Diagnostic Kit for Novel-Coronavirus (2019-nCoV) RNA (Isothermal Amplification-Real Time Fluorescence Assay) Instructions for Use

CE IVD

[PROPRIETARY NAME]
Diagnostic Kit for Novel-Coronavirus (2019-nCoV) RNA (Isothermal Amplification-Real Time Fluorescence Assay)

[COMMON OR USUAL NAME]
EasyNAT SARS-CoV-2 Assay

[SPECIFICATION]
Σ 20

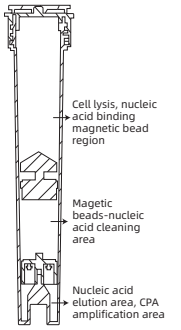
[INTENDED USE]
This kit is used for the *in vitro* qualitative detection of the novel coronavirus (2019-nCoV) ORF1ab gene, N gene in nasopharyngeal and oropharyngeal swabs, sputum, saliva samples from patients with suspected cases of pneumonia with novel coronavirus infection, patients with suspected clustered cases, and others who require a novel coronavirus infection diagnosis or differential diagnosis.
The defnitions of "suspected cases" and "suspected clustered cases" and other groups are implemented with reference to documents such as the "Pneumonitis Diagnosis and Treatment Program for Novel Coronavirus Infection" and "Monitoring Scheme for Pneumonia Cases with Novel Coronavirus Infection." Carrying out the detection of novel coronavirus (2019-nCoV) RNA should meet the requirements of "Technical Guide for Laboratory Testing of Pneumonia of Novel Coronavirus Infection" and take good biological protection measures. The results of the kit are for clinical reference only and should not be used as the sole standard for clinical diagnosis. It is recommended to conduct a comprehensive analysis of the condition in combination with the clinical manifestations of the patient and other laboratory tests.

[MATERIALS PROVIDED]

No	Name	Specification	Quantity	Main compositions
1	COVID-19-Cartridge	1 test/cartridge	20 cartridges	Tris, MgSO4, 2019-nCoV specific primers and probe, human GAPDH specific primers and probes, deoxyribonucleoside triphosphate (dNTP), DNA polymerase
2	COVID-19-RNA Extraction Solution	1 mL/test/tube	20 tubes	Guanidine salt, magnetic beads
3	COVID-19-Positive Control	1.2 mL/tube	1 tube	Pseudovirion contains ORF1ab and N gene
4	COVID-19-Negative Control	1.2 mL/tube	1 tube	Pseudovirion contains human GAPDH gene

Note:
1. The components in different batches of kits cannot be used interchangeably.
2. Material required but not provided: Sampling solution (Youkang Hengye Biotechnology (Beijing) Co., Ltd/JingXieZhuZhun20182400236), proteinase K buffer (not necessary for nasopharyngeal and oropharyngeal swabs or saliva test).

[TEST PRINCIPLE]



The COVID-19-Cartridge in this kit is equipped with multiple hydrophobic separation layers to isolate the lysate, cleaning solution and reaction solution in the cartridge. Controlled by an external instrument, the extraction is chemically lysed at high temperatures to test the sample and release the nucleic acid. Through the magnetic permeability of the external instrument, the nucleic acid of the specimen used for detection passes through different liquid layers, and finally the nucleic acid is eluded in the cartridge leg and the amplification reaction occurs, so as to realize the "one tube" full-automatic nucleic acid analysis, that is, the lysis, binding, cleaning, elution and amplification reaction are completed in a closed cartridge.
This kit uses Cross Priming Amplification (CPA[1]) to detect ORF1 ab and N gene sequences specific to the novel coronavirus (2019-nCoV). By virtue of specific amplification primers, specific fluorescence probes, and highly active reverse transcriptase and DNA polymerase with strand displacement activity, the reaction system can complete the specific amplification process of novel coronavirus fragments at a single time at a constant temperature, and the fluorescence signal is detected by applicable instrument and real-time fluorescence curve is automatically generated.
The kit includes internal controls (IC) which consist of a set of CPA system that specifically detects human GAPDH mRNA to monitor the effectiveness of sampling, extraction, purification, and amplification reactions. COVID-19-Cartridge is pre-loaded with nucleic acid purification reagents, nucleic acid elution reagents and CPA reaction reagents. Before starting the test, the operator only needs to add COVID-19-RNA Extraction Solution and the sample into cartridge, then the RNA in the sample can be purified automatically under the control of the applicable instrument. After the purified RNA and CPA reaction reagent are mixed and heated by the applicable instrument, the constant temperature amplification will be carried out, and the fluorescence probe will specifically bind to the target to produce fluorescence signal. The instrument can collect the fluorescence signal in real time and analyze the change of the fluoresce signal to determine the test result automatically.

[STORAGE AND STABILITY]
1. Storage conditions: 2℃-8℃
2. Validity period: 6 months. For production date and expiry date, please refer to the label.
3. The product performance will not be affected by ambient temperature (-25℃-30℃) transportation within 15 days.

[SAMPLE COLLECTION AND HANDLING]
1.Applicable sample type: Nasopharyngeal and Oropharyngeal swabs, sputum, saliva.
2.Sample collection
(refer to "Technical Guidelines for Laboratory Testing of Pneumonia Due to Novel Coronavirus Infection (Current Edition)")
a) **Nasopharyngeal swab:** Gently insert the sterile swab into the nasal palate of the nasal canal, and then Slowly rotate to collect the secretion. Immerse the swab head in the tube containing 3mL of sampling solution (Youkang Hengye Biotechnology (Beijing) Co., Ltd./JingXieZhuZhun20182400236), discard the tail, and tighten the tube cap tightly.
b) **Oropharyngeal swab:** Gently wipe the bilateral pharyngeal tonsils and the posterior pharyngeal wall simultaneously with two plastic rod swabs of polypropylene fiber head, immerse the swab head in a tube containing 3mL of sampling solution (Youkang Hengye Biotechnology (Beijing) Co., Ltd./JingXie-Zhu-Zhun20182400236), discard the tail, and tighten the tube cap tightly.
c) **Sputum:** After the patient is asked to have a deep cough, collect the coughed sputum in a screw mouth plastic tube containing 3mL of sampling solution (Youkang Hengye Biotechnology (Beijing) Co., Ltd./JingXie-ZhuZhun20182400236), and screw the tube cap tightly.
d) **Saliva:** Spit the saliva into the collection funnel gently until the liquid reach 4mL. Gently turn the saliva collector to make the saliva flow into the collection tube completely, and screw the tube cap tightly.
3.Sample Pre-treatment
Nasopharyngeal and oropharyngeal swabs, saliva:
3.1 Skip this inactivation step if you are using Disposable Sample Collector produced by Ustar to collect swab sample. Only if inactivation is needed, place nasopharyngeal, oropharyngeal swabs and saliva sample in a 56℃ water bath for 30 minutes.
3.2 The sampling solution/sample preservation solution of the nasopharyngeal, oropharyngeal swabs and saliva sample specimen is used as the test sample and can be added to the cartridge directly.
Sputum:
3.3 Add an equal volume of proteinase K buffer (0.4mg/mL) to the sputum sample, vortex or shake for 15s.
3.4 Place sputum sample in a 56℃ water bath for 30 minutes to inactivate.
3.5 Centrifuge the inactivated sputum sample at 13,000 rpm for 3 minutes, and the supernatant is used as the test sample and can be added to the cartridge directly.
4.Storage
According to the "Technical Guide for Laboratory Testing of Pneumonia of Novel Coronavirus Infection (Current Edition)", nasopharyngeal and oropharyngeal swabs, saliva or sputum samples should be submitted for inspection as soon as possible. Specimens that can be tested within 24 hours can be stored at 2℃-8℃; Samples can be stored at -70 ℃ or below for 6 months (if -70℃ storage condition is not available, they can be temporarily refrigerated at -25℃-15℃ for 2 weeks using Ustar VTM). A special library or counter should be set up to keep specimens separately. Avoid repeated freeze-thaw cycles when transporting samples.

5. Transportation
Specimens should be sent to the laboratory as soon as possible after collection. If the specimens need to be transported over long distances, it is recommended to use dry ice for preservation.

[APPLICABLE INSTRUMENT]
Nucleic Acid Amplification and Detection Analyzer (UC0102/UC0104/UC0108/UC0116) produced by Ustar Biotechnologies (Hangzhou) Ltd.

[TEST PROCEDURE]
Please test in accordance with this IFU.
1.Sample testing
1.1 Sample loading
1.1.1 Fully mix the COVID-19-RNA Extraction Solution, and then transfer all the solution into the COVID-19-Cartridge. If there is any remaining liquid, transfer it with a pipette. It is abnormal to find any crystals in the tube.
1.1.2 Add 500μL (nasopharyngeal and oropharyngeal swabs or saliva test sample) or 200μL (sputum supernatant test sample) into the COVID-19-Cartridge, screw the cap tightly, gently mix the lysis mixture (paraffin may float, but it will not influence subsequent steps). Then, the cartridge is ready to be tested.
1.2 Testing
Note: this section lists basic steps for running the test. For more details, please refer to the operator manual of applicable instrument.
1.2.1 Input cartridge information
Put the QR code of the cartridge (located on the cover of the cartridge) prepared in accordance with [*TEST PROCEDURE*] 1.1 in the scanning area of the instrument, and the system will automatically input the cartridge information and run the program CoV10 used for amplification testing. If the QR code cannot be input automatically, click the [*Scan QR code on the cartridge*] button on the touch screen to input it manually.
1.2.2 Input sample information
Put the sample barcode in the scanning area of the same instrument, scan the barcode and the system will automatically read and enter the sample information. If the information cannot be input automatically, click the [*Scan the sample barcode*] button on the touch screen to input it manually.
1.2.3 Start the test
Put the cartridge into a module of applicable instrument, close the module cover, and click the [*Start*] button on the touch screen to start the testing program. The instrument will start the test procedure and the countdown will be displayed on the screen. The total test duration was 49 minutes.
1.3 Result viewing
Test results will be displayed and saved automatically by instrument at the end of testing. Please refer to the [*INTERPRETATION OF RESULTS*] for details.
2.Quality control testing
2.1 Fully mix the COVID-19-RNA Extraction Solution and transfer all the solution into the COVID-19-Cartridge. If there is any remaining liquid, transfer it with a pipette. It is abnormal to find any crystals in the tube.
2.2 Add 500μL COVID-19-Positive Control or COVID-19-Negative Control to COVID-19-Cartridge, cover the cap, gently mix the lysis mixture (paraffin may float, continue to do the next step) and the cartridge is ready to be tested.
2.3 Follow the steps described in [*TEST PROCEDURE*] 1.2-1.3.

[CUT-OFF VALUE]
As soon as the test is completed, the analyzer will report test results automatically.
The analyzer has early termination function, which means test will be terminated and report "Positive" once target fluorescence reaches setting threshold.
In the cartridge, the left amplification area is targeted at ORF1ab gene, and the right amplification area is targeted at N gene.
The analyzer calculates Tt values for the amplification curves of gene ORF1ab, N, and both ICs respectively, and reports positive or negative results according to following criteria:
●If the Tt value of gene ORF1ab is N/A, test result for gene ORF1ab will be "Negative". If the Tt value of gene ORF1ab≤25, test result for gene ORF1ab will be "Positive".
●If the Tt value of gene N is N/A, test result for gene N will be "Negative". If the Tt value of gene N≤25, test result for gene N will be "Positive".
●If the Tt value of an IC is N/A, test result for the corresponding IC will be "Negative". If the Tt value of an IC≤25, test result for the corresponding IC will be "Positive".

[INTERPRETATION OF RESULTS]

Test Result	Interpretation
Positive	Gene ORF1ab and/or N is "Positive", which means 2019-nCoV RNA is detected in the sample. Note: Test result for either IC is not required.
Negative	Both gene ORF1ab and N are reported as "Negative", and ICr and/or ICl is "Positive", which means no 2019-nCoV RNA is detected in the sample.
Invalid	Gene ORF1ab, N, ICr, and ICl are all "Negative", which means the test fails in detecting 2019-nCoV RNA or human GAPDH mRNA.
No Result	Insufficient data for analysis.

1.Conditions that require retesting
If any of the following conditions occur, please retest with a new COVID-19-Cartridge.
●Invalid results. Invalid results may be caused by improper sample collection/processing procedures, inhibition of test reagent, or expired products.
●No results. The test is terminated before the due time.
●Abnormal results of external quality control (COVID-19-Positive Control and COVID-19-Negative Control). For example, if the COVID-19-Negative Control reports a positive result, there may be contamination from the experimental environment.
2.Quality control
The kit provides internal quality control and external quality control.
2.1 Internal quality control
Internal Quality Control is intended to monitor failures in sample collection, sample processing, amplification reagents, and malfunctions of analyzers.
If a positive result is reported, the test results of ICs are not required.
If a negative result is reported, at least one IC must be positive (IC Tt≤25).
The analyzer reports Invalid results if amplification fails in all fluorescence channels.
2.2 External quality control
The COVID-19-Positive Control in the kit is an artificially constructed pseudovirion containing the target gene of novel coronavirus. It is used to monitor the failure of the amplification reagents, and the malfunctions of analyzers. The COVID-19-Negative Control is an artificially constructed pseudovirion containing human GAPDH target fragment to monitor potential contamination from the experimental environment.
The test result of COVID-19-Positive Control should be Positive (Tt≤25 for ORF1ab or N), while the test result of COVID-19-Negative Control should be Negative (Tt=N/A for ORF1ab and N, and Tt≤25 for at least one IC).

[LIMITATIONS]
1. The test results of this assay are for clinical reference only. The clinical diagnosis and treatment of patients should be considered in combination with their symptoms, signs, medical history, other laboratory tests and therapeutic effect.
2. False negative results may come from improper sample collection, transportation, and processing procedures, or samples with low viral load.
3. Other unverified interference or amplification inhibitors may yield false negative results.

[PERFORMANCE CHARACTERISTICS]
1.Consistency with positive reference panels
National positive reference panels: all positive.
Enterprise positive reference panels: all positive.
2.Consistency with negative reference panels
National negative reference panels: all negative.
Enterprise negative reference panels: all negative.
3.Consistency with repeatability reference panels
National repeatability reference panels: all positive.
Enterprise weak positive reference panels: all positive
Enterprise moderate positive reference panels: all positive.
4.Limit of detection (LOD): 200 copies/mL.
Using the national sensitivity reference panels, test results were positive on S1-S7 samples, which meet the acceptance criteria.
5.Repeatability
Three different lots of products were tested on moderate positive samples, weak positive samples, and negative samples, by two operators in twenty days. The inter- and intra-lot repeatability, inter- and intra-day repeatability, as well as inter- and intra-operator repeatability met the acceptance criteria. The consistency rate was 100%.
6.Interfering substances

The following substances have no effects on test results of this kit.

6.1 Endogenous interfering substances

Human plasma (1% v/v), human mucoprotein (1% v/v), human genome DNA (300ng), nasal spray or nose drops: Benfolin, methotrexate, sodium chloride (with preservative) (1% v/v).

6.2 Exogenous interfering substances

Beclomethasone, dexamethasone, flunisolone, triamcinolone acetonide, budesonide, mometasone, fluticasone; histamine hydrochloride; interferon α, zanamivir, ribavirin, oseltamivir, peramivir, arbidol, lopinavir, ritonavir; Mupirocin, levofloxacin, azithromycin, cephalosporin, minocycline, tobramycin.

7.Cross-reactivity

Inactivated cultures or nucleic acid extracts from following respiratory pathogens have no effect on the performance of the kit, including: *human coronavirus (HKU1, OC43, NL63 and 229E), SARS coronavirus, MERS coronavirus; Influenza virus (H1N1, H3N2, H5N1, H7N9), influenza B virus BY, influenza B virus BV, respiratory syncytial virus A, B, parainfluenza virus 1, 2, 3, rhinovirus, adenovirus, Enterovirus, Human Interstitial Pneumovirus (Human Metapneumovirus), Epstein Barr Virus, Measles Virus, Human Cytomegalovirus, Rotavirus, Norovirus, Mumps Virus, Varicella-Zoster Virus; Mycoplasma Pneumoniae, Pneumonia Chlamydia; Legionella, Pertussis, Haemophilus influenzae, Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Klebsiella pneumoniae, Mycobacterium tuberculosis; Aspergillus fumigatus, Candida albicans, Candida smoothus, Cryptococcus neoformans.*

8.Clinical evaluation

Clinical trials of 592 samples were completed in five clinical institutions, compared with comparison kit that have been approved for marketing: positive compliance rate of 98.0%, negative compliance rate of 95.4%, and a total compliance rate of 96.3%.

[PRECAUTIONS]

1.This product contains no human-derived materials.

2. Please read this IFU carefully before use.

3.Before using the product, test COVID-19-Positive Control and COVID-19-Negative Control. For the operation method, please refer to [*TEST PROCEDURES-Quality control testing*].

4. This kit is a disposable product.

5. All samples and other materials should be processed in accordance with [*Medical Waste Management Regulations*] [2] after use.

6.The work table and required items should be regularly disinfected with 1% sodium hypochlorite, 75% alcohol or ultraviolet light.

7.The COVID-19-RNA Extraction Solution contains insoluble particles. Please mix well before pipetting.

8.Please ensure that the QR code of the COVID-19-Cartridge (located above the cover of cartridge) is clean, clear and should not be written or painted, so as not to atect the function of QR code.

9.COVID-19-Cartridge shall be amplified immediately once opening.

10.Do not open the lid of the sample module when running test.

11.The instrument will save test results automatically, please check in the view interface.

12.Do not squeeze the middle and lower part of the COVID-19-Cartridge when operating.

13.Please operate in strict accordance with the instructions. The COVID-19-Cartridge is not allowed to insert first.

14.Operators should use the product in a laboratory with biosafety protection and need to wear protective equipment.

15.All items and samples to be tested in the laboratory shall be considered to be contagious, and all operations in the laboratory shall conform to the [*General Guidelines for Biosafety of Microbiology Laboratory*] [3].

16.Performance changes may happen due to various factors during the storage, transportation and use of reagents. For example, inappropriate storage and transportation, non-conformity of sample collection, processing and testing procedures. Please strictly follow the instructions. Due to the characteristics of the sample collection and virus infection, results may be false negative caused by insufficient sample volume. Other clinical diagnosis and treatment information should be considered for comprehensive determination. Perform retesting if necessary.

[REFERENCES]

1.Xu G, Hu L, Zhong H, *et al.* Cross priming amplification: mechanism and optimization for isothermal DNA amplification. Sci. Rep. 2012; 2:246.

2.Regulations on the management of medical waste: promulgated by the state council of the People's Republic of China on June 16, 2003.

3.General Guidelines for Biosafety in Pathogenic Microbiology Laboratories: Issued by the National Health and Family Planning Commission of the People's Republic of China on July 24, 2017.

[EXPLANATION OF SYMBOLS]

IVD	*In vitro* diagnostic medical device		Do not re-use
	Use-by date		Consult instructions for use
	Caution		Manufacturer
	Temperature limit	LOT	Batch code
EC REP	Authorized representative in the European Community		Keep dry
	Keep away from sunlight		Do not use if package is damaged
	Date of manufacture		Biological risks
	Contains sufficient for <*n*> tests	REF	Catalogue number
CE	The product meets the basic requirements of European *in vitro* diagnostic medical devices directive 98/79/EC.		

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EC REP **Lotus NL B.V.**

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[INSTRUCTION VERSION AND MODIFICATION DATE]

Approved on March 13, 2020. Version: A0

Revised on April 3, 2020. Version: A1

Revised on May 19, 2020. Version: A2

Revised on September 27, 2020. Version: A3

Revised on December 9, 2020. Version: A4

Revised on December 28, 2020. Version: A5

Revised on January 27, 2021. Version: A6

Revised on April 22, 2021. Version: A7

Revised on April 27, 2021. Version: A8

Revised on May 10, 2021. Version: A9

Revised on July 9, 2021. Version: B0

Revised on Aug. 20, 2021. Version: B1

Revised on Aug. 30, 2021. Version: B2

Revised on September 10, 2021. Version: B3