SARS-CoV-2 Detection Kit (RT-PCR)

For B.1.617 Variants

Catalog Number: 02.01.1050

For in vitro Diagnostic (IVD) Use

【Kit Components】

Item No.	Components	Specifications and Quantities	Label
1	SARS-CoV-2 RT-PCR Detection Buffer	672μL×1	
2	Enzyme Mix	96μL×1	
3	SARS-CoV-2 Positive Control	500μL x1	
4	SARS-CoV-2 Negative Control	500μL×1	

【Storage Condition】

Recommend -20 ºC for long term storage. It is stable for 12 months stored at - 20 ºC.

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Instructions for SARS-CoV-2 Detection Kit (RT-PCR) For B.1.617 variants

【Product Name】 SARS-CoV-2 Detection Kit (RT-PCR)

【Package Specifications】 96 Tests

【Intended Usage】

Coronaviruses are a class of RNA viruses with viral cystic membranes and a positive linear single-stranded genome, about 80-120 nm in diameter. Currently, they only infect human, mouse, pig, cat, dog, and avian vertebrates. The new coronavirus (SARS-CoV-2) has been confirmed as a new variant that can cause viral pneumonia, fever and dry cough in mild cases, and breathing difficulties and even shock in severe cases.

This kit is used for RNA detection of SARS-CoV-2, and the results can be used for auxiliary diagnosis of patients with new coronavirus infection or patients suspected of new coronavirus infection, providing molecular diagnosis for infected patients. This kit can identify if the sample is SARS-CoV-2 positive/negative, and for SARS-CoV-2 positive samples, if B.1.617 variants, specially if B.1.617.2 (Delta) variant is present.

[Detection Principle】

The SARS-CoV-2 detection kit (RT-PCR) is a real time reverse transcription followed by polymerase chain reaction (rRT-PCR) test. The SARS-CoV-2 primer and probe sets are designed to detect RNA from SARS-CoV-2 in respiratory specimens from patients who are suspected of SARS-CoV-2 infection by their healthcare provider. This kit is used for qualitative detection of the ORF1ab gene from SARS-CoV-2, and T478K, P681R mutations of the SARS-CoV-2 B.1. 617.variants. P681R mutation is present in all sublineages of B.1.617 (B.1.617.1, B.1.617.2 and B.1.617.3). T478K is specific for B.1.617.2 (Delta) variant.

After PCR reaction, fluorescent signal from target is acquired and further analyzed by a real time PCR instrument. As a result, genes specific to novel coronavirus SARS-CoV-2 and B.1.617 lineage can be detected with high precision.

In particular, ORF1ab probe contains FAM label, T478K probe contains HEX/Joe label, P681R probe contains ROX label, Internal control probe contains Cy5 label.

【Kit Components】

Item No.	Components	Specifications	Label
1	SARS-CoV-2 RT-PCR Detection Buffer	672μl×1	
2	Enzyme Mix	96μl×1	
3	SARS-CoV-2 Positive Control	500μl×1	
4	SARS-CoV-2 Negative Control	500μl×1	

Note: The components of different lots cannot be used interchangeably.
End users need to prepare their own nucleic acid extraction and purification reagents.

【Storage Conditions and Expiration Date】

1. The reagents should be sealed from light and the reagent components should be stored below -18 ℃. The kit is valid for 12 months. Please see the outer box for manufacture date and expiration date.

2. The reagents can be stored stably within a valid period indicated on package. The repeated freeze-thaw cycles should not be more than 5. And unpacked kits should avoid repeated freeze-thaw cycles.

【Instrument Compatibility】

Mic Real-Time PCR Cycler.

【Specimen Requirements】

1. Applicable specimen types: upper respiratory tract specimens (throat swabs or nasal swabs,).

2. Specimen storage and transportation: Specimens is recommended to immediately processed. Specimens should be tested within 24 hours if stored at 2-8℃. Specimens that cannot be tested within 24 hours should be stored at -70℃ or below (in the absence of -70℃ storage conditions, specimens can be stored below -18℃). Multiple freeze-thaw cycles should be avoided. Specimens should be transported in a sealed frozen pitcher with ice or in a sealed foam box with ice. Follow the sample collection and transportation kit manufacturer's instruction for sample storage.

【Testing Method】

1. Sample Collection

Rinsing mouth clean with water for 20-30 seconds before sample collection to remove any food residue and keep mouth clean. The food residue may inhibit PCR reaction and cause false negative results. ***To realize direct PCR without RNA extraction, Swab specimens should be collected only using virus transport medium in Rainamp collection, Transport and processing kit.*** Place swabs immediately into Virus Transport Medium. If specimen is collected in other supplier's VTM, RNA extraction step is required prior to PCR reaction. The SARS-CoV-2 detection kit is compatible with other supplier's oropharyngeal/nasopharyngeal swab collection kits when RNA extraction is performed.

2. Reagent Preparation

Aliquot (n+1) x 7μl of the **SARS-CoV-2 RT-PCR Detection Buffer** and (n+1) x 1 μl **Enzyme Mix** (n is the number of reaction tubes, including specimens, negative control and positive control) into a centrifuge tube, shake and mix thoroughly, and centrifuge at 3000 rpm for 1 minute.

3. Adding Specimen

Aliquot 8 μl of the above mixed solutions into each PCR tube, and then add 17 μl extracted RNA, 17 μl positive control, or 17 μl negative control to each PCR

tube, cover the tubes with their caps, mix and centrifuge. Immediately perform the PCR amplification reaction.

4. PCR Amplification

The PCR reaction tube is placed in a Real Time PCR instrument. The recommended thermal cycling protocol is set as follows:

RT-PCR procedure				
Steps		Temperature	Time	Cycles
1	Reverse transcript	50℃	5min	1
2	Enzyme activation	95℃	1 min	1
3	Denature	95℃	5 s	40
	Annealing/ Extension & fluorescence detection*	58℃	15 s	
4	Cooling	4℃	Infinite	1

* Fluorescence Data Collection

Fluorescent channel selection: Choose FAM, HEX, ROX, Cy5 channels

5. Set baseline and threshold

Take fluorescence signals from 3-24 cycles for baseline adjustment. The threshold setting principle is based on the threshold line just exceeding the highest point of the DEPC-H2O fluorescence detection curve.

6. Quality Control

Both Negative and Positive Controls in the kit must meet the following criteria. If Negative Control shows result other than described in the table below, it indicates contamination of reagents or specimens. All specimen results need to be invalidated and results must not be reported. It is recommended to decontaminate the PCR lab and use a new box of un-opened reagents before repeating specimen testing. If Positive Control shows result other than described in the table below, it indicates the failure of RT-PCR reaction. All specimen results need to be invalidated and results must not be reported. The specimens are required to be re-tested.

	FAM Ct (ORF1ab)	HEX Ct (T478K)	ROX Ct (P681R)	Cy5 Ct (Internal Control)
Negative control	UNDET or >38	UNDET or >38	UNDET or >38	UNDET or >38
Positive control	≤38	≤38	≤38	≤38

7.Examination and Interpretation of Patient Specimen Results

ORF1ab (FAM)	T478K (HEX)	P681R (ROX)	IC (Cy5)	Results
+	-	-	+/-	**SARS-CoV-2 Positive, B.1.617 variant Negative**
+	-	+	+/-	**SARS-CoV-2 Positive, B.1.617.1 or B.1.617.3 variant Positive**
+	+	+	+/-	**SARS-CoV-2 Positive, Delta variant Positive (B.1.617.2)**
-	+	+/-	+/-	**Inconclusive, suggested to repeat the test**
-	+/-	+	+/-	
-	-	-	+	**SARS-CoV-2 Negative**
-	-	-	-	**Invalid, suggested to repeat the test**

Result of (-): Ct value >38 or Undetermined for ORF1ab (FAM), Internal Control (Cy5), P681R (ROX), and T478K (HEX).
Result of (+): Ct value ≤ 38 for ORF1ab (FAM), Internal control (Cy5), P681R (ROX), and T478K (HEX).
Inconclusive results: If P681R(ROX) or T478K (HEX) Ct value ≤ 38 and ORF1ab (FAM) Ct value >38 or Undetermined, this sample should be an inconclusive result. It is suggested to repeat the test. If the result is still inconclusive, it is suggested to increase the sample concentration and repeat the test. To increase sample concentration, double swab collection is recommended.
Invalid Result: If the sample is negative in all 4 channels and there is no typical S-shape amplification curve or Ct value >38 or Undetermined for Internal control (Cy5), indicating that the specimen concentration is below detection limit, or there are interfering substances that inhibit the reaction. If upon retest, the result is invalid again, another fresh sample should be collected and tested.

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted. **Table** above describes the results interpretation of specimens concerning the use of the controls provided with the test. The Ct cutoff value of this kit is set as 38 for ORF1ab (FAM), Internal control (Cy5), P681R (ROX) and T478K (HEX). And the end user is required to review fluorescent curves before final interpretation. All positive curves should be typical S-shape amplification curves.

Interpretation of Specimen Results

【Reference range】 Detection of the novel coronavirus SARS-CoV-2 is a pathogenic microorganism, which does not exist in healthy humans

【Interpretation of test results】 Laboratory environment pollution, reagent contamination, and specimen cross-contamination will cause false positive results; Improper reagent transportation, storage, or inaccurate reagent preparation may result in a decline in the reagent detection efficiency, false negatives or inaccurate quantitative detection. There is no typical S-shape amplification curve or Ct value >38 for ORF1ab (FAM), Internal Control (Cy5), T478K(HEX) and P681R (ROX) of Positive Control, indicating that there are interfering substances that inhibit the reaction or the PCR experiment set up is not correct. It is suggested to check the PCR thermal cycling setting and check if any reagent was contaminated with PCR inhibitor. If Ct value ≤38 for ORF1ab (FAM), Internal Control (Cy5),T478K (HEX) and P681R(HEX) of Negative Control, indicating the reagent or the testing environment is contaminated. Decontamination is required before running new tests. If P681R(ROX) or T478K (HEX) Ct value≤38 and ORF1ab (FAM) Ct value > 38 or Undetermined, this sample should be an inconclusive result. It is suggested to repeat the test, if the result is still inconclusive, it is suggested to increase the sample concentration and repeat the test.

P681R mutation is reported in all three sublineages of B.1.617 variants, T478K is specific to B.1.617.2 (Delta) variant.

Clinical Validation

	Digital PCR B.1.617 Positive	Digital PCR B.1.617 Negative	Total
SARS-CoV-2 (RT-PCR) B.1.617 Positive	43	0	43
SARS-CoV-2 (RT-PCR) B.1.617 Negative	0	53	53
Total	43	53	96

	Digital PCR SARS-CoV-2 Positive	Digital PCR SARS-CoV-2 Negative	Total
SARS-CoV-2 (RT-PCR) SARS-CoV-2 Positive	43	0	43
SARS-CoV-2 (RT-PCR) SARS-CoV-2 Negative	0	53	53
Total	43	53	96

PPA = 43/43 x 100% = 100%

NPA = 53/53 x 100%= 100%

PPA = 43/43 x 100% = 100%

NPA = 53/53 x 100%= 100%

【Limitation of the test method】

1) Test results are affected by how the specimen is collected, processed, transported and stored. Loss of control in any of the steps will lead to incorrect results. Special attention should be paid to the risk of specimen cross contamination during processing which may lead to false positive results.

2) Improper specimen collection, transport and processing may lead to false negative results; mutation of target sequence may lead to false positive or false negative results.

3) This kit is applicable to specified specimen types. Validation is required before applying any new specimen types or detection system.

【Product performance specification】

Detection limit: ORF1ab 504.12copies/mL; T478K 412.94copies/mL; P681R 349.41copies/mL; IC 251.75copies/mL.

【Precautions】

Please read the entire manual carefully before starting test.

1. The entire testing process is suggested to be performed in three separated areas:

a) Reaction system preparation and reagent preparation area;

b) Specimen processing and specimen adding area;

c) PCR amplification, fluorescence detection and result analysis area.

Reagent preparation and specimen processing should use ultra-clean workbenches (negative pressure) or anti-pollution covers to prevent environmental pollution; Instruments, equipment, consumables and work clothes used in each area shall be used independently; Clean the workbench immediately after the experiment. Pipettes, centrifuges, PCR amplifiers and other instruments should be disinfected with 10% hypochlorous acid or 75% alcohol, UV lamps or ozone.

2. Operators should be professionally trained and have corresponding operating skills, certain experimental experience, and good safety precautions.

3. Non-fluorescence contaminated disposable gloves, disposable centrifuge tubes, and disposable pipettes tips with filters should be used throughout experiments. Wastes (such as pipette tips), amplification centrifuge tubes, and specimens that have come into contact with standards and controls during experiments should be decontaminated before disposal.

4. To ensure the success and accuracy of experiment:

Each experiment should include negative and positive controls. Reagents should be equilibrated to room temperature before use, and fully melted and mixed. The prepared PCR reaction mixture should be protected from light. The reaction mix in tubes should be thoroughly mixed and centrifuged to avoid air bubbles as much as possible. Do not mix reagents from different batches. Kits must be used before expiration dates.

【Instruction Approval and Revision Date】

Approval Date: 2021.08.31
Revision Date: 2021.08.31
Date of Issue: 2021.08.31

【Index of Symbols】

IVD	The product is used in vitro, please don't swallow it.	EC REP	European union authorization representative
	Validity		Refer to instruction book
	Warning, please refer to the instruction in the annex		Manufacturer
	Product temperature scope	REF	Catalogue number
LOT	Batch number	Σ	Contains sufficient for <n> tests
	Avoid overexposure to sun		Date of manufacture
CE	The product meets the basic requirements of European in vitro diagnostic medical devices directive 98/79/EC		

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