Novel Coronavirus (2019-nCoV) RT-PCR Detection Kit (Lyophilized) User Manual

【PRODUCT NAME】

Novel Coronavirus (2019-nCoV) RT-PCR Detection Kit (Lyophilized)

【SIZE】

48 Tests/kit，50 Tests/kit

【INTENDED USE】

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. This kit is intended to *in vitro* qualitatively detect the ORF1ab and N genes of 2019-nCoV in the throat swab and nasopharyngeal swab samples collected from cases and clustered cases suspected with novel coronavirus–infected pneumonia and others required for the diagnosis or differential diagnosis of novel coronavirus infection. This product is only limited to auxiliary diagnosis of relevant cases during the outbreak of COVID-19, and can not be used in clinic as a routine *in vitro* diagnostic (IVD) reagent.

【PRINCIPLE OF DETECTION】

This product is a fluorescent probe-based Taqman RT-PCR assay system. Firstly, the RNA of 2019-nCov will be reverse transcribed into cDNA by reverse transcriptase, and then PCR amplification will be performed with cDNA as template. During amplification of the template, the Taqman probe will be degraded due to the 5'-3' polymerase activity and exonuclease activity of Taq DNA polymerase, then the separation of fluorescent reporter and quencher enables the fluorescent signal to be detected by instrument. The ORF1ab gene of 2019-nCoV will be detected qualitatively by FAM channel, the N gene of 2019-nCoV will be detected qualitatively by ROX channel, and the human internal reference gene HPRT1 will be detected by CY5 channel. Internal reference is used in the kit for quality control starting from sample collection to avoid false negative results.

【PRODUCT CONTENTS】

Components	Package	specification	Ingredient
2019-nCoV PCR Mix	1×bottle (Lyophilized powder)	50 Test	dNTPs, MgCl2, Primers , Probes,Reverse Transcriptase,Taq DNA polymerase
	1×bottle (Lyophilized microspheres)	50 Test	
	6×0.2ml 8 well-strip tube (Lyophilized)	48 Test	
Positive Control	2×0.2ml tube (lyophilized)	8 Test	Plasmid or Pseudovirus containing ORF1ab, N, and IC gene specific fragments
Dissolving solution	1.5 ml Cryotube	800 μL	/
Negative Control	1.5 ml Cryotube	200 μL	0.9%NaCl

Note: Do not mix the components from different batches for detection. The positive control of 2019-nCOV and internal reference were constructed artificially, and they were not infectious.

【STORAGE & SHELF LIFE】

(1) The kit can be transported by Normal transport.
(2) All kit components can be stored at 2°C~30°C with protection from light. And the kit is stable for 12 months when stored at the recommended condition.
(3) See label of outside box for production date and expiration date.
(4) The lyophilized powder version reagent should be stored at -20°C after dissolution and the repeated freeze -thaw should be less than 4 times.

【INSTRUMENTS】

Our recommendation for platform to use Novel Coronavirus (2019-nCoV) RT-PCR Detection Kit: Real-time PCR instrument-- Roche LightCycler 480、Life Technologies 7500、Molarray MA-688, Analytikjena qTOWER serials, and other real-time fluorescence PCR instruments with FAM, ROX, CY5 channels.

【SAMPLING & HANDLING】

(1) Throat Swab: Use the plastic rod swab with polypropylene fiber head to wipe the bilateral pharyngeal tonsils and the posterior pharyngeal wall at the same time, immerse the swab head into the tube containing physiological saline or VTM, discard the tail, and tighten the tube cover.
(2) Nasopharyngeal swab: Use the Nasopharyngeal flocking swab to collect the sample correctly through nasal cavity, immerse the swab head into the tube containing physiological saline or VTM, discard the tail, and tighten the tube cover..
(3) Bronchoalveolar Lavage: Collect bronchoalveolar lavage correctly for test.
The collected sample should be used for detection as soon as possible. If the sample need to be transferred cannot be detected immediately, please store it at low temperature.
The sample can be stored for 24 hours at 2~8°C and for a long time below -70°C. It can also be stored in refrigerator at -20°C temporarily. Samples shall be transported at low temperature in accordance with biosafety regulations.

【PROTOCOL】

1. Reagent Preparation
a) Lyophilized powder version kit:Add 750uL dissolving solution to the bottle to dissolve the lyophilized powder. Divide 15uL of the dissolved reagent into each PCR reaction tube.
b) Lyophilized microsphere version kit:Use tweezers (or special tool supplied by kit manufacturer) to place one lyophilized microsphere into each well of 8-well strip tubes, then add 15uL dissolving solution to each well to dissolve the lyophilized microsphere.
c) Lyophilized 8-well strip version kit: Add 15uL dissolving solution to each tube well.
d) Positive Control :Add 20uL dissolving solution to one tube well of positive control.
Shock and centrifuge them at low speed. Then, store the dissolved reagent in 4°C.
*Notes:When using the lyophilized powder version kit, after dissolving thereagent can be stored at -20°C and repeated freeze -thaw should be less than 4 times.

2. RNA Extraction
Extract the nucleic acid(RNA) from the specimen using appropriate nucleic acid extraction kit and following the instructions of extraction kit. After RNA extraction, the extracted RNA shall be added to the PCR reaction tubes within 15 minutes, or transferred to the centrifuge tubes and stored at -15 °C~-25 °C.

3. Template Addition
Add 5 μL Negative Control, 5 μL Positive Control, and Add 5 μL extracted nucleic acid of each specimen into each PCR reaction tube. Shock and centrifuge them at low speed. Then, move them to the Real-time PCR instrument.

4. PCR Amplification
Recommended Setting

Step	Temperature (°C)	Time	Cycle
1 Reverse Transcription	50	10mins	1
2 Pre-denaturation	95	2mins	1
3 Denaturation PCR	95	10s	40
4 annealing/extension	60	20s	

*Note: The signals of FAM, ROX and CY5 fluorescence channels will be collected at 60°C. Select "None" for the passive reference and set annealing/extension time at 30s on operation interface of ABI7500 RT-PCR software.

5. Data Analysis
Test data file need to be saved after PCR reaction. Please set the parameters and analysis the results of FAM, ROX and CY5 channels respectively.
(1) Baseline setting: the baseline can be set automatically or adjusted according to the shape of amplification curve.
(2) Threshold setting: the threshold value should be higher than the highest fluorescence value of negative control in this kit.

6. Quality Control

Negative control and positive control provide the quality control for the assay and shall be conducted for each run of test. The result is valid if ALL the below criteria is met. Otherwise, the test is invalid. In this case, the errors of instruments, reagents, amplification conditions, etc. shall be checked, and the experiment shall be repeated.

Products of Quality Control	Requirements of Quality Control		
	FAM Channel	ROX Channel	CY5 Channel
Positive Control of 2019-nCoV	Ct ≤ 32	Ct ≤ 32	Ct ≤ 32
Negative Control	Undet	Undet	Undet

7.Interpreting Test Results

Channel			Interpretation of results
FAM Channel	ROX Channel	CY5 Channel	
Ct≤38	Ct≤38	Ct≤32	2019-nCoV Positive
Undet or Ct>38	Undet or Ct>38	Ct≤32	2019-nCoV Negative
Undet or Ct>38	Ct≤38	Ct≤32	retest*
Ct≤38	Undet or Ct>38	Ct≤32	
Any	Any	Undet or Ct>32	re-sampling and retest*

*If any retest result of FAM and ROX channels have a Ct value ≤38, the result is interpreted as positive, otherwise it is negative.
*If the result of the internal control(CY5 channel) Ct is Undet or Ct＞32 the test result is invalid and re-sampling and retest should be done.

【CUT-OFF VALUE OR REFERENCE INTERVAL】

The cut-off value of 2019-nCoV is Ct ≤ 38.

【ASSAY EXPLAINATION】

1. The contamination of laboratory environment and reagent, or cross contamination during specimen treatment may lead to false positive result.
2. The decrease of detection effect even the false negative result may occur if there is any mistakes in the transportation, storage and operation of reagents.
3. 2019-nCOV early infection or other respiratory virus infection can't be excluded in patients with negative results. If conditions permit, it is recommended to collect more sensitive samples such as sputum or bronchoalveolar lavage for retest.

【ASSAY LIMITATIONS】

1. The positive result detected by this kit can't indicate whether there is virus in vivo. It is suggested to use other methods for confirmation at the same time.
2. This kit is intended for classification and detection of 2019-nCoV. The result is only for clinical reference, and the clinical management of patients should be considered in combination with their symptoms/-signs, history, other laboratory tests and treatment responses.
3. Although the detected target sequences of this kit are the conservative region of 2019-nCoV's gene, the missed detection of coronavirus types with rare mutations in the conservative region can't be completely avoided in theory.

【PERFORMANCE SPECIFICATIONS】

Conformity rate of Negative Control: detection results of 2019-nCoV were negative in 8 enterprise reference samples (T1-T8), and the conformity rate of negative control (-/-) should be 8/8.
Conformity rate of Positive Control: detection results of 2019-nCoV were positive in 5 enterprise reference samples (Y1-Y5).
Detection limitation: 1000 copies /mL or 5copies/ Reaction
Repeatability: The test results of enterprise reference samples (J1) were all positive after 10 repetitions, and the coefficient of variation (CV) of J1's Ct value is less than 5.0%.
Precision: 2 times of continuous testing of 2 samples, each time 8 repetitions for each sample, and the coefficient of variation (CV) of their Ct value is less than 5.0%.
Specificity: non-specific interference of other related pathogens (Coronavirus (229E, HKU1, OC43, NL63), Influenza A Virus (H1N1, H3N2), Influenza B Virus, Canine coronavirus, Avian influenza H7N9. .

【ATTENTIONS】

1. The kit is only used for in vitro diagnosis.
2. Please read this manual carefully before beginning the experiment.
3. All equipment used in the experiment shall be sterilized.
4. Unreasonable sample collection, transfer, storage and operation may lead to wrong test results.
5. RNA extraction shall be carried out as soon as possible after sample collection to avoid degradation. If it cannot be carried out immediately, it shall be stored in accordance with [SAMPLING & HANDING].
6. After the operation of the nucleic acid extractor, the used consumables shall be sealed. After the instrument is cleaned, turn on the ultraviolet lamp for 30 minutes.
7. As this test involves the extraction of viral RNA and PCR amplification, please take care to avoid contamination of the amplification reaction mixture. Regular monitoring of laboratory contamination is recommended.
8. When using this kit, please strictly follow the instructions. The collection, storage and transfer of samples, the extraction and detection of RNA, and the interpretation of results must be carried out in strict accordance with the requirements of the kit instructions. The processes of sample preparation and addition must be carried out in the biosafety cabinet or other basic protective facilities according to the technical requirements of the clinical gene amplification laboratory.
9. 2019-nCOV has strong transmission ability and high-risk coefficient. Personal protection should be a three-level laboratory level of biosafety. The operator must have professional skills and PCR inspection qualification. During the whole operation process, it is necessary to prevent the infection risk of aerosol pollution, and the operator must add samples and use reagents and consumables accurately.
10. To prevent virus spreading, the 2019-nCOV must be detected in a biosafety level 2 (P2) or above laboratory. Laboratory management should strictly follow the management standard of PCR gene amplification laboratory, and the experimental operation must be strictly partitioned. The instruments, equipment, consumables, work clothes used in each region must be distinguished strictly and can't be used intercross to avoid contamination.
11. All test samples shall be regarded as infectious substances. During the experiment, work clothes shall be worn, disposable gloves shall be worn and replaced frequently to avoid cross contamination between samples. The operation of sample and waste shall meet the requirements of relevant laws and regulations.

【Literature References】

[1] General Office of the National Health Commission of the People's Republic of China, Office of State Administration of Traditional Chinese Medicine, Diagnosis and Treatment Protocol for COVID-19(Trail Verion 7).
[2] World Health Organization: Clinical management of severe acute respiratory infection when Novel coronavirus (nCoV) infection is suspected: Interim Guidance.

【General Information】

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【Date of Approval and Revision of Instructions】 2020-7-6V2.0