WHO Emergency Use Assessment SARS-CoV-2 IVDs
PUBLIC REPORT

Product: SARS-CoV-2 Virus Detection Diagnostic Kit
EUL Number: EUL 0494-189-00
Outcome: Accepted

The EUL process is intended to expedite the availability of in vitro diagnostics needed in public health emergency situations and to assist interested UN procurement agencies and Member States in determining the acceptability of using specific products in the context of a Public Health Emergency of International Concern (PHEIC), based on an essential set of available quality, safety and performance data. The EUL procedure includes the following:

- Quality Management Systems Review and Plan for Post-Market Surveillance: desk-top review of the manufacturer's Quality Management System documentation and specific manufacturing documents;
- Product Dossier Review: assessment of the documentary evidence of safety and performance.

SARS-CoV-2 Virus Detection Diagnostic Kit codes XC25073 and XC25087, CE-mark regulatory version, manufactured by Ningbo Health Gene Technologies Co., Ltd., 396 Mingzhu Road, Hi-Tech Park, Ningbo, Zhejiang Province, China was listed on 28 August 2020.

Intended use:

According to the claim of intended use from Ningbo Health Gene Technologies Co., Ltd., "this *kit is used for in vitro manual 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. The product is for aiding in the diagnosis of SARS-CoV-2 infection for professional use by laboratory personnel specifically trained in RT-PCR. The patients being tested should meet SARS-CoV-2 clinical criteria. Positive results are indicative of active infection. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information."*

Specimen type that was validated:

Sputum, Oropharyngeal swab and Nasopharyngeal swab specimens.

Test kit contents:

Component	50 tests (product code XC25073)	200 tests (product code XC25087)
SARS-CoV-2 Master Mix	770 µL x 1 tube	1540 µL x 2 tubes
SARS-CoV-2 Enzyme Solution	60 µL x 1 tube	240 µL x 1 tube
SARS-CoV-2 Positive Control	700 µL x 1 tube	1400 µL x 2 tubes
SARS-CoV-2 Negative Control	1000 µL x 1 tube	1000 µL x 2 tubes

Items required but not provided:

Specimen collection kits:

Extraction/Purification:

- TANBead extract system (Taiwan Advanced Nanotech, PN SLA32/ Maelstrom 9600) with a TANBead Viral Auto Plate kit (Taiwan Advanced Nanotech, PN 665A46),
- Spin column-based nucleic acid extraction method such as the RNeasy Mini Kit (Qiagen, PN 74104),

Real-Time PCR equipment:

- Applied Biosystems 7500/7500 Fast real-time PCR systems (Real-time PCR software v2.4).

General laboratory equipment and consumables

- Vortex mixer
- Microcentrifuge
- Calibrated micropipettes (10µL, 100µL, 200µL, 1000µL)
- Multichannel micropipettes (1-10µL, 5-50µL)
- Racks for 1.5mL microcentrifuge tubes
- 0.2 mL PCR tubes (DNase/RNase free)
- Disposable powder-free gloves and surgical gowns
- Aerosol barrier pipette tips
- Class II (or higher) biological safety cabinet (BSC)

Storage:

-25 to -15°C.

Shelf-life upon manufacture:

6 months.

Warnings/limitations:

Refer to the instructions for use (IFU)

Product dossier assessment

Ningbo Health Gene Technologies Co., Ltd. submitted a product dossier for the SARS-CoV-2 Virus Detection Diagnostic Kit for detecting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as per the "*Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid (PQDx_0347)"*. The information (data and documentation) submitted in the product dossier was reviewed by WHO staff and external technical experts (assessors) appointed by WHO.

Post listing Commitments for EUL:

As commitments to listing, the manufacturer is required,

1. To determine the limit of detection with the WHO international standard when available.
2. Provide the additional specimen stability studies to WHO by 30 October 2021.

Risk benefit assessment conclusion: acceptable.

Quality Management Systems Review

To establish the eligibility for WHO procurement, Ningbo Health Gene Technologies Co., Ltd. was asked to provide up-to-date information about the status of their quality management system.

Based on the review of the submitted quality management system documentation by WHO staff, it was established that sufficient information was provided by Ningbo Health Gene Technologies Co., Ltd. to fulfil the requirements described in the "*Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid (PQDx_347)*".

Quality management documentation assessment conclusion: acceptable.

Plan for Post-Market Surveillance

Post-market surveillance, including monitoring all customer feedback, detecting and acting on adverse events, product problems, non-conforming goods and processes is a critical component of minimizing potential harm of an IVD listed for emergency use.

The following post-EUL activities are required to maintain the EUL listing status:
1. Notification to WHO of any planned changes to a EUL product, in accordance with "*WHO procedure for changes to a WHO prequalified in vitro diagnostic" (document number PQDx_121*); and
2. Post-market surveillance activities, in accordance with "*WHO guidance on post-market surveillance of in vitro diagnostics" (ISBN 978 92 4 150921 3).*

Ningbo Health Gene Technologies Co., Ltd. is also required to submit an annual report that details sales data and all categories of complaints in a summarized form. There are certain categories of complaints and changes to the product that must be notified immediately to WHO, as per the above-mentioned documents.

The manufacturer has committed to ensure that post-emergency use listing safety, quality and performance monitoring activities are in place which are in accordance with WHO guidance "*WHO guidance on post-market surveillance of in vitro diagnostics*".[1]

Scope and duration of procurement eligibility

SARS-CoV-2 Virus Detection Diagnostic Kit, product codes XC25073 and XC25087, manufactured by Ningbo Health Gene Technologies Co., Ltd. is considered to be eligible for WHO procurement for 12 months from the day of listing. The assay may be used for the detection of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA. This listing does not infer that the product meets WHO prequalification requirements and does not mean that the product is listed as WHO prequalified.

As part of the on-going requirements for listing as eligible for WHO procurement, Ningbo Health Gene Technologies Co., Ltd. must engage in post-market surveillance activities to ensure that the product continues to meet safety, quality and performance requirements. Ningbo Health Gene Technologies Co., Ltd. is required to notify WHO of any complaints, including adverse events related to the use of the product within 7 days and any changes to the product.

WHO reserves the right to rescind eligibility for WHO procurement, if additional information on the safety, quality, performance during post-market surveillance activities, and if new data becomes available to WHO that changes the risk benefit balance.

[1] Available on the web page https://www.who.int/diagnostics_laboratory/postmarket/en/

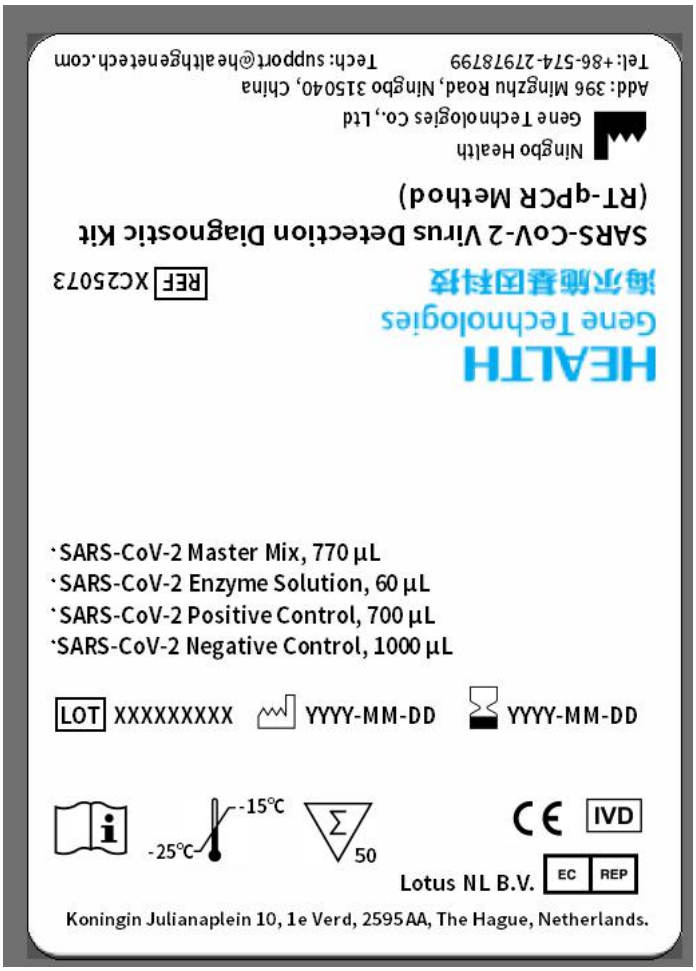
Labelling

1.0 Labels

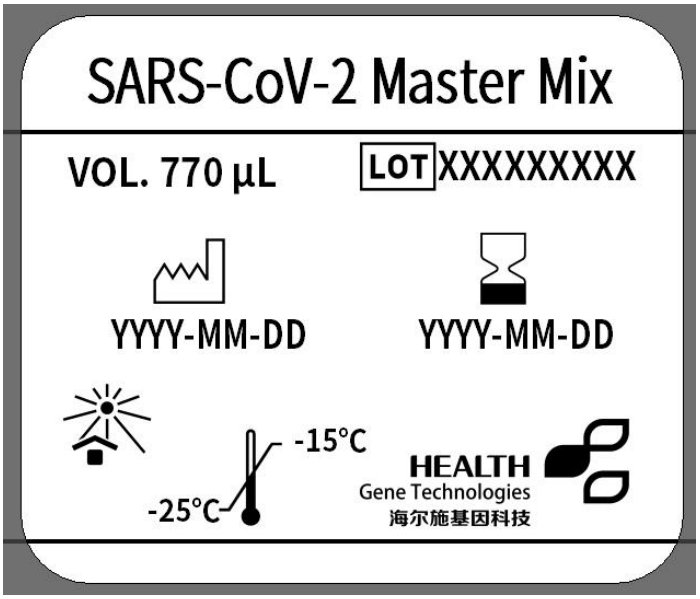
2.0 Instructions for Use (IFU)

1.0 Product labels

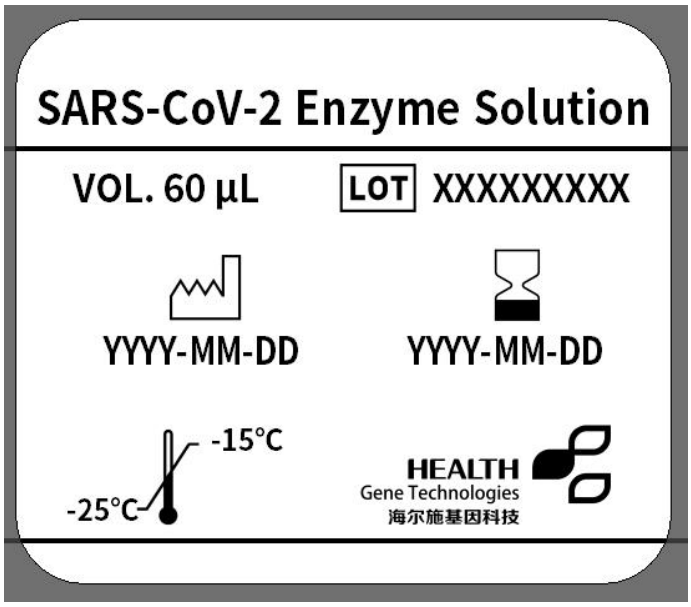
1.1 Outside box lebel (XC25073)



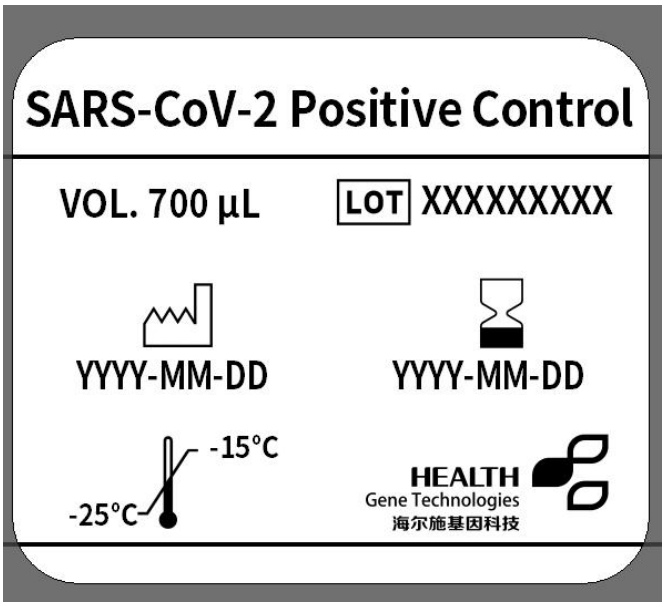
1.2 SARS-CoV-2 Master Mix (XC25073)



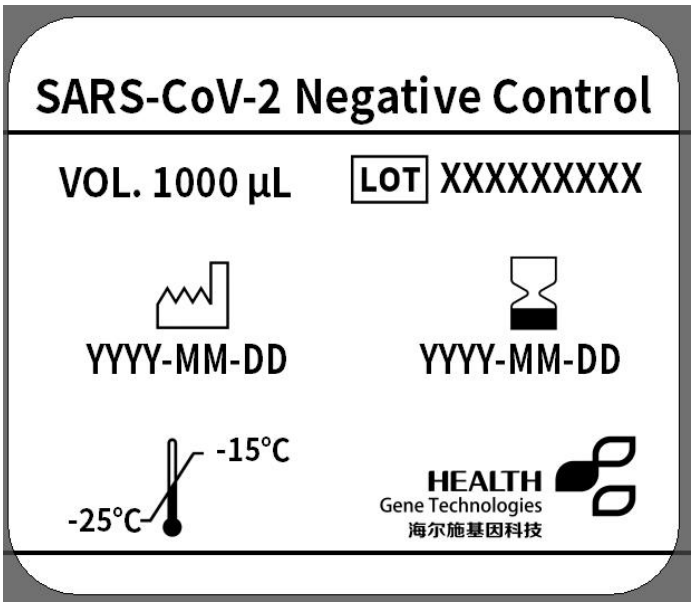
1.3 SARS-CoV-2 Enzyme Solution (XC25073)



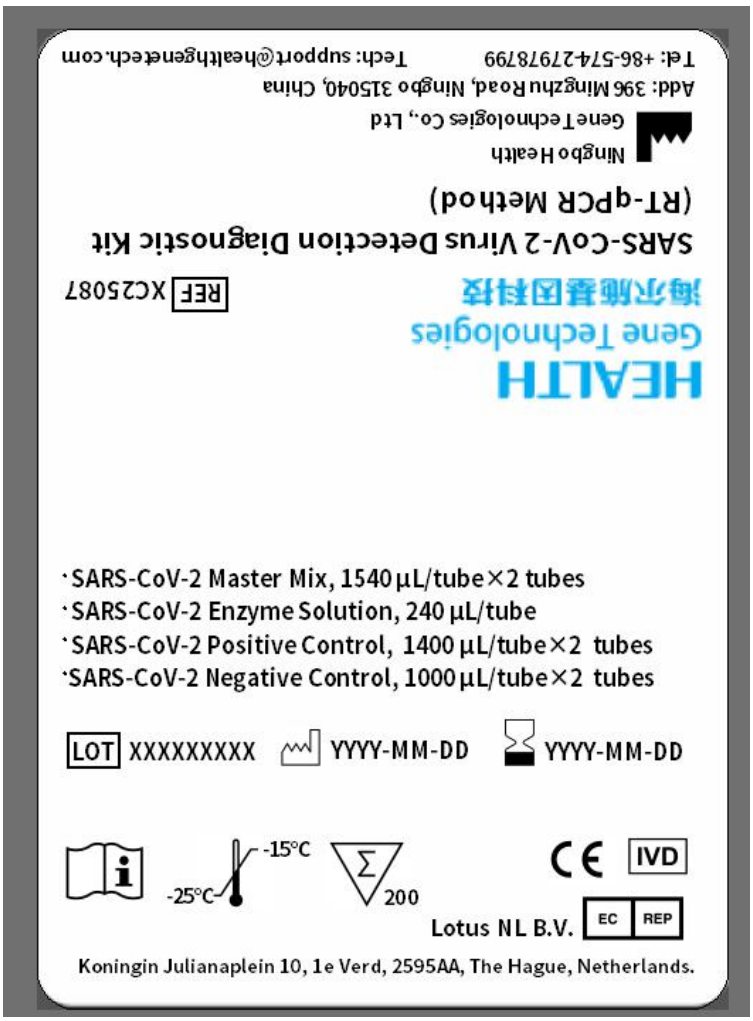
1.4 SARS-CoV-2 Positive Control (XC25073)



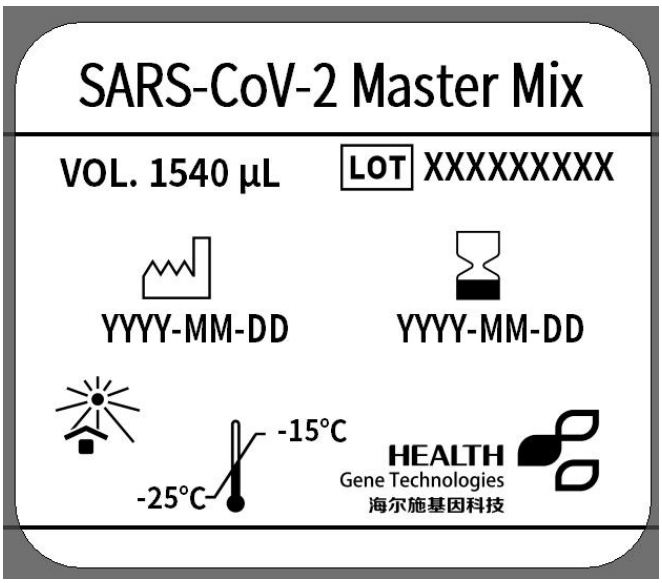
1.5 SARS-CoV-2 Negative Control (XC25073)



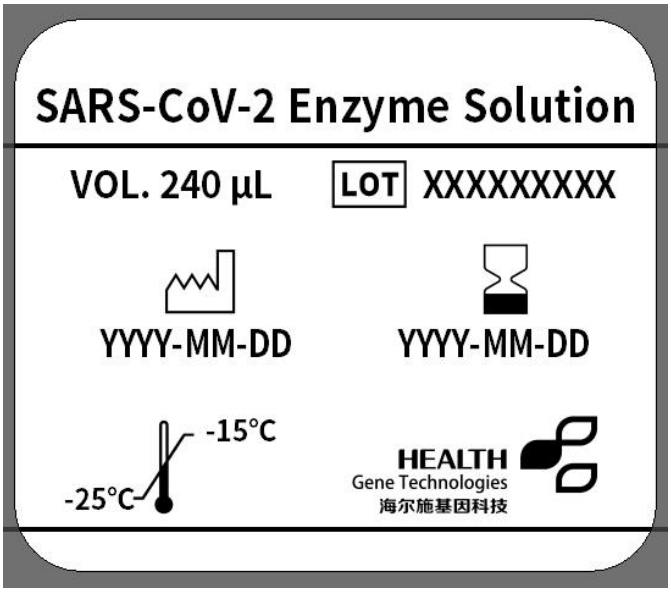
1.6 Outside box label (XC25087)



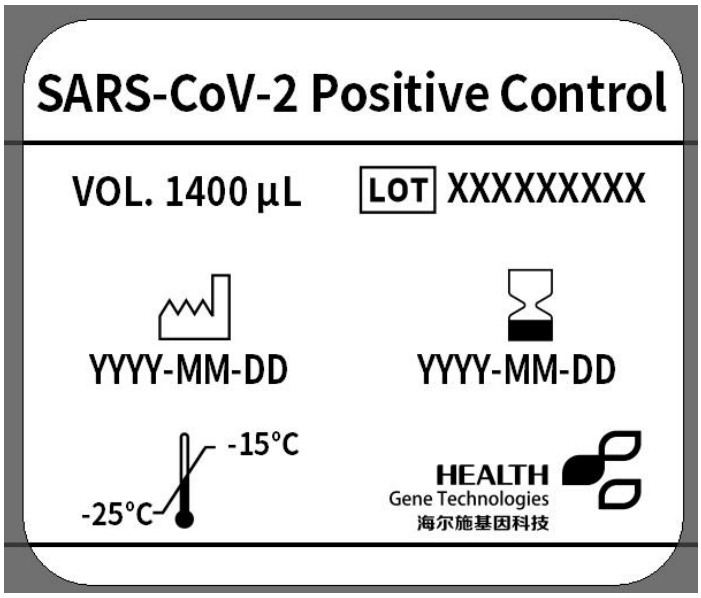
1.7 SARS-CoV-2 Master Mix (XC25087)



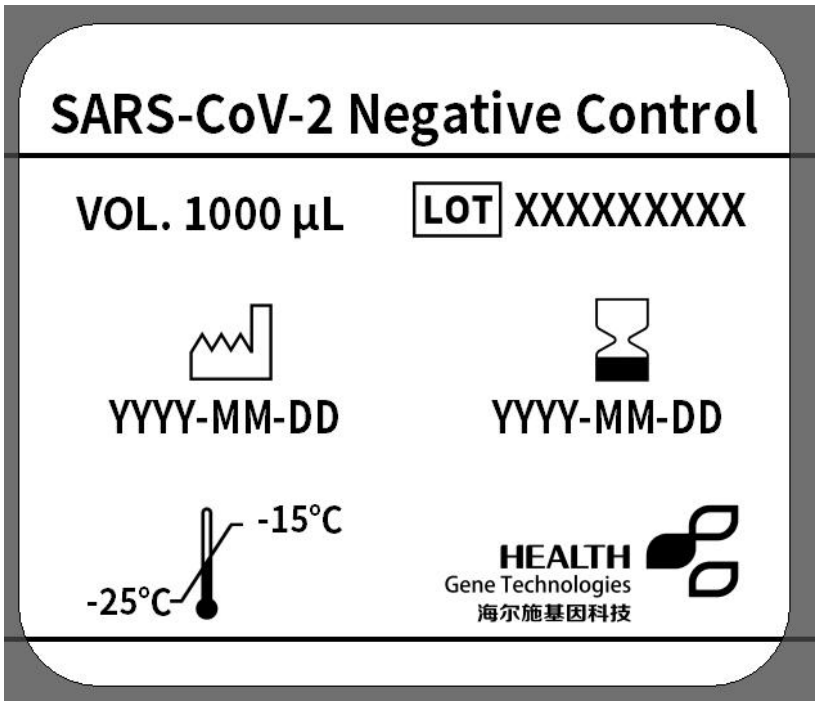
1.8 SARS-CoV-2 Enzyme Solution (XC25087)



1.9 SARS-CoV-2 Positive Control (XC25087)



1.10 SARS-CoV-2 Negative Control (XC25087)



2.0 Instructions for use[2]

[2] English version of the IFU was the one that was assessed by WHO. It is the responsibility of the manufacturer to ensure correct translation into other languages.

Version 1.2

HEALTH
Gene Technologies

PN.XC25073/XC25087

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

CE
IVD

1 Product Name

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

2 Contents

XC25073: 50 Tests/Kit
XC25087: 200 Tests/Kit

3 Intended Use

This kit is used for in vitro manual 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. The product is for aiding in the diagnosis of SARS-CoV-2 infection for professional use by laboratory personnel specifically trained in RT-PCR. The patients being tested should meet SARS-CoV-2 clinical criteria. Positive results are indicative of active infection. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

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	Tube Color	50 Tests/Kit	200 Tests/Kit
SARS-CoV-2 Master Mix	Brown	770 μL, 1 tube	1540 μL, 2 tubes
SARS-CoV-2 Enzyme Solution	Red	60 μL, 1 tube	240 μL, 1 tube
SARS-CoV-2 Positive Control	Yellow	700 μL, 1 tube	1400 μL, 2 tubes
SARS-CoV-2 Negative Control	Transparent	1000 μL, 1 tube	1000 μL, 2 tubes

Note:
(1)The components of different lots of kits cannot be mixed for use.
(2)Equipment and materials required but not listed: Adjustable calibrated micropipettes (2μL,20 μL, 200 μL and 1000 μL), Disposable powder-free gloves, centrifuges, vortex mixers,0.2 mL PCR tubes (DNase/RNase free), 1.5 mL microcentrifuge tubes (DNase/RNase free), etc.

6 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. Freeze-thaw cycles should not be more than three times and 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.
13.Dust may affect the test results of the kit, please carry out relevant test procedures in a Biosafety Cabinet.

7 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. During transportation, it can be kept upto 14 days at a temperature of -80 ~ 2°C and up to 7 days at a temperature of 2 ~ 8°C.

8 Applicable Instruments

Applied Biosystems® 7500/7500 Fast real-time PCR systems (Real-time PCR software v2.4).

9 Specimen Requirements

Respiratory specimens including: nasopharyngeal or oropharyngeal swabs, and sputum. FLOQSwabs® (COPAN, PN 5U027S01.CN) is recommended to use for collecting nasopharyngeal swabs and DNA flocked swab (MIRACLEAN, PN 93050) is recommended to use for collecting oropharyngeal swabs. Cotton swabs with wooden shafts are not recommended. Place swabs immediately into sterile tubes of Viral Transport Medium (Qingdao Hope Bio-Technology Co., Ltd. PNHBPT8661).

1.Sampling Method for Sputum:
Rinse mouth with water, or toothbrush (without toothpaste) before collecting specimens. Those who have dentures should remove the dentures (to reduce the normal flora contamination of the oral cavity). Cough up the sputum, and spit it directly into the sputum cup. The amount of specimen should be more than 1 mL. If one can't cough up sputum by himself/herself, he/she can use a suction machine with a suction tube.
2.Sampling Method for Nasopharyngeal or Oropharyngeal Swabs:
Nasopharyngeal swab: Hold the patient's head lightly with one hand and hold the swab with another hand. Stuck the swab against the nostril to enter slowly along the bottom of the lower nasal passage, because the nasal passage is curved, do not use excessive force to avoid trauma and bleeding. When the tip of the swab reaches the back wall of the nasopharynx, gently rotate it once (in case of reflex cough, stay for a while). Then slowly take out the swab, immerse the swab head in a tube containing 2 to 3 mL of viral transport medium (also can use isotonic saline solution, tissue culture solution or phosphate buffer solution), discard the tail, and screw the tube cap tightly.
Oropharyngeal swab: Hold the tongue of the patient with the tongue depressor in the one hand and extend the swab to the throat area with the other hand, apply moderate pressure on the posterior pharynx wall and the tonsils on both sides and rotate the swab to increase the contact surface, avoid touching the tongue and oral mucosa. After sampling, quickly place the swab in the sampling tube, break off and discard the tail near the top of the sampling tube, and tighten the tube cap.
3.Preservation of Specimens:
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. Specimens should not be frozen and thawed frequently.Specimens must be package, shipped and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential SARS-CoV-2specimens.

10 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 SARS-CoV-2 Positive Control and SARS-CoV-2 Negative Control should be used starting from nucleic acid extraction to monitor the process of extraction and RT-PCR.
2.Reaction mix preparation
Thaw the Master Mix at room temperature 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, or the extracted the positive control and 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 bellow:

Gene Detected	Fluorescence Channel
ORF1ab gene	FAM
S gene	ROX
N 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.

11 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.

12 Quality Control

1.SARS-CoV-2 Negative Control: all channels have no Ct value or Ct>40.
2.SARS-CoV-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.

13 Cut-off Value

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

14 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.

15 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 is no pathogen detected in the sample, but the possibility of infections by other pathogens cannot be completely ruledout.
6.False positive results may occur if contamination is not controlled effectively during sample preparation and RT-PCR setup.

16 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 InfluenzaAvirusH1N1(2009), seasonal H3N2 virus, adenovirus, bocavirus, rhinovirus, parainfluenza virus, metapneumo virus, 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.The minimum detection limit reference of the kit is all positive.
7.Clinical Validation: The test reagent and the comparative reagent were compared and tested in 524 samples. The results of comparative analysis showed that with the comparative reagent as the relative standard, the positive coincidence rate of the test reagent was 96.77% (95%CI:93.47%~98.69%), and the negative coincidence rate was 99.67% (95%CI:98.20%~ 99.99%), the total coincidence rate was 98.47% (95%CI: 97.01% ~ 99.34%). The Kappa value of the consistency test of the two reagents was 0.97, P <0.01, which was statistically significant.The test reagent and the clinical diagnosis were compared and tested in 525 samples. The results of comparative analysis showed that with the clinical diagnosis result as the relative standard, the positive coincidence rate of the test reagent was 95.91% (95%CI：92.38%~98.11%), and the negative coincidence rate was 100.00% (95% CI: 98.80% ~ 100.00%), the total coincidence rate was 98.29% (95% CI: 96.77% ~ 99.21%). The Kappa value of the consistency test of the two reagents was 0.96, P <0.01, which was statistically significant.

17 Description of the logo

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

18 Approval And Modification Date

2020-09-10

19 Contact Information

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