



PN.XC25073

SARS-CoV-2 Virus Detection Diagnostic Kit (RT-qPCR Method)

Instructions for Use

1 Product Name

SARS-CoV-2 Virus Detection Diagnostic Kit (RT-qPCR Method)

2 Contents

50 Tests/Kit

3 Intended Use

This kit is used for in vitro qualitative detection of SARS-CoV-2 *ORF1ab* gene, *N* gene and *S* gene in specimens of sputum, nasopharyngeal or oropharyngeal swabs from COVID-19 suspected cases, suspected clustered cases, and others who need to be diagnosed or differentiated.

4 Assay Principle

This kit uses one-step reverse transcription-polymerase chain reaction (One Step RT-PCR) method and Taqman probe technology to qualitatively detect SARS-CoV-2 RNA. Specific primers and probes were designed to target highly conserved regions of *ORF1ab* gene, *N* gene and *S* gene sequences.

5 Kit Components

Components	Main Ingredients	Contents
SARS-CoV-2 Master Mix	RT-PCR primer/probe sets, dNTP, Mg ²⁺ , dUTP, Buffer	770 μL, 1 tube
SARS-CoV-2 Enzyme Solution	Reverse transcriptase, Uracil-DNA Glycosylase, Hotstart DNA, Polymerase, Glycerol	60 μL, 1 tube
SARS-CoV-2 Positive Control	Recombinant plasmids including the targets and human RNA fragments	50 μL, 1 tube
SARS-CoV-2 Negative Control	Nuclease Free-Water	1000 μL, 1 tube

Note:
(1) The components of different lots of kits cannot be mixed for use.
(2) Equipment and materials required but not listed: PCR tubes, centrifuges, vortex mixers, pipettes, etc.

6 Storage

Expiration: Temporarily 6 months from the date of manufacture.
Production dates: see box label or tube label.
All reagents can be used until the expiration date indicated on the kit label.
All reagents should be stored at -25 ~ -15°C.
Freeze and thaw for less than 3 times.
Kits should be cold-chain shipped with gel pack or dry ice.

7 Applicable Instruments

Applied Biosystems® 7500/7500 Fast real-time PCR systems.

8 Specimen Requirements

Respiratory specimens including: nasopharyngeal or oropharyngeal swabs, and sputum. Specimens should be detected immediately. If extraction is delayed up to 24h, specimens should be stored at 4°C or lower. If extraction is delayed more than 24h, specimens should be stored at -70°C or lower. Extracted nucleic acids should be stored at 2 ~ 8°C for less than 3 days, or -25 ~ -15°C for less than 7 days.
Specimens should not be frozen and thawed frequently.

9 Testing Procedure

1. Nucleic acid extraction
Nucleic acid extraction should be performed using either bead-based nucleic acid extraction method such as the TANBead® extract system (Taiwan Advanced Nanotech, PN SLA32/ Maelstrom 9600) with a TANBead Viral Auto Plate kit (Taiwan Advanced Nanotech, PN 665A46), or spin column-based nucleic acid extraction method such as the RNeasy Mini Kit (Qiagen, PN 74104), following the manufacturer's instructions for the extraction procedure, respectively.
The negative control should be used starting from nucleic acid extraction to monitor the process of extraction and RT-PCR. The SARS-CoV-2 positive control does not need to go through extraction but is used for quality control of PCR reagents.
2. Reaction mix preparation
Thaw the Master Mix and mix well. Centrifuge the Master Mix and Enzyme Solution for 10 seconds and place on ice. For each sample, add 14 μL of Master Mix and 1 μL of Enzyme Solution to the reaction mixture.
3. Add samples
Add 5 μL of nucleic acid of each sample, the positive control, or the extracted negative control to the above reaction tubes. Centrifuge the reaction tubes for 10 seconds and place the tubes vertically on ice.
4. RT-PCR amplification
Place the reaction tubes on a fluorescent PCR instrument with a reaction system of 20 μL.
Recommended cycling and parameter settings as below:

Gene detected	Fluorescence channel
<i>ORF1ab</i> gene	FAM
<i>S</i> gene	ROX
<i>N</i> gene	CY5
Internal reference (human RNA)	VIC

Temperature (°C)	Time	Number of cycles
25	2 min	1
50	30 min	1
95	2 min	1
95	15 s	45
60	60 s (fluorescence collection)	

Note: Select "none" for "Passive Reference" and "Quencher" when using ABI brand fluorescence PCR instrument.

10 Baseline and Threshold Settings

1. Baseline setting: The region, where the fluorescence signal of all samples is stable before the exponential amplification (the fluorescence signal of all samples does not fluctuate greatly), should be selected as baseline. The start point (Start) should avoid signal fluctuations of the initial phase of fluorescence collection, and the end point (End) should be reduced by 1 ~ 3 cycles to the Ct value of the earliest exponentially amplified sample.
2. Threshold setting: Set the threshold line just above the highest point of the negative amplification curve.

11 Quality Control

1. Negative control: all channels have no Ct value or Ct>40.
2. Positive control: The fluorescence signal of all channels increased significantly, and the amplification curve showed a clear S-shaped curve, with Ct≤35.
3. The test for the reference samples in the kit must meet the above standards, otherwise the experiment is judged as invalid.

12 Cut-off Value

The cut-off value of Ct for the target genes and internal reference is 40.

13 Interpreting of Test Results

1. If the Ct value of the internal reference (VIC) channel is less than or equal to 40, and there is no Ct or Ct> 40 in other fluorescent signal channels, the sample is judged as negative.
2. When two or three channels of FAM/ROX/CY5 showed obvious S-shaped curve with Ct≤ 40, and the Ct value of the internal reference (VIC) channel is less than or equal to 40, the sample is judged as positive.
3. When only one channel of FAM/ROX/CY5 has an obvious S-shaped amplification curve and Ct ≤ 40, and the Ct value of the internal reference (VIC) channel is less than or equal to 40, it is recommended to repeat the RT-PCR test. If the repeated result is still Ct ≤ 40 from the same channel of FAM/ROX/CY5, and the repeated Ct value of the internal reference (VIC) channel is still less than or equal to 40, the sample is judged as positive.
4. When all channels have no Ct value or the Ct value is more than 40, the results cannot be interpreted, indicating that there is a problem in sample quality or extraction. It is recommended to re-extract or re-collect specimen.

14 Assay Limitations

1. The test results are only for clinical reference, and cannot be used as the only criteria for the diagnosis or exclusion of cases. The clinical diagnosis and treatment of patients should be combined with their symptoms, signs, medical history, other laboratory tests and treatment reactions.
2. A false negative result may occur if inadequate amount of target RNA is present in the specimen due to improper sample collection, transport or handling.
3. The variation of targeted sequence caused by mutations may lead to false negative results.
4. False negative results may occur due to the degradation of reagent caused by improper transport or storage of reagent, or inaccurate reagent preparation.
5. Negative results only indicate that the concentration of pathogens in the sample is lower than the limit of detection, or there is no pathogen detected in the sample, but the possibility of infections by other pathogens cannot be completely ruled out.
6. False positive results may occur if contamination is not controlled effectively during sample preparation and RT-PCR setup.
7. False results may occur if samples contain excessive drugs.

15 Key Performance Index

1. Limit of Detection: 1,000 copies/mL for each target gene and human RNA in simulated samples.
2. Cross-Reactivity: The assay does not cross-react with human genomic DNA and other respiratory pathogens including Influenza A virus H1N1 (2009), Seasonal H3N2 virus, Adenovirus, Boca virus, Rhinovirus, Parainfluenza virus, Metapneumovirus, Influenza B virus, Coronavirus OC43, Coronavirus HPU1, Coronavirus 229E, Coronavirus NL63, Respiratory syncytial virus A group, Respiratory syncytial virus B group, Chlamydia, Mycoplasma pneumoniae, Staphylococcus epidermidis, Staphylococcus aureus, Enterococcus faecalis, and Klebsiella pneumoniae.
3. External Interfering Substances: Common drugs such as 1.2 mg/mL salbutamol sulfate, 5 mg/mL dexamethasone acetate, 0.15 mg/mL oxymetazoline hydrochloride, 166 mg/mL azithromycin, 166 mg/mL cefixime do not interfere with the assay.
4. Internal interfering substances, 20 mg/mL mucin and 5% blood, do not interfere with the assay.
5. Precision: Coefficient of variation of Ct value (CV%) for batch to batch, operator to operator and day to day is ≤5.0%.
6. Clinical Validation: Total 215 clinical samples were collected and tested at Zhejiang Provincial CDC, No.1 Affiliated Hospital of Zhejiang University, and Huamei Hospital of University of Chinese Academy. 127 positive results and 88 negative results were 100% in concordance with the conclusions of clinical diagnosis.

16 Precautions

1. For *in vitro* diagnostic use.
2. Appropriate operation training is required before using the kit. The assay must be proceeded with strict adherence to the procedure guidelines.

3. Laboratories should follow good laboratory practices and comply with all applicable regulatory requirements. Maintain separate areas and dedicated equipment (e.g., pipettes, microcentrifuges) and supplies (e.g., microcentrifuge tubes, pipette tips, gowns and gloves) for assay reagent setup and handling of extracted nucleic acids.
4. Personnel must be familiar with the protocol and instruments used. Wear appropriate personal protective equipment (e.g. gowns, gloves, eye protection) when working with clinical specimens. Specimen processing should be performed in a certified class II biological safety cabinet following biosafety level 2 or higher guidelines.
5. Wear clean disposable gowns and new, previously unworn, powder-free gloves during assay reagent setup and handling of extracted nucleic acids. Change gloves whenever contamination is suspected.
6. Use nuclease-free, sterile disposable aerosol barrier pipette tips for each addition and transfer to avoid cross-contamination in pre-PCR procedures. Use nuclease-free, disposable polypropylene tubes for preparing the reaction mixes.
7. Make sure the reagents are completely thawed and thoroughly mixed before usage. Centrifuge for 5 to 10 seconds to collect contents at the bottom of the tube.
8. The SARS-CoV-2 Master Mix is light and heat sensitive. Frequent freeze-thaw cycles should be avoided. Maintain on ice when thawed.
9. After the nucleic acid extraction procedure, the extracts should be used for amplification immediately. Retain residual specimen and nucleic extract and store immediately at -70°C or lower. Frequent freeze-thaw cycles should be avoided. Maintain cold when thawed.
10. Use fresh pipette tips to aliquot samples.
11. Specimens should be disposed according to local regulatory requirements. Product components (reagents, packing) can be considered as laboratory waste.
12. If the outside package is damaged but vials remain intact upon reception, the kit can still be used without compromising performance. If the package and vials are both damaged, the kit should not be used.

17 Description of the logo

logo	Description
	Symbol for "CONSULT INSTRUCTIONS FOR USE"
	Symbol for "CATALOGUE NUMBER"
	Symbol for "AUTHORISED REPRESENTATIVE IN THE EUROPEAN COMMUNITY"
	Symbol for "IN VITRO DIAGNOSTIC MEDICAL DEVICE"
	Symbol for "TEMPERATURE LIMITATION"
	Symbol for "KEEP AWAY FROM SUNLIGHT"
	Symbol for "BATCH CODE"
	Symbol for "DATE OF MANUFACTURE"
	Symbol for "MANUFACTURER"
	Symbol for "USE BY"
	Symbol for "NEGATIVE CONTROL"
	Symbol for "POSITIVE CONTROL"

18 Approval And Modification Date

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19 Contact Information

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