Dengue virus, Zika virus, and Chikungunya virus Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing)

Product Identification

Product Name:Dengue virus, Zika virus, and Chikungunya virus Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing)
Reference Number:S3333E-24, S3333E-48, S3333E-12-S
Package Specification:24 tests/kit, 48 tests/kit, Pre-packaged 12 tests/kit

Intended Use

The Dengue virus, Zika virus, and Chikungunya virus Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) is used to detect Dengue virus (DENV), Zika virus (ZIKV), and Chikungunya virus (CHIKV) RNA in serum. The test results can only be used for auxiliary detection of DENV, ZIKV, and CHIKV infection, and should not be used as the sole basis for patient management decisions.

The Dengue virus, Zika virus, and Chikungunya virus Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) is intended for use by professional, qualified, trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.

For in vitro diagnostic use only. For professional use only.

Test principle summary and explanation

Summary

The DENV, ZIKV, and CHIKV RNA are generally detectable in serum from infected individuals[1]. Positive results are indicative of the presence of DENV, ZIKV, and CHIKV, clinical correlation with medical history and other diagnostic information is necessary to determine patient infection status[2]. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Negative results do not preclude DENV, ZIKV, and/or CHIKV infection. Negative results must be combined with clinical observations, medical history, and epidemiological information.

Test Principle

The Dengue virus, Zika virus, and Chikungunya virus Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) is a real-time reverse transcription polymerase chain reaction test. The kit employed DENV, ZIKV, and CHIKV's primers and probes sets to detect DENV, ZIKV, and CHIKV RNA from serum of suspected patients. The kit is used for detection of DENV, ZIKV, and CHIKV RNA qualitatively.

A specifically designed primers and probe set is targeting the human GAPDH gene as internal control, which monitors the sample collection, sample handling and qPCR process to avoid false-negative results.

Materials provided

This kit is an amplification reaction reagent and contains the following components:

No.	Reagent Name	Spec. & Qty.			Main Ingredients
		24 T	48 T	Pre-packaged 12T	
1	DENV/ZIKV/CHIKV PCR Mix	624 μL/tube × 1 tube	1248 μL/tube × 1 tube	26 μL/tube x 12 tubes	Primers, Probes, dNTPs (T), Mg^{2+}, PCR buffer
2	DENV/ZIKV/CHIKV Enzyme Mix	96 μL/tube × 1 tube	192 μL/tube × 1 tube	4 μL/tube x 12 tubes	Reverse transcriptase, Taq DNA polymerase
3	DENV/ZIKV/CHIKV Negative Control	1000 μL/tube × 1 tube	1000 μL/tube × 1 tube	1000 μL/tube x 1 tube	Normal Saline
4	DENV/ZIKV/CHIKV Positive Control	1000 μL/tube × 1 tube	1000 μL/tube × 1 tube	1000 μL/tube x 1 tube	Synthetic sequences contain targets of interest

Materials required but not provided

1. All contents in this package are prepared and validated for the intended testing purpose. Replacement or modification of any of the package contents will affect the testing performance of the kit. Components contained within a kit are intended to be used together. Do not mix or exchange components from different kits.
2. Materials required but not provided: 1.5 mL DNase-free and RNase-free microcentrifuge tubes, 0.2 mL PCR tubes or strips, various models of pipettes and pipette tips (10 μL, 200 μL and 1000 μL tips with filters), microcentrifuge, vortex mixer.
3. Reagent required but not provided: Sample Release Reagent (Reference Number: S1014E series) ,Nucleic Acid Extraction-Purification Kit (Reference Number: S1006E series) or Nucleic Acid Extraction-Purification Kit(Reference Number: S50016E-12A)manufactured by Sansure Biotech Inc. for nucleic acid extraction.

Warnings and precautions

Warnings

1. All contents in this package are prepared and validated for the intended testing purpose.
2. Replacement or modification of any of the package contents will affect the testing performance of the kit.
3. Components contained within a kit are intended to be used together. Do not mix or exchange components from different kits.

Precautions

1. For *in vitro* diagnostic use only. Please read the product manual carefully before operation.
2. Please learn and be familiar with the operation procedures and precautions for each instrument before test. Please make sure quality control is performed for each test.
3. Laboratory management shall strictly follow management practices of PCR gene amplification laboratory, laboratories personnel must receive professional training, test processes must be performed in separated regions, all consumables should be for single use only after sterilization, special instruments and devices should be used for every process, all lab devices required in different processes and regions should not be cross-used.
4. All samples for detection should be handled as potentially infectious. Wear laboratory coats, protective disposable gloves and change the gloves often to avoid cross-contamination between samples. Handling of samples and waste must meet relevant requirements outlined in local, state and national regulations.
5. Note: Improper operation during the storage, transportation and use of the reagent may affect the test results. For example, improper storage and transportation, sample collection, sample processing and test process are not standardized. Please strictly follow the instructions.
6. Due to the characteristics of swab and other sample collection process and viral infection process itself, false negative results may be caused by insufficient sample volume, which should be combined with other clinical diagnosis and treatment information for comprehensive judgment, retest when necessary.
7. After the addition of the sample in the tube the resulting solution is to be considered potentially biohazardous, handle the reagent with appropriate precautions and good laboratory practice.
8. The safe disposal of the reagents supplied must be carried out according to the instruction contained in the specific Safety Data Sheets and in compliance with the national regulations on disposal of potentially hazardous waste.
9. Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established; If you have any questions about the test or the results, please contact Sansure`s customer service hotline +86-731-88883176-6116 or send an email to info@sansure.com.cn/ support@sansure.com.cn.

IVD storage, operating conditions and stability

1. The shelf life of the kit is 12 months at -25°C to -15°C and protected from light.
2. Please refer to the date of manufacture and expiry date printed on the outside of the box.
3. Unopened reagents are valid and stable until the expiry date.
4. Once the reagents are opened, the maximum number of freeze/thaw cycles should not exceed three.
5. The reagents keep valid and stable before the expiry date on the outer package when transporting for up to 7 days in a sealed foam box containing coolant with the temperature lower than 20°C.

Instrumentation

The kit is compatible to Fluorescence Quantitative Analysis System containing FAM, ROX and CY5 channels such as:

1. Applied Biosystems/7500 Real-Time PCR System,
2. Applied Biosystems/QuantStudio 5 Dx Real-Time PCR System,
3. Hongshi/SLAN®-96P Real-Time PCR System,
4. Molarray/Real-Time Quantitative Thermal Cycler (Model: MA-6000),
5. Roche/LightCycler® 480 instrument II
6. Sansure/Portable Molecular Diagnostic System (S-Q37A/S-Q37B).

Collecting and preparing specimens

1. Applicable specimen type: serum.
2. Collection of specimens

Serum: Use a sterilized syringe to draw 2 mL of venous blood from the patient to a sterilized collection tube. Incubate the tube under room temperature for up to 4 hours to separate serum from blood cells, or centrifuge it at 4000 rpm for 5 minutes. Transfer the serum to a 1.5 mL sterilized micro centrifuge tube for later use.

3. Storage and delivery of specimens:

Specimens to be tested can be immediately processed, specimens to be tested within 24 hours can be stored at 4°C. Specimens that cannot be detected within 24 hours should be stored at -70°C or below (in the absence of -70°C storage conditions, specimens to be tested can be stored at -20°C for 10 days, nucleic acid can be stored at -25°C to -15°C for 15 days). Multiple freeze-thaw cycles should be avoided. Specimens should be transported in a sealed frozen container with ice or in a sealed foam box with ice packs.

Test procedure

1. Preparation of reagent (performed at "reagent preparation room")

1.1 Take out all the components out off the kit and equilibrate at room temperature,then vortex each of them respectively for later use .

1.2 Prepare the DENV/ZIKV/CHIKV PCR Master Mix according to following table. The volume required is based on the total number of specimens, plus a DENV/ZIKV/CHIKV Negative Control and a DENV/ZIKV/CHIKV Positive Control. Mix thoroughly then centrifuge it for later use. The remaining reagent must be stored at -20°C immediately.

	1 test	24 tests	48 tests
DENV/ZIKV/CHIKV PCR Mix (μL)	26	624	1248
DENV/ZIKV/CHIKV Enzyme Mix (μL)	4	96	192

Note: The above configuration is for reference only.Not applicable to prepackaging specifications

2. Processing and loading of specimens (performed at "specimen processing room")

2.1 Use Sample Release Reagent (Reference Number: S1014E series) or Nucleic Acid Extraction-Purification Kit (Reference Number: S1006E series) manufactured by Sansure Biotech Inc. to extract the nucleic acid according to corresponding manual.

2.2 Add 20 μL of the extracted RNA to the PCR tubes in the following order: DENV/ZIKV/CHIKV Negative Control, patient specimen(s), and DENV/ZIKV/CHIKV Positive Control.

2.3 Add 30 μL of DENV/ZIKV/CHIKV PCR Master Mix into each well. Cover each well, centrifuge at 2000 rpm for 10 seconds, and place into the Real-Time PCR system.

3. PCR Amplification (Refer to user manual of each instrument to adjust the settings.)

3.1 Place PCR tubes into the specimen wells of the amplification equipment. Set up the DENV/ZIKV/CHIKV Negative Control, DENV/ZIKV/CHIKV Positive Control and specimens to be tested in order and input specimen name.

3.2 Select PCR test channel:

1) Select FAM channel (Reporter: FAM, Quencher: None) to test DENV RNA, 2) Select ROX channel (Reporter: ROX, Quencher: None) to test ZIKV RNA, 3) Select CY5 channel (Reporter: CY5, Quencher: None) to test CHIKV RNA, 4) Select HEX or VIC channel (Reporter: HEX/VIC, Quencher: None) to test Internal Control, 5) Set passive reference: none. Set sample volume: 50.

3.3 Set cycle parameters

	Steps	Temperature	Time	Cycles
1	Reverse transcription	50°C	30 min.	1
2	cDNA pre-denaturation	95°C	1 min.	1
3	Denaturation	95°C	15 sec.	45
	Annealing, extension and fluorescence collection	60°C	30 sec*.	
4	Device cooling	25°C	10 sec.	1

When the settings are completed, save the settings and carry out the reaction procedure. (*Note: Due to the setting of ABI7500 instrument, it cannot be set to 30 seconds, but can be set to 31 seconds or 32 seconds.)

4. Please process according to the following steps for Portable Molecular Diagnostic System (S-Q37A/S-Q37B):

4.1 Pre-run preparation

4.1.1 Load the amplification reagent component assembly into the extraction reagent component (Nucleic Acid Extraction-Purification Kit, Reference Number: S50016E-12A) to compose the test reagent cartridge;

4.1.2 Open the seal plug of the sample loading hole, add 350μL sample or DENV/ZIKV/CHIKV-Positive Control or DENV/ZIKV/CHIKV-Negative Control into the sample loading hole (To ensure Diagnostic System have 300μL samples for nucleic acid extraction); or use transfer pipet from the extraction reagent kit to pipette sample into the sample loading hole (When sample enter the lower bubble of transfer pipet indicates enough sample has been taken). Then close the seal plug.

4.2 Test Procedure

4.2.1 Click the "Specimen" button on the instrument display screen to open the door of the instrument and enter the new experiment task setting interface.

4.2.2 Put the prepared consumables into the designated position of the instrument.

4.2.3 Enter specimen information, select the required experimental project in the drop-down menu of Experimental project, enter the corresponding task name in the Task Name bar, and input and select other items that should be input or selected.

4.2.4 Click "Submit" to submit the experimental task and "OK" to run the instrument.

Reading test results

1. Result Analysis (Refer to user manual of instrument to adjust the settings.)

Results will be saved automatically when reactions are completed. Analyze amplification curve of target of detection and internal control. Adjust Start, End and Threshold values of Baseline of the graph according to analysis result (Users can adjust the values according to the actual situation. Start value can be set between 3-15, and End value between 5-20. Adjust the amplification curve of negative control to be flat or below threshold). Click "Analyze" to implement the analysis, make sure each parameter satisfies the requirements given in "2. Quality Control". Go to "Plate" window to record qualitative results.

2. Quality Control

A valid test result where the test meet the conditions described in the table below in the same test. Otherwise the test result is treated as invalid and needs to be re-tested.

Results	DENV/ZIKV/CHIKV Positive Control	DENV/ZIKV/CHIKV Negative Control
DENV (FAM) Ct	≤35	>40 or No Ct
ZIKV (ROX) Ct	≤35	>40 or No Ct
CHIKV (CY5) Ct	≤35	>40 or No Ct
IC (HEX/VIC) Ct	≤35	>40 or No Ct

Reference Range

Through the research on reference values, the Ct reference value of target gene and internal control is determined to be 40.

Interpretation of test results

Conclusion	Ct value of sample	Ct value of internal control
Positive	⩽ 40	⩽ 40
Negative	> 40	⩽ 40
Out of limit (concentration < 6.00E+02 copies/mL)	> 40	⩽ 40
Invalid*		> 40 or N/A

Note: *This suggests an investigation should be carried out to find out the reasons when Ct value of the internal control is > 40 or N/A and retest it. (If repeated tests still produce invalid results, please contact Sansure Biotech at info@sansure.com.cn)

1. For the FAM channel with Ct value ≤40, with internal control detected Ct value ≤40, it should be reported as DENV positive. For specimens which are detected with Ct value >40 or No Ct, and internal control detected Ct value ≤40 reported as DENV negative.
2. For the ROX channel with Ct value ≤40, with internal control detected Ct value ≤40, it should be reported as ZIKV positive. For specimens which are detected with Ct value >40 or No Ct, and internal control detected Ct value ≤40 reported as ZIKV negative.
3. For the CY5 channel with Ct value ≤40, with internal control detected Ct value ≤40, it should be reported as CHIKV positive. For specimens which are detected with Ct value >40 or No Ct, and internal control detected Ct value ≤40 reported as CHIKV negative.
4. If HEX/VIC channel with Ct value >40 or No Ct, then the specimen's detection result is invalid. An investigation should be performed to find out reasons and then retest the specimens. (If repeated tests still produce invalid results, please contact Sansure Biotech Inc.)

Limitations of the procedure

1. False negative can be caused by poor specimen quality, improper sample collection, improper transportation, improper laboratory processing, or a limitation of the test method.
2. Mutation in the target sequence of DENV/ZIKV/CHIKV may lead to false negative results.
3. Improper reagent storage may lead to false negative results.
4. Use of this assay is limited to personnel who are trained in the procedure.
5. Test results of the diagnostic kit can only be used as an aid in clinical diagnosis. Symptoms and physical signs, medical history, other laboratory examinations and therapeutic reactions of the patients should be comprehensively considered for the clinical diagnosis and treatment.
6. Unverified interfering substances or PCR inhibitors may lead to false negative or invalid results.

Performance characteristics

1. Specificity

The Dengue virus, Zika virus, and Chikungunya virus Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) has no cross-reactions with Japanese encephalitis virus, West nile virus, Mumps virus, Norovirus, Yellow fever virus, and Rubella virus.

2. Limit of detection

The limit of detection of this kit is 600 copies/mL.

3. Precision

The coefficient of variation (CV%) of Ct value of the precision is ≤5%.

4. Possible interfering substances in specimens

Hemoglobin (≤2 mg/dL), total bilirubin (≤28 mg/dL), triglyceride (≤3 g/dL), total IgG (≤40 mg/mL), Interferon α (≤9 ng/mL), and Ribavirin (≤300 μg/mL) have no significant interference with the detection results of the kit.

List of references

1. Detection of zika, dengue and chikungunya viruses using single-reaction multiplex real-time RT-PCR. Diagn Microbiol Infect Dis. 2018.
2. An updated RT-qPCR assay for the simultaneous detection and quantification of chikungunya, dengue and zika viruses. Infect Genet Evol. 2021.

Symbol key

Symbols	Meanings	Symbols	Meanings
IVD	*In Vitro* Diagnostic Medical Device		Date of Manufacture
	Use-by date		Consult Instructions for Use
	Temperature Limit		Manufacturer
LOT	Batch Code	REF	Reference Number
Σ	Contains sufficient for <n> tests	EC REP	Authorized representative in the European Community
	Caution	CE	This product fulfills the requirements of the European Directive 98/79/EC for *in vitro* diagnostic medical devices.
21 PAP	PAP21: Not corrugated cardboard		Do not re-use
PCR MIX	PCR Mix	ENZYME MIX	Enzyme Mix
CONTROL –	Negative Control	CONTROL +	Positive Control
VERSION	Version		Keep away from light
PRE	Prepackaging	UDI	Unique device identifier

Contact information

Sansure Biotech Inc.

Add.: No. 680, Lusong Road, Yuelu District, 410205 Changsha, Hunan Province, PEOPLE'S REPUBLIC OF CHINA

Tel.: +86-731-88883176

Fax: +86-731-88884876

Web: www.sansureglobal.com

Obelis s.a.

Bd. Général Wahis 53, 1030 Brussels, BELGIUM

Tel: + (32) 2.732.59.54

Fax: + (32) 2.732.60.03

E-Mail : mail@obelis.net

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