RunMei

Please read the instructions carefully before use and strictly follow the instructions.

# SARS-CoV-2, Influenza A Virus, Influenza B Virus, and RSV Virus Nucleic Acid Detection Kit (Fluorescence RT-PCR Method)

## VERSION 1.0

Approval Date: 8/10/2022 Revision Date: 8/10/2022 Date of Issue: 8/10/2022

## PRODUCT NAME

SARS-CoV-2, Influenza A Virus, Influenza B Virus, and RSV Virus Nucleic Acid Detection Kit (Fluorescent RT-PCR Method)

## SPECIFICATION

50 tests / kit & 200 tests / box

## INTENDED USE

This kit is used for the detection of SARS-CoV-2, Influenza A Virus, Influenza B, and RSV Virus Nucleic Acid and assisted diagnosis and epidemiological surveillance of SARS-CoV-2, Influenza A Virus, Influenza B, and RSV Virus.

## TEST PRINCIPLE

This kit is based on the principle of fluorescent PCR technology, designed specific primers and Taqman probes for SARS-CoV-2, Influenza A Virus, Influenza B, and RSV Virus, and detect them with a fluorescent PCR instrument to achieve detection of SARS-CoV-2, Influenza A Virus, Influenza B, and RSV Virus nucleic acids.

In addition, the PCR detection system contains internal control primers and probes to monitor the sample collection and the extraction process by detecting whether the internal standard is normal, to avoid false negative results.

## MAIN CONTENTS

| Number | Composition | 50 tests / kit | 200 tests / box |
|---|---|---|---|
| 1 | SARS-CoV-2, FluA, FluB, RSV PCR Reaction Solution | 725μl × 1 tube | 725μl× 4 tube |
| 2 | SARS-CoV-2, FluA, FluB, RSV Enzyme Mixture | 275μl × 1 tube | 275μl× 4 tube |
| 3 | SARS-CoV-2, FluA, FluB, RSV Negative Control | 100μl × 1 tube | 100μl × 4 tube |
| 4 | SARS-CoV-2, FluA, FluB, RSV Positive Control | 100μl × 1 tube | 100μl × 4 tube |
| 5 | Instructions | 1 serving | 1 serving |

**Note**

1. Different batches of reagents cannot be mixed or interchanged.
2. The SARS-CoV-2, FluA, FluB, RSV Negative Control is saline, and the SARS-CoV-2, FluA, FluB, RSV Positive Control is Plasmid.

## STORAGE CONDITIONS AND EXPIRING DATE

The kit should be stored at -20 ± 5℃. The expiring data is tentatively set at 12 months.

The production date and the expiration date can be seen in the outer packaging.

This kit should not be frozen and thawed more than 5 times. Please use the reagent within 1 month after opening.

## APPLICABLE INSTRUMENT

This kit is suitable for ABI series, Bio-Rad series, Agilent Stratagene MX series, Roche LightCycler R480, Cepheid SmartCycler, Rotor-Gene series and other multi-channel real-time quantitative PCR instruments.

## SAMPLE REQUIREMENTS

- **Sample types:** Upper respiratory tract specimens (including throat swabs, nasal swabs, nasopharyngeal extracts, deep cough sputum), lower respiratory tract specimens (including respiratory tract extracts, bronchial lavage fluid, alveolar lavage fluid, lung tissue biopsy specimens), Tissue culture and other samples.
- **Storage conditions:** The collected specimens should be submitted for inspection in a timely manner, and the specimens should be stored at 4ºC within 24 hours. It is best to store at -70ºC for more than 24 hours, and avoid repeated freeze-thaw cycles.

## TESTING METHOD

**Reagent Preparation (reagent preparing area)**

Melt the components of the kit at 4ºC, protected from light, and mix thoroughly before centrifugation. Calculate the number of reagents used N (N= number of samples + 1 positive control + 1 negative control), configure the reaction system mix according to the following table, add an appropriate volume of centrifuge tube, fully mix and centrifuge immediately, aliquot according to 20μL to a PCR reaction tube/plate and transfer to the sample processing area.

| Component | Volume (μL) |
|---|---|
| SARS-CoV-2, FluA, FluB, RSV PCR Reaction Solution | 14.5 |
| SARS-CoV-2, FluA, FluB, RSV Enzyme Mixture | 5.5 |
| Total capacity (reaction system mix) | 20 |

- **Processing and Extraction of The Sample (sample processing area)**

This kit does not contain extraction. It is recommended to use RNA extraction kits produced by QIAGEN and Roche or Hunan Runmei Gene Technology Co., Ltd. To extract viral RNA. The specific operation is in accordance with its kit instructions.

- **Loading**

Add 5μL each of the processed sample nucleic acid, the SARS-CoV-2, FluA, FluB, RSV Negative Control, and the SARS-CoV-2, FluA, FluB, RSV Positive Control to the PCR reaction tubes/plates to which the reaction mix has been added. The final volume is 25μL. Cap the tube tightly or seal the membrane, and then immediately amplify by low-speed centrifugation and a fluorescence PCR detector.

- **PCR Amplification (Nucleic Acid Amplification Area)**

1.Take the PCR reaction tube to be detected, place it in the corresponding position of the sample tank of the real-time fluorescence quantitative PCR instrument, and record the placement sequence.

2.Set the instrument nucleic acid amplification related parameters according to the following table for PCR amplification.

| Steps | Temperature | Time | Cycles |
|---|---|---|---|
| 1 | 50ºC | 10min | 1 |
| 2 | 95ºC | 3min | 1 |
| 3 | 95ºC | 10s | 40 |
| | 55ºC | 30s | |

**Note**

1) The fluorescence collection is set at “Step 3: 55ºC, 30s” . Selection of detection channels: FAM, HEX, ROX, Cy5 and Q705 where FAM channel is SARS-CoV-2 gene, HEX channel is FluA gene, ROX channel is FluB gene, Cy5 channel is RSV gene, Q705 channel is IC(RNase P)gene and the reaction system is set to 25 μL.

2) ABI series fluorescent PCR instruments do not select ROXcalibration and select None for the quenching group.

## POSITIVE JUDGEMENT VALLUE OR REFERENCE INTERVAL

**● Condition Setting for Result Analysis**

The adjustment principle of Baseline and Threshold is generally based on the results of the automatic analysis of the instrument. When the overall slope of the curve appears, the Start, End, and Threshold values of the Baseline can be adjusted according to the image. Usually, the user can adjust it according to the actual situation. The Start value can be set to 3–15, and the End value can be set to 5–20. Adjust the amplification curve of the negative control to make it straight or below the threshold line.

**● The Validity of The Kit**

| Channels | Target | Negative | Positive |
|---|---|---|---|
| FAM | SARS-CoV-2 | No Ct value or Ct value >38 | Ct values ≤32 |
| HEX | Flu A | No Ct value or Ct value >38 | Ct values ≤32 |
| ROX | Flu B | No Ct value or Ct value >38 | Ct values ≤32 |
| Cy5 | RSV | No Ct value or Ct value >38 | Ct values ≤32 |
| Q705 | IC | No Ct value or Ct value >38 | Ct values ≤32 |

**● The Positive Judgment Value**

Through the study of reference values, it was determined that the Ct reference value of the target gene and the internal control gene detected by this kit was both 38.

## THE SAMPLE RESULT JUDGMENT

● The following table interprets the experimental results and there is a typical S-type amplification curve in the Internal Control channel (Q705).

| Channels | Target | Negative | Positive |
|---|---|---|---|
| FAM | SARS-CoV-2 | No Ct value or Ct value >38 | Ct values ≤35 |
| HEX | Flu A | No Ct value or Ct value >38 | Ct values ≤35 |
| ROX | Flu B | No Ct value or Ct value >38 | Ct values ≤35 |
| Cy5 | RSV | No Ct value or Ct value >38 | Ct values ≤35 |

● If the test sample yields a Ct value range is 35-38 in the FAM, VIC , ROX or Cy5 channel and there is a typical S-type amplification curve in the Internal Control channel (Q705), the results need to retest. If the results repeated are consistent, and has a typical S-type amplification curve, the sample can be judged positive, otherwise, the sample can be judged as negative.

● If no typical S-type amplification curve (No Ct value) or Ct value > 38 is detected in the Q705 channel of the test sample, it means that there is a problem with the quality of the sample or a problem with the operation. If the result is invalid, you should find and eliminate the cause, collect the sample again, and repeat the test (if the test result is still invalid, please contact the company).

## LIMITATIONS OF INSPECTION METHODS

(1)The test results of this kit are for clinical reference only. The clinical diagnosis and treatment of patients should be considered in conjunction with their symptoms, signs, medical history and other related conditions.

(2)False negative results may occur when the concentration of the detected nucleic acid in the test sample is below the minimum detection limit of this kit.

(3)Improper handling of the tested sample during collection, transportation, storage, and processing can easily result in RNA degradation and false negative results.

When samples are cross-contaminated during collection, transportation, storage, and processing, it is easy to get false positive results.

## PRODUCT PERFORMANCE INDEX

● **LOD:**The limit of detection is 500 copies/ml.

● **Precision:** Coefficient of variation (CV%) of precision Ct value within batch ≤3%.

● **Specificity:**There is no cross reaction between the kit and positive samples, such as human coronavirus HCoV-NL63, human coronavirus HCoV-OC43, SARS coronavirus, MERS coronavirus, adenovirus(type 2), Mycoplasma pneumoniae, Chlamydia pneumoniae, pertussis, Streptococcus pneumoniae, rhinovirus (Type A) etc.

## PRECATIONS

1.The entire detection process should be performed strictly in accordance with the requirements of this manual in the reagent preparation area,sample processing area, and PCR amplification area, and the experimental clothes, instruments, and consumables in each area should be used independently and cannot be mixed. The experimental tips use filter tips, the sample processing area should be equipped with a biological safety cabinet, the sample processing should be performed in the biological safety cabinet, and three areas should be equipped with ultraviolet sterilization devices.

2.To avoid RNA degradation, the sample processing process should be operated at 0-4ºC, and the test should be performed immediately after the experiment is completed. Utensil consumables used in sample processing should be nuclease-free.

3.Negative and positive controls should be set for each experiment.

4.All reagents in the kit should be fully thawed and mixed at room temperature and centrifuged immediately before use.

5.All negative and positive controls in the kit should be transferred to the sample preparation area and stored separately before the first use.

6.To prevent fluorescence interference, avoid touching the PCR reaction tube directly with bare hands, and avoid any marking on the PCR reaction tube.

7.The instrument amplification related parameters should be set in accordance with the relevant requirements of this manual, and different batches of reagents cannot be mixed.

8.The product waste during the experiment should be detoxified before being discarded.

9.During transportation, ensure that the product temperature is below 0ºC, which will not affect product performance.

## BASIC INFORMATION

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| IVD | For in vitro diagnostic use | | Manufacturer | | Biohazard material | CE | CE marking |
| -25℃ -15℃ | Store at -25 °C~-15°C | | Expiry data | | Do not re-use | | Avoid light |
| | Date of Manufacture | LOT | Lot number | EC REP | EU representative | | Keep dry |
| | Be careful and fragile | | Please consult instruction for use | | Can only be placed in this direction, keep upright | | Don't use the product when the package is damaged |

**HuNan RunMei Gene Technology Co., Ltd.**
Add: Room 401-1, Building 3, Shanhe Medical and Health Industrial Park, No. 1048, Zhongqing Road, Shaping Street, Kaifu District, Changsha, HuNan Province China, 410153
Tel: (+86)-731-89919680 / (+86)-755-86537353 / (+86)-18601451556
Website : www.crocomed.com www.runmeigene.com
E-mail : sales@crocomed.com sales@runmeigene.com

**CMC Medical Devices & Drugs S.L**
Address: C/Horacio Lengo No 18 CP 29006, Málaga-Spain.
Tel: +34951214054
Fax: +34952330100
E-mail: info@cmcmedicaldevices.com