Dengue virus/Chikungunya virus/Zika virus Diagnostic Kit (PCR-Fluorescence)

REF 106-0044-01

Product Name
Dengue virus/Chikungunya virus/Zika virus Diagnostic Kit (PCR-Fluorescence)

Specification
12 reactions/kit

Intended Use
Vector-borne diseases are infectious disease carried by arthropods and transmitted to susceptible vertebrate hosts through contact or bite. Currently, the global outbreak of multiple emerging and re-emerging vector-borne diseases, such as paludism, yellow fever, chikungunya fever, dengue fever, West Nile fever, and Zika virus infection-related microcephaly that has caused worldwide panic, brings a heavy public health burden worldwide.
This kit uses real-time RT-PCR method for in vitro qualitative detection and analysis of Chikungunya virus (CHIKV), Dengue virus (DENV), and Zika virus (ZIKV) in serum. The test results are for clinical reference only.

Test Principle
The Automated Fully Enclosed qPCR Instrument adopts the iCassette technology to automatically perform nucleic acid extraction, nucleic acid amplification test and data reading and result analysis. The instrument consists of two parts: nucleic acid extraction and multiplex fluorescent PCR. At the same time, a built-in QR code scanner can automatically identify the execution process corresponding to this kit. A uniquely designed software of the kit is used to perform nucleic acid extraction, whole-process PCR, result display and analysis. The Kit contains twelve sets of CV/DV/ZV iCassette, which include nucleic acid extraction reagents and PCR reagents. It is applicable to the Automated Fully Enclosed qPCR Instrument. Since this system is fully enclosed and performs automatic nucleic acid extraction and PCR reactions, it reduces direct cross-contamination of samples. For a complete description of the instrument, please refer to the instruction for use of the corresponding instrument.
The Kit adopts the real-time multiplex fluorescent real-time PCR technology to qualitatively detect the CHIKV, DENV and ZIKV in patients' serum samples in vitro.This product designs primers and fluorescent probes for detection based on Chikungunya virus E2 gene (HEX marker), Dengue virus 3'NC gene (FAM marker), Zika virus E gene (TEXAS RED marker), and also designs primers and probes for internal control (CY5 marker). Through the detection of the quality control sample, confirm whether the target virus is fully processed, and detect whether there are PCR reaction inhibitors.

Components

Kit Components			Content	Quantity
CV/DV/ZV iCassette (12 pcs)	Nucleic acid extraction reagent	CV/DV/ZV lyophilization A	Proteinase K	1 pc/iCassette
		CV/DV/ZV lyophilization B	Pseudovirus lyophilization containing internal control fragments	1 pc/iCassette
		Virus lysate solution	Guanidinium isothiocyanate	450 μL/iCassette
		Virus binding solution	Guanidinium isothiocyanate	175 μL/iCassette
		Virus washing solution	Sodium Chloride	900 μL/iCassette
		Virus eluent	Tris-HCl	100 μL/iCassette
		Magnetic beads	Magnetic Microspheres	10 μL/iCassette
		CV/DV/ZV PCR cosolvent solution	PCR Buffer, $MgCl_2$	40 μL/iCassette
	CV/DV/ZV PCR reagent	CV/DV/ZV lyophilization	Specific primer, probe, dNTP, enzyme	1 pc/iCassette
Control	CV/DV/ZV positive control		Pseudovirus containing target fragment	1tube(1000μL)
	CV/DV/ZV negative control		Sterilized purified water	1tube(1000μL)

Note:Components in kits with different batch numbers are not interchangeable

Storage condition and Shelf life
1. The kit can be stored at 2-8℃ and the shelf life is 9 months.
2. The transportation temperature range of the kit should be kept at 2-8℃.
3. Please do not open the iCassette lid before adding the sample. If you open the iCassette lid, it should be used within 30 minutes.

Applicable instruments
Automated Fully Enclosed qPCR Instrument: Galaxy Lite and Galaxy Pro.

Materials Required but Not Provided
- Automated Fully Enclosed qPCR Instrument : Galaxy Lite or Galaxy Pro.
- Leak-proof, sterile, screw-capped specimen collection containers.
- Disposable gloves, eye protection, laboratory coats, and labels or permanent marking pen.
- Centrifuge.
- Pipettes.
- Sterile pipette tips.

Sample Requirements
1. Applicable sample types: human serum sample
2. Sample collection
Human serum samples: Use sterile vacuum drying tubes to collect non-anticoagulated blood from patients, separate serum in time, and store in separate packages in cryopreservation tubes with screw caps and gaskets inside, and store them at low temperature after labeling.
3.Sample storage and transportation
Samples used for virus isolation and nucleic acid testing should be tested as soon as possible. Samples that can be tested within 24 hours can be stored at 2-8°C; samples that cannot be tested within 24 hours should be stored temporarily in a -20±5°C refrigerator. Blood samples can be stored below -20±5°C for not more than 1 week). A special warehouse or a special cabinet should be established to store samples separately. Repeated freeze-thaw cycles of samples during transportation should be avoided.
4.Principles of Biosafety Protection
All operations should comply with relevant local laws and regulations.

Test Method and Operation
1. Prepare CV/DV/ZV iCassette
1.1. Processing samples or control materials in the samples preparation room, Serum samples were collected from patients with 5 mL of non-anticoagulated blood. Centrifuge at 3000rpm for 5 minutes, then carefully transfer the supernatant to cryovials with a sterile pipette.
1.2 Open the package of CV/DV/ZV lyophilization, observe whether the CV/DV/ZV lyophilization is intact, replace the empty PCR tube on the iCassette with the PCR tube containing the CV/DV/ZV lyophilization, and make sure the PCR tube is screwed up.
1.3 Open the lid of the iCassette, and pipette 900 μL of sample or controls to the CV/DV/ZV iCassette sample compartment as shown in Figure 1. slowly, close the lid tightly.
1.4 Place the iCassette into the instrument. (Note: After the sample is added to the iCassette, the test needs to start running within 30 minutes)

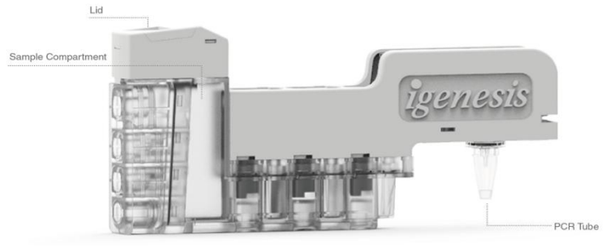

Figure 1. CV/DV/ZV iCassette (Lateral View)

2. Test Operation
2.1 Turn on the power, press the switch button on front side of the instrument, and the blue light is on, i.e. "the instrument is on". After the instrument is turned on, click the control software icon on the desktop to enter the login interface.
2.2 Log in to the software for the first time with the administrator account (Admin/123456), click "OK" to complete the login.
2.3 Click the "Open" button in the initial interface to open the compartment door of the instrument.
2.4 Put the iCassette that has been added with sample into the instrument and click the "Close" button in the initial interface to close the compartment door. (For multi-channel instrument, iCassette tray holder is required to load the iCassette. Input the sample information at the corresponding channel position of the control software while loading the iCassette)
2.5 After the compartment door is closed, the instrument scans the corresponding QR code on the iCassette automatically.
2.6 After scanning, click the "Run" button, the software will automatically load the program corresponding to the QR code of the iCassette, and click "OK" to start the program.
2.7 After the program starts , the progress of the instrument running will be displayed in the main interface.
2.8 After the amplification is completed, the compartment door will open automatically.
For the detailed steps of test operation, please refer to user manual of the instrument.

3. Result Analysis
After the experiment is finished, the instrument will automatically save the results, and output the Ct value and amplification curves with results interpretation in the interface.

Quality Control
Internal control: CY5 channel is positive (internal control may be detected as negative when the sample to be tested is positive due to the specific competition between the internal control and the sample. When the clinical sample is negative, the internal control must be positive, otherwise it may be due to extraction failed).
Probe quality control: if the fluorescence signal measurement of the probe displays "probe check fail", it indicates that there is a problem with the probe in the iCassette and the iCassette is unavailable; If the PCR step is performed after the iCassette self-test, it indicates that the iCassette probe is qualified.
The above requirements must be met at the same time in the same test. Otherwise, the test is invalid and needs to be repeated.
Test results of positive control: FAM channel, HEX channel, TEXAS RED channel and CY5 channel are all positive;
Test results of negative control: FAM channel, HEX channel, TEXAS RED channel are all negative;CY5 channel is positive;

Interpretation of test results

Negative	No Ct value or Ct > 39
Positive	Ct ≤ 37
Suspicious	If the Ct value falls 37-39, it is recommended to repeat the test. If the Ct < 39 again and the amplification curve has obvious peaks, the sample is judged as positive, otherwise it is negative.

The sample to be tested will be determined according to the standards in the table below:

FAM Channel	HEX Channel	TEXAS RED Channel	CY5 Channel	Results
√	×	×	√	DENV positive(Figure 2)
×	√	×	√	CHIKV positive(Figure 3)
×	×	√	√	ZIKV positive(Figure 4)
√	√	×	√	DENV and CHIKV positive
√	×	√	√	ENV and ZIKV positive
×	√	√	√	CHIKV and ZIKV positive
√	√	√	√	DENV, CHIKV, and ZIKV positive
×	×	×	√	Negative control(Figure 5)
√	√	√	√	Positive control

Note:"√" means that the result "has an obvious logarithmic amplification curve";"×" indicates the result "no logarithmic amplification curve". The CY5 channel is an internal control channel.

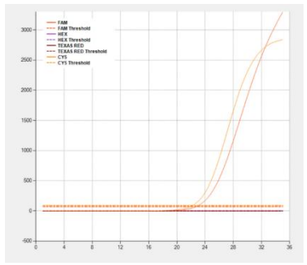

Figure 2.DENV Positive

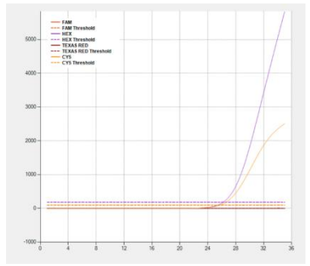

Figure 3. CHIKV Positive

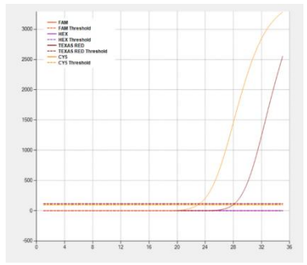

Figure 4. ZIKA Positive

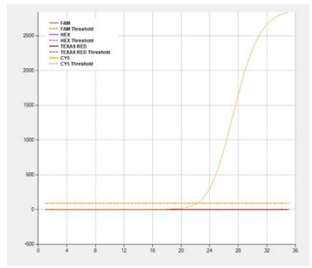

Figure 5. Negative

Limitations of Test Method

1. Improper sample collection, transportation and processing, too low virus content in the sample, excessive nucleic acid degradation or target concentrations below the LOD in the amplification reaction system may lead to false negative results.
2. The test results of this product cannot be used directly as the basis for clinical diagnosis or case exclusion. However, they should be analyzed comprehensively in combination with other relevant medical test results.

Product Performance Index

1. The lower limit of detection of the Kit is 1×10^3 copies/mL.
2. Analysis specificity

The specificity test results show that there is no cross reaction with pathogenic microorganisms (Japanese encephalitis virus, tick-borne encephalitis virus, yellow fever virus, Hepatitis C virus; influenza B virus lineages Yamagata and Victoria; influenza A virus subtypes H1N1, H3N2, H5N1, H7N9; EBV, measles virus, rubella virus) same as those at the infection site or causing the similar symptoms of infection.

3. Potential interfering substances

Endogenous substances in human serum such as whole blood and mucus don't interfere with the test results of the Kit. Substances (anticoagulants, coagulants, separating gels) in the blood collection tubes and exogenous drugs (ribavirin, amoxicillin, dexamethasone, paracetamol, ibuprofen, amantadine, etc.) don't interfere with the test results of the Kit.

4. Precision:

The coefficient of variation of intra-batch precision is ≤ 5%.

Precautions

1. The kit should be used within the validity period.
2. If the iCassette is leaked after adding the sample, do not use the iCassette.
3. Experimenters should take protection and wear disposable gloves and masks.
4. Each iCassette is single-use, please do not reuse it.
5. In order to avoid any potential biological hazards in the samples, the test samples should be regarded as infectious substances and avoid contact with skin and mucous membranes; sample handling and processing must comply with relevant regulatory requirements.

References

Kanti Pabbaraiu, Sallene Wong, Kara Gill, Kewin Fonseca, Graham A. Tipples, Raymond Tellier. Simultaneous detection of Zika, Chikungunya and Dengue viruses by a multiplex real-time RT-PCR assay [J]. Joumal of Clinical Virology, 2016,83.

Instruction Version

Version: A/0
Date of Issue:May,2022

Symbols

The following symbols may appear on the product labeling:

IVD	In vitro diagnostic medical device		Do not re-use
	Use-by date		Consult instructions for use or consult electronic instructions for use
	Caution		Manufacturer
	Temperature limit	LOT	Batch code
	Contains sufficient for <n>		Keep dry
	Keep away from sunlight		Do not use if package is damaged and consult instructions for use
	Date of manufacture		Biological risks
REF	Catalogue number	CE	CE marking of conformity
EC REP	Authorized representative in the European Community		

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