BioPerfectus

# Zika Virus/Dengue Virus/Chikungunya Virus

# Real Time PCR Kit

# IFU

Revision History

| No. | Version | Reviser | Revised Sections and Content | Revision Date |
|---|---|---|---|---|
| 1 | V1.0 | Cui Dongming | First release | 2021.05.12 |
| 2 | V1.1 | Wang Zhengfeng | Upgrade IFU to version 1.1<br>" For Professional Use Only " has been added. | 2021.06.12 |
| 3 | V1.2 | Li Lily | Upgrade IFU to version 1.2<br>Update the version format;<br>Add testing instruments. | 2022.04.23 |
| 4 | V1.3 | Li Lily | Upgrade IFU to version 1.3<br>Added interpretation of IC results at Result Interpretation. | 2022.06.15 |
| 5 | V1.4 | Wang Wenqing | Upgrade IFU to version 1.4<br>Update the address, and added E-mail;<br>Upgrade LOGO. | 2022.11.16 |
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Prepared by: 王雪婧 Date:2022.11.16

Reviewed by: Date:2022.11.16

Approved by: 李名林 Date:2022.11.16

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# INSTRUCTIONS FOR USE

IVD CE

## Product Name:
## Zika Virus/Dengue Virus/Chikungunya Virus Real Time PCR Kit

For use with Bioperfectus STC-96A, STC-96A PLUS, Applied Biosystems 7500, QuantStudio™ 5, Roche LightCycler®480, Bio-Rad CFX96™, QIAGEN Rotor-Gene Q, Analytik Jena qTOWER³ and other applicable Bioperfectus machines.

REF JC30302NW-25T/JC30302NW-50T Σ 25T/50T

**Jiangsu Bioperfectus Technologies Co., Ltd**
No. 837, Yaocheng Avenue, 225300 Taizhou City, Jiangsu Province, PEOPLE'S REPUBLIC OF CHINA.
**www.bioperfectus.com Tel: +86-21-34637616**

EC REP MedNet EC-REP GmbH
Borkstrasse 10•48163 Muenster•Germany

## 1. Intended Use

The Bioperfectus Zika Virus/Dengue Virus/Chikungunya Virus Real Time PCR Kit is intended for the *in vitro* qualitative detection and differentiation of RNA from dengue, chikungunya and zika viruses in human serum, whole blood(EDTA), cerebrospinal fluid (CSF) and for the detection of zika virus RNA in human urine, and amniotic fluid. The use of RT-PCR method is designed to facilitate simultaneous testing for the presence of dengue, chikungunya and zika viruses using a single sample.

## 2. Kit Components

| Components | Number of Vials | Volume (μL/Vial) 25T | Volume (μL/Vial) 50T |
|---|---|---|---|
| RT-PCR Buffer | 1 | 188 | 375 |
| RT-PCR Enzyme Mix | 1 | 125 | 250 |
| Reaction Mix | 1 | 188 | 375 |
| Positive Control | 1 | 500 | 500 |
| Blank Control (RNase-free Water) | 1 | 500 | 500 |

NOTE: Components from different batch number kit can't be used interchangeably.

## 3. Storage

- All reagents should be stored at -20±5°C until the expiration date listed on the outer kit box.
- Protect Reaction Mix from light during storage.
- Repeated freezing and thawing (more than five times) of reagents should be avoided.
- Manufacturing date and expiry date: see outer packing box.

## 4. Materials and Devices Required but Not Provided

- Appropriate real time PCR instrument: Bioperfectus STC-96A, STC-96A PLUS, Applied Biosystems 7500, QuantStudio™ 5, Roche LightCycler®480, Bio-Rad CFX96™, QIAGEN Rotor-Gene Q, Analytik Jena qTOWER³ and other applicable Bioperfectus machines.
- Appropriate Nucleic acid extractor： SSNP-2000B (32 channels), SSNP-3000A (64 channels), SSNP-9600A (96 channels), SMPE-960 (96 channels), SAW-96 (96 channels), SAW-48 (48 channels) and other applicable Bioperfectus machines.
- Magnetic grate for 1.5 mL centrifuge tubes.
- Centrifuge tube shelf.
- Centrifuge with a rotor for 1.5 mL reaction tubes.
- Centrifuge with a rotor for 0.2 mL reaction tubes or plate.
- Vortex mixer.
- Calibrated adjustable pipettes or multi-channel pipette.
- Pipette tips with filters.
- 1.5mL centrifuge tubes.
- 0.2 mL PCR tubes or plates.
- Disposable particle-free gloves and operating gown.
- 10% sodium hypochlorite or pasteurized disinfectant.
- Biological safety cabinet or PCR hood.

## 5. Background Information

Zika virus (ZIKV) belongs to flavivirus and is the pathogen of Zika virus disease. After being bitten by mosquitoes with Zika virus, the disease occurs about 3~7 days later. most people have mild symptoms, such as fever, rash, conjunctivitis, muscle and joint pain, general fatigue, and headache, which can last 2~7 days and heal itself. Recent studies have shown that the virus infection may lead to neurological and autoimmune system complications in a few people, and maternal infection may lead to fetal microcephaly. Dengue virus (DENV) belongs to the genus flaviviruses and is a dengue pathogen capable of A mosquito-borne infectious disease is caused by rapid transmission, high incidence and low mortality. However, dengue hemorrhagic fever syndrome can occur in dengue fever endemic areas, with a high mortality rate of 10~15. The main clinical manifestations of chikungunya fever, a pathogen of chikungunya virus (CHIKV), are fever, arthralgia / arthritis and rash. Although the mortality rate is very low, it is easy to form large scale in areas with high mosquito vector density Outbreaks and epidemics.

The Zika Virus/Dengue Virus/Chikungunya Virus Real Time PCR Kit is an IVD reagent replying on fluorescent PCR technology and aiming at qualitatively detect Zika virus, Dengue virus and Chikungunya virus. The kit contains oligonucleotides primer, double-labeled hydrolysis probe (Taqman) and quality controls for PCR reaction.

## 6. Technical Principle

The kit is based on real-time PCR technology. Specific primers and probes are designed based on specific gene areas of Zika virus (Polyprotein), Dengue virus (3'UTR) and Chikungunya virus (NSP1). Probes consist of a reporter dye at 5' and quenching dye at 3'. The fluorescent signals emitted from reporter dye are absorbed by the quencher, so it doesn't emit signals. During amplification, probes bonded to templates are cut off by Taq enzyme (5'-3'exonuclease activity), separating reporter dye from the quencher, and generating fluorescent signals. The PCR instrument will then automatically draw a real-time amplification curve based on the signal change, and finally realizing the qualitative detection of Zika, Dengue and Chikungunya virus at the nucleic acid level.In addition, the kit also contains human RNase P gene (labeled by CY5) for clinical sample detection.

## 7. Warnings and Precautions

- For in vitro diagnostic use only. For professional use only.
- Do not use components of the kit after expiration date.
- Test results only serve as clinical reference and can not be used as the basis of confirmed or excluded cases of illness individually.
- Use of this product is limited to personnel specifically instructed and trained in the techniques of Real Time PCR and in vitro diagnostic procedures.
- Wear protective disposable powder-free gloves, laboratory coat and eye protection when handling specimens and kit reagents.
- Avoid microbial and nuclease (DNase/RNase) contamination of the specimen and the components of the kit.
- Always use DNase/RNase-free disposable aerosol-blocking pipette tips.
- Use separated and segregated working areas for (i) reaction set-up, (ii) specimen preparation and (iii) amplification/detection activities. Workflow in the laboratory should proceed in unidirectional manner. Wear separate coats and gloves in each area.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Prepare the Master Mix on ice or in the cooling block.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not open the reaction tubes/plate post amplification to avoid contamination with amplicons.
- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
- Discard sample and assay waste according to your local safety regulations.
- Do not eat, drink, or smoke in the laboratory working area.
- Carefully read this instruction before starting the procedure.

## 8. Sample Preparation

### 8.1 Sampling

**For whole blood**

- For adults, a minimum volume of 4 mL whole blood is preferable.
- For pediatric samples, a minimum of 1 mL whole blood should be collected in pediatric-sized collection tubes.
- Blood must be collected in plastic collection tubes.
- Whole blood preserved with EDTA is preferred, but whole blood preserved with sodium polyanethol sulfonate, citrate or with clot activator is also acceptable.

**For serum**

- For each serum specimen, collect 5 ml of whole blood into a serum separator tube (marble or tiger top SST).
- A minimum of 1 ml of whole blood is needed for testing of pediatric patients.
- Allow whole blood to clot at room temperature for a minimum of 30 minutes and centrifuge.

Other types of specimen should be collected according to clinical laboratory guidelines.

### 8.2 Transportation

Specimen packaging and transportation follows regulations.

- Pack 3 layers according to class A or B infectious articles if external transportation involves.
- Specimen collected from suspected cases should be preserved using 2-8°C ice bags or -70°C dry ice and sent to qualified laboratories within 24 hours.

### 8.3 Sample transport and 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 preserves at -70°C or colder.

## 9. Procedure

### 9.1 RNA Extraction

Blank and positive controls should fully involve nucleic acid extraction process.

For reproducible isolation of nucleic acid, the following nucleic acid extraction systems and kits are recommended:

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

### 9.2 Master Mix Preparation

The Master Mix volume for each reaction should be pipetted as follows:

| Components | Volume |
|---|---|
| RT-PCR Buffer | 7.5 μL |
| RT-PCR Enzyme Mix | 5 μL |
| Reaction Mix | 7.5μL |
| Total Volume (Master Mix) | 20 μL |

Determine the number of extracted specimens to be tested, thaw the components. For maximal recovery of contents, briefly spin vials in centrifuge before opening. Mix carefully



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and thoroughly by pipetting up and down.

**9.3 RT-PCR Set-up Procedure**

Place your samples on ice. Follow the procedure below to prepare the RT-PCR Master Mix.
a. Pipette 20 µL of the Master Mix into each required reaction tubes/plate.
b. Add 5 µL isolated RNA or 5 µL the controls (Positive Control or Blank Control).
c. Make sure that every run including at least one Positive Control and one Blank Control.
d. Cap or seal the reaction tubes/plate and centrifuge using an appropriate centrifuge for 30 seconds at approximately 2,000 rpm.
e. Ensure that all liquid is at the bottom of the tubes/plate.
f. Perform the following protocol in the instrument.

| Step | | Temperature | Time | Cycle |
|---|---|---|---|---|
| 1 | Reverse transcription | 50℃ | 10 min | 1 cycle |
| 2 | Initial denaturation | 95℃ | 5 min | 1 cycle |
| 3 | Denaturation | 95℃ | 10 sec | 45 cycles |
| | Anealing, extension and fluorescent signal collection* | 60℃ | 30 sec | |

* Fluorescent signal should be collected during this step through the FAM,VIC,ROX and CY5 channels.

## 10. Real Time PCR System Operation

The following amplification protocol was developed for use on the Bioperfectus STC-96A, STC-96A PLUS. See the instrument operator's manual for detail. Other appropriate real time PCR instruments refer to the corresponding instrument operator's manual.

**10.1 Bioperfectus STC-96A/96A PLUS Real-Time PCR System Amplification Protocol**

1. Switch on Bioperfectus STC-96A/96A PLUS Real-Time PCR System.
2. Launch the Bioperfectus STC-96A/96A PLUS Real-Time PCR System software Version 1.0.
3. Click on "Experiment Wizard", and set up proper parameters in "Project" and "Setup".
4. Set up "Plate".
5. Set up "Sample".
6. Starting the PCR
a. Insert the 96 well PCR plate or reaction tubes into the machine.
b. Select the "Start Run" button,
7. Post PCR Analyze the data by pressing the "Analysis" button on left side of the menu and analyze the data using the "Analyze".

## 11. Data Analysis and Interpretation

**11.1 Threshold setting**

Above the maximum level of Blank Control.

**11.2 Quality control**

Prior to evaluating the specimen results, the Positive Control and Blank Control should be interpreted using the table below.

| Channels / Controls | Threshold cycle (Ct) value | | | |
|---|---|---|---|---|
| | FAM | VIC | ROX | CY5 |
| Blank Control | UNDET | UNDET | UNDET | UNDET |
| Positive Control | Ct≤30 | Ct≤30 | Ct≤30 | Ct≤30 |

- NOTE: Internal Control is specially designed to detect in fluorimeter channel CY5.
- The Positive Control and Blank Control should be included per PCR run.
- If the Positive Control and Blank Control do not meet the criteria, the entire run is invalid and results should not be reported. Repeat the entire process (specimen and control preparation, amplification and detection). If the repeat run is still invalid, please contact Technical Support.
- Viral transport media or previously characterized negative specimen may be used as an external negative control. This must be treated as a patient specimen in every extraction and PCR run.
- Additional controls may be used in accordance with local, state, federal accrediting organizations, as applicable.

**11.3 Limitations**

- Negative results do not preclude infection with Zika virus, Dengue virus and Chikungunya virus and should not be the sole basis of a patient treatment decision.
- Reliable results are dependent on the adequate specimen collection, transportation, storage and processing procedures.
- Inhibitors present in the sample and/or errors in the following assay procedure may lead to false negative results.
- A trained health care professional should interpret assay results in conjunction with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests.
- Potential mutations within the target regions of the virus genome covered by the tests primers and/or probes may result in failure to detect the presence of the pathogens.
- There is a risk of false positive values resulting from cross-contamination by target organisms, their nucleic acids or amplified product, or from non-specific signals in the assay.

**11.4 Result Interpretation**

The following results are possible: (FAM channel for Dengue virus, VIC channel for Zika virus, ROX channel for Chikungunya virus, CY5 channel for the internal control.)
A sample is considered positive if the Ct value obtained is less than or equal to 40 and the internal control shows or not an amplification signal. Sometimes, the detection of internal control is not necessary because a high copy number of target can cause preferential amplification of target-specific nucleic acids.
A sample is considered negative, if the sample shows no Ct value, or a Ct value of greater than 40 cycles in the detection system but the internal control is positive. An inhibition of the PCR reaction can be excluded by the amplification of internal control.
For RNaseP (CY5): Ct value ⩽40 is considered positive (+); Ct value >40 or UNDET is considered negative (-)
Using the following table read and analyze the results:

| | FAM Ct Value | VIC Ct Value | ROX Ct Value | CY5 Ct Value | Result Interpretation |
|---|---|---|---|---|---|
| 1 | + | — | — | +/- | Dengue virus Positive |
| 2 | — | + | — | +/- | Zika virus Positive |
| 3 | — | — | + | +/- | Chikungunya virus Positive |
| 4 | — | — | — | + | Negative |
| 5 | — | — | — | — | Invalid |

Invalid results should be taken consideration of repeat extraction and amplification and repeat sampling from patient if necessary.

## 12. Performance Evaluation

**12.1 Analytical Sensitivity**

The limit of detection of Zika Virus/Dengue Virus/Chikungunya Virus Real Time PCR Kit for the detection of dengue, chikungunya and Zika viruses RNA from serum, whole blood (EDTA), cerebrospinal fluid (CSF) and for the detection of Zika virus RNA from urine, and amniotic fluid was determined to be 5 copies/reaction.

**12.2 Analytical Specificity**

The analytical specificity of Zika Virus/Dengue Virus/Chikungunya Virus Real Time PCR Kit was evalu¬ated by testing a panel of genomic DNA/RNA extracted from other virus. The kit did not cross-react with Hepatitis C virus, Salmonella bongori, Salmonella typhi, Enterovirus, Marburg virus (Ci67), Japanese encephalitis virus, Saint Louis encephalitis virus, West Nile virus, Crimean-Congo Hemorrhagic Fever virus (Dubai), Lassa virus (Pinneo), Rift Valley Fever virus (SA51), Yellow fever virus, Hantaan virus, Seoul virus, Xinjiang hemorrhagic fever virus, Severe Fever with Thrombocytopenia Syndrome Bunyavirus, RSV, Adenovirus, Influenza virus type A and B, Group A Streptococcus, Leptospira, Haemophilus influenzae, Streptococcus pneumoniae, Pseudomonas aeruginosa, Measles virus.

**12.3 Precision**

1. Repeatability - The specimens with both 10×LOD and 100×LOD concentrations were detected as positive in all the 10 times of run, and the CV of the Ct value among those 10 tests were all less than 5% (%CV<5%).
2. Reproducibility - Results showed that inter/inner batch, inter/inner day and inter/inner operator were all less than 5% (%CV<5%).

## 13. Reference


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## 14. Appendix

**Index of Symbols**

| | | | |
|---|---|---|---|
| CE | CE certification | EC REP | Authorized representative in the European Community |
| IVD | In vitro diagnostic Medical device | | Use-by date |
| | Manufacturer | | Date of manufacture |
| REF | Catalogue number | Σ | Contains sufficient for <n> tests |
| | Consult instructions for use | | Temperature limit |
| LOT | Batch code | | This side up |

## 15. Contact and Support

For more information about Bioperfectus Technologies, please visit our web-site at: http://www.bioperfectus.com or contact at E-mail: info@bioperfectus.com.
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.