Convergys® COVID-19 real-time PCR Kit

COVID-19 RNA Detection Kit
(Real-time Fluorescent PCR method)

Instructions for Use

Version: V1.1, May, 2020

Product name: Convergys® COVID-19 real-time PCR Kit

Packing specifications: 96 tests /kit

Contents

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Intended use:

This kit is used for the detection of SARS-CoV-2 RNA in human nasopharyngeal swabs, sputum and alveolar lavage fluid samples. It qualitatively detects the SARS-CoV-2 specific S gene and N gene.

This kit is for clinical reference only, and cannot be used as the sole criterion for diagnosis. Please refer to the clinical symptoms of the patient or other lab testing results to define the infection condition.

For research use only.

About COVID-19:

Coronaviruses (CoV) are a large family of RNA viruses that cause illness ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV).

The novel coronavirus is a new strain that has not been previously identified in humans, which has thus been named “Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)”, while the disease associated with it is now referred to as COVID-19. Common signs of infection include respiratory symptoms, fever, cough, shortness of breath and breathing difficulties. In more severe cases, infection can cause pneumonia, severe acute respiratory syndrome, kidney failure and even death.

Working principle:

For the detection via real-time PCR, the kit’s primers target conserved S gene and N gene sequences from SARS-CoV-2, to carry out the FAM and ROX dual fluorescence channel dual target gene design. At the same time, it uses an endogenous human target sequence as a non-competitive internal control, detected via Cy5. The one-step method for PCR detection uses TaqMan probes which can specifically bind to a cDNA template in the middle of the primer amplification region. The exonuclease activity of rTth DNA polymerase cuts the 5′-end fluorescent group from the probe and dissociates it from the reaction system, thereby breaking the shield of the 3′-end fluorescent quenching group. The induced emission of fluorescence can be detected by the instrument, thereby realizing the detection of target nucleic acids of COVID-19.

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Main components:

Composition	Ingredients	Specification
Nucleic acid amplification reagents:		
RT-PCR main reaction solution	Buffer,dNTPs,rTth DNA polymerase and antibodies	4 tubes
Manganese ion solution	Mn^{2+} solution	1 tube
Fluorescent probe	primers and probes	2 tubes
DEPC water	Rnase and Dnase free deionized water	2 tubes
Reference:		
Negative control	Main ingredient is TE solution	500ul
Positive control	Main ingredient is pseudovirus containing the target gene	500ul

Required Materials:

Extraction Reagents:

QIAGEN's column extraction or magnetic beads extraction reagents are recommended, such as Qiagen52904QIAamp Viral RNA Mini Kit, Cat No: 52904

Instruments:

- High-speed desktop refrigerated centrifuge
- Pipettes
- Consumable Material (enzyme free tips, centrifuge tubes, etc.)
- Personal protective equipment

Real-time PCR device:

The kit has been tested with ABI-7500 real-time PCR machine and Bio-rad CFX96 real-time PCR machine.

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Storage Conditions and Validity:

Reagents must be stored at -20°C and protected from light. They are valid for 12 months from the date of production. The expiry dates are printed on the labels. Avoid repeated freeze and thaw.

Sample requirements:

1. Sample types: human nasopharyngeal swabs, sputum, alveolar lavage fluid.
2. Pre-treatment of specimens: Add 1 ml of sterile normal saline solution to the test tube of the swab specimen (the frozen specimen should be thawed and thoroughly mixed at room temperature before use), shake well, pipet the washing solution into a 1.5 ml centrifuge tube, 12000 rpm Centrifugation for 5 minutes, remove a portion of the supernatant, and leave 100 µl of the dissolved pellet to isolate and purify the viral nucleic acid.
3. Sample storage: The test sample should be stored for less than 24 h at 2-8°C, for up to three months at -20°C, or for longer periods at -70°C or less.
4. Avoid repeated freeze-thaw samples (max. three times).
5. Sample transportation: Transport in frozen state.

How to use:

1. Sample processing (Sample processing area)

Isolation and purification of the nucleic acids of the virus according to the instructions of the used kit.

2. Amplification reagent preparation (Reagents preparation area)

Take the RT-PCR main reaction solution, Manganese ion solution and Fluorescent probe from the kit. Before use, the reagents should be thawed completely at room temperature, mixed thoroughly and then spun down.

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Each single test reaction is composed as follows:

Reagent	RT-PCR main reaction solution	Manganese ion solution	Fluorescent probe
Volume [µl]	20	5	5

Calculate the required total amount for each reagent, add to an centrifuge tube of appropriate size, mix thoroughly, centrifuge briefly, and add to the set of n (n=sample number+1 positive control+1 negative control) PCR reaction tubes, 30 µl each. Transfer to sample processing area.

3. Adding samples (Sample processing area)

Add 10 µl of the RNA solution prepared in step 1 to each PCR reaction tube, close the tube tightly, centrifuge briefly, place the reaction tube in the fluorescence RT-PCR device, and note the order of sample placement.

4. RT-PCR reaction (PCR detection area)

Step	Cycles	Temperature [°C]	Reaction time (min:sec)
1	1	94	2:00
2	1	56	20:00
3	1	95	01:00
4	40	95	00:10
		58	00:20

The detection of fluorescence signals must be set to FAM and ROX. The signal of the internal control is detected via Cy5. The data collection is set at 58°C.

Results analysis condition setting:

When using the ABI PRISM 7500 fluorescence PCR machine for result analysis, the baseline takes 6-10 or 6-15 cycles of fluorescence signals, and the threshold setting principle is based on the baseline just exceeding the highest point of the negative control amplification curve. And Ct value is not higher than 38 shall prevail.

Quality control standards:

1. The negative control test result is 0, the Ct value column is displayed as undet, and the Ct value of the internal standard test is less than 38.

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2. The positive control Ct result is less than 38.

Interpretation of test results:

The threshold setting principle is that the threshold line just exceeds the highest point of the normal negative control. The Ct value of the negative control should have no value, and the Ct value of the positive control should be less than 38. The fluorescence curve above the threshold should have an obvious S-type shape, otherwise the experiment is considered invalid. In such case, any error messages of the instrument, reagents, and amplification conditions should be checked.

1. The Ct value of the test samples FAM and ROX are both not higher than 38.0, which is regarded as positive;
2. If only one of the Ct values of FAM and ROX in the test sample is not higher than 38.0, it is recommended to retest. If the retested result is still only one of the Ct values of FAM and ROX not higher than 38.0, it is determined to be positive;
3. Samples with a Ct value higher than 38.0 are recommended to be redone. Those with a rechecked Ct value less than 40 are positive, otherwise they are negative.

Limitations of detection methods:

It cannot be used as a basis for clinical diagnosis, and it is for reference only by clinicians.

Product performance index:

1. The minimum detection amount is 500 copies/ml.
2. This kit has no non-specific amplification for endemic human coronavirus (HKU1, OC43, NL63 and 229E), SARS coronavirus, MERS coronavirus, H1N1, H3N2, H7N9, influenza B, Respiratory syncytial virus, Parainfluenza virus type 1, 2, 3, Adenovirus, Rubella virus, Vesicular stomatitis virus, Haemophilus influenzae, Staphylococcus aureus, Streptococcus pneumoniae, Mycobacterium tuberculosis, Candida albicans, Candida glabrata and human genome.
3. Interference: Mucin, Phenylephrine hydrochloride, Oxymetazoline, Sodium chloride, Beclomethasone, Dexamethasone, Triamcinolone acetonide, mometasone, fluticasone, Histamine dihydrochloride, α-Interferon, Ribavirin, Oseltamivir, Arbidol, levofloxacin, Azithromycin, Ceftriaxone, Tobramycin will not affect the assay of this kit.
4. Intra- and inter-assay imprecision: CV values are less than 5%.

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PRECAUTIONS:

1. Relevant laboratory management specifications should be strictly implemented in accordance with the management specifications of genetic amplification laboratories issued by your local industry administrative department.
2. This kit is only for *in vitro* testing.
3. Strictly follow up the PCR laboratory areas operation:
   1st area: Reagents preparation area-preparing reagents for amplification
   2nd area: Sample processing area-processing of samples and reference to be tested
   3rd area: PCR detection area-PCR amplification detection
4. The articles in each area are for exclusive use, and should not be exchanged and used to avoid contamination. Clean the workbench immediately after each experiment.
5. Use non-fluorescent disposable gloves, disposable centrifuge tubes, self-loading pipettes, and filter tips.
6. Bubbles should be avoided when the reaction solution is dispensed. Check whether the reaction tubes are tightly closed before loaded to the machine to prevent the leakage of fluorescent substances to contaminate the instrument
7. When adding the sample, the sample should be completely added to the reaction solution, and no sample should adhere to the tube wall. The tube cap should be closed as soon as possible after the sample is added.
8. Immediately remove the reaction tube after the amplification, seal it in a special plastic bag, place it in the designated place, and wait for unified processing.
9. Please dispose the tip used in the experiment directly into the waste tank containing 1% sodium hypochlorite, and sterilize it with other waste products before discarding it.
10. The workbench and various experimental supplies should be regularly disinfected with 1% sodium hypochlorite or 75% alcohol or UV lamps.
11. The PCR reaction mixture should be kept at low temperature and away from light.
12. Do not mix reagents of different batches. Please use them within the validity period.
13. It is suggested to use fresh samples to isolate and purify viral RNA. Repeated freezing and thawing of specimens will lead to a reduction in the sensitivity of detection, which is manifested by the larger Ct value or a false negative result.
14. Nucleic acid extraction steps are also required for negative and positive controls.

Manufacturer:

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