Dengue Virus Nucleic Acid Detection Kit (Fluorescent PCR)

(For research use only)

INTENDED USE

This kit is used for in vitro qualitative detection of dengue virus RNA in human serum and/or plasma only.

Dengue Virus (DENV) is a single plus-stranded RNA virus with spherical particles with a diameter of about 55nm, consisting of four serotypes(DENV 1-4), that is members of the *Flaviviridae* family, genus flavivirus.

Dengue fever, dengue haemorrhagic fever and dengue shock syndrome with high morbidity and mortality are caused by dengue virus, mainly transmitted by vector insects such as Aedes aegypti and Aedes albopictus. The classical clinical manifestations of dengue fever are sudden onset, high fever, headache, severe aches and pains of muscles, bones and joints, rash, bleeding tendency, lymph node enlargement, decreased white blood cell count, and thrombocytopenia in some patients. The existing detection technologies in the laboratory mainly include virus isolation, Serotype detection and Nucleic acid test. This product can be used to assist the clinical diagnosis of dengue-related diseases caused by DENV 1-4.

The test results are for clinical reference only and cannot be used as the sole basis for diagnosing or excluding suspected cases of the patient's condition. A comprehensive evaluation of the patient's condition must be conducted in conjunction with clinical manifestations and other laboratory testing indicators.

DETECTION PRINCIPLE

This kit utilizes real-time fluorescent PCR technology, targeting the highly conserved region of the dengue virus gene, and designs specific primers and TaqMan probes for qualitative detection of dengue RNA in serum and/or plasma samples. At the same time, internal gene was act as a non-competitive internal control during the extraction and detection process.

COMPONENTS

Item No.		BSJ43M1	BSJ43L1	Main components
Product specifications		48 tests	96 tests	
Amplification reagent	PCR Buffer	685 μL	1370 μL	Tris-HCl, KCl, MgCl, dNTP, specific primer probe
	Enzyme mixture	65 μL	130 μL	Hotstart Taq DNA Polymerase, RT enzyme, etc
Control	Positive control	1000 μL	1000 μL	Solution containing DENV gene fragment plasmid and internal reference gene plasmid
	Negative control	1000 μL	1000 μL	Solution containing the only internal reference gene plasmid

1.The positive control and negative control need to be set to monitor the test body and the operating environment; the negative control and positive control have been packaged in the kit.

2.The components of different lots cannot be mixed for use.

3.Equipment or materials required but not provided: specimen collection kits, nucleic acid extraction kits; PCR tubes and caps, etc.

STORAGE AND PERIOD OF VALIDITY

1. The kit should be stored at -25°C ~ -15°C away from light, and avoid repeated freeze-thaw. The kit can be stored for 5 days at 2 ~ 8°C after opening

2. The kit can be stored for up to 12 months if all components are kept in the manner above. Do not use after the stated expiry date.

3. The kit can be transported in foam box sealed with ice bags or dry ice at 2 ~ 8°C or lower.

APPLIED INSTRUMENT

The kit can be applied to Fluorescent Quantitative Detection System from Bioer, QuantGene 9600 (FQD-96C) , LineGene 9600 Plus (FQD-96A) and Bioer's fully automatic nucleic acid purification and real-time fluorescence PCR analysis system (FQD-A1600). The instrument should contain at least three channels of FAM,and CY5.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORTATION

1. Applicable specimens:Human serum and/or plasma
2. Collection: Specimens of all types are collected by conventional methods
3. Storage: It is recommended that specimens be processed as soon as possible after collection. If specimens are not processed immediately, they should be stored at 2-8 °C for up to 5 days. If a delayed processing is expected, the specimens should be stored at -25°C ~ -15°C for no more than 3 months. Specimens should not be frozen and thawed frequently.

USING OF THE KIT PCR REACTION (PCR TEST AREA)

1. Reagent prepares

Thaw out the reagents at room temperature. Gently mix and centrifuge all reagents for a few seconds.

Make PCR reagents according to the quantity of specimens and controls as below (N means the number of specimens and controls, An extra blank control is highly recommended to prevent the loss of reaction mix):

Amplification reagent	μL/test
PCR Buffer	13.7
Enzyme mixture	1.3
Total Volume	15

Distribute 15 μL mixed PCR reagents into each PCR tubes, and then transfer the reaction plate to sample processing area.

2. Adding sample

Add 10 μL negative control, 10 μL extracted product, 10 μL positive control into different PCR tubes. The final volume should be 25μL per tube. Cap the PCR tubes immediately to prevent cross contamination

After extraction, take 10μL add the extracted products of the sample to be tested, negative control and positive control to the prepared detection reagent, cover the tube tightly, centrifuge to remove bubbles, and transfer to the amplification analysis area.

3. PCR reaction

Place the reaction tubes on a PCR instrument.

It is recommended to choose FAM and CY5 channels to collect fluorescent signals.

Set fluorescent signals detecting at 60°C, liquid volume is 25μL.

Set reaction procedure as below:

Step	Temperature	Duration	Number of cycles
1	50 ℃	5min	1cycel
2	95 ℃	1 min	1cycel
3	95 ℃	5 sec	45cycels
	60 ℃	10 sec	

QUALITY CONTROL STANDARDS

Control	FAM	CY5	Detection result
Positive control	Ct≤35，with "S" curve	Ct≤35，with "S" curve	All requirements should

Negative control	No Ct	Ct≤35，with “S” curve	meet in the same experiment, indicating that the experiment is valid, otherwise it is invalid.

RESULT ANALYSIS AND JUDGMENTS

Expected performances of specimens are as below:

	FAM（DENV）	CY5（IC）	Result interpretation
1	CT≤40	CT≤40	DENV（+）
2	CT＞40 or no Ct Value.	CT≤40	DENV（-）

Limitations

1. This kit is only used for in vitro assisted diagnosis, and the test results should be comprehensively analyzed and interpreted in combination with the patient's symptoms/signs, medical history, and other laboratory diagnostic results, and should not be used as the sole basis for clinical diagnosis, treatment or management of patients.

2. Analysis of false negative results:

① Unreasonable sample collection, processing, transportation, and storage conditions;

② The concentration of target genes in the sample is too low;

③ Mutations in the genetic sequence of dengue fever virus or other causes;

④ Unverified other interfering substances, such as endogenous or exogenous substances introduced into the sample;

⑤ Collecting samples from the same patient at different times or multiple times can reduce the likelihood of false negative results.

4. Analysis of false positive results:

① If cross contamination occurs during transportation and processing of samples, it may lead to false positive results;

② Cross contamination between samples;

③ If the experimental environment is contaminated with aerosols such as PCR products, it may lead to false positive results;

④ Other cross reactive substances that have not been verified;

⑤ Contamination of consumables, equipment, etc. used during the experiment may result in false positive results.

5. The test result is negative, and it cannot be determined that there is no relevant pathogen infection in the clinical sample. This may be due to the detected nucleic acid concentration in the sample being lower than the detection limit.

PERFORMANCE INDICATORS

1. Limit of Detection (LoD): The minimum detection limit is 500 copies/mL.

2. Analytical sensitivity: The positive coincidence rate was 100%, and the negative coincidence rate was 100 %.

3. Analytical specificity: No cross reactivity has been observed by testing the clinical positive specimens such as Herpes simplex virus type I, Herpes simplex virus type II, Herpes simplex virus type IV, hepatitis B virus, HIV virus, hepatitis C virus, Measles morbillivirus, rubella virus, Influenza A virus, Influenza B virus, etc;

4. Precision: The product coefficient of variation (CV) for factors such as intra day/inter day, intra batch/inter batch, and between different equipment is less than 5%.

WARNINGS AND PRECAUTIONS

- For research use only.
- Read the Instructions for Use carefully before operation. The appropriate operations from specimen

collection, storage and transportation, and laboratory test should be strictly manipulated in line with relevant regulations of biosafety and molecular laboratory management.

- Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Perform all manipulations of live virus samples within a Class II (or higher) biological safety cabinet (BSC). Handling samples in the biosafety cabinet, to ensure operator safety and avoid environmental pollution. Place harmful samples and reagents properly. Discard the waste in special containers. Wipe the table, centrifuge, and equipment frequently with 1.0% sodium hypochlorite or 70 % ethanol. The laboratory and the ultra-clean workbench need UV-treated periodically and after each experiment.
- All the articles in each district are for special use which cannot allow to be exchanged for avoiding pollution. The workbench should be cleaned immediately after the completion of each experiment.
- Use disposable gloves without fluorescent substances, disposable special centrifuge tubes, etc.
- Use personal protective equipment such as (but not limited to) gloves, eye protection, and lab coats when handling kit reagents, while performing this assay and handling materials including samples, reagents, pipettes, and other equipment and reagents.
- The false positive or negative testing result can be led by poor quality of specimen, incorrect operations in sample collection, transportation or laboratory processing, or limitation of the technology. Operator should understand well the principles of the procedures and its limitation in performance in advance and avoid any potential mistakes intentionally.
- Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the real-time reagents used in the amplification step become contaminated by accidental introduction of amplification product.
- Separate laboratory areas are recommended to performing predefined procedures of the assay. Area I: Reagent preparation area-reagent required for preparing amplification. Area II: Sample processing area-processing of tested samples and controls. Area III: PCR detection region-PCR amplification detection.
- The separation of the reaction solution should avoid the generation of air bubbles as far as possible. Before the amplification, pay attention to check whether the caps of each reaction tube are tightened to avoid contaminating instrument.
- Samples should be completely put into the reaction solution when adding samples. No samples should adhere to the tube wall and the cap should be tightened as soon as possible after adding samples.
- Both the kit and nucleic acid products are all stored at -20°C. Before using, they should be fully thaw out at room temperature, mixed and then instantaneous briefly centrifugation. DNA should be maintained on cold-block or on ice during preparation and use to ensure stability.
- After amplification, please take out the reaction tube immediately, seal it in the special plastic bag, put it in the designated place, and wait for unified treatment.
- Dispose of used / unused kit reagents and human specimens according to local, state, and federal regulations.

REFERENCES

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Manufacturer

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