2019-nCoV/FluA/FluB Real-time PCR Kit

Instructions for Use

For Research Use Only. Not for use in diagnostic procedures

【PRODUCT NAME】

2019-nCoV/FluA/FluB Real-time PCR Kit

【PACK-SIZE】

32 tests/kit

【PRODUCT INTRODUCTION】

This product is intended for the rapid detection of 2019-nCoV, Influenza A and Influenza B Virus in human throat swab.

The common signs of people infected with coronavirus are respiratory symptoms, fever, cough, shortness of breath and dyspnea. In more serious cases, infection can lead to pneumonia, severe acute respiratory syndrome, renal failure, and even death. The "2019-nCoV" was named by WHO in January 12, 2020.

Influenza virus is a common and important respiratory virus in clinic. It is located in the respiratory tract and can be infected by adults and children. The prevalence rate of influenza virus is high. Influenza virus is highly infectious and spreads rapidly. The typical clinical symptoms are: sudden onset of high fever, general pain, significant fatigue and mild respiratory symptoms. Generally, autumn and winter are the high incidence period of influenza, and the complications and deaths caused by it are very important.

【PRINCIPLE OF DETECTION】

This product is a fluorescent probe-based TaqMan real-time PCR assay system. During the amplification of the template, the TaqMan probe will be degraded due to the 5'-3' polymerase activity and exonuclease activity of Taq RNA 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 VIC channel, the Influenza A virus will be detected qualitatively by ROX channel, the Influenza B virus will be detected qualitatively by CY5.5 channel, and the internal reference will be detected by CY5 channel.

dUTP and UNG enzyme are used in the kit to prevent contamination of the amplified products.

Internal control is used in the kit for quality control starting from sample collection.

【PRODUCT CONTENTS】

Components	Amount	Amount per reaction
	32 Tests/kit	
Lyophilized Reaction Reagent	32 balls	1 ball
Positive Control	1 tube	-
Negative Control	1 tube	-
Control Diluent	1.8 mL	-

Note: Do not mix the components from different batches for detection. The positive control, negative control of 2019-nCoV, Influenza A and Influenza B Virus and IC were constructed artificially, and they were not infectious.

【STORAGE & SHELF LIFE 】

All reagents should be stored at 2℃~25℃ with sealed protection from light, and the reagents are stable for 12 months (to be determined) when stored at the recommended condition. See label for production date and expiration date.

After each individual package in the kit is opened, it can be stored at 2℃~25℃ away from light, and should be used within 24 hours.

The positive control and the negative control lyophilized powder can be stored for 30 days at -15℃~-25℃ after redissolution.

The kit can be transported at 2℃~25℃.

【INSTRUMENTS】

ABI QuantStudio® 5.

【SAMPLING & HANDING】

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, discard the tail, and tighten the tube cover.

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℃, 4 days at -20℃ and for a long time below -70℃.

Samples shall be transported at low temperature in accordance with biosafety regulations.

【PROTOCOL】

1. Redissolution of positive control and negative control

Remove the sealing bag of Positive Control and Negative Control, take out the tube, and add 900 μL control diluent to each tube, shake and mix well. After the freeze-dried powder is dissolved, it can be used as a Positive Control and Negative Control, or it should be stored at - 15 ℃~- 25 ℃ for standby.

2. RNA Extraction

It is recommended to use the Nucleic Acid extraction and purification reagent (general type) produced by our company to extract RNA from sample, Positive and Negative control.

The volume of sample to be extracted is 400 μL. after RNA extraction, the extracted RNA shall be added to the reaction tubes within 10 minutes, or transferred to the centrifuge tubes and stored at -15 ℃~-25 ℃.

3. Template Addition

Remove the sealing bag of Lyophilized Reaction Reagent, take out the tubes and add 20 μL of extracted Negative Control, 20 μL of extracted Positive Control, and 20 μL of extracted RNA from sample to different PCR reaction tubes. Gently flick for several times, and centrifuge at low speed for several seconds after all freeze-dried balls are dissolved. Then, move them to the Real-time PCR instrument.。

4. PCR Amplification

QuantStudio® 5

Step1: 50℃ for 5 minutes, 1 cycle；

Step2: 95℃ for 1 minutes, 1 cycle；

Step3: 93℃ for 1 seconds to 60℃ for 10 seconds, 5 cycles；

Step4: 93℃ for 1 seconds to 60℃ for 10 seconds, 37 cycles. The signals of FAM, VIC, ROX, CY5.5 and CY5 fluorescence channels will be collected at 60℃.

Note: In the QuantStudio5 software operation interface, select "fast" from the run mode menu..

5. Data Analysis

Test data file need to be saved after PCR reaction. Please set the parameters and analysis the results of FAM, VIC, ROX, CY5.5 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 calibration for the kit, and shall be set for each 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	VIC Channel	ROX Channel	CY5.5 Channel	CY5 Channel
Positive Control of MPXV	Ct≤32	Ct≤32	Ct≤32	Ct≤32	No requirement
Negative Control	Undet or Ct>36	Undet or Ct>36	Undet or Ct>36	Undet or Ct>36	Ct≤32

Note: "Undet" means "not detected".

7. Interpreting Test Results

【CUT-OFF VALUE OR REFERENCE INTERVAL】

The cut-off value of each target is Ct≤36.

【ASSAY EXPLAINATION】

After the test is completed, under the normal quality control condition, test results is determined according to the Ct

values detected by FAM, VIC, ROX, CY5.5 and CY5 channels of the instrument

1. When the Ct value of at least one channel of FAM and VIC is≤36, it is determined that 2019-nCoV is detected. When the Ct value of FAM and VIC channel is>36 or Undet, and the Ct value of CY5 channel is≤32, it is determined that 2019-nCoV is undetected.

2. When the Ct value of the ROX channel test sample is≤36, it is determined that influenza A virus is detected. When the Ct value of ROX channel is>36 or Undet, and the Ct value of CY5 channel is≤32, it is determined that influenza A virus is undetected.

3. When the Ct value of the CY5.5 channel test sample is≤36, it is determined that influenza B virus is detected. When the Ct value of ROX channel is>36 or Undet, and the Ct value of CY5 channel is≤32, it is determined that influenza B virus is undetected.

4. When the Ct values of FAM, VIC, ROX and CY5.5 channel are all >36 or Undet, and the Ct values of CY5 channel are>32 or Undet, the test results are invalid and need to be resampled and retested.

【ASSAY LIMITATIONS】

1. The detection of the target 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 detection of 2019-nCoV, Influenza A and Influenza B.

3. Although the detected target sequences of this kit are the conservative region of 2019-nCoV, Influenza A and Influenza B's gene, the missed detection of 2019-nCoV, Influenza A and Influenza B with rare mutations in the conservative region can't be completely avoided in theory.

【PERFORMANCE SPECIFICATIONS】

1. Detection limitation: 2019-nCoV, Influenza A and Influenza B Virus is 200 copies/mL.

2. This kit have no cross reaction with other related pathogens, including endemic human coronavirus (HKU1, OC43, NL63 and 229E), SARS coronavirus, MERS coronavirus, Respiratory syncytial virus A, B, Parainfluenza viruses 1, 2 and 3, Rhinoviruses A, B and C, Adenovirus type 1, 2, 3, 4, 5, 7, 55, Enterovirus group A, B, C, D, Human metapneumovirus (Human lung virus), EB virus, Measles virus, Human cytomegalovirus, Rotavirus, Norovirus, Mumps virus, Varicella zoster virus, Mycoplasma pneumoniae, Chlamydia pneumoniae, Legionella, Pertussis bacillus, Haemophilus influenzae, Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Klebsiella pneumoniae, Mycobacterium tuberculosis, Aspergillus fumigatus, Candida albicans, Candida glabrata, Cryptococcus neoformans, Human genome DNA, etc.

【ATTENTIONS】

1. The kit is for Research Use Only. Not for use in diagnostic procedures.

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, Influenza A and Influenza B 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 such as The general guidelines for biosafety of microbiological biomedical laboratories and The regulations on the management of medical wastes issued by the Ministry of Health.

【Reference】

[1] Alan J. Hay, Victoria Gregory, Alan R. Douglas, etal. The evolution of human influenza viruses. Phil. Trans. R. Soc. Lond. B, 2001(356): 1861-1870..

[2] Shapiro, G.I., Krug, R.M., 1988. Influenza virus RNA replication in vitro: synthesis of viral template RNAs and virion RNAs in the absence of an added primer. J. Virol. 62(7): 2285–2290. WHO. Clinical management of severe acute respiratory infection when novel coronavirus (nCoV) infection is suspected. 2020..

【General Information】

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