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Zika Virus (ZIKV) Real Time RT-PCR Kit User Manual

LT020640RE

For use with ABIPrism® 7000/7300/7500/7900/Step One Plus, iCycler iQ™4/iQ™5; Smart Cycler II, Bio-Rad CFX 96; Rotor Gene ™6000; Mx3000P/3005P; MJ-Option2/Chromo4; LightCycler®480 Instrument

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1.Intended Use

The Zika Virus (ZIKV) Real Time RT-PCR Kit is used for in vitro qualitative detection of Zika virus RNA in specimens of serum, saliva, urine. Specific RNA fragments of Zika virus are detected by utilizing the combination of the polymerase chain reaction (PCR) and Taqman technology, which is configured with a PCR instrument. The detection results are used to aid in diagnosis of Zika virus infection.

2.Principle of Real-Time PCR

The principle of the real-time detection is based on the fluorogenic 5'nuclease assay. During the PCR reaction, the DNA polymerase cleaves the probe at the 5'end and separates the reporter dye from the quencher dye only when the probe hybridizes to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored in real-time fashion by the PCR detection system. The PCR cycle at which an increase in the fluorescence signal is detected initially (Ct) is proportional to the amount of the specific PCR product. Monitoring the real-time fluorescence intensities allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

3.Product Description

Zika virus is icosahedral and enveloped with a non-segmented, single-stranded, positive sense RNA genome. It is most closely related to the Spondweni virus and is one of the two viruses in the Spondweni virus clade. The virus was first isolated in 1947 from a rhesus monkey in the Zika Forest of Uganda, Africa and was isolated for the first time from humans in 1968 in Nigeria. Common symptoms of infection with the virus include mild headaches, maculopapular rash, fever, malaise, conjunctivitis, and arthralgia. In 2009, it was proved that Zika virus can be sexually transmitted between humans.

The Zika Virus (ZIKV) real time RT-PCR Kit contains a specific ready-to-use system for the detection of the Zika Virus using RT-PCR (Reverse Transcription Polymerase Chain Reaction) in the real-time PCR system. The master contains a Super Mix for the specific amplification of the Zika Virus RNA. The action is done in one step real time RT-PCR. The first step is a reverse transcription (RT), during which the Zika Virus RNA is transcribed into cDNA. Afterwards, at thermo stable DNA polymerase is used to amplify the specific gene fragments by means of PCR (polymerase chain reaction). Fluorescence is emitted and measured by the real time systems´ optical unit during the PCR. The detection of amplified Zika Virus DNA fragment is performed in fluorimeter channel 530 nm with the fluorescent quencher BHQ1. In addition, the kit contains a system to identify possible PCR inhibition by measuring the 560 nm fluorescence of the internal control(IC). An external positive control defined as 1×10^7copies/ml is supplied, which allow the determination of the gene load.

4. Kit Contents

Ref.	Type of reagent	Presentation 25rxns
1	ZIKV Super Mix	1 vial, 480µl
2	RT-PCR Enzyme Mix	1 vial, 28µl
3	Molecular Grade Water	1 vial, 400µl
4	Internal Control (IC)	1 vial, 30µl
5	ZIKV Positive Control	1 vial, 30µl

Analysis sensitivity:1×10^3copies/ml

Note: Analysis sensitivity depends on the sample volume, elution volume, nucleic acid extraction methods and other factors. If you use the RNA extraction kits recommended, the analysis sensitivity is the same as it declares. However, when the sample volume is dozens or even hundreds of times greater than elution volume by some concentrating method, it can be much higher.

5. Storage

•All reagents should be stored at -20 ℃. Storage at + 4°C is not recommended.
•All reagents can be used until the expiration date indicated on the kit label.
• If the reagents are to be re-used, repeated thawing and freezing (> 3x) should be avoided, as this may reduce the sensitivity of the assay. Storage of aliquots is recommended.
•Cool all reagents during the working steps.
•Super Mix should be stored in the dark.

6. Additionally Required Materials and Devices

•Biological cabinet, RNA extraction kit, real time PCR reaction tubes/plates
•Desk top microcentrifuge for "eppendorf" type tubes (RCFmax.16,000 xg)
• Real time PCR system
•Cryo-container, biohazard waste container
•Pipets (0.5 µL–1000 µL), sterile filter tips for micropipets
•Sterile micro tubes, tube racks, vortex mixer, disposable gloves, powderless
•Refrigerator and freezer

7.Warnings and Precaution

•Carefully read this instruction before starting the procedure.
•For *in vitro* diagnostic use only.
•This assay needs to be carried out by skilled personnel.
•Clinical samples should be regarded as potentially infectious materials and should be prepared in a laminar flow hood.
•This assay needs to be run according to Good Laboratory Practice.
•Do not use the kit after its expiration date.
•Avoid repeated thawing and freezing of the reagents, this may reduce the sensitivity of the test.
•Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use.
•Prepare quickly the Reaction mix on ice or in the cooling block.
•Setup two separate working areas: 1) Isolation of the RNA/DNA and 2) Amplification/detection of amplification products.
•Pipets, vials and other working materials should not circulate among working units.
• Always use sterile pipette tips with filters.
•Wear separate coats and gloves in each area.
•Disposal of the experiment waste needs follow the applicable local, state, provincial and/or national regulations. Appropriate protective goggles, clothing, and gloves should be equipped.
•Do not pipette by mouth. Do not eat, drink, smoke in laboratory.
•Avoid aerosols

8. Sample Collection, Storage and transport

• Collected samples in sterile tubes.
• Specimens can be extracted immediately or frozen at -20°C to -80°C.
• Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents.

9.Procedure

9.1RNA-Extraction

RNA extraction kits are available from various manufacturers. You may use your own extraction systems or the commercial kit based on the yield. For the RNA extraction, please comply with the manufacturer's instructions. The recommended extraction kit is as follows:

RNA Isolation Kit GEN 52-904 LT

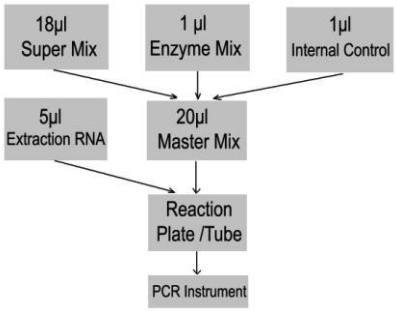
9.2 Internal Control

It is necessary to add internal control (IC) in the reaction mix. The IC allows the user to identify specimens that contain polymerase inhibitors. Use of the IC is an option for testing specimens routinely, testing only negative specimens, or designated specimen types in conformance with laboratory practices.

Add the internal control (IC) 1 µL/rxn and the result will be shown in the HEX/VIC/JOE.

9.3RT-PCR Protocol

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



※PCR system without HEX/VIC/JOE channel may be treated with 1µl Molecular Grade Water instead of 1µl IC.

1) The volumes of Super Mix and Enzyme Mix per reaction multiply with the number of samples, which includes the number of controls, standards, and sample prepared. Molecular Grade Water is used as the negative control. For reasons of unprecise pipetting, always add an extra virtual sample. Mix completely then spin down briefly in a centrifuge.
2) Pipet **20 µL** Master Mix with micropipets of sterile filter tips to each of the real time PCR reaction plate/tubes. Separately add **5 µL** RNA sample template, positive and negative controls to different plate/tubes. Immediately close the plate/tubes to avoid contamination.
3) Spin down briefly in order to collect the Master Mix in the bottom of the reaction tubes.
4) Perform the following protocol in the instrument:

45 °C for 10 min	1 cycle
95 °C for 15 min	1 cycle
95 °C for15 sec, 60 °C for 1 min (Fluorescence measured at 60 °C)	45 cycles

Selection of fluorescence channels	
FAM	Target Nucleic Acid
HEX/VIC/JOE	IC

5)⚠If you use ABIPrism®system, please choose **"none"** as **passive reference** and **quencher**.

10. Threshold setting: Just above the maximum level of molecular grade water.

11. Quality control: Negative control, positive control, internal control and QS curve must be performed correctly, otherwise the sample results is invalid.

Channel / Control	Ct value	
	FAM	HEX/VIC/JOE
Molecular Grade Water	UNDET	25~40
Positive Control(qualitative assay)	≤35	——
QS（quantitative detection）	Correlation coefficient of QS curve≤—0.98	

11. Data Analysis and Interpretation

The following results are possible:

	Ct value		Result Analysis
	FAM	HEX/VIC/JOE	
1#	UNDET	25~40	Below the detection limit or negative
2#	≤43	——	Positive; and the software displays the quantitative value
3#	43～45	25~40	Re-test; if it is still 43~45, report as 1#
4#	UNDET	UNDET	PCR Inhibition; no diagnosis can be concluded.