

Dengue virus type I/II/III/IV Detection Kit (Real-Time PCR Method)

Product Number: DTK600

Shipping and Storage

-20 °C± 5 °C, stored in the dark, transported, and subjected to repeated freeze-thaw cycles no more than 5 times, with a validity period of 12 months.

Component

| Component | 25T | 50T |
|-----------------------------------|-------|---------|
| DENV-1/-2/-3/-4 reaction solution | 500μL | 500μL×2 |
| DENV-NB reaction solution | 500μL | 500μL×2 |
| Enzyme solution | 50μL | 50μL×2 |
| Positive control substance | 50μL | 50μL |
| Negative quality control product | 250μL | 250μL |

Note: Different batches of reagents cannot be mixed.

Description

This kit uses TaqMan probe method for real-time fluorescence PCR technology, designs a pair of dengue virus specific primers, combines with a specific probe, and uses fluorescence PCR technology for in vitro amplification and detection of dengue virus nucleic acid, which is used for pathogen diagnosis of suspected infectious materials in clinical practice.

Application

This kit is suitable for detecting dengue virus in human serum and mosquito samples, and is used for auxiliary diagnosis of dengue virus infection.

Applicable instruments

ABI , Agilent MX3000P/3005P, LightCycler, Bio-Rad, Eppendorf and other series of fluorescence quantitative PCR detectors.

Specimen collection

It is recommended to follow the steps in Appendix B.1.3 of WS/T 216-2018 "Diagnosis of Dengue Fever" for sample collection and processing.

The collected or processed samples should be stored at 2°C~8°C for no more than 24 hours; if long-term storage is required, they should be stored at -70°C or below, with no more than 3 freeze-thaw cycles.

Protocol

1. Reagent Preparation (Reagent Preparation Area)

1.1. Sample pre-processing

It is recommended to follow the steps in Appendix B.1.3 of WS/T 216-2018 "Diagnosis of Dengue Fever" for sample collection and processing.

1.2. nucleic acid extraction

We recommend using our company's nucleic acid extraction or purification reagents (magnetic bead method or centrifugal column method) for nucleic acid extraction. Please follow the reagent instructions for operation.

2. Sample processing (sample processing area)

Based on the total number of samples to be tested, the required number of PCR reaction tubes is N (N=number of samples+1 negative control tube+1 positive control tube); For every 10 portions of reaction tubes, an additional 1 portion is prepared. The

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preparation of each test reaction system is shown in the following table:

| Reagent | DENV-1/-2/-3/-4 reaction solution/DENV-NB reaction solution | Enzyme solution |
|---------|---|-----------------|
| Usage | 19 μ L | 1 μ L |

Transfer the mixed test reaction solution into a PCR reaction tube at a concentration of 20 μ L per tube.

3. Sample addition (sample processing area)

Take 5 μ L of the nucleic acid, positive control sample, and negative control sample extracted in step 1, and add them to the corresponding reaction tubes. Cover the tubes, mix well, and briefly centrifuge.

4. PCR amplification (nucleic acid amplification zone)

4.1. Place the reaction tube to be tested in the reaction tank of the fluorescence quantitative PCR instrument;

4.2. Set the channel and sample information, and set the reaction system to 25 μ L;

Fluorescence channel selection: Detection channels (Reporter Dye) FAM, VIC, ROX, CY5, quenching channel (Quencher Dye) NONE, ABI series instruments do not select ROX reference fluorescence, select None.

4.3. Recommended loop parameter settings:

| step | Cycles | Temperature | Time | Collect fluorescence signals |
|------|-----------|-------------|-------|------------------------------|
| 1 | 1 cycle | 50°C | 10min | No |
| 2 | 1 cycle | 95°C | 2min | No |
| 3 | 45 cycles | 95°C | 15sec | No |
| | | 60°C | 30sec | Yes |

5. Result analysis and judgment

5.1. Result Analysis Condition Setting

(Please refer to the user manuals of each instrument for setting up, taking the ABI7500 instrument as an example)

After the reaction is complete, the results will be automatically saved. Based on the analyzed image, adjust the Start value, End value, and Threshold value of the baseline (users can adjust them according to their actual situation, with Start value set between 3-15 and End value set between 5-20, so that the threshold line is in the exponential period of the amplification curve, and the amplification curve of negative quality control products is flat or below the threshold line). Click Analyze to automatically obtain the analysis results.

5.2. Result judgment

Positive: The Ct value of the detection channel is ≤ 40 , and the curve shows a significant exponential growth curve;

Negative: The Ct value of the sample test result is greater than 40 or there is no Ct value.

| Multiple Projects | Fluorescence Channel | Test Item |
|-------------------|----------------------|----------------------------------|
| DENV-1/-2/-3/-4 | FAM | Dengue virus type I |
| | VIC | Dengue virus type II |
| | ROX | Dengue virus type III |
| | Cy5 | Dengue virus type IV |
| DENV-NB | FAM | Human derived internal reference |

Quality control

Negative quality control product: no specific amplification curve or Ct value display;

Positive quality control product: The amplification curve shows a significant exponential growth period, and the Ct value is ≤ 32 ;

The above conditions should be met simultaneously, otherwise the experiment will be considered invalid.

Limitations of detection methods

1. The results of sample testing are related to the quality of sample collection, processing, transportation, and preservation;
2. Failure to control cross contamination during sample extraction can result in false positive results;
3. Leakage of positive controls and amplification products can lead to false positive results;
4. Genetic mutations and recombination of pathogens during epidemics can lead to false negative results;
5. Different extraction methods have differences in extraction efficiency, which can lead to false negative results;
6. Improper transportation, storage, or preparation of reagents can lead to a decrease in reagent detection efficiency, resulting in false negatives or inaccurate quantitative testing results;



MEBEP TECH(HK) Co., Limited

Email: sales@mebep.com Website: www.mebep.com

Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

7. The test results are for reference only. If a diagnosis is required, please combine clinical symptoms and other testing methods.

Note

1. All operations must be strictly carried out in accordance with the instructions;
2. The various components in the reagent kit should be naturally melted, completely mixed, and briefly centrifuged before use;
3. The reaction solution should be stored away from light;
4. Try to avoid the presence of bubbles during the reaction, and cover the tube tightly;
5. Use disposable suction tips, disposable gloves, and specialized work clothes for each area;
6. Sample processing, reagent preparation, and sample addition should be carried out in different areas to avoid cross contamination;
7. After the experiment is completed, treat the workbench and pipette with 10% hypochlorous acid, 75% alcohol, or a UV lamp;
8. All items in the reagent kit should be treated as contaminants and handled in accordance with the "Biosafety Guidelines for Microbial Biomedical Laboratories".