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

(48 tests per kit)

For Prescription Use

For In Vitro Diagnostic Use Only

For Use Under Emergency Use Authorization Only

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1. Intended Use

The Wantai SARS-CoV-2 RT-PCR Kit is a real-time reverse transcription-PCR assay (RT-PCR) intended for the qualitative detection of nucleic acid from SARS-CoV-2 in oropharyngeal swab specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C.§263a, that meet requirements to perform high complexity tests.

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. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

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.

Testing with the Wantai SARS-CoV-2 RT-PCR Kit is intended for use by qualified laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The Wantai SARS-CoV-2 RT-PCR Kit is intended for use only under the Food and Drug Administration's Emergency Use Authorization.

2. Summary and Explanation

Coronavirus disease 2019 (COVID-19) is a respiratory disease caused by infection with the SARS-CoV-2 virus. Common signs of infection include respiratory symptoms, fever, cough, shortness of breath and breathing difficulties. In severe cases, infection can cause pneumonia, acute respiratory distress syndrome (ARDS), kidney failure and death.

Coronaviruses (CoV) are a large family of viruses that cause illness ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV). The 2019 Novel Coronavirus, formerly known as 2019-nCoV and now known as SARS-COV-2, is a new strain of coronavirus that was first identified during 2019-2020 pandemic.

3. 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 SARS-COV-2. This kit has integrated quality control (IC, human housekeeping gene: β-actin gene) intended for monitoring of the test run to avoid false-negative result. After collecting specimens, Beijing Wantai Nucleic Acid Extraction Kit (catalog No. ZCT1246) and QIAGEN QIAamp Viral RNA Mini Kit (catalog No. 52094) can be used as extraction methods. Extraction specimen volumes of 200μL and elution volumes of 50μL are considered for the extraction kits. Then 10μL of extracted RNA is added to 30μL of PCR reaction mix containing RT-PCR master mix, $Mn(OAc)_2$ and primer and probe. Finally, amplification is conducted on ABI7500 or BIO-RAD CFX96 platforms to determine whether clinical specimens are positive or negative.

4. Kit Components and Storage

Amplification and controls	RT-PCR master mix	1.25mL×1	dNTPs, rTth enzyme, UDG enzyme	Store at -15°C
	$Mn(OAc)_2$	125μL×1	$Mn(OAc)_2$ solution	Store at -15°C
	Primer and probe	125μL×1	Primer and probe solution	Store at -15°C
	Positive control[1]	1 mL×1	Artificial virus containing SARS-CoV-2 amplification target sequence; supplied at 4X LoD (200 copies/mL)	Store at -15°C
	Negative control	1 mL×1	Nuclease free Distilled Water	Store at -15°C

[1] Developed by the National Institute of Diagnostics and Vaccine Development in Infectious Diseases (Xiamen University)

5. Materials Required but Not Provided

Sample collection kits	This kit does not contain materials for collection , storage and transportation of human oropharyngeal swabs. Validated commercial VTM kits: Wantai SARS-CoV-2 VTM (Cat # ZCT1261) manufactured by Beijing Wantai Biological Pharmacy Enterprise Co. Ltd. and VTM&UTM (Cat # MT0301) manufactured by Yocon Biotechnology Co., Ltd.

	Validated OP swabs: Flocked swabs from HuaChengYang (wwww.hcyusa.com) (Cat # CY-93050)
Nucleic acid extraction kits	This kit does not contain RNA extraction reagents. Validated commercial extraction kits include the Wantai Nucleic Acid Extracting Reagent (Cat # ZCT1246) and QIAGEN QIAamp Viral RNA Mini Kit (Cat # 52094). Validated initial specimen volumes of 200 µL and elution volumes of 50 µL.
PCR tubes and caps	When using the BIO-RAD CFX-96 platform, use the Low-Profile PCR Tubes (Cat # TLS0851) and Optical Flat 8-Cap Strips for 0.2ml tube strips/plates (Cat # TCS0803) of BIO-RAD. When using the ABI7500 platform, use the PCR STRIP TUBES (Cat # PCR-0208-C) and PCR STRIP CAPS (Cat # PCR-2CP-RT-C) from Axygen or PCR consumables from ABI.

6. Storage and Shelf-life

- Store the kit under -15°C. 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.
- Do not use reagents past their expiration date.

7. RT-PCR Instruments Validated for Use

Fluorescent qPCR Instrument	Software version
BIO-RAD CFX-96	Bio-Rad CFX Manager 3.1
ABI 7500	7500 software v2.3

8. Warnings and Precautions

1) For in vitro diagnostic use (IVD) only.
2) For Emergency Use Authorization only.
3) For Prescription Use only.
4) The Wantai SARS-CoV-2 Test RT-PCR Kit has not been FDA cleared or approved.
5) The Wantai SARS-CoV-2 RT-PCR Kit has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high complexity tests.
6) 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.
7) The Wantai SARS-CoV-2 RT-PCR Kit is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
8) This kit should be used only by qualified laboratory professionals.
9) Reagents from different lots are not interchangeable.
10) Do not mix with reagents from other commercially available kits.
11) Samples 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.

12) Lab management should strictly follow established national molecular biology laboratory and clinical gene amplification laboratory management standards.

13) Laboratory personnel who perform the test must undergo professional training.

14) The workflow of the Wantai SARS-CoV-2 RT-PCR Kit should be carried in different areas (kit preparation area, sample 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.

15) Follow the manufacturer's procedures for nucleic acid extraction for the validated extraction kits. Otherwise, there may be differences between their extraction efficiencies.

16) Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.

17) Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.

18) Separate laboratory areas, dedicated to performing predefined procedures of the assay, are required. a) 1st area: preparation area--prepare testing reagent; b) 2nd area: specimen processing--process the specimens and controls; c) 3rd area: amplification area--PCR conducted.

19) Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples.

9. Sample Collection, Storage and Transport

- Collect oropharyngeal swabs from individuals suspected of having COVID-19 by their healthcare provider. Flocked swabs from HuaChengYang in viral transport media/universal transport media from Beijing Wantai and Yocon Biotechnology Co., Ltd. are acceptable for processing with the workflow of the Wantai SARS-CoV-2 RT-PCR Kit.

- **Sample storage and transportation:** Samples that will be tested within 12 hours of collection can be stored at 2-8°C if necessary. For long-term storage, keep samples at -70°C. Samples should be transported to the testing laboratory at -15°C. Before testing, equilibrate the samples to room temperature. The frozen samples should be mixed well before testing.

- This product follows the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulations and Guidance of the Centers for Disease Control and Prevention (CDC) for packing and shipping samples.

10. 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.

- **STEP. 2 - PREPARE THE PCR REACTION MIX:** One test requires 30 µL of PCR reaction mix. Depending on how many specimens will be tested, mix the required volumes of reagents as per the table below. Mix thoroughly by vortexing and centrifuge immediately. It is advised to prepare one additional RT-PCR reaction each time to prevent the loss of reaction mix due to pipetting error.

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 30 µL of the PCR reaction mix into a PCR reaction tube. For BIO-RAD CFX-96 platform, apply Low-Profile PCR Tubes (Catalog No.TLS0851) and Optical Flat 8-Cap Strips for 0.2ml tube strips/plates (Catalog No.TCS0803) from BIO-RAD. For ABI7500 platform, apply PCR STRIP TUBES (PCR-0208-C) and PCR STRIP CAPS (PCR-2CP-RT-C) from Axygen or PCR consumables from ABI accessories.

- **STEP. 4 - ADD THE RNA TEMPLATE:** Add 10 µL of RNA template or controls to the PCR amplification tube. Close the tube and centrifuge immediately. Transfer to the amplification and analysis area for PCR amplification.

Amplification:

AMPLIFICATION AND ANALYSIS AREA

- Place the PCR amplification tube into the RT-PCR instrument.
- Label using the instrument software to indicate the controls and clinical samples.
- Select FAM for ORF1ab gene, VIC /HEX for the N gene, and ROX for the IC (β-actin).
- Set the PCR reaction mix volume to 40 µL
- Set the cycles according to the tables below:

BIO-RAD CFX-96				
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	

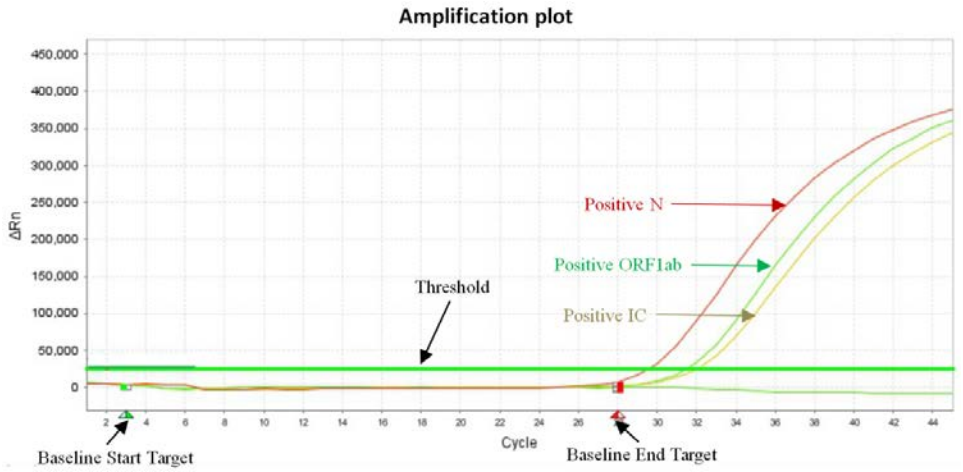
ABI 7500				
Steps		Temperature	Time	Cycles
1	UDG enzyme action	37°C	2 min	1
2	RNA denaturation	90°C	30 sec	1
3	RNA reverse transcription	61°C	15 min	1
4	Denaturation	95°C	3 sec	45
	Annealing, fluorescence signal gathering	60°C	30 sec	

Note: Annealing times between BIO-RAD CFX-96 and ABI 7500 amplification instruments are different.

Result analysis:

- Baseline setting: Automatic optimization of the instrument for BIO-RAD CFX-96. 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 about 1/10th of the End point fluorescence value.
- Analyze the curves of SARS-CoV-2 and internal control respectively. All positive curves need to be S-shaped, except for positive internal control.

11. Assay Control Results/Quality Control



Assay Control Results Interpretation

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 (β-actin)	No Ct or Ct=45	No requirements*

* No requirements (Ct ≤ 35/No Ct or Ct > 35) are given to the internal control (β-actin) when a SARS-CoV-2 target is detected (ORF1ab and/or N); Internal control must be positive when a clinical sample is negative for both SARS-CoV-2 targets (both ORF1ab and N) for the run to be valid.

The following assay controls are provided with the Wantai SARS-CoV-2 RT-PCR Kit:

- A negative control contains nuclease free distilled water and is intended to evaluate potential cross contamination that could occur during nucleic acid extraction and assay preparation. The negative control is processed like a clinical specimen, beginning with nucleic acid extraction, and 10 µL must be run in one tube per assay.
- A positive control (PC) is intended to evaluate enzyme activity, and analytical and clinical performance of the assay. The positive control consists of armored RNA for ORF1ab and N genes that was developed and produced by the National Institute of Diagnostics and Vaccine Development in Infectious Diseases (Xiamen University). The positive control is supplied at 4X LoD (200 copies/mL) and therefore, no additional dilution by the user is necessary. The positive control is processed through the extraction procedure and 10 µL of this control must be added to one tube per assay.
- All test controls should be examined before results interpretation.
- If the ORF1ab or N are positive (Ct ≤ 40) in the negative control, the RT-PCR assay run is invalid. If the β-actin control is positive (Ct ≤ 35) in the negative control, the RT-PCR assay run is invalid.
- If the ORF1ab or N are negative in the positive control, the RT-PCR assay run is invalid.
- If controls are invalid, results cannot be interpreted, and the test needs to be repeated using residual nucleic acid from clinical samples and fresh controls.
- The internal control does not need to be positive if ORF1ab and/or N are detected; however, if SARS-CoV-2 targets are both negative, the internal control must be positive for the run to be valid.

12. Result Interpretation for Clinical Samples

Testing Scenario	ORF1ab (FAM)	N (VIC)	β-actin (ROX)	Interpretation	Action
1	Ct ≤ 40	Ct ≤ 40	/*	SARS-CoV-2 positive	Report the result to sender.
2	Ct ≤ 40	40 < Ct < 45	/	Inconclusive	Re-extraction and retest are needed. During retest, if one of the targets have a Ct < 45, then the sample is SARS-CoV-2 positive. If the two targets have no Ct (or Ct=45) and internal control has a Ct of ≤35, then the sample is negative for SARS-CoV-2.
3	40 < Ct < 45	Ct ≤ 40	/		
4	Ct < 45	No Ct or Ct = 45	/		
5	No Ct or Ct = 45	Ct < 45	/		
6	40 < Ct < 45	40 < Ct < 45	/		
7	No Ct or Ct = 45	No Ct or Ct = 45	Ct ≤ 35	SARS-CoV-2 negative	Report the result to sender.
8	No Ct or Ct = 45	No Ct or Ct = 45	No Ct or Ct > 35	Invalid result	Sample is repeated once using new extracted nucleic acid from residual clinical sample and tested again. Poor RNA yield or RT-PCR inhibition is suspected. If the repeated result is still invalid, report the result to the sender and recommend that a new specimen is collected.

* Detection of the internal control (β-actin) in the ROX detection channel is not required (Ct ≤ 35 No Ct or Ct >35) when a SARS-CoV-2 target is detected in a clinical sample (ORF1ab and/or N). A high copy number of target-specific gene can lead to reduced or absent β-actin.

13. Limitations

- The Wantai SARS-CoV-2 RT-PCR Kit is for prescription use, in vitro diagnostic use, and for under FDA Emergency Use Authorization only. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.
- 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.
- Validation of this kit was completed using only oropharyngeal swabs. Other upper and lower respiratory tract specimens have not been validated and are not considered acceptable specimen types for use with this kit.
- Validation of this kit was only completed on the CFX-96 and the ABI7500 RT-PCR instruments.
- This kit was validated for use with the QIAamp Viral RNA Mini Kit and the Wantai Nucleic Acid Extracting Reagent.

14. Conditions of Authorization for the Laboratory

The Wantai SARS-CoV-2 RT-PCR Kit Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website:

https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas.

However, to assist clinical laboratories using the Wantai SARS-CoV-2 RT-PCR, the relevant Conditions of Authorization are listed below:

A. Authorized laboratories[1] using the Wantai SARS-CoV-2 RT-PCR will include with result reports of the test, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

B. Authorized laboratories using the Wantai SARS-CoV-2 RT-PCR Kit will perform the test as outlined in the Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use the Wantai SARS-CoV-2 RT-PCR Kit are not permitted.

C. Authorized laboratories that receive the Wantai SARS-CoV-2 RT-PCR Kit will notify the relevant public health authorities of their intent to run the test prior to initiating testing.

D. Authorized laboratories using the Wantai SARS-CoV-2 RT-PCR Kit will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E. Authorized laboratories will collect information on the performance of the Wantai SARS-CoV-2 RT-PCR Kit and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Beijing Wantai Biological Pharmacy Enterprise Co., Ltd (email: wtexport@ystwt.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.

F. All laboratory personnel using the Wanati SARS-CoV-2 RT-PCR Kit must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the Wantai SARS-CoV-2 RT-PCR Kit in accordance with the authorized labeling.

G. You , authorized distributors, and authorized laboratories using your product will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1]For ease of reference, this refers to, "laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests as "authorized laboratories."

15. Performance Characteristics

- The performance validation of the kit has been conducted with the Applied Biosystem® 7500 Real-Time PCR system (Software version is 7500 software v2.3) and Bio-Rad CFX 96 instruments (Software version is Bio-Rad CFX Manager 3.1). For sample extraction, the Wantai Nucleic Acid Extraction Kit (Cat #. ZCT1246) and the QIAGEN QIAamp Viral RNA Mini Kit (Cat # 52094) with Initial specimen volume of 200 µL and elution volume of 50 µL have been validated.

Limit of Detection (Sensitivity):

- LoD studies determine the lowest detectable concentration of SARS-CoV-2 at which ≥ 95% of all (true positive) replicates test positive. The LoD was determined by limiting dilution studies using characterized samples. The analytical sensitivity was determined by spiking negative oropharyngeal swab matrix with SARS-CoV-2 RNA that was extracted from positive SARS-CoV-2 specimens obtained from the Academy of Military Medical Sciences (College of Medical Research). RNA from the positive clinical specimens was extracted using the Nucleic Acid Extracting Reagent from Wantai Biological Pharmacy (Cat # ZCT1246) as well as the Qiagen QIAamp Viral RNA Mini Kit and quantitated using ddPCR. Four different concentrations were prepared and tested with 20 independent extraction replicates. The testing results demonstrated that the analytical sensitivity of the kit is 50 copies/mL using samples extracted with the Wantai and Qiagen extraction kits and tested on both the CFX-96 and ABI7500 RT-PCR platforms.

Summary Results for LoD Confirmatory Study Performed on the CFX-96 Platform Using Samples Extracted with the Wantai Nucleic Acid Extracting Reagent

Oropharyngeal swab					
Concentration (copies/mL)	ORF1ab		N		Overall Detection rate
	Detection rate	Mean Ct	Detection rate	Mean Ct	
100	20/20	37.06	20/20	35.14	100%
50	19/20	38.30	19/20	35.95	95%
25	12/20	38.39	12/20	38.31	60%
10	2/20	39.72	2/20	37.63	10%

Summary results for LoD confirmatory Study Performed on the ABI7500 Platform Using Samples Extracted with the Wantai Nucleic Acid Extracting Reagent

Oropharyngeal swab					
Concentration (copies/mL)	ORF1ab		N		Overall Detection rate
	Detection rate	Mean Ct	Detection rate	Mean Ct	
100	20/20	35.96	20/20	35.17	100%
50	19/20	36.62	19/20	36.20	95%
25	11/20	37.20	11/20	37.67	55%
10	2/20	37.55	2/20	38.99	10%

Summary results for LoD confirmatory Study Performed on the CFX-96 Platform Using Samples Extracted with the QIAamp Viral RNA Mini Kit

Oropharyngeal swab					
Concentration (copies/mL)	ORF1ab		N		Overall Detection rate
	Detection rate	Mean Ct	Detection rate	Mean Ct	
100	20/20	36.99	20/20	35.82	100%
50	19/20	38.23	19/20	36.57	95%
25	12/20	38.30	12/20	38.96	60%
10	2/20	39.65	2/20	38.22	10%

Summary results for LoD confirmatory Study Performed on the ABI7500 Platform Using Samples Extracted with the QIAamp Viral RNA Mini Kit

Oropharyngeal swab					
Concentration (copies/mL)	ORF1ab		N		Overall Detection rate
	Detection rate	Mean Ct	Detection rate	Mean Ct	
100	20/20	36.12	20/20	35.34	100%
50	19/20	36.78	19/20	36.27	95%
25	12/20	37.35	12/20	37.85	60%
10	1/20	39.16	1/20	39.45	5%

Inclusivity:

- *In silico* inclusivity analyses of the SARS-CoV-2-specific ORF1ab and N oligonucleotides were performed by a BLASTn analysis using 9968 publicly available SARS-CoV-2 sequences obtained from the GenBank on August 5, 2020. Primer and probe sets for the ORF1ab and N targets exhibited 100% sequence homology to the evaluated SARS-CoV-2 sequences. Note that degenerate base error phenomenon exists within the gene sequences of SARS-CoV-2 in the NCBI database, which will affect the comparison results of primers and probes but will not affect their conservation.

Homology to Human SARS-CoV-2 Circulating Strains

Target	Primer or Probe	Number of Sequences	Percent Homology
ORF1ab region	Forward	9968	100%
	Reverse	9968	100%
	Probe	9968	100%
N gene	Forward	9968	100%
	Reverse	9968	100%
	Probe	9968	100%

- Also, the *in silico* inclusivity study showed that oligonucleotide sequences for the SARS-CoV-2 ORF1ab and N genes have 100% homology to several bat and pangolin SARS-CoV-2 viral genome sequences within GenBank. The hosts of these viruses are not human and the probability of infecting humans is very low; therefore, the probability of the Wantai SARS-CoV-2 RT-PCR Kit generating a false positive result is low. Detailed data is shown in the following table for the homology of the Wantai oligonucleotides to bat and pangolin genome sequences.

Homology to Bat and Pangolin Genome Sequences

Target	Primer or Probe	Number of Sequences	Percent Homology (# with mismatch)
ORF1ab region	Forward	3348	98.9247% (36)
	Reverse	4306	99.6749% (14)
	Probe	3822	99.9738% (1)
N gene	Forward	4908	100% (0)
	Reverse	6192	99.9193% (5)
	Probe	5160	99.8837% (6)

Analytical Specificity:

- Cross-reactivity of the Wantai SARS-CoV-2 RT-PCR Kit was evaluated using both *in silico* analysis and wet testing against 6 types of Coronavirus, 28 common respiratory viruses, 13 respiratory bacteria/fungi, and 7 enteroviruses. During the *in silico* analysis, the ORF1ab and N primer and probe sequences were compared to the gene sequences of these pathogens using DNA MAN 8.0, which showed that no homology was above 80%. During wet testing, these pathogens were extracted with Wantai Nucleic Acid Extracting Reagent and 3 consecutive lots of the Wantai SARS-CoV-2 RT-PCR Kit were tested on both the BIO-RAD CFX-96 and ABI7500 PCR instruments. Results were all negative and there was no predicted assay cross reactivity with any of the tested pathogens.

***In silico* Cross-Reactivity Study Results**

Microorganism	GenBank Accession	ORF1ab % Homology			N gene % Homology		
		F	R	Probe	F	R	Probe
Human coronavirus229E	NC-002645.1	76	42	39	22	27	25
Human coronavirus OC43	NC_006213.1	61	21	42	9	27	30
Human coronavirus HKU1	NC_006577.2	48	63	38	36	41	45
Human coronavirus NL63	NC_005831.2	48	47	38	41	45	50
SARS-coronavirus	NC_004718.3	76	53	96	91	68	75
MERS-coronavirus	NC_019843.3	48	47	38	45	41	70
Adenovirus C1	KF429744.1	48	63	36	41	59	55
Adenovirus 71	KF268207.1	43	68	36	50	45	55
Human Metapneumovirus (hMPV)	NC_039199.1	52	47	32	36	45	55
Parainfluenza virus 1	NC_003461.1	43	63	29	45	45	40
Parainfluenza virus 2	KM190939.1	43	47	36	45	45	50
Parainfluenza virus 3	NC_001796.2	62	42	32	36	36	45
Parainfluenza virus 4	NC_021928.1	43	53	32	36	45	45
Influenza A	NC_002023.1	38	47	29	41	41	40
	NC_002022.1	38	42	29	45	36	45
	NC_002021.1	33	42	25	0	45	35
	NC_002020.1	33	0	25	32	32	40
	NC_002019.1	33	42	29	41	32	40
	NC_002018.1	33	37	25	0	32	40
	NC_002017.1	38	0	29	32	36	35
	NC_002016.1	33	0	29	0	32	50
Influenza B	NC_002204.1	38	47	50	32	45	40
	NC_002209.1	33	37	54	32	64	0
	NC_002208.1	33	42	46	32	41	55
	NC_002207.1	38	37	54	36	36	45
	NC_002206.1	33	37	50	41	45	40
	NC_002205.1	38	37	54	41	41	40
	NC_002211.1	33	37	50	32	36	35
	NC_002210.1	43	37	50	36	36	40
Enterovirus 68	NC_038308.1	43	47	32	41	36	65
Respiratory syncytial virus	NC_001803.1	43	42	32	36	41	45
Rhinovirus	FJ869955.1	43	37	25	0	0	0

Microorganism	GenBank Accession	ORF1ab % Homology			N gene % Homology		
		F	R	Probe	F	R	Probe
Chlamydia pneumoniae	NC_005043.1	57	63	43	64	59	65
Haemophilus influenzae	NZ_LN831035.1	57	63	54	55	59	65
Legionella pneumophila	NZ_LR134380.1	62	63	43	59	59	65
Mycobacterium tuberculosis	NC_000962.3	52	68	54	55	50	70
Streptococcus pneumoniae	NZ_LN831051.1	71	63	43	68	55	60
Streptococcus pyogenes	LN831034.1	57	68	43	55	59	85
Bordetella pertussis	NC_005357.1	38	47	36	41	41	55
Mycoplasma pneumoniae	CP039772.1	57	58	46	50	55	60
Pneumocystis jirovecii (PJP)	EU979570.1	33	0	0	36	32	0
Candida albicans	CM016738.1	62	79	43	59	59	75
Pseudomonas aeruginosa	CP029707.1	52	63	46	59	50	65
Staphylococcus epidermis	MT125873.1	33	37	0	0	32	40
Streptococcus salivarius	CP013216.1	62	63	50	59	73	60
Novel A H1N1	DI250803.1	52	63	54	59	50	65
H1N1(2009)	GQ221691.1	33	63	36	24	55	35
	KC782028.1	29	37	18	59	45	35
	GQ303338.1	33	37	11	59	36	35
	CY052047.1	19	58	39	27	27	35
	GQ338331.1	26	21	29	64	32	45
	KC782027.1	24	63	29	36	41	40
	KC782031.1	38	16	32	50	45	20
	KC782031.1	29	63	21	27	36	35
H3N2	AF153241.1	33	32	14	64	45	32
	AF153245.1	24	26	32	59	36	40
	AF153249.1	24	32	25	59	18	35
	CY040462.1	62	21	21	41	36	45
	KX412348.1	38	42	21	59	32	35
	JQ290171.1	24	47	25	27	55	35
	AF153257.1	19	0	29	32	0	65
	AF153262.1	14	21	57	14	23	5
H5Nl	AY950282.1	24	21	21	27	5	50
	AY950278.1	14	26	25	41	27	45
	AY950268.1	48	53	18	27	27	35
	AY950236.1	24	32	57	0	23	20
	AY950255.1	19	0	32	0	18	65
	AY950250.1	33	26	0	0	50	20
	AY950241.1	0	0	29	50	0	0
	AY950262.1	43	0	0	0	41	20
H7N9	KF001507.1	19	26	32	9	36	75
	KF001508.1	19	26	57	18	41	35
	KF001509.1	55	16	0	36	27	10
	KF001516.1	0	63	0	36	23	25
	KF001510.1	19	37	0	27	64	20
	KF001517.1	0	47	0	23	18	10
	KC853764.2	29	0	32	0	0	15
	KF001511.1	0	0	0	59	14	0
Adenovirus 1	MH183293.1	19	58	29	27	50	50
Adenovirus 2	AC_000007.1	71	21	29	27	32	5
Adenovirus 3	DQ086466.1	43	63	32	32	50	45
Adenovirus 4	MG030483.1	24	21	36	64	36	25
Adenovirus 5	AC_000008.1	29	74	25	45	36	50
Adenovirus 7	MN164629.1	29	58	29	27	54	50
Adenovirus 55	MN052861.1	71	37	21	36	36	25
EB virus	V01555.2	76	37	21	32	59	30
Human cytomegalovirus	K02988.1	19	68	21	36	36	30
Rotavirus	AB741657.1	24	74	25	32	41	55

Microorganism	GenBank Accession	ORF1ab % Homology			N gene % Homology		
		F	R	Probe	F	R	Probe
Norovirus	NC_029645.1	71	32	36	27	23	25
Varicella zoster virus	X02132.1	62	37	46	32	32	30
Enterovirus A	NC_001612.1	28	63	32	31	31	35
Enterovirus B	NC_001472.1	38	57	21	50	45	30
Enterovirus C	HQ738303.1	33	57	21	45	36	40
Enterovirus D	NC_001430.1	23	47	14	50	45	50
Measles virus	NC_001498.1	28	63	39	27	40	35
Mumps virus	NC_002200.1	33	42	21	63	50	30

Cross-Reactivity Wet Testing Results with 3 Consecutive Production Lots Performed on the ABI 7500

Pathogens	Concentration	Lot #					
		nCoVP20200101		nCoVP20200102		nCoVP20200103	
		Mean Ct Value					
		ORF1ab＆N	Internal Control	ORF1ab＆N	Internal Control	ORF1ab＆N	Internal Control
Novel A H1N1	≥10^5pfu/ml	Negative	25.17	Negative	25.35	Negative	25.48
Human coronavirus229E	≥10^5pfu/ml	Negative	24.97	Negative	25.14	Negative	25.26
Human coronavirus OC43	≥10^5pfu/ml	Negative	24.87	Negative	25.04	Negative	25.16
Haemophilus influenzae	≥10^6cfu/ml	Negative	24.85	Negative	25.00	Negative	25.12
Streptococcus pneumoniae	≥10^6cfu/ml	Negative	24.90	Negative	25.06	Negative	25.18
Klebsiella pneumoniae	≥10^6cfu/ml	Negative	24.80	Negative	24.96	Negative	25.07
H1N1(2009)	≥10^5pfu/ml	Negative	25.16	Negative	25.34	Negative	25.46
Seasonal H1N1[1]	≥10^5pfu/ml	Negative	24.99	Negative	25.20	Negative	25.33
H3N2	≥10^5pfu/ml	Negative	25.01	Negative	25.21	Negative	25.34
H5N1	≥10^5pfu/ml	Negative	25.16	Negative	25.34	Negative	25.47
H7N9	≥10^5pfu/ml	Negative	25.19	Negative	25.37	Negative	25.50
Influenza B virus	≥10^5pfu/ml	Negative	25.32	Negative	25.50	Negative	25.63
Respiratory syncytial virus A	≥10^5pfu/ml	Negative	25.34	Negative	25.52	Negative	25.64
Respiratory syncytial virus B	≥10^5pfu/ml	Negative	25.20	Negative	25.38	Negative	25.51
Parainfluenza virus	≥10^5pfu/ml	Negative	25.16	Negative	25.35	Negative	25.48
Adenovirus 1	≥10^5pfu/ml	Negative	25.09	Negative	25.27	Negative	25.40
Adenovirus 2	≥10^5pfu/ml	Negative	25.05	Negative	25.22	Negative	25.34
Adenovirus 3	≥10^5pfu/ml	Negative	25.11	Negative	25.29	Negative	25.42
Adenovirus 4	≥10^5pfu/ml	Negative	25.15	Negative	25.33	Negative	25.46
Adenovirus 5	≥10^5pfu/ml	Negative	25.22	Negative	25.40	Negative	25.54
Adenovirus 7	≥10^5pfu/ml	Negative	25.25	Negative	25.44	Negative	25.57
Adenovirus 55	≥10^5pfu/ml	Negative	25.15	Negative	25.32	Negative	25.45
Human partial lung virus	≥10^5pfu/ml	Negative	25.14	Negative	25.32	Negative	25.46
EB virus	≥10^5pfu/ml	Negative	24.99	Negative	25.21	Negative	25.34
Human cytomegalovirus	≥10^5pfu/ml	Negative	25.17	Negative	25.35	Negative	25.48
Rotavirus	≥10^5pfu/ml	Negative	25.18	Negative	25.36	Negative	25.49
Norovirus	≥10^5copies/μl	Negative	25.25	Negative	25.44	Negative	25.57
Varicella zoster virus	≥10^5pfu/ml	Negative	25.19	Negative	25.37	Negative	25.50
Mycoplasma pneumoniae	≥10^6cfu/ml	Negative	25.15	Negative	25.34	Negative	25.46
Chlamydia pneumoniae	≥10^6cfu/ml	Negative	24.99	Negative	25.22	Negative	25.35
Rhinovirus	≥10^5pfu/ml	Negative	24.94	Negative	25.15	Negative	25.27
Enterovirus A	≥10^5pfu/ml	Negative	25.08	Negative	25.26	Negative	25.38
Enterovirus B	≥10^5pfu/ml	Negative	25.13	Negative	25.30	Negative	25.43
Enterovirus C	≥10^5pfu/ml	Negative	25.09	Negative	25.27	Negative	25.40
Enterovirus D	≥10^5pfu/ml	Negative	25.06	Negative	25.24	Negative	25.37
Human lung virus	≥10^5pfu/ml	Negative	25.09	Negative	25.27	Negative	25.40
Measles virus	≥10^5pfu/ml	Negative	24.91	Negative	25.12	Negative	25.25
Mumps virus	≥10^5pfu/ml	Negative	24.73	Negative	24.90	Negative	25.03
Legionella	≥10^6cfu/ml	Negative	24.92	Negative	25.11	Negative	25.23
Staphylococcus aureus	≥10^6cfu/ml	Negative	24.94	Negative	25.11	Negative	25.24
BCG vaccine	/	Negative	25.05	Negative	25.23	Negative	25.36

[1]Seasonal H1N1 strain that circulates in China during fall and winter

/ concentration not provided

Cross-Reactivity Wet Testing Results with 3 Consecutive Production Lots Performed on the CFX-96

Pathogens	Concentration	Lot #					
		nCoVP20200101		nCoVP20200102		nCoVP20200103	
		Mean Ct Value					
		ORF1ab & N	Internal Control	ORF1ab & N	Internal Control	ORF1ab & N	Internal Control
Novel A H1N1	≥10^5pfu/ml	Negative	26.25	Negative	26.38	Negative	26.05
Human coronavirus229E	≥105pfu/ml	Negative	25.89	Negative	26.04	Negative	25.64
Human coronavirus OC43	≥105pfu/ml	Negative	26.09	Negative	26.20	Negative	25.86
Haemophilus influenzae	≥106cfu/ml	Negative	25.72	Negative	25.88	Negative	25.48
Streptococcus pneumoniae	≥106cfu/ml	Negative	26.10	Negative	26.21	Negative	25.89
Klebsiella pneumoniae	≥106cfu/ml	Negative	26.05	Negative	26.15	Negative	25.81
H1N1(2009)	≥10^5pfu/ml	Negative	25.86	Negative	26.02	Negative	25.62
Seasonal H1N1[1]	≥10^5pfu/ml	Negative	25.97	Negative	26.09	Negative	25.71
H3N2	≥10^5pfu/ml	Negative	25.87	Negative	26.02	Negative	25.63
H5N1	≥10^5pfu/ml	Negative	25.86	Negative	26.02	Negative	25.62
H7N9	≥10^5pfu/ml	Negative	25.83	Negative	26.00	Negative	25.59
Influenza B virus	≥10^5pfu/ml	Negative	25.95	Negative	26.07	Negative	25.70
Respiratory syncytial virus A	≥10^5pfu/ml	Negative	25.91	Negative	26.05	Negative	25.65
Respiratory syncytial virus B	≥10^5pfu/ml	Negative	26.20	Negative	26.33	Negative	26.01
Parainfluenza virus	≥10^5pfu/ml	Negative	25.99	Negative	26.11	Negative	25.73
Adenovirus 1	≥10^5pfu/ml	Negative	25.90	Negative	26.04	Negative	25.65
Adenovirus 2	≥10^5pfu/ml	Negative	26.01	Negative	26.12	Negative	25.76
Adenovirus 3	≥10^5pfu/ml	Negative	26.00	Negative	26.11	Negative	25.75
Adenovirus 4	≥10^5pfu/ml	Negative	25.98	Negative	26.09	Negative	25.73
Adenovirus 5	≥10^5pfu/ml	Negative	25.88	Negative	26.03	Negative	25.64
Adenovirus 7	≥10^5pfu/ml	Negative	26.06	Negative	26.17	Negative	25.82
Adenovirus 55	≥10^5pfu/ml	Negative	26.12	Negative	26.24	Negative	25.91
Human partial lung virus	≥10^5pfu/ml	Negative	25.85	Negative	26.01	Negative	25.60
EB virus	≥10^5pfu/ml	Negative	25.85	Negative	26.01	Negative	25.61
Human cytomegalovirus	≥10^5pfu/ml	Negative	25.91	Negative	26.05	Negative	25.66
Rotavirus	≥10^5pfu/ml	Negative	25.88	Negative	26.03	Negative	25.63
Norovirus	≥10^5copies/μl	Negative	25.92	Negative	26.06	Negative	25.67
Varicella zoster virus	≥10^5pfu/ml	Negative	25.85	Negative	26.01	Negative	25.60
Mycoplasma pneumoniae	≥10^6cfu/ml	Negative	26.01	Negative	26.11	Negative	25.75
Chlamydia pneumoniae	≥10^6cfu/ml	Negative	26.05	Negative	26.16	Negative	25.80
Rhinovirus	≥10^5pfu/ml	Negative	25.98	Negative	26.10	Negative	25.72
Enterovirus A	≥10^5pfu/ml	Negative	25.96	Negative	26.08	Negative	25.70
Enterovirus B	≥10^5pfu/ml	Negative	25.95	Negative	26.08	Negative	25.70
Enterovirus C	≥10^5pfu/ml	Negative	25.93	Negative	26.06	Negative	25.68
Enterovirus D	≥10^5pfu/ml	Negative	25.97	Negative	26.08	Negative	25.71
Human lung virus	≥10^5pfu/ml	Negative	26.00	Negative	26.10	Negative	25.74
Measles virus	≥10^5pfu/ml	Negative	26.05	Negative	26.16	Negative	25.82
Mumps virus	≥10^5pfu/ml	Negative	26.06	Negative	26.18	Negative	25.82
Legionella	≥10^6cfu/ml	Negative	26.00	Negative	26.11	Negative	25.74
Staphylococcus aureus	≥10^6cfu/ml	Negative	25.92	Negative	26.06	Negative	25.67
BCG vaccine	/	Negative	26.01	Negative	26.11	Negative	25.75

[1]Seasonal H1N1 strain that circulates in China during fall and winter

/ concentration not provided

Interfering Substances:

- An interfering substances study was performed to determine if common interferents that could be present in respiratory samples could impact device performance. Each endogenous/exogenous interfering substance was evaluated at the highest medically relevant concentration (worst case) with samples spiked at 3X LoD (positive contrived sample consisting of spiked inactivated virus in pooled negative oropharyngeal swab clinical matrix). Prepared samples with each interfering substance were extracted with the Wantai Nucleic Acid Extracting Reagent and three replicates were tested using the Bio-Rad CFX-96 System.

Endogenous/Exogenous Interfering Substances Evaluated in Interference Testing

Interfering substance	Description	Lot	Mean Ct Value		
			ORF1ab	N	Internal Control
0.2 mg/ L of beclomethasone	Anti - inflammatory and anti - allergenic	nCoVP20200101	36.41	34.92	26.25
		nCoVP20200102	36.08	34.01	26.30
		nCoVP20200103	36.20	33.57	26.35
0.15 mg/L of dexamethasone	Corticosteroid	nCoVP20200101	36.98	33.96	26.32
		nCoVP20200102	36.09	33.97	26.37
		nCoVP20200103	36.24	33.99	26.23
12 mg/L of triamcinolone	Corticosteroid	nCoVP20200101	36.69	34.29	26.26
		nCoVP20200102	35.75	34.02	26.40
		nCoVP20200103	36.33	34.51	26.37
0.4 mg/L of budesonide	Corticosteroid	nCoVP20200101	35.71	33.94	26.29
		nCoVP20200102	36.67	34.01	26.28
		nCoVP20200103	36.03	34.04	26.44
0.05 mg/L of mometasone	Anti-inflammatory	nCoVP20200101	36.00	34.19	26.52
		nCoVP20200102	35.85	33.98	26.35
		nCoVP20200103	36.30	33.94	26.24
0.5 mg/L of fluticasone nasal spray (Flonase Allergy)	Corticosteroid	nCoVP20200101	36.53	34.82	26.33
		nCoVP20200102	36.34	34.83	26.32
		nCoVP20200103	36.44	35.00	26.37
75 mg/L of benzocaine	Liposoluble topical anesthetic	nCoVP20200101	36.33	34.42	26.35
		nCoVP20200102	36.06	33.80	26.38
		nCoVP20200103	35.75	34.03	26.51
5 mg/L of zanamivir	Antiviral	nCoVP20200101	36.06	33.94	26.52
		nCoVP20200102	36.79	34.14	26.46
		nCoVP20200103	36.48	34.12	26.54
37.5 mg/L of oseltamivir	Antiviral	nCoVP20200101	36.70	34.74	26.71
		nCoVP20200102	36.56	33.98	26.56
		nCoVP20200103	35.89	33.97	26.46
75 mg/L of tobramycin	Antibiotic	nCoVP20200101	36.02	34.28	26.34
		nCoVP20200102	36.97	34.62	26.28
		nCoVP20200103	35.73	34.13	26.31
50 mg/L of amantadine	Antiviral	nCoVP20200101	35.49	34.15	26.35
		nCoVP20200102	36.08	33.95	26.47
		nCoVP20200103	36.36	34.05	26.60
75 mg/L of sulfur	Nasal gel	nCoVP20200101	36.37	33.70	26.49
		nCoVP20200102	35.97	33.71	26.32
		nCoVP20200103	36.24	34.27	26.19
150 mg/L of thryallis	For relieving respiratory allergies (Chinese traditional medicine)	nCoVP20200101	35.91	33.53	26.27
		nCoVP20200102	36.49	33.99	26.36
		nCoVP20200103	36.39	34.25	26.57
50 mg/L of Amantadine Hydrochloride	Antiviral	nCoVP20200101	36.97	34.32	26.88
		nCoVP20200102	36.62	33.98	27.07

Interfering substance	Description	Lot	Mean Ct Value		
			ORF1ab	N	Internal Control
		nCoVP20200103	36.74	33.70	26.84
0.125 mg/L of adrenaline	Hormone	nCoVP20200101	36.15	34.78	26.40
		nCoVP20200102	36.13	34.02	26.41
		nCoVP20200103	36.49	33.89	26.67
25m g/L of menthol	Saturated cyclic alcohol	nCoVP20200101	37.13	34.04	26.94
		nCoVP20200102	36.14	33.35	26.64
		nCoVP20200103	36.36	33.69	26.31
0.05% of hydroxymethazoline	Nasal spray	nCoVP20200101	35.68	33.89	26.22
		nCoVP20200102	36.64	33.77	26.20
		nCoVP20200103	36.13	34.41	26.40
500 mg/L of flunisolide nasal spray	Corticosteroids	nCoVP20200101	36.74	33.98	26.36
		nCoVP20200102	36.09	34.51	26.52
		nCoVP20200103	37.28	33.82	26.68
500 mg/L of mupirocin	Antibiotic	nCoVP20200101	36.34	33.61	26.47
		nCoVP20200102	36.62	33.42	26.27
		nCoVP20200103	36.03	33.54	26.09
400 mg/L of purified mucin	Mucin	nCoVP20200101	36.19	34.71	26.31
		nCoVP20200102	36.49	34.25	26.12
		nCoVP20200103	35.70	34.81	26.26
200 µL of hemolytic blood	Blood	nCoVP20200101	36.07	33.60	26.21
		nCoVP20200102	36.35	33.65	26.28
		nCoVP20200103	36.02	33.86	26.34

Clinical Evaluation:

- To evaluate the clinical performance of the Wantai SARS-CoV-2 RT-PCR Kit confirmed negative and positive oropharyngeal swab specimens were evaluated with both the Wantai kit and another EUA authorized molecular comparator assay. All 76 specimens (36 positive and 40 negative clinical samples) were collected at the Third People's Hospital of Shenzhen, China and tested with both assays. Samples tested with the Wantai SARS-CoV-2 RT-PCR Kit were extracted with the Wantai Nucleic Acid Extracting Reagent and run on the ABI7500 platform. From initial testing there were 17 inconclusive results. Upon repeat testing using new extracted nucleic acid from residual clinical specimens (as indicated in the result interpretation table), all 17 results were considered positive for SARS-CoV-2. The evaluation demonstrated 100% positive and negative percent agreement between the Wantai SARS-CoV-2 RT-PCR Kit and the EUA authorized comparator assay.

Summary of Oropharyngeal Swab Evaluation of the Wantai SARS-CoV-2 RT-PCR Kit Compared to Another EUA Authorized Assay

Oropharyngeal Swabs		Comparator - EUA Authorized Assay		
		Positive	Negative	Total
Wantai SARS-CoV-2 RT-PCR Kit	Positive	36	0	36
	Negative	0	40	40
	Total	36	40	76
Positive Percent Agreement (PPA)		36/36; 100% (95% CI 90.36-100%)[1]		
Negative Percent Agreement (NPA)		40/40; 100% (95% CI 91.24-100%)[1]		

[1]Two-sided 95% score confidence intervals

16. References

- **Association between Clinical, Laboratory and CT Characteristics and RT-PCR Results in the Follow-up of COVID-19 patients**

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- **Application and optimization of RT-PCR in diagnosis of SARS-CoV-2 infection**

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- **Highly accurate and sensitive diagnostic detection of SARS-CoV-2 by digital PCR**

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- **Household Secondary Attack Rate of COVID-19 and Associated Determinants**

  Qin-Long Jing, Ming-Jin Liu, Jun Yuan, Zhou-Bin Zhang, An-Ran Zhang, Natalie E Dean, Lei Luo, Meng-Meng Ma, Ira Longini, Eben Kenah, Ying Lu, Yu Ma, Neda Jalali, Li-Qun Fang, Zhi-Cong Yang, Yang Yang.

- **Viral Kinetics and Antibody Responses in Patients with COVID-19**

  View ORCID ProfileWenting Tan, Yanqiu Lu, Juan Zhang, Jing Wang, Yunjie Dan, Zhaoxia Tan, Xiaoqing He, Chunfang Qian, Qiangzhong Sun, Qingli Hu, Honglan Liu, Sikuan Ye, Xiaomei Xiang, Yi Zhou, Wei Zhang, Yanzhi Guo, Xiu-Hua Wang, Weiwei He, Xing Wan, Fengming Sun, Quanfang Wei, Cong Chen, Guangqiang Pan, Jie Xia, Qing Mao, Yaokai Chen, View ORCID ProfileGuohong Deng.

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Marking Symbols:

IVD	**In Vitro Diagnostic Medical Device**		**<-15°C Storage Conditions**
	Use By	LOT	**Batch**
Σ	**Content Sufficient For <n> Tests**		**Instructions For Use**
REF	**Catalog Number**		**Manufacturer**

Version: V. 2020-01 [Eng.]

Issuing Date: March 4, 2020

Wantai Molecular Diagnostics

WANTAI Nucleic Acid Extraction Kit (Magnetic Beads Method)

INSTRUCTIONS FOR USE

REF ZCT1246 V. 2020-01 [Eng.] Σ 48 IVD

Read the package insert carefully and completely before performing the assay. Follow the instructions and do not modify them. Only by strict adherence to these instructions, the erroneous results can be avoided and the optimal performance of the WANTAI Nucleic Acid Extraction Kit (Magnetic Beads) achieved.

INTENDED USE

WANTAI Nucleic Acid Extraction Kit (Magnetic Beads) is used for extraction and purification of viral nucleic acid (DNA or RNA) from serum, plasma, oropharyngeal and, nasopharyngeal swabs, sputum, endotracheal aspirate, bronchoalveolar lavage fluid, urine and other biological samples. The extracted nucleic acids can be used directly for molecular testing.

PRINCIPLE OF THE EXTRACTION METHOD

WANTAI Nucleic Acid Extraction Kit (Magnetic Beads) uses magnetic beads to extract and purify viral DNA or RNA from biological samples. The virus is lysed and the released viral nucleic acid is specifically adsorbed to the surface of magnetic beads. Proteins and salt ions on the surface of the magnetic beads are removed by washing and the viral nucleic acid are then eluted and enriched.

COMPONENTS

IVD In Vitro Diagnostic Use Only

Components	Specification	Description
LH Buffer	19mL x1 vial	Guanidine thiocyanate solution for sample lysis
Wash Buffer A	7mL x1 vial	Saline solution for washing of the magnetic beads
Wash Buffer B	7mL x1 vial	Saline solution for washing of the magnetic beads
W/E Buffer	3mL x1 vial	Low saline solution for nucleic acid elution
Bictex	1mL x1 vial	A magnetic bead suspension used to adsorb nucleic acids
Protease K	0.5mL x1 vial	For protein degradation / viral lysis

MATERIALS REQUIRED BUT NOT PROVIDED

Anhydrous ethanol and isopropyl alcohol which are required for the preparation of the reagents, are not provided in this kit.

Recommended instruments for automatic extraction: Thermo Kingfisher Flex 96, TANBead SLA-32, NEXOR32, or other magnetic beads extractor with acceptable quality.

STORAGE AND STABILITY

This kit can be stored at 2-8°C for 12 months from the date of manufacture. Avoid heavy pressing, humidity, heat and light during storage.

PRECAUTIONS AND SAFETY

TO BE USED ONLY BY QUALIFIED PROFESSIONALS

1. For in vitro diagnostic use (IVD) only.
2. For Emergency Use Authorization only.
3. For Prescription Use only.
4. The Wantai Nucleic Acid Extraction Kit has not been FDA cleared or approved.
5. The Wantai Nucleic Acid Extraction Kit has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high complexity tests.
6. The Wantai Nucleic Acid Extraction Kit has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
7. The Wantai Nucleic Acid Extraction Kit is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
8. Please read the instructions carefully before use and operate in strict accordance with the requirements.
9. This reagent is only used for in vitro testing, and the operation should be carried out in strict accordance with the instructions. Make sure that the reagents are not expired (EXP Date indicated on the kit box). Components from different batch numbers should not be mixed.Do not use the components of any other type of test kit as a substitute for the components in this kit.
10. All the waste and specimens should be treated in case of transmitting disease and must be properly disinfected (autoclaving is preferred) before disposal.
11. Use routine laboratory precautions. Do not eat, drink or smoke in the area where samples and kit reagents are handled. Avoid any contact between hands, eyes or mouth during sample collection and testing.
12. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when handling patient samples. Wash hands thoroughly after handling specimens and kit reagents.
13. Make sure that anhydrous ethanol and isopropyl alcohol have been added to the corresponding solutions and stored at the required temperature.
14. Do not change the sample volume.
15. All laboratory personnel using the kit must be appropriately trained in molecular diagnostic techniques and use appropriate laboratory and personal protective equipment when handling this kit.

PROCEDURE

1. Reagents preparation:

- Add 6mL of isopropyl alcohol to 19mL of LH Buffer for final volume of 25mL **LH Buffer**. Mix well.

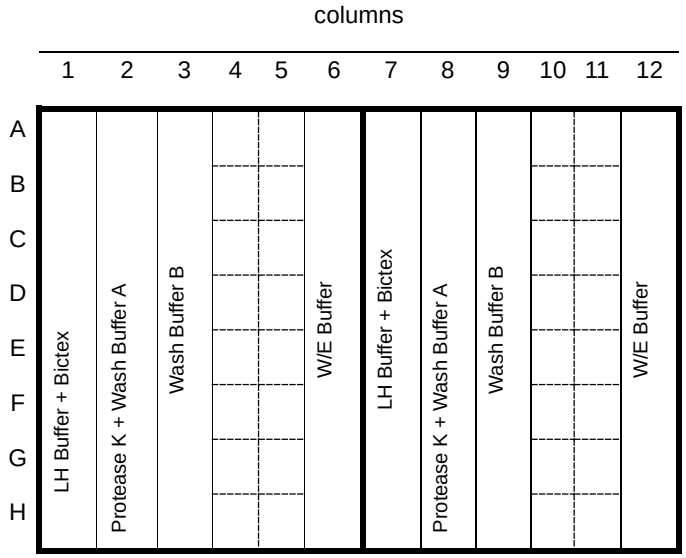
- Add 8mL of isopropyl alcohol to 17mL of Wash Buffer A for a final volume of 25mL **Wash buffer A**.Mix well.
- Add 18 mL of anhydrous ethanol to 7 mL of Wash Buffer B for a final volume of 25 mL **Wash Buffer B**. Mix well.

After ethanol and alcohol have been added, the reagents are stable within the kit's shelf-life when stored at 2-8°C.

2. Automatic Extraction:

Extraction plate preparation:

- For 16 extractions, in a 96 deep well plate, group 6 columns to the left and 6 to the right of the plate as shown in the scheme below. For 8 extractions, use only 6 left columns.



- Add the reagents to each well of the columns.

Column 1 (7)	Add 400μL of LH Buffer Add 20μL of Bictex
Column 2 (8)	Add 10μL of Protease K Add 500μL of Wash Buffer A *Protease K and Wash buffer A can be mixed in advance and stored for later use.Add 510 μL/well.*
Column 3 (9)	Add 500μL of Wash Buffer B
Column 6 (12)	Add 50μL of W/E Buffer

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Specimen extraction:

- Add 200μL of specimen to each well of columns 1 or 7.
- Place the plate inside the extraction instrument. Insert the magnetic rod.
- Turn on the instrument and execute the extraction program with the following settings:

Column 1	Low speed blending for 5min Magnetic absorption for 40s
Column 2	Low speed washing for 4min， Magnetic absorption for 40s;
Column 3	Low speed washing for 1min Magnetic absorption for 40s, Place still for 3min;
Column 6	Moderate speed washing at 55℃ for 2min, Magnetic absorption for 30s

- Take the supernatant from column 6 or 12 as a template for PCR amplification.

3. Manual extraction:

Step 1	Add 20μL of Bictex and 400μL of LH Buffer to a 1.5 mL centrifuge tube and mix well. Add 200μL of specimen and mix for 5min by turning the tube upside down.
Step 2	Place the tube on magnetic rack for 2min then thrown away the supernatant after magnetic bead deposition. Add 10μL of protease K and 500μL of Wash Buffer A. Mix by turning upside down for 5min.
Step 3	Place the tube on the magnetic rack for 2 min then thrown away the supernatant after magnetic bead deposition. Add 500μL of Wash Buffer B and mix.
Step 4	Place the tube on the magnetic rack for 2 min then thrown away the supernatant after magnetic bead deposition. Instant centrifugation for 30s is conducted and supernatant is thrown away again. Then it is left to dry out at room temperature for 5min.
Step 5	Add 50μL of W/E Buffer and mixed by turning upside down, then heat at 55°C for 3 min.
Step 6	Place the tube on the magnetic rack for 2 min. The supernatant is then taken as a template for PCR amplification.

Note: make sure that there is no residual liquid after the supernatant is thrown away in each step. Centrifuge for 30s if needed.

PERFORMANCE CHARACTERISTICS

Purity and recovery of viral nucleic acid with this kit:

Sample type	Purity (A_{260}/A_{280})	Extraction efficiency
DNA	1.8-1.9	≥95%
RNA	1.9-2.0	≥95%

LIMITATIONS

1. Solid tissue samples, such as liver, feces, leaves, etc., shall be pretreated accordingly before nucleic acids are extracted with this kit.

2. If the sample volume is less than 200μL, the sample should be diluted accordingly.

CE MARKING SYMBOLS:

In Vitro Diagnostic Medical Device

+2°C~+8°C Storage Conditions

Use By

Batch

Content Sufficient For <n> Tests

Instructions For Use

CE Marking – IVDD 98/79/EC

EU Authorized Representative

Catalog Number

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