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SARS-CoV-2 Variant Omicron (B.1.1.529) Real Time PCR Kit（RUO）

Product Name SARS-CoV-2 Variant Omicron (B.1.1.529) Real Time PCR Kit (Research Use Only)
Cat. No. JC10501NW-25T, JC10501NW-50T
Packaging Specification 25 tests/kit, 50 tests/kit

1. Intended Use

This kit is used for the in-vitro qualitative detection of ORF1ab and S-gene of SARS-CoV-2 in throat swabs, nasopharyngeal swabs and sputum. This kit can also be used to detect E484A, N679K, L981F, 69-70del and H655Y mutations on S-gene of SARS-CoV-2.

2. Background Information

The variation of B. 1.1.529 is quite different from other variants, with more than 30 mutations in the spike protein (S protein) and about 50 mutations in the entire genome. It is likely to be more infectious, toxic and immune to escape.

3. Product Description

This kit adopts the amplification refractory mutation system (ARMS) technique to detect the mutations. Primers for ORF1ab gene and mutations E484A, N679K, L981F, 69-70del and H655Y of S gene are specifically designed according to the SARS-CoV-2 genome. Probes for ORF1ab, E484A, N679K, L981F, 69-70del and H655Y are labeled with different fluorescent dyes respectively and the mutations will be detected by examining the ΔCt between the targeted mutation gene and the ORF1ab.
This kit contains an internal control (RNase P) to monitor the collection, transportation, and extraction of specimens to avoid a false negative result.

4. Kit Components

Component	Specifications		Ingredients
	25T	50T	
RT-PCR Buffer	750μL/vial ×1	750μL/vial ×2	Tris Hydroxy Methyl Aminomethane, potassium chloride, magnesium chloride, and dNTPs
RT-PCR Enzyme Mix	500μL/vial ×1	1000μL/vial ×1	Reverse transcriptase, RNase inhibitor, and Taq DNA polymerase
Reaction Mix A	125μL/vial ×1	250μL/vial ×1	Primers and probes of E484A, N679K and ORF1ab
Reaction Mix B	125μL/vial ×1	250μL/vial ×1	Primers and probes of L981F, 69-70del, H655Y and RNase P
Positive control	500μL/vial ×1	500μL/vial ×1	Virus-like particles of ORF1ab, S and RNase P
Negative control	500μL/vial ×1	500μL/vial ×1	Virus-like particles of RNase P

5. Materials and Devices Required but Not Provided

5.1 Extraction Kit

Manufacturer	Nucleic Acid Extraction Kit	Cat. No.
Bioperfectus Technologies	Viral Nucleic Acid Extraction Kit (Silica-Based Spin Column)	SDK60102
	Nucleic Acid Extraction Rapid Kit (Magnetic Bead Method)	SDKF60101
	Viral Nucleic Acid Extraction Kit (Magnetic Bead Method)	SDK60104
Qiagen	QIAamp Viral RNA Mini Kit	52904/52906

5.2 Instruments and Consumables

- Vortex mixer.
- Centrifuge
- Calibrated adjustable pipettes (10μL, 100μL, 200μL, 1000μL)
- Calibrated Adjustable Multi-channel pipette (5-50μL）
- 1.5 mL centrifuge tube shelf
- Magnetic grate for 1.5 mL centrifuge tube
- Specimen preservation fluid (Vial transfer medium)
- Appropriate real-time PCR system: Applied Biosystems 7500 (software version V2.3 and V2.4), QuantStudio™ 5 (software version V1.4.3 and V1.5.1), Roche LightCycler®480 (software version V1.5.1.62), Bio-Rad CFX96™ (software version V3.1), Bioperfectus STC-48A/96A/96A PLUS (software version V1.0.0).
- Nucleic acid extraction system: SSNP-2000B (32 channels), SSNP-3000A (64 channels), SSNP-9600A (96 channels), and SMPE-960 (96 channels)
- RNase-free Water, molecular grade
- 10% sodium hypochlorite or Pasteurized disinfectant
- Disposable particle-free gloves and operating gown
- Pipette tips with filter
- 1.5 mL centrifuge tube (No DNase/RNase)
- 0.2 mL PCR plate (Applied Biosystems)
- 0.2 mL PCR tube (Applied Biosystems)
- Biological safety cabinet or PCR hood

6. Warnings and Precautions

- For research use only.
- Usage of this product is limited to personnel being trained with real-time PCR operation and in vitro diagnostic procedures.
- Tested results only serve as clinical reference and cannot be solely used for decision-making of confirmed or excluded cases.
- All patient specimens should be inactivated at 56℃ for 30 minutes and processed in accordance with laboratory biosafety requirements. For more information, refer to
- https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html
- Nucleic acid extraction of SARS-CoV-2 should be manually carried out in biosafety cabinet or by automatic nucleic acid extraction system.
- Wear personal protective equipment (PPE), including (but not limited to) disposable clean powder-free gloves, masks, and goggles.
- Working zones in laboratory should be strictly separated. Use separated and segregated working areas for (i) Reagent preparation, (ii) Specimen preparation and (iii) Amplification. The workflow in the laboratory should proceed in unidirectional manner. The experiment processes shall comply with the Good Clinical Laboratory Practice (GCLP) for Molecular Based Tests Used in Diagnostic Laboratories. https://cdn.intechopen.com/pdfs-wm/23728.pdf
- Clean work benches, pipettes and centrifuge by using 10% sodium hypochlorite and 70% ethanol.
- The use of sterile disposable pipettes and nuclease-free pipette tips is recommended.
- Use applicable real-time PCR instrument and nucleic acid extraction system to ensure optimal test performance.
- Do not open the reaction tubes/plate after the amplification to avoid potential contamination with amplicons.
- Use reagents before expiry date. DON'T replace or interchange reagents from different batches or manufactures.
- Carefully read this instruction before starting the procedure
- Discard specimens and assay waste according to your local safety regulations. http://www.gov.cn/gongbao/content/2003/content_62236.htm

7. Storage

- Store at -20±5℃condition.
- Always check expiry date before use and do not use expired reagent.
- Keep probe away from light.
- Properly thaw and mix before reagent preparation.
- Avoid repeatedly freeze-thaw.
- Avoid exposure of the kit to excessive temperature or humidity.
- Manufacturing date and expiry date: see outer packing box.
- Ensure that it is stored on a flat surface.

8. Specimen Type

- Upper respiratory tract specimens: throat swab, nasopharyngeal swab.
- Lower respiratory tract specimens: sputum.

9. Specimen Collection, Transportation and Storage

Inappropriate sampling, storage and transportation may lead incorrect detection results. Following trainings are suggested.

9.1 Sampling

- For sampling, please refer to https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html
- Follow product instructions of instruments and consumables.
- Swab specimen should be collected using plastic swab with polypropylene fiber head. Wooden swab with cotton is NOT allowed. Specimen preserved at virus transport media after collection.
- Sputum specimen is collected into a 50mL plastic container with viral transfer medium. A digesting buffer (with 1g/L proteinase K in PBS) is added in the specimen tube at a 1:1 ratio (v/v), mixed with shaking, hold for 5 minutes before the nucleic acid extraction.
- Recommended specimen collection kit.

Manufacturer	Product Code	Contents
Yocon Biotech Co., Ltd	Jing 20182400236	1 × viral transfer medium 1 × plastic swab (sterile)
Jiangsu Kangjian Medical Apparatus Co., Ltd	Su 20180169	1 × viral transfer medium
Health Gene Technologies Co., Ltd	Zhe 20190062	1 × viral transfer medium
Jiangsu Bioperfectus Technologies Co., Ltd	Su 20200071	1 × viral transfer medium
Jiangsu Bioperfectus Technologies Co., Ltd.	Su 20200114	1 × viral transfer medium
Jiangsu Bioperfectus Technologies Co., Ltd.	N/A	Physical saline containing 0.9% Sodium chloride

9.2 Transportation

- Specimen packaging and transportation follows https://www.who.int/publications/i/item/9789240019720
- Pack 3 layers according to class A or B infectious articles if external transportation involves.
- Specimen collected from suspected SARS-CoV-2 cases should be preserved using 2-8°C ice bags or -70°C dry ice and sent to qualified laboratories within 24 hours.

9.3 Storage

- Specimen preserves at 2-8°C up to 24 hours after received
- Specimen preserves at -70°C or colder if extraction is arranged after 24 hours.

- Extracted RNA preserved at -70°C or colder for 6 months.

10. Reagent Setup (in the Reagent Preparation Area)

- Take out all the kit contents, thaw them at ambient temperature. Vortex and centrifuge briefly, and calculate the number of reagents to be prepared (N = the number of specimens + 2 control tubes). All reaction solutions A and B should be tested for each specimen. The reagents for each test are listed as follows:

Reagent Table for Reaction mix (Per Test)

Reagent	Volume/Test
RT-PCR Buffer	15 μL
RT-PCR Enzyme Mix	10 μL
Reaction Mix A/B	5 μL
Total Volume	30 μL

Calculate the total volume of reaction mix A and B separately, adding them into appropriate centrifuge tubes. After mixing, Pipette 30μL reaction mix A and B into different PCR tubes, respectively.

11. Specimen processing (in the Specimen Operations Area)

11.1 Nucleic acid extraction
Extraction follows instructions from kit manufacturer. Negative and positive controls shall fully be involved in the nucleic acid extraction process.
11.2 Add extraction sample eluate
Add 20 μL of nucleic acid specimens, positive control, and negative control to the prepared PCR tubes respectively, making each tube contain final volume of 50 μL mixture), tighten the tube cap, and centrifuge them instantaneously at low speed.

12. Amplification and Detection (in the Amplification Area)

12.1 Software Settings for manual interpretation of sample test results

- Place the PCR tubes into a real-time PCR system to run the amplification test.
- Follow the instructions for use of the ABI 7500 Real-Time PCR System.
- Cycling parameter setting

Step		Temperature	Time	Number of Cycles
1	Reverse transcription	50°C	10 min	1
2	Pre-denaturation	97°C	1 min	1
3	Denaturation	97°C	5s	45
	Annealing, extension and fluorescence detection	58°C	30s	
Fluorescence detection at 58°C in Step 3, channels: FAM, VIC, CY5 and ROX.				

Note: When setting ABI 7500 Real-Time PCR System, choose **None** for **Quencher**, and select **None** for **passive reference**.

- Result analysis

ABI 7500: The results are automatically saved after the reaction, and the **Start** value, **End** value, and **Threshold** value of **Baseline** should be adjusted as needed (e.g. **Start** value: 3-15, **End** value: 5-20) based on the image after analysis. The analysis results can be obtained by clicking **Analysis**, and viewed on the **Report** screen.

STC-48A/96A/STC 96A Plus: The results are automatically saved after the amplification. Baseline Start value, End value and Threshold value are set to 6, 12 and 0.12 respectively by default. All values can be manually updated. The analysis results can be viewed by clicking "Analyze".

12.2 Software Settings for automatic interpretation of sample test results

STC-48A/96A/STC 96A Plus：In the Project interface, click the **Import** option to import the project file containing the test result interpretation criteria for this product, which can realize the automatic interpretation of the test result and output in the Sample Conclusion of Well Information Table. Please contact support@bioperfectus.com for the details.

13. Cut-off Value

- Positive: Ct ≤ 40.0 and S-shaped amplification curve.
- Negative: Ct > 40.0 or Undet.
- Site mutation

ΔCt of mutation X of a specimen equals the Ct value of the specimen (Mx) minus the Ct value of the ORF1ab: ΔCt (x) = Ct (Mx) – Ct (ORF1ab); ΔCt can be negative. The cut-off ΔCt values of E484A, N679K, L981F, 69-70del and H655Y mutations are determined using the ROC curve analysis.

Mutation site	E484A	N679K	L981F	H655Y	69-70del
Cut-off ΔCt Value	8.0	8.0	6.0	8.0	8.0

Note: The specimen can be directly determined to be the wild-type of E484A, N679K, L981F, 69-70del and H655Y, if only the amplification curve of ORF1ab is observed during the detection.

14. Quality control

Reaction Tube	Channel	Mutation site	Positive control	Negative control
A	FAM	E484A	Ct≤30.0,ΔCt＜8.0	UNDET
	VIC	ORF1ab	Ct≤30.0	UNDET
	ROX	N679K	Ct≤30.0,ΔCt＜8.0	UNDET
B	FAM	L981F	Ct≤30.0,ΔCt＜6.0	UNDET
	VIC	69-70del	Ct≤30.0,ΔCt＜8.0	UNDET
	ROX	H655Y	Ct≤30.0,ΔCt＜8.0	UNDET
	CY5	RNaseP	Ct≤37.0	Ct≤37.0

15. Result interpretation and reporting

The results should be interpreted when the system, positive control, and negative control all work properly.

Reaction Tube	Channel	mutation site	Ct	ΔCt	Result Interpretation
A	FAM	E484A	≤40.0	<8.0	E484A, otherwise wild type
	VIC	ORF1ab	≤40.0	/	If the Ct value ≤ 40.0, the specimen can be considered to be positive for SARS-CoV-2. If not, the specimen can be considered to be negative for SARS-CoV-2.
	ROX	N679K	≤40.0	<8.0	N679K, otherwise wild type
B	FAM	L981F	≤40.0	<6.0	L981F, otherwise wild type
	VIC	69-70del	≤40.0	<8.0	69-70del, otherwise wild type
	ROX	H655Y	≤40.0	<8.0	H655Y, otherwise wild type
	CY5	RNaseP	≤37.0	/	The Ct value of CY5 channel (internal control) should be ≤ 37.0, otherwise new specimens need to be recollected.

When ORF1ab of a specimen is positive (Ct value ≤ 40.0) but the Ct value of the internal control > 37.0 or not detected, the result is still valid.

Variants are identified according to the above results interpretation of different sites.

E484A	N679K	L981F	69-70del	H655Y	Variant
Mutation	Mutation	Mutation	Mutation	Mutation	B.1.1.529

Note: The SARS-CoV-2 Variant.B.1.1.529 has mutations at the above sites, but if one of these sites is wild-type, it is considered as a special case and is still B.1.1.529 .

16. Limitations

- All operator, data analysis staff and results reporting staff should be trained and proven to have competence for doing test and explaining results independently. User of the kit is limited to staff who have successfully passed the training.
- The kit is only used for throat swab, nasopharyngeal swab, sputum, other upper and lower respiratory tract specimens (nasal swab, respiratory tract extract, bronchial perfusate and bronchoalveolar lavage fluid) were not validated.
- Negative results can neither straightforwardly exclude SARS-CoV-2 infection, nor the only decision-making evidence for treatment and patient management. Optimized specimen type and time of peek virus value induced by SARS-CoV-2 infection has not been finalized, and therefore multiple specimen collection (type and time) is necessary.
- Improper specimen collection, transportation and processing may lead to false negative results. Inhibitors or insufficient viral load may also lead to false negative results.
- SARS-CoV-2 may be undetectable if target genes of the virus mutate.
- Inhibitors and other interferences may lead false negative results.
- Epidemiological or clinical features of SARS-CoV-2 induced infection have not been fully understood and therefore the performance of the kit might be affected. For example, optimized specimen type and sampling time of which containing best viral RNA are still unknown.
- Detected viral RNA may not straightforwardly show infection, or SARS-CoV-2 is not the root cause of clinical symptoms.
- Detection using the kit cannot exclude other bacteria or pathogen induced diseases.

17. Performance characteristics

- Coincidence with internal standardized positive references: 100%
- Coincidence with internal standardized negative references: 100%
- Limit of detection (LoD): 500 copies/mL.
- Interfering substances: The test results are not affected by these interfering substances, including 5% fresh human blood, 5% nasal secretions, 5% mucus,

2g/dL mucoprotein, antiviral drugs (100 units/mL α-interferon, 5mg/L zanamivir, and 0.2g/L ribavirin), antibiotics (0.2% mupirocin, and 50mg/L aspirin) and systemic antibiotic (10mg/mL tobramycin).

- Precision: The coefficient of variation (CV, %) of the Ct values for 10 replicates of Bioperfectus's standardized reference controls was less than 5%.

18. References

[1] Clinical Management of Severe Acute Respiratory Infection When Novel Coronavirus (2019-nCoV) Infection is Suspected. WHO. 2020.
[2] Interim Measures for the Management of Clinical DNA Amplification Testing Laboratories YWF [2002] No.10.
[3] Omicron (B.1.1.529): Infectivity, vaccine breakthrough, and antibody resistance.2021.

19. Contact and Support

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For detailed programming instructions regarding the use of the Bioperfectus Technologies Real Time PCR Kits on specific Real Time PCR instruments please contact our Technical Support at E-mail: support@bioperfectus.com.