to false positive, and the FAM or HEX/VIC or ROX channel detection results of the negative control showed an amplification curve.

PRODUCT PERFORMANCE

1. The limit detection of the kit is 500 copies•mL^{-1}.
2. Coincidence rate of enterprise references: The coincidence rate of 12 negative references is 12/12 of 5 positive references is 5/5.
3. Precision: The coefficient of variation of Ct values of precision references is not higher than 5% within-batch and not higher than 10% inter-batch.
4. Specificity

4.1. There are no cross-reactivities with other viruses that infect the same position of the body or have similar infectious symptoms (*e.g.* Human coronavirus 229E, Human coronavirus OC43, Human coronavirus HKU1, Human coronavirus NL63, SARS-coronavirus, MERS-coronavirus, Adenovirus, Human Metapneumovirus (hMPV), Parainfluenza virus 1-3, Influenza A & B, Enterovirus, Respiratory syncytial virus, Rhinovirus, *Chlamydia pneumoniae*, *Haemophilus influenza*, *Legionella pneumophila*, *Streptococcus pneumonia*, *Streptococcus pyogenes*, *Bordetella pertussis* and *Mycoplasma pneumonia*).

4.2. 1mg•mL^{-1} mucin and 10% blood in the tested sample will not influence the detection. However, try to avoid such substances which may have a potential influence on PCR reaction during sampling.

4.3. Impact of medicine: 0.5mg•L^{-1} Hydroxymethylazoline, 10mg•mL^{-1} Phenylephrine, 0.9% sodium chloride, 0.15mg•L^{-1} Dexamethasone, 1.1mg•mL^{-1} Triamcinolone acetonide spray, 0.25mg•mL^{-1} Beclomethasone, 38nmol•L^{-1} Histamine hydrochloride, 2mg•L^{-1} ribavirin, 65ng•mL^{-1} Oseltamivir, 30mg/•mL^{-1} Pa Rami Vee, 1mg•mL^{-1} Levofloxacin, 18mg•L^{-1}Cefatriaxone, 20mg•L^{-1} Meropenem and 5μg•mL^{-1} Tobramycin will have no influence on the detection.

PRECAUTIONS

1. This kit is only for emergency use during the pandemic of 2019-nCoV. The test is manually operated. Experimental personnel who perform this test should have received professional training in gene amplification or molecular biology diagnostics and be qualified for relevant experimental operations. There should be reasonable biosecurity precautions and protective procedures in the laboratories. The test should only be performed in laboratories that follow safety practices according to the applicable Biosafety Regulations in Microbiological and Biomedical Laboratories.
2. The whole detection process should be carried out in three areas: the first area is for reagent preparation. The second area is for specimen processing and reaction system preparation. The third area is for amplification, fluorescence detection and results analysis. Instruments, equipment and lab coats should be used independently in each area to prevent contamination.
3. In the testing process, should always take care to avoid RNase contamination, wear disposable gloves without fluorescent substances (Frequent replacement is recommended), use the disposable thin-walled 200μL PCR tube (or 96-well PCR plate with optical film) and pipette tips with filter. Never touch the reaction tube directly with bare hands.
4. The handling of Clinical Specimens should be performed in the biosafety cabinet to ensure the safety of laboratory staff and prevent environmental pollution. Harmful and/or toxic specimens and reagents in the experiment should be properly placed and stored, and in charge by an assigned person. Waste should be disposed of properly in special containers. Lab bench, equipment such as operator's stations, pipettes, centrifuges, and PCR thermocyclers etc., should be regularly wiped and disinfected with 1.0% sodium hypochlorite and/or 70% ethanol. Laboratory room, an ultra-clean bench should be treated with an ultraviolet lamp regularly and after each experiment.
5. Prior to the experiment, reagents should be fully thawed, mixed well, and centrifuged for a few seconds to bring down all the liquid to the bottom of the centrifuge tubes. When preparing the reaction solution, attention should be paid to mixing all liquids on the vortex mixer, no blowing with the pipette to avoid bubbles and centrifuging the reaction mixture solution for a few seconds. Use the kit before the expiration date and do not combinate the reagents with different batch numbers.

Manufacturing date and expiration date: view on label

INDEX OF SYMBOLS

	Consult Instructions for Use	Σ	Contain <n> tests	EC REP	Authorized Representative in the European Community
IVD	In vitro diagnostic medical device		Use-by date	-25℃ -15℃	Temperature limit -25 to -15℃
REF	Catalogue #	LOT	Lot Number	CE	CE conformity marking
	Manufacture Date		Manufacturer		

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