Sprise India Pvt. Ltd

SENSIT REAL TIME RT-PCR KIT

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Product Description

Corona virus belongs to the family of Corona viridae, in the order of Nidovirales, which is a positive-sense single-stranded RNA and it usually appears spherical with a size of 80 – 120 nm, and with crown-like spikes on the surface. This large family of virus is commonly circulating among vertebrates, such as camels, cats and bats. Novel corona virus (COVID-19) has been identified as a new strain of corona virus. It can cause viral pneumonia and dyspnea in humans. ViraGEN RT-PCR is designed for the qualitative detection of nucleic acid from SARS-CoV-2 in upper and lower respiratory specimens (such as anterior nasal swabs, mid-turbinate nasal swabs, nasopharyngeal swabs, oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate or nasal aspirate) from individuals suspected of COVID-19. The results can be used to assist diagnosis of patients with COVID-19 infection, and provide molecular diagnostic basis for infected patients. The test results of this product are for clinical reference only and should not be used as the only standard for clinical diagnosis. It is recommended to conduct a comprehensive analysis by combining the test results with clinical symptoms presented by the patient and other laboratory tests.

PRINCIPLE

The primer and probe mix adopts the dual-target gene design, which targets the specific conserved sequence encoding the ORF lab gene and the nucleoprotein N gene. With the PCR reaction mix provided, the amplification of template can be quantitatively monitored by the increasing fluorescence signal detected by a real time PCR instrument.

The PCR detection system includes an endogenous internal control primer and probe mix. The result of internal control provides the accuracy of sampling and extraction process, in order to avoid false negative results.

Kit Contents, 96 Tests

ViraGEN COVID-19 Real Time PCR Kit contains amplification reagents, composed of the following:

Description	Quantity	Storage
1. Lyophilised Enzyme Mix	96 tests/bottle	-20^{0}C
2. Enzyme Mix Buffer (5X)	400µL/vial	-20^{0}C
3. Primer Probe Mix	100µL/vial	-20^{0}C
4. Positive Control of SARS-CoV-2 (Plasmid)	90µL/vial	-20^{0}C
5. Negative Control (DEPC-treated H_2O)	90µL/vial	-20^{0}C

Instrument Compatibility

ViraGEN COVID-19 Real Time PCR Kit is compatible with with:

Real time PCR instruments
• FAM
• HEX/VIC
• RED/ROX channels

Storage And Shelf-life

1. Shelf – life of components in 12 Months. Manufacture date is indicated on the box.
2. Reagents should be stored in the dark at -20 ±5°C.
3. Repeated thawing and freezing should be no more than 10 times.
4. The reconstituted liquid reagent should be used up at once. Leftover reagents should be stored at 4°C for no longer than 1 week.

Specimen Requirement

1. Sample Type: Serum, throat swabs, virus preservation buffer and others
2. Sample Collection: Collect in accordance with conventional sample collection methods
3. Sample Storage & Transportation: Sample to be tested can be processed immediately, or stored at -20 ±5°C for 3 months, -70°C for long term.
   Avoid repeated thawing and freezing. Sample should be transported with refrigerant packs in sealed Styrofoam box or ice chest.

Preparation Before Testing

Please follow user manual instructions to extract virus RNA from clinical sample using RNA extraction kit. Extracted RNA can be used directly for PCR detection. Otherwise, keep RNA sample at -70°C, if not in use. Avoid repeated thawing and freezing.

Note: This product does not contain an RNA extraction kit, and is compatible with ViraGEN RNA Extraction Kit and other commercial kits.

Detection Method

1. Reagent Preparation (Perform in Reagent Processing Area)

   1.1. Master Mix Preparation:

Take out the components and let it thaw at room temperature until equilibrated. Resuspend the Lyophilized Enzyme Mix in 400µL Enzyme Mix Buffer. Add 500 µL RNase-free water and gently pipette up and down. Avoid generating air bubbles. Wash the wall of the tube by pipetting to prevent lyophilized powder from remaining. Place the tube aside for 30 min.
Note: The reconstituted liquid reagent should be used up at once. Leftover reagents should be stored at 4°C for no longer than 1 week.

1.2. Reaction Mix Preparation:

The recommended sample volume used in the reaction is 5 µL or 10 µL .Refer to one of the columns below to prepare the reaction mix:

1x volume required		
	For 5 µL Sample	For 10 µL Sample
Resuspended master mix	9 µL	9µL
ORF Iab /N/ICON Primer& probe (FAM/HEX/ROX)	1µL	1µL
RNase –free water	5 µL	-
Total volume	15 µL	10µL

- Multiply the numbers according to the number of tests.

1.3. Aliquot 15 µL (or 10µL, depending on sample volume) of the above reaction mix into the PCR platform. Aliquot into wells according to the number of samples to be tested, include one well for the positive control and one well for the negative control. Transfer the reaction mix to Sample Processing Area.

2. Sample Adding (Perform in Sample Processing Area)

2.1 For 5 µL sample:

Add 5 µL of the following into the appropriate wells according to plate setup:

Sample(s), Positive Control, Negative Control

2.2 For 10 µL sample:

Dilute positive control with 5 µL DEPC-treated water to total volume of 10 µL. Add 10 µL of the following into the appropriate wells according to plate setup:

Sample(s), Diluted Positive Control, Negative Control

2.3 After adding the samples, cover the lid immediately. Spin down briefly using a centrifuge to remove air bubbles. Transfer the mixture to amplification area.

3. PCR Amplification (Perform in Amplification and Analysis Area)

3.1 Place the tubes on the sample holder in the instrument. Set up the test panel according to the positions of positive control, negative control and RNA samples.

3.2 Select the detection channels as following:

a) Select FAM (ORF-lab gene) and HEX (N gene) channels to detect COVID-19 RNA.

b) Select ROX channel to detect internal control.

3.3. Enter the amplification program. Recommended as below:

	Step	Temperature	Time	Cycle
1	Reverse Transcription	50°C	15 min	1
2	cDNA Initial Denaturation	95°C	3min	1
3	Denaturation	95°C	15 sec	45~50
4	Annealing, Extension and Fluorescence measurement	55°C	40 sec	
	Cooling	25°C	10 sec	1

Save the file after settings and run the reaction. Please set the fluorescence internal control of the instrument to “None”. For example, for ABI series instruments –set “Passive Reference” to “None”.

4. Result Interpretation (Please refer to the user manual of instrument for setting, the following analysis uses ABI series instruments as an example)

4.1. After the reaction is completed, the results are automatically saved and the amplification curves of the detected target DNA and the internal control are analyzed separately.

4.2. According to the analysis, the amplification plot will adjust the Start value, End value and Threshold value of the Baseline (Users can adjust the values according to the actual situation. Start value can be set within 3~15, End value can be set within 5~20; Users can adjust the amplification curve of negative control to make it linear or below the threshold line). Click

"Analyze" to perform the analysis and the parameters should meet the following requirements mentioned in "Section 5. Quality Control". Lastly, record the qualitative results in the Plats window.

Quality Control

COVID-19 PCR Negative Control:

None of the FAM, HEX & Internal Control (ROX) channels a Ct value or Ct>40.

COVID-19 PCR Positive Control:

FAM, HEX & Internal Control (ROX) channels a Ct≤35

The above requirements must be met at the same time in the same experiment. Otherwise, this experiment is invalid and needs to be repeated.

Positive Threshold

According to the study of the reference value, the Ct reference value for the target gene detected by this product is 40, and the Ct reference value of internal control is 40.

Result Analysis

Internal Control	ORF lab gene	N gene	Conclusion	Remark
Ct<40	Has amplification curve; Ct<40	Has amplification curve; Ct<40	Positive	
Ct<40	No amplification curve	No amplification curve	Negative	
Ct<40	Has amplification curve; Ct<40	No amplification curve	Suspected ; need retesting	If again getting one gene positive result, then need collecting the sample
Ct<40	No amplification curve	Has amplification curve; Ct<40	Suspected ; need retesting	If again getting one gene

				positive result ,then need collecting the sample
Ct<40	Has amplification curve; Ct>40	Has amplification curve; Ct>40	Suspected ; need retesting	If again getting the same result, then the result is positive
Ct>40	-	-	Invalid : Need collecting sample again	

1. First to analyze the amplification curve internal control ROX channel. If Ct≤40, it indicates that the detection is valid, and users can continue the subsequent analysis:

   a) If a typical S-type amplification curve is detected by the FAM and HEX channel, with Ct≤40, it indicates that COVID-19 virus is positive.

   b) If FAM and HEX channels do not detect a typical S-type amplification curve (No Ct), it indicates that COVID-19 virus is negative.

   c)If only FAM channel or only HEX channel detects a typical S-type amplification curve with Ct≤40, Users should repeat the experiment.

2. If the internal control ROX channel failed to detect Ct or Ct>40, it indicates that the concentration of the tested sample is too low or there is an inhibitory reaction from the interfering substance. Users have to repeat the experiment.

3. For virus cultures (not from human body), there is no requirement of the internal control results. For negative samples, the internal control should be positive. If the internal control is negative, the test result of the sample is invalid. The cause should be found and eliminated. Users should redo sampling and repeat the experiment. (If the result is still invalid, please contact the manufacturer.)

4. Determination of grey area results: If the fluorescence signal of a sample has a significant increase in the FAM and HEX channels, but the Ct value is greater than 40, the sample is in the grey area and needs to be re-examined. If the retest result is still in the greyarea, it is judged as positive.

Warnings And Precautions

1. This product is only used for in vitro diagnostic detection; for use only by laboratory trained professionals. Please read this manual carefully below use.
2. The contamination of laboratory environment and reagent, or cross contamination during specimen treatment may lead to false positive result.
3. Operation procedure and precautionary warnings of this instrument to be well-understood before conducting the test. Quality control should be performed for each test.
4. The decrease of detection effect even the false negative result may occur if there are any mistakes in the transportation, storage and operation of reagents. COVID-19 early infection or other respiratory virus infection can't be excluded in patients with negative results.
5. Handle all specimens as if infectious using safe laboratory procedures. Refer to official ICMR guidelines on handling specimen for SARS-CoV-2. All samples should be regarded as potentially infectious materials. Laboratory workers should wear appropriate personal protective equipment (PPE) including disposable gloves, laboratory coat/gown, etc . . . . Gloves should be changed after handling each sample, to avoid contamination and false results. Laboratory management should be strictly in accordance with the regulations of PCR gene amplification laboratories. Laboratory personnel must be professionally trained and the experiment process should be strictly divided into sections/ organized. All consumables should be used only once; properly sterilized. Instruments and equipment should be assigned to each stage of the experiment and prevent alternative use of the same.
6. Inappropriate sample collection, transfer, storage and operation may lead to inaccurate test results. 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 suitable specimen storage procedure. As this test involves the extraction of viral RNA and PCR amplification, please take care to avoid contamination of the amplification reaction mixture. Also, regular monitoring of laboratory contamination is recommended.
7. 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 regulatory standards.

8. The operation of sample and waste shall meet the requirements of relevant laws and regulations. Discard all materials in a safe and acceptable manner, in compliance with all legal requirements. If exposure to skin or mucous membranes occurs, immediately wash the area with large amounts of water. Seek medical advice immediately. Do not use components beyond the expiration the date printed on the kit boxes. Do not mix reagents from different lots. Return all components to the appropriate storage condition after preparing the working reagents. Do not interchange vial or bottle caps, as cross-contamination may occur. Reagents supplied are formulated specifically for use with this kit. Make no substitutions in order to ensure optimal performance of the kit. Further dilution of the reagents or alteration of incubation time and temperature may result in erroneous or discordant data.

LIMITATIONS

1. The test results of this product are for clinical reference only. The clinical diagnosis and treatment of patients should be considered in combination with their symptoms, medical history, other laboratory tests and treatment response.

2. Analysis of possibility of false positive & negative results:

2.1 Improper sample collection, processing & transportation and low sample concentration may cause false negative results.

2.2 Variations in the target sequence of the novel corona virus (COVID-19) or sequence changes caused by other reasons may lead to false negative results.

2.3 Improper reagent storage can lead to false negative results.

2.4 Other un proven interferences or PCR inhibitors may cause false positive results.

2.5 During sample processing may cause false positive results.

2.6 This assay should be performed according to Good Laboratory Practice (GLP) regulation. Operators should strictly follow the manufacturer's instructions in performing the test.

Product Performance

Specificity

1. The primer and probe provided is designed based on the conserved sequence of the novel coronavirus (COVID -19) and has high detection rate of the target gene fragment. This product has no cross- reactions among positive samples of coronavirus (NL63, HKU1, 229E,

OC43), Influenza A virus, Influenza B virus, Respiratory syncytical virus, Adenovirus, Parainfluenza virus, Klebsiella pneumoniae, Haemophilus influenza, Pseudomonas aeruginosa, Chlamydia pneumonia. The negative and positive rates of detecting commercial reference materials were 100%.

2. Minimum detection limit: 500 copies/ml

Thank you

Manufactured by

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