**Instruction for Use**

**Novel Coronavirus (COVID-19) & Influenza A/B Multiplex Nucleic Acid Detection Kit (PCR-fluorescent Probe)**

【**Product Name**】

**Novel Coronavirus (COVID-19) & Influenza A/B Multiplex Nucleic Acid Detection Kit (PCR-fluorescent Probe)**

【**Intended Use**】

The product is a real-time reverse-transcriptase polymerase chain reaction (RT-PCR) test that detects and differentiates RNA from COVID-19, influenza A virus and influenza B virus. Samples can be obtained via nasopharyngeal or pharyngeal swab or sputum.

【**Packaging Specification**】24 tests/kit, 48 tests/kit, 96 tests/kit

【**Kit Components**】

|  |  |  |  |
| --- | --- | --- | --- |
| Components | Volume (μl/Vial) 24T Kit | Volume (μl/Vial) 48T Kit | Volume (μl/Vial) 96T Kit |
| nCoV/InfluABReaction Mix | 400 μL | 800 μL | 1600 μL |
| RT Enzyme Mix | 11.5 µL | 23 µL | 46 µL |
| Positive control | 50μL | 50μL | 50μL |
| Nuclease-free water | 1.0mL | 1.0mL | 1.0mL |

**NOTIFICATION:**

1. nCoV/InfluAB Reaction Mix is the key component of this detection kit, which contains specific primers and probes:

Virus nucleocapsid (N) gene for specific detection of 2019nCOV (COVID-19)

Nucleoprotein(NP) gene for specific detection of influenza A/B virus

RNase P gene (RP) for specific detection of human nucleic acid that serves as an internal control

|  |  |  |
| --- | --- | --- |
|  | Specific Genes | Fluorescent Dyes |
| 2019nCOV | N Gene | VIC |
| Influenza A virus | NP Gene | FAM |
| Influenza B virus | NP Gene | Tex Red |
| Internal control  | RNase P | CY5 |

1. The positive control is the mix of single-stranded RNA. It is required to be dispensed into 200 μL PCR tubes with 5μL per reaction according to the required number of reactions. Please avoid repeated frozen-thawed cycles to avoid the degradation of RNA templates.
2. The components in different batches of kits are not interchangeable.

【**Storage**】

1. All reagents should be stored at -20±5℃ until the expiration date listed on the outer kit box.

2. Reaction Mix should be stored away from light.

3. Repeated freezing and thawing (more than five times) of reagents should be avoided.

4. The kit is valid for 9 months.

【**Materials and Devices Required but Not Provided**】

1. Biological cabinet.

2. Appropriate real time PCR instrument: ABI7500、ABI QuantStudio 6/7/12K; Roche Lightcycler®480/1536/Nano; Agilent Mx3000P/3005P; Qiagen Rotor-Gene 6000 / Q; Bio-Rad CFX384/CFX96, Bio-Rad Touch / iQ5; Cepheid Smartcycler/Smartcycler II; Eppendorf Mastercycler.

3. Appropriate nucleic acid extraction system or kit.

4. Desktop centrifuge (suitable for 96-well plate or 8-strip tube).

5. Centrifuge with a rotor for 0.2ml reaction tubes or plate.

6. Vortex mixer.

7. Adjustable pipettes (with maximum capacity of 2μL, 10μL, 50μL, 100μL, and 200μL, respectively)

8. Disposable pipette tips with filters.

9. Disposable powder-free gloves.

10. RNase free 1.5mL centrifuge tube.

11. PCR reaction tube / PCR reaction plate.

**NOTE:** Please ensure that instruments have been installed, calibrated, checked, and maintained according to the manufacturer’s instructions and recommendations.

【**Background Information**】

Symptoms of COVID-19 and influenza may look the same. It can be difficult for clinicians to identify based on signs & symptoms alone and if left undiagnosed.

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.

Influenza is an acute respiratory tract infection caused by influenza virus with strong infectivity and high transmission speed. It is mainly transmitted through airborne droplets, person-to-person contact or contact with contaminated objects. Typical clinical symptoms are: acute high fever, systemic pain, significant fatigue and mild respiratory symptoms. Generally speaking, the disease is highly prevalent in autumn and winter, which can cause serious complications and death. Influenza virus is A human and animal influenza RNA virus and can be divided into 3 types: influenza A, influenza B and influenza C.

This detection kit can detect Novel Coronavirus, influenza A virus and influenza B virus in one reaction. It is a quadruplex assay that includes: One primer mix, one probe mix and a positive control confirms all four targets in the assay are working correctly. Primers and probes target: Virus nucleocapsid (N) gene for specific detection of 2019nCOV (COVID-19), Nucleoprotein(NP) gene for specific detection of influenza A/B virus, RNase P gene (RP) for specific detection of human nucleic acid that serves as an internal control.

【**Detection Principle**】

The kit is based on real-time fluorescent probe Quantitative RT-PCR technology. RT-PCR（Reverse Transcription-Polymerase Chain Reaction）is a method in which RNA reverse transcription (RT) is combined with polymerase chain reaction of cDNA. First, via reverse transcriptase, RNA fragment in COVID-19 will be synthesized to cDNA, then with cDNA as the template, target fragment will be synthesized via amplification by DNA Polymerase.

In PCR-Fluorescent Probe method, the probe with specific binding to target sequence is added based on the forward primer and the reverse primer, Specific primers and probes are designed based on specific gene areas of Novel Coronavirus (COVID-19). Probes consist of a reporter fluorophore at 5’ and quenching fluorophore at 3’. The fluorescent signals emitted from reporter fluorophores are absorbed by the quenchers, so it doesn’t emit signals. During amplification, probes bonded to templates are cut off by Taq enzyme (5’-3’ exonuclease activity), separating reporter dye from the quencher, generating fluorescent signals, the PCR instrument will then automatically draw a real-time amplification curve based on the signal change, finally realizing the qualitative detection of Novel Coronavirus (COVID-19) at the nucleic acid level.

【**Warnings and Precautions**】

1. For in vitro diagnostic use only.

2. Carefully read this instruction **before the experiment.** Components from different batch number kit cannot be used interchangeably.

3. Once each component within the kit is thawed, it is suggested to use them up within one operation based on examination demand, and the component remained should be restored at -20±5℃. Repeated freezing and thawing (more than five times) of reagents should be avoided.

4. Viral RNA and RT-PCR premix are sensitive to temperature. Once Sample RNA and RT-PCR pre-mix are taken out of -20±5℃ freezer, prepare the Master Mix on ice or in the cooling block.

5. Avoid microbial and nuclease (DNase/RNase) contamination of the specimen and the components of the kit.

6. Always use DNase/RNase-free disposable aerosol-blocking pipette tips.

7. Use of this product is limited to personnel specifically instructed and trained in the Wear protective disposable powder-free gloves, laboratory coat and eye protection when handling specimens and kit reagents.

8. Use separated and segregated working areas for (i) reaction set-up, (ii) specimen preparation and (iii) amplification/detection activities. Workflow in the laboratory should proceed in unidirectional manner. Wear separate coats and gloves in each area.

9. All biological samples and materials with the contact with the product should be treated as infectious biohazard, and related local regulations shall be followed for the disposal. Prevent the exposure to skin and mucosa.

10. The detection test with this kit should be conducted by medical staff and technician with professional technical training.

11. Store positive and/or potentially positive material separated from all other components of the kit.

12. Do not open the reaction tubes/plate post amplification to avoid contamination with amplicons.

13. Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.

14. Discard sample and assay waste according to your local safety regulations.

15. Do not eat, drink, or smoke in the laboratory working area.

16. Do not use components of the kit after the expiration date.

【**Recommendation on Specimen** **according to the WHO Guideline**】

1. Applicable sample type: nasopharyngeal or pharyngeal swab, sputum.

2. Requirements on sample collection: the sample collection shall be conducted with polyester swab or polyester flocked swab.

(1) Nasopharyngeal swab: extend the sterile nasopharyngeal swab (metal swab with one curved end) from the oral cavity to nasopharynx, swab the posterior wall of the pharynx to get the sample.

(2) Oropharyngeal swab: The sampling technician shall fix the tongue with a spatula, and use the polyester swab or calcium alginate swab to cross the root of the tongue to reach the positions such as pharynx posterior wall and tonsillar crypts, side wall, swab 3 to 5 times to collect mucosa cells; take out the swab gently to avoid the contact with the tongue, horst, oral mucosa or saliva, and insert the swab back into the sampling apparatus.

3. Requirements on sample transportation and storage: It is required to ship the sample at 4℃ to the lab, and store at 4℃ if the storage time is less than 5 days, while it is required to store at -70 °C if the storage time is longer than 5 days.

4. Precautions:

(1) Nasopharyngeal swab and Oropharyngeal swab shall be placed into one tube in order to increase the viral load;

(2) For the transportation of virus detection sample, WHO guideline suggested to use VTM (Viral Transport Medium) with antifungal and antibiotic supplement.

5. **SPUTUM SPECIMEN**: Recommend stored in sterile container. The sample should be stored at 4℃ during transportation to the lab; store at 4℃ if the storage time is less than 48 hours, store at -70 °C if the storage time is longer than 48 hours. PLEASE **ENSURE THE MATERIAL IS FROM THE LOWER RESPIRATORY TRACT**.

**【Procedure】**

1. Collect clinic sample RNA with QIAamp viral RNA mini kit or other RNA isolation kit (see the instructions).

2. Formulation of RT-PCR One-step Mix

2.1 Determine the amounts of samples to be tested first; RNase-free reaction tubes shall be provided with each sample.

2.2 Thaw the nCoV/InfluAB Reaction Mix, RT Enzyme Mix, positive control, and RNA samples on ice. Shake Reaction Mix, positive control, and RNA samples with inching on the shaker, then centrifuge bridfly. Briefly spin the RT Enzyme Mix in the centrifuge, flick 3-5 times, then briefly spin again in the centrifuge. The positive control is the mix of single-stranded RNA and should be dispensed into 200 μL PCR tubes with 5μL per reaction according to the required number of reactions. Please avoid repeated frozen-thawed cycles to avoid the degradation of RNA templates.

2.3 The nCoV/InfluAB Reaction Mix contain RT-PCR primers, probes and reaction reagents (except enzymes). Set up the following ingredients in order:

|  |  |
| --- | --- |
| **Reagents** | **Amount per test (μL)** |
|  nCoV/InfluAB Reaction Mix | 14.6 |
| RT Enzyme Mix | 0.4 |
| RNA sample | 5 |
| Total volume | 20 |

2.4 Prepare 10% more reagent for waste.

 **NOTE: Avoid repeated freezing and thawing of the reagent for more than 5 times during the period of validity. During the period of use, each component of each kit can be distributed and mixed for a maximum of 5 times to reduce the wastage.**

2.5 Aliquot 15 μL of final Reaction Mix( 14.6 μL Reaction Mix +0.4 μL RT Enzyme Mix) into each reaction well and mix.

2.6 The total RT-PCR reaction volume is 20 μL:

（1）RNA sample: Add 5μL of RNA sample to the corresponding reaction well in Reaction Mix.

（2）Positive control: Add 5μL of positive control to the reaction well in Reaction Mix as positive control well.

（3）Negative control: Add 5μL of Nuclease-free water to the reaction well in Reaction Mix as negative control well.

2.7 Seal the tube cap and shake with inching on the shaker several times, followed by short spin on the centrifuge.

3. RT-PCR protocol as below:

|  |  |  |
| --- | --- | --- |
| **Temp (℃)** | **Time** | **Cycles** |
| 50 | 5 min | 1 |
| 95 | 20 sec | 1 |
| 95 | 5 sec | 45 |
| 60 | 30 sec |

Fluorescence Dyes:

FAM(Influenza A); VIC(COVID-19);

Tex Red(Influenza B); CY5 (IC-RNaseP).

4. Result Interpretation

4.1 Quality Check for the Test Results

The following requirements on value Ct of positive control well and negative control well on the reaction plate within the same reaction plate/batch:

|  |  |
| --- | --- |
|  | **Quality control requirement** |
| Positive Control reaction well | Ct≤37 |
| Negative Control reaction well | Ct>37 or Undet |

4.2 If the positive control and/or negative control does not meet the criteria set above, then the experiment is invalidated and should be repeated.

4.3 The analysis of the Ct value of the COVID-19, Influenza A, Influenza B and IC reaction wells are as follows:

4.3.1 When IC(CY5) Ct≤40:

|  |  |  |
| --- | --- | --- |
| **Virus** | **Fluorescent Dyes** | **Result Interpretation** |
| Ct≤37 | Ct>40 or Undet  | 40>Ct>37 |
| 2019nCOV | VIC | Positive | Negative | Weak positive; Retest to confirm |
| Influenza A virus | FAM | Positive | Negative | Weak positive; Retest to confirm |
| Influenza B virus | Tex Red | Positive | Negative | Weak positive; Retest to confirm |

4.3.2 When IC(CY5) Ct>40 or undetected:

|  |  |  |  |
| --- | --- | --- | --- |
| **Virus** | **Fluorescent Dyes** | **Ct Value** | **Result Interpretation** |
| 2019nCOV | VIC | Ct>40 or Undet  | Resampling and test |
| Influenza A virus | FAM | Ct>40 or Undet  | Resampling and test |
| Influenza B virus | Tex Red | Ct>40 or Undet  | Resampling and test |

**【Performance Evaluation】**

**1.** Limit of Detection (LOD): 400 copies/mL.

2. Interference reaction : The five potential reference(Dexamethasone, Azithromycin, Tobramycln, Levofloxacin, Ceftriaxone) will not interfere with the detection results of the kit.

3. Cross-reactivity: No cross reaction with 16 viruses(Human BK polyomavirus, Human adenovirus C serotype 5, Human adenovirus A/B1/C/D/E, Human herpesvirus 1/2/3/4/5/7, Human parvovirus B19, Human JC polyomavirus, Simian vacuolating virus 40) and human genome DNA.

4. Internal precision: repeatability : cv% < 10%, between-run precision : cv% < 10%, between-day precision : cv% < 10%, total precision: cv% < 10%..

5. External precision: repeatability : cv% < 10%, between-run precision : cv% < 10%, total precision: cv% < 10%.

**【Performance Limitation】**

1. Test results only serve as clinical reference and comprehensive judgments based on clinical symptoms and other laboratory tests method should be considered by clinicians. Negative results must be combined with clinical observations, patient history, and/or epidemiological information.

2. Negative results do not preclude SARS-CoV-2, influenza A, and/or influenza B infection and should not be used as the sole basis for diagnosis, treatment or other patient management decisions. Improper sample collection, improper transportation, improper processing and insufficient initiation VL(viral load) may influences the experimental results.

3. Other unverified interferences or PCR inhibitors may cause false negative results.

**【Explanation of Marks】**

|  |  |
| --- | --- |
| Diagram and symbol used on kit label | remarks |
|  | Manufacturer |
|  | Authorized representative in the European Community |
|  | Consult instructions for use |
|  | In vitro diagnosis reagent |
| C:\Users\Lenovo\AppData\Roaming\Tencent\Users\904097501\QQ\WinTemp\RichOle\G)NB[0@OGXN9PRL[(LC$@F3.png | Contains sufficient for <n> tests |
|  | Date of manufacture |
|  | Use-by date |
|  | Do Not Reuse |
|  | Batch code |
|  | Biological risks |
|  | Storage temperature |
| C:\Users\Lenovo\AppData\Roaming\Tencent\Users\904097501\QQ\WinTemp\RichOle\7$O{Y92LUHQ923D9K3XQJW7.png | Keep dry |
| C:\Users\Lenovo\AppData\Roaming\Tencent\Users\904097501\QQ\WinTemp\RichOle\1JG[ESZUT}EX2~KACFK68(4.png | Keep away from sunlight |
| C:\Users\Lenovo\AppData\Roaming\Tencent\Users\904097501\QQ\WinTemp\RichOle\@LY8X~8W}I(FPXTSPD2$ZPA.png | Fragile, handle with care |
|  | Recoverable PAP material |
| 1584780032 | Recoverable PP material |
|  | Recycled recyclable |

**【Manufacturing Date and Expiration Date】**

See details on packaging label.

**【Basic Information】**

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**【User Manual Information】**

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