Covid-19 Delta & Omicron Variant Detection Kit

The *Covid-19 Omicron & Delta Variant Real Time Detection Kit* is designed for specific and qualitative detection of SARS-CoV-2 RNA in specimens such as oropharyngeal swabs, nasopharyngeal swabs or sputum suspected of SARS-CoV-2. The product is intended for use by qualified laboratory personnel well trained in nucleic acid amplification techniques and in vitro diagnostic procedures. Positive results do not rule out bacterial infection or co-infection with other viruses. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Test results must be combined with clinical observations, patient history, and epidemiological information.

SUMMARY AND EXPLANATION OF THE TEST

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.

The *SARS-CoV-2 Real-Time RT-PCR Assay* is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasopharyngeal or oropharyngeal swabs, sputum.

PRINCIPLE

The primers and probes are selected from the regions of *SARS-CoV-2* ORF1ab and S genes, human RNase P gene. Three primer/probe sets are designed for specific detection of SARS-CoV-2's ORF1ab gene and specific S gene mutations of Delta and Omicron Variants. An additional primer/probe set to detect human RNase P gene (as an internal control) is also included. RNA extracted from respiratory specimens is reverse transcribed and amplified in a Real-Time PCR Thermocycler (such as Applied Biosystems 7500 Real-Time PCR Instrument). The fluorescent signal is emitted when the 5' nuclease active domain of Taq polymerase degrades the probe anneals to target sequence between the forward and the reverse primers. During each cycle, the fluorescence intensity is increased with the additional reporter dye molecules released from their respective probes.

The *SARS-CoV-2 Real-Time RT-PCR Assay* also contains a positive control (Plasmid DNA) and a negative control for testing process monitoring.

REAQENTS AND MATERIALS PROVIDED

Item	Component	Quantity		Volume /T
		100 Tests / Box	500 Tests / Box	
1	COVIDELTA OMICRON PCR Mix	1.5 mL/ tube × 1	1.5 mL/ tube × 5	14 µL
2	RT-PCR Enzyme Mix	110 µL/ tube × 1	550 µL/ tube × 1	1 µL
3	SARS-CoV-2 Positive Control	50 µL/ tube × 1	250 µL/ tube × 1	5 µL
4	SARS-CoV-2 Negative Control	50 µL/ tube × 1	250 µL/ tube × 1	5 µL

MATERIALS MAY REQUIRED BUT NOT PROVIDED

1. Real-Time PCR Thermocycler compatible with JOE (***VIC or HEX is equivalent to JOE)***, FAM and Cy5 channels.
2. Specimen Collection Containers
3. RNA extraction reagent
4. PCR tubes compatible with Thermocycler.
5. 1.5 mL centrifuge tubes (RNase-Free)
6. Vortex
7. High-speed centrifuge
8. Micropipette (0.5-2 µL, 1-10 µL)
9. Pipette tips with filters
10. Personal protective material

WARNING AND PRECAUTIONS

1. For use by professionals specifically trained in nucleic acid amplification techniques and in vitro diagnostic procedures.
2. Check the expiration date.
3. Follow Good Laboratory Practices: wear appropriate protective clothing and use disposal gloves and protective eyewear. Do not eat, drink, or smoke in designated work areas. Wash hands thoroughly after handling specimens and kit reagents.
4. The testing workflow must be one-directional to minimize contamination risks (allocate segregated areas for each step): it should started from RNA Purification Area, then move to the Reaction Setup Area, followed by Amplification and Detection Area. Do not bring samples, equipment, and reagents to the area in which the previous step was performed and always change gloves when changing areas.
5. Regular decontamination of commonly used equipment is recommended, especially for micropipettes and work surfaces.
6. Specimens must be treated as potentially infectious sources as well as all reagents and materials that have been exposed to the samples and handled in the same manner as an infectious agent. Take appropriate precautions during specimen collection, storage, handling, and disposal in accordance with the guidance from country or region authorities.
7. To ensure optimal performance of the test, always follow appropriate procedures for specimen collection, transport, storage, and processing. Improper procedures may lead to false negative results.
8. Nucleic acids must first be extracted from specimens using a RNA Extraction Kit prior testing. Do not perform a RT-PCR Assay directly using specimens without extraction.
9. Appropriate precautions should be exercised to monitor contamination and preserve the purity of kit components and reactions. Avoid microbial and nuclease (RNase/DNase) contamination of specimens and kit components. Avoid the spread of aerosols when handling or uncapping specimens.
10. To minimize cross-contamination, open only one tube at a time in the process.
11. Always change pipette tips between liquid transfers. To minimize cross contamination, it's recommended to use aerosol-barrier pipette tips.
12. Always use disposable gloves and regularly check that they are not contaminated with sample materials. Discard gloves if they become contaminated.

TRANSPORT AND STORAGE INSTRUCTIONS

Store the kit frozen at -20±5°C and avoid repeated freeze-thaw cycles (less than 5 frozen-thaw times allowed). The product is valid for 12 months, and the expiry date of the product is shown on the label.

SPECIMEN TYPE and PRESERVATION

Specimen type: oropharyngeal swabs, nasopharyngeal swabs and sputum.

Specimen preservation: process specimens for viral RNA extraction and nucleic acid detection as soon as possible. Specimens can be stored at 4°C for 24 hours, or frozen at -80°C for longer storage, if not processed immediately. Avoid repeated freeze-thaw cycles during transport and storage of the specimens.

ASSAY PCR REACTION SETUP (at reagent preparation area)

A. Reagent Preparation

To calculate the number (n) of PCR reaction tubes (n= number of specimens to be tested + number of positive controls + number of negative controls), add n x14 µL of SARS-CoV-2 PCR Mix and n x 1 µL of RT-PCR Enzyme Mix to a clean tube, vortex to mix well, centrifuge and aliquot 15 µL of the mix into each PCR reaction tube.

B. Addition of Viral RNA

Add 5 µL of viral RNA extracts of the specimen or positive/negative control to each PCR tube according to the reaction preparation table below for testing. Then immediately cap the tube tightly, briefly spin the mixture and move it to the amplification area for RT-PCR detection.

PCR Reaction Preparation:

Component	Volume per test
PCR Mix	14 µL
Enzyme Mix	1 µL
Negative Control / Positive Control / or RNA of specimen to be tested	5 µL
Total Reaction Volume	20 µL

C. RT-PCR amplification (at Amplification Area, use Applied Biosystems 7500 Real-Time PCR System as an example)

1. Place the complete PCR reaction tubes into the fluorescent quantitative PCR analyzer and label positive control, negative control and testing specimen on the Thermocycler software.
2. Enter the PCR reaction volume: 20 μL.
3. Select fluorescence detection channels: JOE, FAM, Rox and Cy5 channels. FAM is the ORF1ab (RdRp) indicator channel, and JOE is the RNase P gene indicator channel (internal control). Rox and Cy5 are the S gene mutation indicator channels.
4. Select PCR cycle parameter setting following the instructions below, save the file and start the PCR run.
5. Analyze the results according to your thermocycler manufacturer's instructions. If the positive and negative control samples do not meet their respective requirements, the PCR run must be repeated.

Note: The positive and negative control can be used directly without purification.

Cycles	Step	Time	Temperature
1	Reverse transcription	15 minutes	50 ºC
1	Initial denaturation	30 seconds	95 ºC
40	Denaturation	30 seconds	95 ºC
	Annealing/Extension	40 seconds	60 ºC

QUALITY CONTROL

Quality control requirements must be performed in conformance with local, accreditation requirements and the user's laboratory's standard quality control procedures.

NC (negative control) material: no obvious amplification curve for Cy5 and FAM detection channels, and no obvious amplification curve for JOE channel;

PC (positive control) material: obvious amplification curves for Cy5 and FAM detection channels (Ct value ⩽37), and obvious amplification curve for JOE channel;

Pay attention that the above requirements must be met each time when the experiment is performed; otherwise, the experiment is considered as invalidate. The reason of invalidation needs to be resolved and the test result needs to be repeated using residual specimens storaged appropriately.

DETERMINATION OF RESULTS

1. The results can only be determined when the results of both controls meet their requirements.
2. Criterion for a positive specimen: a specimen can be reported as positive when FAM, Cy5 and JOE channels are all positive.
3. Criterion for a negative specimen: a specimen can be reported as negative when the JOE channel is positive and the FAM and Cy5 channels are negative.

Channel	Gene	Results Interpretation
Cy5	S (Omicron Variant)	PCOVID-19 Positive: If FAM has an exponential increase curve with Ct ⩽ 37. COVID-19 Negative: If FAM has a Ct value >40. Omicron: If Cy5 and Fam have exponential increase curves with Ct ⩽ 37. Delta: If Rox and FAM have exponential increase curves with Ct ⩽ 37. Inconclusive: If Cy5 or Rox have exponential increase curves with Ct ⩽ 37 and FAM has a Ct value>38.
Rox	S (Delta Variant)	
FAM	ORF1 ab	
JOE	RNase P	It's a valid result if Ct ⩽37, otherwise it's invalid.

COMPATIBILITY OF REAL-TIME PCR THERMOCYCLERS

Manufacturer	Model
Applied Biosystems	7500 Real-Time PCR System
	7300 plus Real-Time PCR System

PRODUCT PERFORMANCE

Product analysis performance evaluation results:

1. The analytical sensitivity of this kit is 200 copies/mL.
2. Cross-reaction: no cross-reaction with other pathogens such as seasonal influenza A (H1N1) virus, novelinfluenza A (H1N1-2009) virus, influenza AH3N2, H5N1, H7N9,influenza B Yamagata,influenza B Victoria, RSV A, RSV B, parainfluenza I, parainfluenza II, parainfluenza III, adenovirus types 1, 2, 3, 4, 5, 7 & 55, enterovirus types A, B, C and D, hMPV (human metapneumovirus), EB virus, measles virus, human cytomegalovirus, rotavirus, norovirus, mumps virus, varicella zoster virus, *mycoplasma pneumoniae, chlamydia pneumoniae, legionella, bordetella pertussis, haemophilus influenzae, staphylococcus aureus, streptococcus pneumoniae, streptococcus pyogenes, klebsiella pneumoniae, mycobacterium tuberculosis, aspergillus fumigatus, candida albicans, candida glabrata, cryptococcus neoformans*, coronavirus (HKU1, OC43, NL63, 229E), SARS-CoV-1, MERS coronavirus.

LIMITATIONS OF TEST

1. This test provides a presumptive diagnosis of SARS-CoV-2 infection. Negative test results do not preclude SARS-CoV-2 infection. All test results should be evaluated by healthcare professionals in the context of clinical symptoms, epidemiological information, patient history, and other diagnostic test results as the basis for patient management decisions.
2. Factors that may lead to false negative results must be excluded, including poor specimen quality; specimens collected too early or too late; specimens not properly stored, transported or processed; virus variation, PCR inhibitors , etc.
3. If test results are negative but clinical symptoms persist, follow up with additional serological diagnostic testing.
4. The Assay Procedure and the Interpretation of Test Results sections must be followed closely when testing. Failure to follow the procedure may lead to inaccurate results.
5. This test should be used only with respiratory specimens. The use of other specimen types has not been validated.
6. The quality of the sample impacts the quality of the test; improper specimen collection, storage and/or transport, and improper RNA purification may yield false negative results.
7. In some samples, extremely low levels of target (below the limit of detection) may yield an amplification signal, but results may not be reproducible.
8. Cross-contamination by samples containing high copies of SARS-CoV-2, SARS-CoV-2 particles or amplification products from previous reactions can yield false positive results. Take proper precautions to monitor contamination and preserve the purity of the kit component

REFERENCE

1. National Health Commission & State Administration of Traditional Chinese Medicine. Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 7). [R]. March 3, 2020

Symbols used

Symbol	Meaning
	Manufacturer
	Authorized representative in the European Community
	In vitro diagnostic medical device
	Meet the requirements of EC Directive 98/79/EC
	Catalogue number
	Batch code
	The number of test
	Caution
	Use by date
	Consult instruction for use
	Date of manufacture
	Storage temperature limit

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