WHO Emergency Use Assessment Coronavirus disease (COVID-19) IVDs PUBLIC REPORT

Product: Wantai SARS-CoV-2 RT-PCR Kit
EUL Number: EUL-0500-005-00
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.

Wantai SARS-CoV-2 RT-PCR Kit code WS-1248, CE-mark regulatory version, manufactured by Beijing Wantai Biological Pharmacy, No.31 Kexueyuan Road, Changping District Beijing 102206, China was listed on 14 August 2020.

Intended use:

According to the claim of intended use from Beijing Wantai Biological Pharmacy, "*this kit is intended for qualitative detection of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARSCoV-2) RNA extracted from oropharyngeal swab, nasopharyngeal swab, sputum, endotracheal aspirate and bronchoalveolar lavage fluid specimens of patients suspected for infection with COVID-19. Target genes detected by the assay are the N and ORF1ab genes of SARS-CoV-2. Testing may be conducted manually or automatically. Results are for the identification of SARS-CoV-2 RNA, which is generally detected in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. This kit is only for in vitro diagnostic use (IVD) to aid the diagnosis of COVID-19. It is intended to be used by laboratory professionals or qualified staff in laboratories that are biosafety level 2 or above.*

Infection with SARS-CoV-2 can cause the respiratory disease COVID-19. Its symptoms include fever, weakness and dry cough, and breathing problems that appear gradually. In severe cases,

acute respiratory distress syndrome (ARDS), septic shock, metabolic acidosis that is hard to correct, coagulation disorders and death may occur."

Specimen type that was validated:

Oropharyngeal swab, Nasopharyngeal swab, sputum, endotracheal aspirate and bronchoalveolar lavage fluid specimens.

Test kit contents:

Component	**48 tests (product code WS-1248)**
RT-PCR master mix	1.25mL×1 vial
$Mn(OAc)_2$	125μL×1 vial
Primer and probe	125μL×1 vial
Positive control	1 mL×1 vial
Negative control	1 mL×1 vial

Items required but not provided:

Specimen collection, storage and transportation materials:

Specimen collection kits:

- Validated commercial VTM kits: "Wantai SARS-CoV-2 VTM"(catalog No. ZCT1261) manufactured by Beijing Wantai Biological Pharmacy Enterprise Co. Ltd. \
- VTM&UTM (catalog No. MT0301) manufactured by Yocon biotechnology Co.,Ltd.

Extraction/Purification:

Extraction kits:

- *Wantai Nucleic Acid Extraction Kit (catalog No. ZCT1246).*
- *GenMagBio (catalog No. NA007-1).*
- *QIAGEN (catalog No. 57704).*

Extraction equipment:

- Kingfisher Flex 96 (manufactured by Thermo Fisher).
- SLA-32 (Manufactured by Taiwan Advanced Nanotech Inc. (TANBead))

Real-Time PCR equipment:

- BIO-RAD CFX96 (software version Bio-Rad CFX Manager 3.1)
- ABI 7500 (software version 7500 version 2.3).

General laboratory equipment and consumables

- PCR tubes and caps
  - When using the BIO-RAD CFX96 amplificator, it is necessary to include the Low-Profile PCR Tubes (Catalog No. TLS0851) and Optical Flat 8-Cap Strips for 0.2ml tube strips/plates (Catalog No. TCS0803) of BIO-RAD.

  - When using the ABI7500 amplificator, it is necessary to include the PCR STRIP TUBES (PCR-0208-C) and PCR STRIP CAPS (PCR-2CP-RT-C) of Axygen or PCR consumables of ABI accessories.

- Class II (or higher) biological safety cabinet (BSC).
- Benchtop centrifuge
- Vortex mixer
- Adjustable calibrated pipettes
- Aerosol barrier pipette tips
- Powder free disposable gloves

Storage:

Store Wantai SARS-CoV-2 RT-PCR Kit below -15 °C. Avoid exposing the kit to direct sunlight.

Shelf-life upon manufacture:

12 months.

Warnings/limitations:

Refer to the instructions for use (IFU)

Product dossier assessment

Beijing Wantai Biological Pharmacy submitted a product dossier for the Wantai SARS-CoV-2 RT-PCR 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 version 4)"*. 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 Commitment for EUL:

As commitments to listing, the manufacturer is required to review the limit of detection with the WHO international standard when available.

Risk benefit assessment conclusion: acceptable.

Quality Management Systems Review

To establish the eligibility for WHO procurement, Beijing Wantai Biological Pharmacy 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 Beijing Wantai Biological Pharmacy 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).*

Beijing Wantai Biological Pharmacy 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]

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

Scope and duration of procurement eligibility

Wantai SARS-CoV-2 RT-PCR Kit, product code WS-1248 manufactured by Beijing Wantai Biological Pharmacy 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, Beijing Wantai Biological Pharmacy must engage in post-market surveillance activities to ensure that the product continues to meet safety, quality and performance requirements. Beijing Wantai Biological Pharmacy 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.

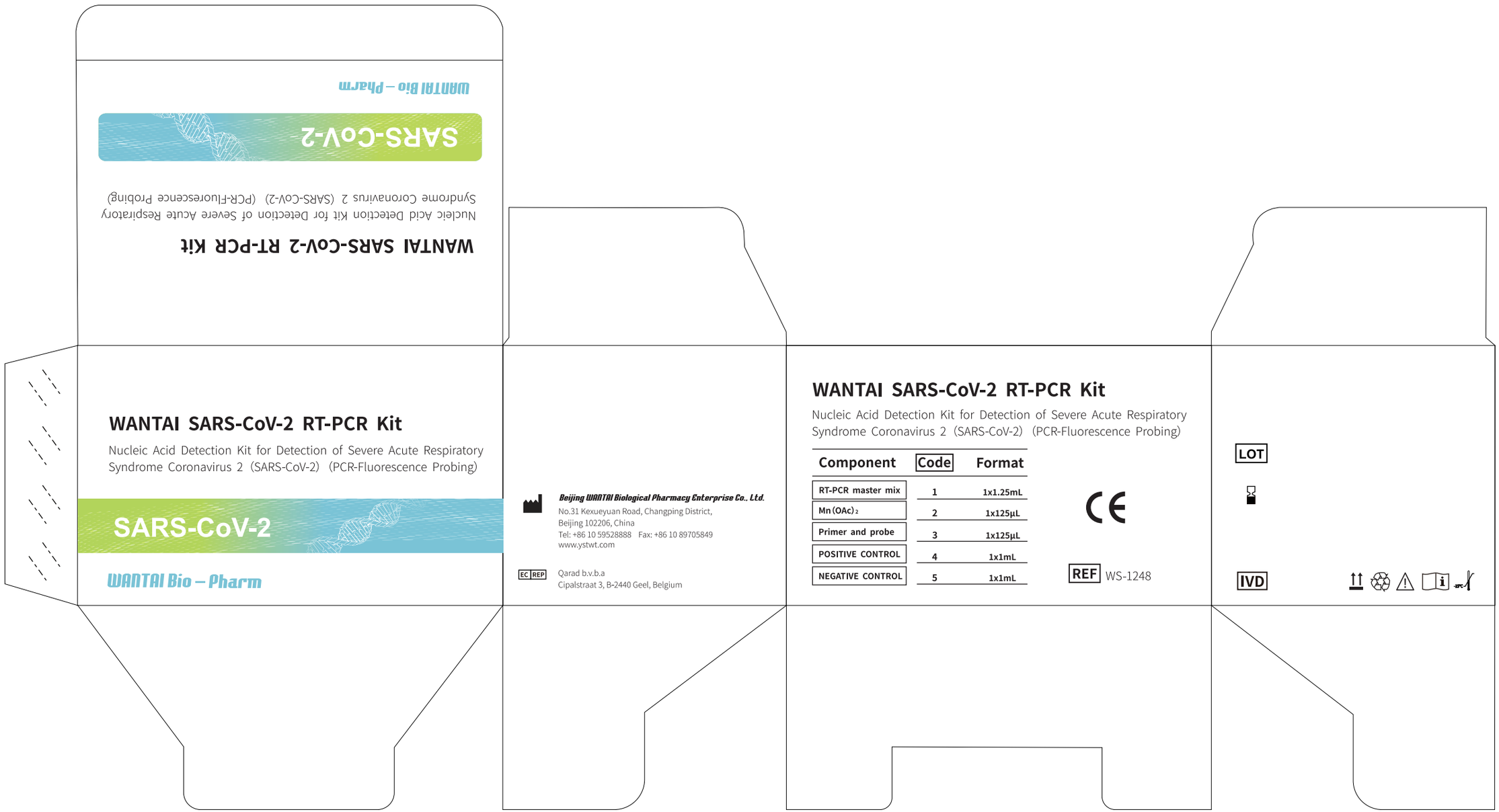
Labelling

1.0 Labels

2.0 Instructions for Use (IFU)

1.0 Product labels

9*5.5*6


WANTAI SARS-CoV-2 RT-PCR Kit
Nucleic Acid Detection Kit for Detection of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) (PCR-Fluorescence Probing)
SARS-CoV-2
WANTAI Bio – Pharm
Beijing WANTAI Biological Pharmacy Enterprise Co., Ltd.
No.31 Kexueyuan Road, Changping District,
Beijing 102206, China
Tel: +86 10 59528888 Fax: +86 10 89705849
www.ystwt.com
EC REP
Qarad b.v.b.a
Cipalstraat 3, B-2440 Geel, Belgium
WANTAI SARS-CoV-2 RT-PCR Kit
Nucleic Acid Detection Kit for Detection of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) (PCR-Fluorescence Probing)
Component
Code
Format
RT-PCR master mix
1
1x1.25mL
Mn(OAc)2
2
1x125μL
Primer and probe
3
1x125μL
POSITIVE CONTROL
4
1x1mL
NEGATIVE CONTROL
5
1x1mL
CE
REF WS-1248
LOT
IVD

Kit box, 48T label

Wantai SARS-CoV-2 RT-PCR Kit

Nucleic Acid Detection Kit for Detection of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)
(PCR-Fluorescence Probing)

Components	Code	Format
RT-PCR master mix	1	1 x 1.25ml
NEGATIVE CONTROL	5	1 x 1ml
POSITIVE CONTROL	4	1 x 1ml
Mn(OAc)2	2	1 x 125μL
Primer and probe	3	1 x 125μL

REF WS-1248

Reagents labels:

Code	SARS-CoV-2 RT-PCR	1.25ml
1	PCR Master Mix LOT	IVD -15°C

Code	SARS-CoV-2 RT-PCR	125μl
2	Mn(OAc)2 LOT	IVD -15°C

Code	SARS-CoV-2 RT-PCR	125μl
3	Primer probe LOT	IVD -15°C

Code	SARS-CoV-2 RT-PCR	1ml
4	Positive Control LOT	IVD -15°C

Code	SARS-CoV-2 RT-PCR	1ml
5	Negative Control LOT	IVD -15°C

Revision History:

Version	Reason of Revision	Revised Content	Revision Date	Revised by	Approved by
1.0	International version	Major revision, adding of new pictograms and hazard markings	February 26,2020	Delemir Delev	Zhao Lingzhi

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.

Wantai SARS-CoV-2 RT-PCR Kit

Nucleic Acid Detection Kit for Detection of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) (PCR-Fluorescence Probing)

Instructions for Use

REF WS-1248 | V. 2020-09 [Eng.] | 48 | IVD

【Intended use】

This kit is intended for qualitative detection of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) RNA extracted from oropharyngeal swab, nasopharyngeal swab, sputum, endotracheal aspirate and bronchoalveolar lavage fluid specimens of patients suspected for infection with COVID-19. Target genes detected by the assay are the N and ORF1ab genes of SARS-CoV-2. Amplification for the test is semi-automatic using BIO-RAD CFX96 (Bio-Rad CFX Manager 3.1) or ABI 7500 (7500 software v2.3) whereas extraction may be automatic, semi-automatic or manual using corresponding extraction instruments. Results are for the identification of SARS-CoV-2 RNA, which is generally detected in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. This kit is only for in vitro diagnostic use (IVD) to aid the diagnosis of COVID-19 and it is intended to be used by qualified laboratory professionals in biosafety level 2 or above laboratories.

Infection with SARS-CoV-2 can cause the respiratory disease COVID-19. Its symptoms include fever, weakness and dry cough, and breathing problems that appear gradually. In severe cases, acute respiratory distress syndrome (ARDS), septic shock, metabolic acidosis that is hard to correct, coagulation disorders and death may occur.

【Test principle】

This kit is a qualitative, real-time fluorescent PCR in which specific primers and fluorescent probes are designed to detect the highly conservative regions of the ORF1ab and N genes of the virus. This kit has integrated quality control (IC, human housekeeping gene) intended for monitoring of the test run.

【Warnings and precautions】

- For in vitro diagnostic use (IVD) only.
- For Prescription Use only.
- The Wantai SARS-CoV-2 RT-PCR Kit has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- Store the product according to the stated storage conditions.
- This kit should be used only by qualified laboratory professionals.
- Reagents from different lots are not interchangeable.
- Do not mix with reagents from other commercially available kits.
- Specimens and disposables left after the testing are potentially infectious. Before disposing, discard used pipette tips into the biological waste container containing disinfectant. After testing, in order to avoid lab contamination, use 75% ethanol to clean the work station. Disinfect with an ultraviolet lamp. Handling should follow the established guidelines for biosafety of microbiological biomedical laboratories, management of medical waste, and other related normative guidelines.
- After nucleic acid extraction, immediately take off the 8 sleeve groove tubes from the instrument. The extracting plate should be sealed after use in order to avoid aerosol pollution.
- Lab management should strictly follow established national molecular biology laboratory and clinical gene amplification laboratory management standards.
- The workflow of the Wantai SARS-CoV-2 RT-PCR Kit should be carried in different areas (kit preparation area, specimen preparation area, amplification and analysis area.) Each phase of the test uses special-purpose instruments and equipment. Cross-use of equipment from different phases and areas is prohibited. Staff and air circulation should be strictly regulated. Avoid cross-contamination as much as possible. Test disposable items should be thoroughly disinfected and inspected in order to avoid contamination or false negative results caused by amplification reaction inhibitor.
- Follow the manufacturer's procedures for nucleic acid extraction for the validated extraction kits. Otherwise, there may be differences between their extraction efficiencies.
- Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious specimens.
- If sputum and bronchoalveolar lavage fluid are not clear, try the following approaches with the specimens:
  ① After centrifugation (12000rpm/min for 5 minutes), extract the clarified part;
  ② After centrifugation (12000rpm/min for 5 minutes) with proper dilution of no more than 2x, extract the clarified part;
  ③ Resample

【Components】

Amplification and controls	RT-PCR master mix	1.25mL×1	dNTPs, rTth enzyme, UDG enzyme
	$Mn(OAc)_2$	125μL×1	$Mn(OAc)_2$solution
	Primer and probe	125μL×1	Primer and probe solution
	Positive control	1 mL×1	Artificial virus containing SARS-CoV-2 amplification target sequence
	Negative control	1 mL×1	Normal Saline

【Materials required but not provided】

Specimen collection kits	This kit does not contain materials for collection , storage and transportation of human oropharyngeal and nasopharyngeal specimens.Validated commercial VTM kits: "Wantai SARS-CoV-2 VTM" (catalog No. ZCT1261) manufactured by Beijing Wantai Biological Pharmacy Enterprise Co. Ltd. and VTM&UTM (catalog No. MT0301) manufactured by Yocon biotechnology Co. Ltd.
Nucleic acid extraction kits	This kit does not contain RNA extraction reagents. Validated commercial extraction kits: Wantai Nucleic Acid Extraction Kit (catalog No. ZCT1246) , GenMagBio (catalog No. NA007-1) and QIAGEN (catalog No. 57704) . Validated initial specimen volumes of 200μL and elution volumes of 50μL.
PCR tubes and caps	When using the BIO-RAD CFX96 amplificator, it is necessary to include the Low-Profile PCR Tubes (Catalog No.TLS0851) and Optical Flat 8-Cap Strips for 0.2ml tube strips/plates (Catalog No.TCS0803) of BIO-RAD. When using the ABI7500 amplificator, it is necessary to include the PCR STRIP TUBES (PCR-0208-C) and PCR STRIP CAPS (PCR-2CP-RT-C) of Axygen or PCR consumables of ABI accessories.
Others	PCR hood, Benchtop centrifuge, Vortex mixer, Single and multichannel adjustable pipettes (1.00 μL to 1,000.0 μL), Aerosol barrier pipette tips, Powder free disposable gloves, Thermostatic equipment (water bath) and ABI 7500 Pure dye calibrator plus FPPM (cat no. 4409561).

【Storage and shelf-life】

- Store the kit under -15℃ . Avoid exposing the kit to direct sunlight. Do not press the package.
- Shelf-life 12 months.
- After opening, the kit can be stored at -15° C for 6 weeks, freeze-thaw no more than 4 times.
- The kit can be transported at -15° C packed into a foam box with ice bags or dry ice.
- See the label for production and expiration date.

【RT-PCR Instruments】

PCR amplificator and software versions

Fluorescent qPCR	Software version
BIO-RAD CFX96	Bio-Rad CFX Manager 3.1
ABI 7500	7500 software v2.3

Nucleic Acid Extractor

Nucleic Acid Extractor model	Manufacturer
Kingfisher Flex 96	Thermo
SLA-32	Taiwan Advanced Nanotech Inc. (TANBead)

【Specimen requirements】

Upper respiratory tract specimen: Oropharyngeal and nasopharyngeal swab. The swab should be a special purpose microbial swab (do not use common swabs). The head of the swab should be of medical grade artificial fiber, the material of the shaft should be plastic.

【Specimen collection】

Nasopharyngeal swab: Use a microbial swab to collect specimens in the nasal area. Softly rotate and push the swab, insert the head of the microbial swab deep into the nasopharyngeal at the root of the nasal cavity, rotate a few times to obtain an abundant specimen. See image 1.
Oropharyngeal swab: Use a microbial swab to wipe the posterior pharyngeal wall and tonsil on both sides with moderate force. Avoid touching the tongue.

Specimen processing: After collecting the specimen, insert the microbial swab into the sterilized VTM tube. Rotate several times against the inner wall of the tube to dissolve the specimen in the solution as much as possible.
Lower respiratory tract specimen: Sputum, endotracheal aspirates, bronchoalveolar lavage fluid. Add 4% NaOH in 2:1 proportion to the collected sputum or tracheal aspirate specimen.Vortex to mix well then place at room temperature for 20 minutes to liquify. Liquification time can be increased if there are too many viscous substances. Transfer 1 mL of liquified specimen to a 1.5 mL centrifuge tube and then vortex again. If bronchoalveolar lavage fluid is clear, it can be directly used for nucleic acid extraction.

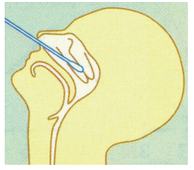


Image 1: nasopharyngeal sampling

Specimen storage and transportation: Specimens tested within 12 hours after collection can be stored at 2-8 ° C. For long-term storage, keep under -70 ° C. Avoid multiple freeze-thaw cycles (no more than 3 times). specimen should be transported under -15° C. Before testing, balance the specimens at room temperature. The frozen specimens should be mixed well before testing.

【Testing Method】

【Reagents preparation】PREPARATION AREA

- **STEP. 1 - PREPARE THE REAGENTS:** Open the kit and remove the components from the box. Thaw at room temperature, shake to mix for 1 minute then centrifuge immediately. Place the RT-PCR master mix, Mn(OAc)2 and primers & probes at 2~8°C to refrigerate for later use.
- **STEP. 2 - PREPARE THE PCR REACTION MIX:** One test requires 30μL of PCR reaction mix. Depending on how many specimens will be tested, mix the required volumes of reagents as per the table below. Centrifuge intermediately after mixing thoroughly. It is advised to prepare one additional test reagent each time to prevent the loss of reaction mix due to splitting.

Component	Volume per 1 reaction(μL)	Volume for 16 reactions(μL)	Volume for 32 reactions(μL)	Volume for 48 reactions(μL)	Volume for n reactions(μL)
RT-PCR master mix	25	425	825	1225	25×（n+1）
Primer probe	2.5	42.5	82.5	122.5	2.5×（n+1）
Mn $(OAc)_2$	2.5	42.5	82.5	122.5	2.5×（n+1）
Total	30	510	990	1470	30×（n+1）

- **STEP. 3 - TRANSFER TO PCR REACTION TUBE:** Pipette 30μL of the PCR reaction mix into a PCR reaction tube (choose a PCR reaction tube compatible with the extractor instrument).
- **STEP. 4 - ADD THE RNA TEMPLATE:** Add 10μL of RNA template or controls to the PCR amplification tube. Close the tube and centrifuge instantly. Transfer to the amplification and analysis area for PCR amplification. *(This kit does not contain RNA extraction reagents. Suggested extraction kits and equipment: Beijing Wantai, GenMagBio or QIAGEN and commercialized RNA extractor reagent kit with reliable quality.)*

【Amplification】 AMPLIFICATION AND ANALYSIS AREA

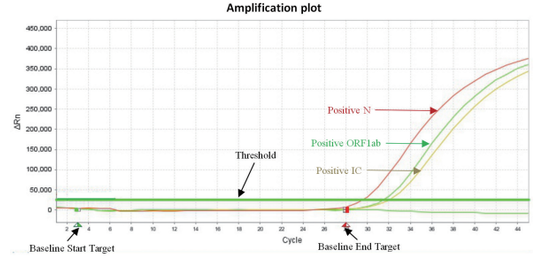
- Place the PCR amplification tube into the RT-PCR instrument.
- Label to indicate the controls and specimen positions.
- Select FAM for ORF1a gene, VIC /HEX for the N gene, and ROX for the IC.
- Set the PCR reaction mix volume to 40μL
- Set the cycles according to the table below:

	Steps	Temperature	Time	Cycles
1	UDG enzyme action	37° C	2 min	1
2	RNA denaturation	90° C	30 sec	1
3	RNA reverse transcription	61° C	15 min	1
4	Denaturation	95° C	3 sec	45
	Annealing, fluorescence signal gathering	60° C	10 sec	

Remarks: When using ABI 7500 series amplification instrument, time for step 4 (annealing, fluorescence signal gathering) should be set as 30 sec. and choose "None" in ROX dye correction settings.

【Result analysis】

- Baseline setting: Automatic optimization of the instrument for BIO-RAD CFX96. Set manually for ABI7500: Open Analysis Plot → Plot Setting SELECT Graph Type, Linear → Options SELECT Target, N, SET manually Threshold and Baseline, Baseline Start Target and Baseline End Target to 3~8 Cycle and 24~30 Cycle → Target SELECT ORF1ab and IC, SET same as above.
- Threshold setting: Automatically by the instrument, or adjust manually according to the baseline that just exceeded the highest point of the amplification curve of the negative control. Manually set the threshold line at the about 1/10th of the End point fluorescence value.
- Analyze the curves of SARS-CoV-2 and internal control respectively.



【Test Run Criteria】

Channel	Testing target	Negative control	Positive control
FAM	ORF1ab	No Ct or Ct=45	Ct ≤ 40
VIC	N	No Ct or Ct=45	Ct ≤ 40
ROX	Internal control	No Ct or Ct=45	No requirement

- The above criteria should be met, otherwise the test run is invalid.

【Result Interpretation】

No.	FAM(ORF1ab)	VIC(N)	ROX(IC)	Result determination and action
1	Ct ≤ 40	Ct ≤ 40	No requirement	SARS-COV-2 positive
2	No Ct or Ct=45	No Ct or Ct=45	≤ 35	SARS-COV-2 negative
3	Ct ＞ 40 or No Ct	Ct ＜ 45	No requirement	Re-extraction and retest are needed. During retest, if any target has a Ct<45, then it is judged as positive. If the two targets have no Ct (or Ct≥45) and internal control has a Ct of ≤ 35, then it is judged as negative. If internal control is >35, then judge the results according to No. 5.
4	Ct ＜ 45	Ct ＞ 40 or No Ct	No requirement	
5	No Ct or Ct=45	No Ct or Ct=45	>35 or No Ct	Test is invalid. Re-extraction and retest are needed. If retest results are still >35 (or No Ct), then judge as Specimen inhibition.

【Limitations】

- This kit is only used for the qualitative detection of SARS-CoV-2 RNA.
- Do not rely solely on the results of this kit for a diagnosis. For a final diagnosis, the results of this kit should be considered in conjunction with the patient's symptoms, physical signs, medical history, other laboratory examinations and reactions to the treatments.
- The primers & probes have been designed to detect the highly conservative regions of the ORFlab and N genes of the virus. However, due to the high mutation rates of the RNA viruses, low possibility of mutation within the conservative regions still exists, which may lead to false negative results with this kit.
- Improper sampling, transportation, storage and handling may cause errors in the results.
- The clinical lab should strictly follow the related clinical molecular diagnostic tests regulations and guidelines. Strictly follow the manual when carrying out the test.
- This kit is limited to the detection of oropharyngeal swab, nasopharyngeal swab, sputum, endotracheal aspirate and bronchoalveolar lavage fluid specimens.
- Nasopharyngeal wash/aspirate or nasal aspirates are acceptable for testing but performance with our assay has not been validated.
- This kit is only applicable for the specified instruments.
- Since the internal control is used for monitoring the complete process of sampling, nucleic acid extraction and amplification, it is not possible to determine where the error lies when there are abnormal internal control results, so it is necessary to do the complete process again. If results are still abnormal after this, test results need to be reported as "Specimen inhibition".

[Performance Indicators]

- The performance validations of the kit have been conducted with the Applied Biosystem® 7500 Real-Time PCR system and Bio-Rad CFX 96 instruments. For specimen extraction, Wantai Nucleic Acid Extraction Kit with Initial specimen volume of 200μL and elution volume of 50μL has been used.
- Sensitivity: The analytical sensitivity was determined by spiking negative oropharyngeal specimens with RNA template. 4 control levels were prepared. The testing results demonstrated that the analytical sensitivity of the kit is 50 copies/ml of SARS-CoV-2 RNA (CI ≥ 95%). The analytical sensitivity was further validated by retesting each of the 20 replicates of 50 copies/ml control level on 3 kit lots.

Establishing of the analytical sensitivity of the kit using Wantai nucleic acid extraction reagent

Level	copies/ml	ORF1ab Gene			N Gene		
		Detection	Mean Cq	Cq SD	Detection	Mean Cq	Cq SD
1	100	100%	37.06	0.63	100%	35.14	0.51
2	50	95%	38.39	0.94	100%	35.91	0.51
3	25	90%	38.48	0.88	70%	38.65	1.11
4	10	25%	40.42	0.79	30%	39.23	1

Analytical sensitivity validation results on Applied Biosystem® 7500 Real-Time PCR system, Lot-1,2,3 using Wantai nucleic acid extraction reagent

Specimen	Lot-1					
	ORF1ab Gene			N Gene		
	% Detection	Mean Cq	Cq SD	% Detection	Mean Cq	Cq SD
1	100%	35.74	0.54	100%	34.44	0.32
2	100%	35.95	0.51	100%	34.17	0.37
3	100%	36.18	0.51	100%	34.23	0.37
4	100%	36.06	0.54	100%	34.76	0.31
5	100%	35.57	0.54	100%	34.29	0.32
6	100%	36.20	0.52	100%	34.26	0.37
7	100%	35.80	0.55	100%	34.52	0.32
8	100%	36.04	0.54	100%	34.72	0.31
9	100%	36.05	0.51	100%	34.38	0.37
10	100%	36.12	0.54	100%	34.82	0.31

Specimen	Lot-2					
	ORF1ab Gene			N Gene		
	% Replicate Detection	Mean Cq	Cq Standard Deviation	% Replicate Detection	Mean Cq	Cq Standard Deviation
1	100%	35.38	0.53	100%	34.52	0.37
2	100%	35.86	0.57	100%	34.28	0.36
3	100%	36.07	0.58	100%	34.33	0.36
4	100%	35.69	0.53	100%	34.80	0.36
5	100%	35.22	0.53	100%	34.37	0.38
6	100%	36.11	0.54	100%	34.34	0.36
7	100%	35.44	0.53	100%	34.58	0.36
8	100%	35.67	0.53	100%	34.77	0.36
9	100%	35.95	0.58	100%	34.48	0.36
10	100%	35.75	0.53	100%	34.86	0.36

Specimen	Lot-3					
	ORF1ab Gene			N Gene		
	% Replicate Detection	Mean Cq	Cq Standard Deviation	% Replicate Detection	Mean Cq	Cq Standard Deviation
1	100%	35.65	0.45	100%	34.51	0.51
2	100%	36.12	0.38	100%	34.34	0.55
3	100%	36.36	0.38	100%	34.40	0.55
4	100%	35.97	0.45	100%	34.82	0.49
5	100%	35.48	0.45	100%	34.35	0.52
6	100%	36.34	0.40	100%	34.46	0.53
7	100%	35.71	0.45	100%	34.59	0.51
8	100%	35.95	0.45	100%	34.79	0.49
9	100%	36.23	0.38	100%	34.56	0.53
10	100%	36.03	0.46	100%	34.89	0.49

Analytical sensitivity validation results of CFX 96 (Bio-Rad®) Real-Time PCR system, Lot-1,2,3 using Wantai nucleic acid extraction reagent

Specimen	Lot-1					
	ORF1ab Gene			N Gene		
	% Replicate Detection	Mean Cq	Cq Standard Deviation	% Replicate Detection	Mean Cq	Cq Standard Deviation
1	100%	35.80	0.39	100%	34.39	0.47
2	100%	35.85	0.39	100%	34.59	0.46
3	100%	35.89	0.39	100%	34.77	0.46
4	100%	35.93	0.39	100%	34.18	0.49
5	100%	35.87	0.39	100%	34.31	0.47
6	100%	35.91	0.40	100%	34.48	0.47
7	100%	36.01	0.40	100%	35.02	0.46
8	100%	36.10	0.40	100%	34.89	0.46
9	100%	36.06	0.40	100%	34.72	0.45
10	100%	35.76	0.39	100%	34.22	0.48

Specimen	Lot-2					
	ORF1ab Gene			N Gene		
	% Replicate Detection	Mean Cq	Cq Standard Deviation	% Replicate Detection	Mean Cq	Cq Standard Deviation
1	100%	36.05	0.30	100%	34.74	0.39
2	100%	36.09	0.30	100%	34.92	0.39
3	100%	36.13	0.30	100%	35.09	0.39
4	100%	36.17	0.30	100%	34.53	0.40
5	100%	36.11	0.30	100%	34.66	0.39
6	100%	36.16	0.30	100%	34.81	0.39
7	100%	36.26	0.31	100%	35.32	0.41
8	100%	36.34	0.31	100%	35.21	0.40
9	100%	36.31	0.31	100%	35.04	0.39
10	100%	36.00	0.31	100%	34.57	0.40

Specimen	Lot-3					
	ORF1ab Gene			N Gene		
	% Replicate Detection	Mean Cq	Cq Standard Deviation	% Replicate Detection	Mean Cq	Cq Standard Deviation
1	100%	35.87	0.40	100%	34.58	0.55
2	100%	35.91	0.41	100%	34.75	0.55
3	100%	35.95	0.40	100%	34.91	0.55
4	100%	35.99	0.40	100%	34.39	0.55
5	100%	35.93	0.40	100%	34.51	0.55
6	100%	35.98	0.40	100%	34.65	0.55
7	100%	36.09	0.40	100%	35.14	0.56
8	100%	36.18	0.41	100%	35.03	0.55
9	100%	36.13	0.41	100%	34.87	0.55
10	100%	35.82	0.40	100%	34.43	0.55

- **Analytical Specificity:** No cross reactivity has been observed after the testing of specimens from individuals infected with influenza and parainfluenza virus, H1N1, HN1(2009), H3N2, H5N1, H7N9, EBV, CMV, Adenovirus 1,2,3,4,5,7, RSV A, RSV B, HCoV-229E, HCoV-OC43, HCoV-HKU1, HCoV-NL63, MERS, Rotavirus, Norovirus, Mycoplasma pneumoniae and Chlamydia.
- **Precision:** The precision of the kit was evaluated by testing of RNA-spiked negative oropharyngeal specimens. 2 laboratory technicians tested 3 lots of kits with 10 replicates of 3 different concentrations (positive 1000 copies/ml, low positive 150 copies/ml and negative 0 copies/ml) for 5 days. The study was conducted using ABI7500and CFX-96 PCR instruments. The results from the study demonstrated very good precision of the kit with CV<5%.

Technician -1, ABI7500

Days	Lot nCoVP20200101			Lot nCoVP20200102			Lot nCoVP20200103		
	POS	Border	NEG	POS	Border	NEG	POS	Border	NEG
1	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
2	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
3	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
4	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
5	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10

Technician- 1,CFX-96

Days	Lot nCoVP20200101			Lot nCoVP20200102			Lot nCoVP20200103		
	POS	Border	NEG	POS	Border	NEG	POS	Border	NEG
1	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
2	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
3	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
4	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
5	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10

Technician-2, ABI7500

Days	Lot nCoVP20200101			Lot nCoVP20200102			Lot nCoVP20200103		
	POS	Border	NEG	POS	Border	NEG	POS	Border	NEG
1	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
2	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
3	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
4	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
5	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10

Technicians 2,CFX-96

Days	Lot nCoVP20200101			Lot nCoVP20200102			Lot nCoVP20200103		
	POS	Border	NEG	POS	Border	NEG	POS	Border	NEG
1	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
2	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
3	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
4	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
5	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10

- **Accuracy:**Oropharyngeal and nasopharyngeal from healthy individuals, 100 each were tested on 3 lots to validate the specificity of the kit and human DNA cross-reactivity. The results from the study given below indicate very good specificity of the kit with no cross-reactivity due to the presence of human DNA.

Testing of 3 lots on negative specimens

RT-PCR	Specimen	Type nCoV	Lot nCoVP20200101 (NEG/Total)	Lot nCoVP20200102 (NEG/Total)	Lot nCoVP20200103 (NEG/Total)
ABI7500	Nasopharyngeal	NEG	100/100	100/100	100/100
	Oropharyngeal	NEG	100/100	100/100	100/100
CFX-96	Nasopharyngeal	NEG	100/100	100/100	100/100
	Oropharyngeal	NEG	100/100	100/100	100/100

- **Clinical studies:** This kit has been evaluated at Beijing Ditan Hospital (Capital Medical University), Beijing Youan Hospital (Capital Medical University) and Institute for Microbial Epidemiology (Academy of Military Medical Sciences). A total of 296 cases were studied and the evaluated reagent and clinically confirmed/excluded results were compared. Sensitivity is 101/107=94.39% (95% CI 88.10%~97.91%) and specificity is 189/189=100% (95% CI 98.07%~100%). Overall, precision is 290/296=97.97% (95% CI 95.64%~99.25%). When the performance of the kit was compared against other commercially available nucleic acid detection kit (China CFDA-EUA approved assay), Wantai SARS-CoV-2 RT-PCR demonstrated positive and negative agreements with the comparison tests of 106/106=100% (95% CI 96.58%~100%) and 195/195=100% (95% CI 98.13%~100%) respectively. Total agreement rate is 301/301=100 (95% CI 98.78%~100%). Within the scope of the this study, Wantai SARS-CoV-2 RT-PCR demonstrated better sensitivity than the comparison test and also very high detection rate in specimens from patients clinically confirmed for COVID-19.
- **Interfering substances:** The following substances do not affect test results. 0.2mg/L of beclomethasone, 0.15mg/L of dexamethasone, 12mg/L of triamcinolone, 0.4mg/L of budesonide, 0.05mg/L of mometasone, 0.5mg/L of fluticasone, 75mg/L of benzocaine, 5mg/L of zanamivir, 37.5mg/L of oseltamivir, 75mg/L of tobramycin, 50mg/L of amantadine, 75mg/L of sulfur, 150mg/L of thryallis, 50mg/L of methyljinamine, 0.125mg/L of adrenaline, 25mg/L of menthol, 0.05% of hydroxymethazoline, 500mg/L of flunisolide, 500mg/L of mupirocin, 400mg/L of purified mucin, 200μl of hemolytic blood.

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[CE Marking Symbols]

IVD	In Vitro Diagnostic Medical Device		<-15° C Storage Conditions
	Use By	LOT	Batch
n	Content Sufficient For <n> Tests		Instructions For Use
CE	CE Marking – IVDD 98/79/EC	EC REP	EU Authorized Representative
REF	Catalog Number		Manufacturer

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