EasyNAT SARS-CoV-2 Omicron Variant Assay

Instructions for Use

[PRODUCT NAME]

EasyNAT SARS-CoV-2 Omicron Variant Assay

[SPECIFICATIONS]

Σ 20

[INTENDED USE]

This assay is intended for qualitative detection of SARS-CoV-2 Omicron variant. It detects characteristic mutations of SARS-CoV-2 Omicron variant using oropharyngeal swab, nasopharyngeal swab samples from patients who have been diagnosed of COVID-19.

The SARS-CoV-2 belongs to the β virus genus. COVID-19 is an acute respiratory infectious disease. People are generally susceptible. Currently, the patients infected by the SARS-CoV-2 are the main source of infection; asymptomatic infected people can also be an infectious source. Based on the current epidemiological investigation, the incubation period is 1 to 14 days, mostly 3 to 7 days. The main manifestations include fever, fatigue and dry cough. Nasal congestion, runny nose, sore throat, myalgia and diarrhea are found in a few cases.

When using the assay, please comply with “Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia”, “Control Protocol for Novel Coronavirus Pneumonia”. Detection of novel coronavirus (2019-nCoV) RNA should be carried out in accordance with "Technical Guideline for Laboratory Testing of Novel Coronavirus Pneumonia", and ensure sufficient protection measures for biological safety.

The results of the kit are for clinical reference only and shall 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 patients and other laboratory tests.

[MATERIALS PROVIDED]

No.	Name	Specification	Quantity	Main components
1	SARS-CoV-2 Omicron Variant-Cartridge	1 test/cartridge	20 cartridges	Specific primers and probe, human GAPDH specific primers and probes, deoxyribonucleoside triphosphate (dNTP), DNA polymerase, reverse transcriptase
2	SARS-CoV-2-RNA Extraction Solution	1 mL/test/tube	20 tubes	Guanidine salt, magnetic beads

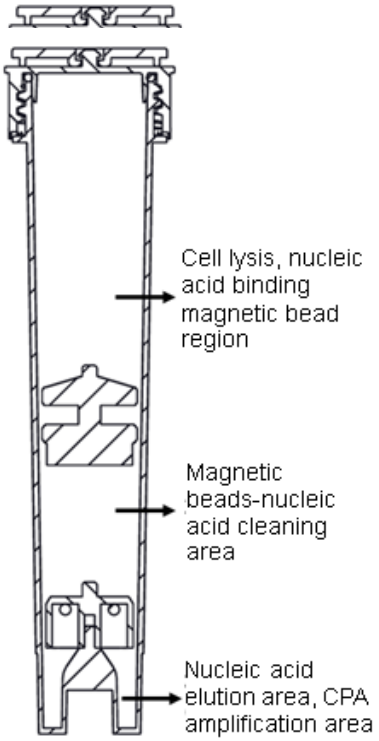
3	SARS-CoV-2 Omicron Variant-Positive Control	1.2 mL/tube	1 tube	Nucleic acid target fragment with characteristic mutations of SARS-CoV-2 Omicron variant
4	SARS-CoV-2-Negative Control	1.2 mL/tube	1 tube	Human GAPDH target fragment

The materials provided in different batches of kits cannot be used interchangeably.

[MATERIALS REQUIRED BUT NOT PROVIDED]

Disposable sample collector

[TEST PRINCIPLE]

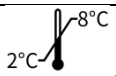


The SARS-CoV-2 Omicron Variant-Cartridge in this assay is equipped with multiple hydrophobic separation layers to separate the lysis buffer, washing solution and reaction solution. Heated by an external applicable instrument, the pathogen in the extraction solution will be chemically lysed and releases nucleic acids. Then, under the magnetic conductivity of the instrument, the released nucleic acids will pass through different layers respectively and finally be eluted in the cartridge legs for subsequent amplification reaction. Thus, the "one tube" automatic nucleic acid analysis is realized, that is, the lysis, washing, elution and amplification reactions are completed in a closed cartridge.

This assay uses cross priming amplification technology (CPA) to detect characteristic mutations of SARS-CoV-2 Omicron variant. It contains specific amplification primers, specific fluorescent probes and highly active reverse transcriptase and DNA polymerase with strand displacement activity. The CPA system can amplify the target fragments at a constant temperature. The fluorescence signal is automatically detected by the instrument and the amplification curve is automatically generated.

The SARS-CoV-2 Omicron Variant-Cartridge contains an internal control (IC) that consists of a CPA system to specifically detect human GAPDH mRNA and monitor the effectiveness of sampling, extraction, purification, and amplification reactions. The cartridge is pre-loaded with nucleic acid purification reagents, nucleic acid elution reagents and CPA reaction reagents. Before starting the test, the user only needs to add SARS-CoV-2-RNA Extraction Solution and the sample into cartridge. Then the RNA in the sample will be extracted automatically under the control of external applicable instrument. After the purified RNA and CPA reaction reagent are mixed and heated by the instrument, the CPA reaction will be carried out, and the fluorescence probe will specifically bind to the amplicon to produce fluorescence signal. The instrument can detect the fluorescence signal in real time and analyze the change of fluorescence signal to determine the test result automatically.

[STORAGE AND STABILITY]

1. Storage: store the assay at 

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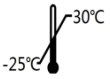
2. Validity Period: 6 months (provisional). See the label for the production and expiry date.

3. Transportation: Transport the assay at ambient temperature (

) within 15 days.

[SAMPLE COLLECTION AND HANDLING]

1. Sample types

Oropharyngeal swab, nasopharyngeal swab

2. Sample collection

a) Oropharyngeal swab

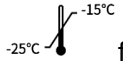
Gently wipe the bilateral pharyngeal tonsils and the posterior pharyngeal wall simultaneously with two plastic rod swabs of polypropylene fiber head, and immerse the swab head in a tube containing sample preservation solution. Then, discard the tail, and tighten the tube cap tightly.

b) Nasopharyngeal swab

Hold a disposable sterile nasal swab into the nasal cavity through the nasal lateral wall to the nasal palate (about 2.0-2.5 cm from the nostril), gently rotate the swab and then slowly remove it while wiping.

3. Storage

Samples should be sent for testing as soon as possible after collection. If not, they should be refrigerated at 

for not more than 24 hours. The samples can be stored at 

for one month and below - 70℃ for three months. Repeated freezing and thawing should be avoided.

4. Transportation

Transport samples with dry ice or in cold chain for not more than 7 days.

[APPLICABLE INSTRUMENT]

Nucleic Acid Amplification and Detection Analyzer (UC0102, UC0104, UC0108, UC0116) produced by Ustar Biotechnologies (Hangzhou) Ltd.

[TEST PROCEDURES]

1. Sample testing

Collect samples following the instructions of [*SAMPLE COLLECTION AND HANDLING*] 2 a) and 2 b).

1.1 Sample pretreatment

Add 1mL sterile saline into the sample storage tube, shake and mix thoroughly, and use it as the sample to be tested.

a) Inactivation: SKIP this step if you use the Disposable Sample Collector produced by Ustar to collect swab sample (Catalogue#: U40032 or U40035). If inactivation is needed, place oropharyngeal swab in a 56℃ water bath for 30 minutes.

b) The sample preservation solution of the oropharyngeal swab sample is used as the test sample and can be added to the cartridge directly.

1.2 Sample loading

1.2.1 Thoroughly mix the SARS-CoV-2-RNA Extraction Solution until there is no visible brown sediment nor crystals, and then transfer it to the SARS-CoV-2 Omicron Variant-Cartridge.

1.2.2 Add 500μL samples to the SARS-CoV-2 Omicron Variant-Cartridge, screw the cap tightly, gently mix the lysis mixture (paraffin may float, but it will not influence subsequent steps), and the cartridge is ready to be tested.

1.3 Testing

⚠ This section lists the basic steps for running a test. Refer to the Operator Manual of applicable instrument for details.

1.3.1 Input cartridge information

Put the QR code of the cartridge (located on the cover of the cartridge) prepared in accordance with [*TEST PROCEDURE*] 1.2 in the scanning area of the instrument, and the system will automatically input the cartridge information. If the QR code cannot be input automatically, click the [*Scan QR code on the cartridge*] button on the touch screen to manually input it.

1.3.2 Input sample information

Put the sample barcode in the scanning area of the same instrument, and the instrument will automatically input the sample information. If the information cannot be input automatically, click the [*Scan the sample barcode*] button on the touch screen to manually input it.

1.3.3 Start the test

Put the cartridge into the module of applicable instrument, close the cover of the module, and click [*START*] on the touch screen. The instrument will then start testing and the countdown will be displayed on the interface.

1.4 Result viewing

After testing, the results will be displayed and saved automatically. For details, please refer to *[INTERPRETATION OF RESULTS*].

2. Quality control testing

2.1 Thoroughly mix the SARS-CoV-2-RNA Extraction Solution, and then transfer it to the SARS-CoV-2 Omicron Variant-Cartridge.

2.2 Add 500μL SARS-CoV-2 Omicron Variant-Positive Control or SARS-CoV-2-Negative Control to SARS-CoV-2 Omicron Variant-Cartridge, screw the cap tightly.

2.3 Other steps are the same as [*TEST PROCEDURES*] Step 1.3-1.4.

[CUT-OFF VALUE]

As soon as the test program is completed, the instrument will report test results automatically.

The qualitative test results should display a typical S-type amplification curve (including the S-curve that has not reached the plateau phase).

- If the Tt value of target gene≤30, test result is **Positive;** otherwise, it is **Negative.**

- If the Tt value of an IC≤30, test result for the corresponding IC is **Positive;** Otherwise, it is **Negative.**

[INTERPRETATION OF RESULTS]

Test Result	Interpretation
Positive	SARS-CoV-2 Omicron variant RNA was detected in the sample. Note: When a positive result is detected, there is no requirement for the IC result.
Negative	SARS-CoV-2 Omicron variant RNA was not detected in the sample, and the internal control testing met the acceptance criteria.
Invalid	SARS-CoV-2 Omicron variant RNA or internal control testing did not meet the acceptance criteria. Test failed.
No Results	Insufficient data for analysis

1. Conditions that require retesting

If any of the following results occur, please retest with a new SARS-CoV-2 Omicron Variant-Cartridge.

- **Invalid results:** inappropriate handling or collecting of samples, test reagent is inhibited, or product is expired.
- **No results:** the test is terminated before the due time.
- **Abnormal results of external quality control** (SARS-CoV-2 Omicron Variant-Positive Control and SARS-CoV-2-Negative Control): for example, if the SARS-CoV-2-Negative Control reports a positive result, there may exist contaminants from the experimental environment.

2. Quality control

This assay contains Internal Control and External Control.

2.1 Internal control

Internal control is intended for monitoring failures of sample collection, sample processing, amplification, and malfunction of instrument.

If a SARS-CoV-2 Omicron variant positive result is reported, the test results of IC are not required. However, if a SARS-CoV-2 Omicron variant negative result is reported, at least one IC must be positive.

If amplification fails in all fluorescence channels, the instrument will report invalid results.

2.2 External control

External control of the assay includes SARS-CoV-2 Omicron Variant-Positive Control and SARS-CoV-2 Negative Control.

The SARS-CoV-2 Omicron Variant-Positive Control is an artificially constructed nucleic acid fragment containing the characteristic mutations of Omicron variant. It is used to indicate amplification failure and

instrument malfunction.

The SARS-CoV-2-Negative Control is an artificially constructed nucleic acid fragment containing human GAPDH target fragment to monitor potential contaminants from the experimental environment.

The test result of SARS-CoV-2 Omicron Variant-Positive Control should be positive, while the test result of SARS-CoV-2-Negative Control should be negative.

[LIMITATIONS]

1. Test results from 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. Inappropriate collection, transport and treatment of samples or low viral load may yield false negative result.

3. Other unverified interfering substances or amplification inhibitors may cause false negative results.

[PERFORMANCE CHARACTERISTICS]

1. Consistency with positive reference panels

Enterprise positive reference panels: all positive

2. Consistency with negative reference panels

Enterprise negative reference panels: all negative

3. Consistency with repeatability reference panels

Enterprise weak positive reference panels: all positive

Enterprise moderate positive reference panels: all positive

4. Limit of Detection (LOD)

The LOD of this assay is 1000 copies/mL.
Use the assay to test the enterprise sensitivity reference panels, and results were positive on S1-S3 samples, which meets the acceptance criteria.

5. Repeatability

Three different batches of assays were tested by two operators in twenty days on moderate positive samples, weak positive samples, and negative samples. The inter- and intra-batch repeatability, inter- and intra-day repeatability, as well as inter- and intra-operator repeatability met the acceptance criteria.

6. Interfering substances

No interfering effect was seen with the following interfering substances.

- **Endogenous interfering substances**

  Human plasma (1% v/v), human mucoprotein (1% v/v), human genomic DNA (1 x 10^4 copies, based on quantification of GAPDH gene)

- **Nasal spray or nose drops**

  Phenylephrine, sodium chloride (with preservative) (1% v/v)

- **Exogenous interfering substances**

Beclomethasone, dexamethasone, triamcinolone acetonide, budesonide, mometasone, fluticasone; histamine hydrochloride; interferon α, zanamivir, ribavirin, oseltamivir, peramivir, arbidol, lopinavir, ritonavir; levofloxacin, azithromycin, ceftriaxone, and tobramycin

7. Cross-reactivity

No cross-reactivity was seen with the inactivated cultures or nucleic acid extracted from the following pathogens: *Human coronavirus (HKU1, OC43 and NL63), SARS coronavirus, MERS coronavirus; Influenza virus H1N1, 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,* and *Cryptococcus neoformans*.

[PRECAUTIONS]

1. Please read this IFU carefully before use.

2. This assay does not contain human-derived materials.

3. Before use, the SARS-CoV-2 Omicron Variant-Positive Control and SARS-CoV-2-Negative Control should be tested. For the operation method, please refer to [*TEST PROCEDURES-Quality control testing*].

4. This assay is a disposable product, please do not reuse.

5. All samples and other materials should be disposed of in compliance with local regulations and standards.

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

7. The SARS-CoV-2-RNA Extraction Solution contains insoluble particles. Please mix well before pipetting.

8. Please ensure that the QR code of the SARS-CoV-2 Omicron Variant-Cartridge (located on the cap of cartridge) is clean and clear. Do not scribble or cover it to ensure the code can be read.

9. The SARS-CoV-2 Omicron Variant-Cartridge should be used immediately once opened.

10. Do not open the module when the test is running.

11. Test results will be saved automatically in the instrument, please check on the [View] interface.

12. Do not squeeze the middle and lower part of the SARS-CoV-2 Omicron Variant-Cartridge during operation.

13. Please operate in strict accordance with the instructions. The SARS-CoV-2 Omicron Variant-Cartridge is not allowed to insert first.

14. Conduct the testing in a laboratory equipped with biosafety protection facilities. Operators should

wear protective equipment.

15. All items and samples to be tested in the laboratory should be considered contagious, and all operations in the laboratory should conform to the “General Guidelines for Biosafety of Microbiology Laboratory”.

16. Performance alteration during the storage, transportation, and use of reagents may happen due to improper storage, transportation, collection, processing of samples and non-standard test procedures. Please strictly follow the instructions. The collection methods of samples may lead to virus infection or insufficient sample volume, which may yield false negative results. Hence, other issues should be comprehensively taken into consideration together with clinical diagnosis and treatment. Conduct retesting if necessary.

[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

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[INSTRUCTION VERSION AND MODIFICATION DATE]

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