One-step Detection Kit for Zika Virus (QPCR-Probe)

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Research Use Only

[Product Name]

One-step Detection Kit for Zika Virus (QPCR-Probe)

[Packing Specification] 24 rxns/kit

[Intended Use]

This kit is used for the detection Zika Virus in blood, serum, urine and saliva samples. It offers auxiliary means for the diagnosis of the Zika Virus infected patients without nucleic acid extraction and purification. Test results should be combined with clinical diagnosis.

[Detection Principle]

This kit adopts PCR method combined with fluorescence probe in vitro amplification technology.

In this method, The Zika Virus probe contains a fluorescent reporter dye FAM at the 5' end of the probe and a quencher dye BHQ at the 3' end of the probe. When the probe is intact, the proximity of the reporter dye to the quencher dye suppresses the reporter fluorescence. Probe cleavage during the PCR reaction spatially separates the reporter dye from the quencher dye, thereby allowing detection of the reporter dye fluorescence. The fragments of reporter dye are displaced from the target, resulting in an increase in fluorescence. This step, which enables the fluorescence signal accumulation and PCR products formed synchronous, thus to achieve qualification detection the Zika Virus in the infected patients' in serum samples, which provide auxiliary means for the Zika Virus in the treatment of the patient.

[Kit Contents]

Ref.	Type of reagent	24 rxns
1	Zika-PCR Reaction Mix	1 vial,1200μL
2	Enzyme mix	1 vial,50μL
3	Positive Control	1 vial,50μL
4	Negative Control	1 vial,500μL
5	User Manual	1

[Storage]

• All reagents should be stored at below -20°C. Storage at +4°C is not recommended.

• All reagents are valid for one year, can be used until the expiration date indicated on the kit label.

[Warnings and Precaution]

• Repeated thawing and freezing (>4x) should be avoided, as this may reduce the sensitivity of the assay.

• Cool all reagents during the working steps.

• Super Mix should be stored in the dark.

• Carefully read this instruction before starting the procedure.

• 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.

• Pipets, vials and other working materials should not circulate among working units.

• Use always sterile pipette tips with filters.

• Wear separate coats and gloves in each area.

• Avoid aerosols.

[Sample Requirements]

• Sample source: whole blood, serum, urine and saliva samples

• Storage: samples can be frozen at -20°C to -80°C.

[Applicable instrument]

Coyote Bio Mini8 Real-Time PCR System

[Recommended consumable]

0.2mLPCRTube:

(1)Axygen® 0.2mL Polypropylene PCR Tube Strips (Product #PCR-0208-C) with PCR 1 x 8 Strip Flat Caps (Product #PCR-2CP-RT-C) / (Product #PCR-02-FCP-C).

(2)Axygen® 0.2mL Thin Wall PCR Tubes with Flat Cap(Product #PCR-02-L-C)/ (Product #PCR-02-C)

[Procedure]

1. Sample Preparation/Treatment

This kit does not need sample nucleic acid extraction and purification.

2. Preparation of amplification reagent

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

• Zika-PCR mix 43 μL+ Enzyme mix 2μL

Mix the components above before adding sample or controls

3.Adding samples and controls to the reaction tubes

• Separately add the samples from step 1, positive control and blank control to different tubes:

1)Separately add 2μL serum or 0.5μL blood or 2μL urine, or 2μLsaliva sample into the sample reaction tube.

2)Separately add 2μL Positive control into the reactions as positive control.

3)Separately add 2μL Negative control to the reaction as negative control.

4.PCR Amplification

Perform the following protocol in the instrument:

Pro.	Tem.	Time	Cycle No.
1	42℃	5min	1cycle
2	95℃	5 second	
	50℃	5 second	15 cycles
3	95℃	1min	1 cycle
4	95℃	5 second	
	50℃	10 second	40 cycles
	(acquire fluorescence)		

Selection of fluorescence channels:

530nm channel(Reporter FAM): Zika Virus;

Please refer to the instrument manual for specific channel set detection.

[Quality control]

Negative control: FAM detection channel should not have the logarithmic growth period;

Positive control: FAM detection channel should have the logarithmic growth period, and the Ct value ≤ 30;

Above condition should be all applied, this test is valid,or the test is invalid.

[Results interpretation]

a. if the sample Ct value ≤30, report positive;

b. if the sample Ct value 35>Ct>30, Please retest the sample. If the repeat result still<35, and the negative control is no value, the result is positive sample, and if the repeat sample result is no value, report as negative.

c. if the sample Ct value is ≥35 or no value, report negative.

[Limitations of the assay]

1. Detection result of this kit is only for clinical reference, clinical diagnosis and treatment to patients should be considered other factors as symptoms, medical history, other laboratory tests and therapeutic reaction.

2. False positive result is easy to caused by the contamination of amplification product and cross-contamination of specimens.

3. Negative result could not means the patient is non-infected, the details should be combined with other clinical findings to determine specific diagnosis. The reason lead to false negative result maybe:

- Unreasonable sample collection, transportation and treatment, low viral titer in a sample;
- Variation of virus detection target sequences;
- Unauthenticated other factors such as taking antiviral drugs;
- Infections caused by other viruses or bacteria.

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