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Measles virus (MV) nucleic acid detection kit (fluorescence PCR method)

Product Number: DTK809

Shipping and Storage

1. -20℃±5℃, stored in the dark, transported, and subjected to repeated freeze-thaw cycles no more than 5 times, with a validity period of 12 months.
2. The above specimens can be stored at -20℃ in the short term and -70℃ in the long term, but cannot exceed 6 months. The specimens should be transported in 2-8℃ ice packs, and repeated freezing and thawing are strictly prohibited.

Component

Component	50T
MV reaction solution	500μL×2
Enzyme solution	50μL
MV Positive control	250μL
Negative control	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 measles virus specific primers, and combines with a specific probe to amplify and detect the RNA of measles virus in vitro using fluorescence PCR technology, which is used for pathogen diagnosis of suspected infectious materials in clinical practice.

Application

This kit is suitable for detecting measles virus RNA in throat swab specimens and other samples, and is suitable for auxiliary diagnosis of measles virus infection. The test results are for reference only.

Applicable instruments

Apply to ABI 7500, Bio-Rad CFX96, Roche Lightcycler480I, Lightcycler480II, cobas Z480, Real time fluorescence quantitative PCR instruments such as Hongshi SLAN-96S and SLAN-96P.

Protocol

1. Sample processing (sample processing area)

1.1. Sample pre-processing

Take 100μL of throat swab sample directly into a 1.5mL sterilized centrifuge tube.

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. Reagent Preparation (reagent preparation 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 samples, an additional 1 sample is prepared. The preparation of each test reaction system is shown in the following table:

Reagent	MV reaction solution	Enzyme solution
Usage(The sample size is N)	19μL	1μL

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

3. Sample addition (sample processing area)

Take 5μL 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 25μL;

Fluorescence channel selection: Detection channel (Reporter Dye) FAM, 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℃	10min	No
2	1 cycle	95℃	2min	No
3	45 cycles	95℃	15sec	No
		60℃	30sec	Yes

5. Result analysis

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

5.2.1. If this kit is used for qualitative testing:

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

Negative: The sample test result shows no Ct value and no specific amplification curve.

Suspicious: If the sample test result is 40<Ct value ≤ 45, it is recommended to repeat the test. If the detection channel is still 40<Ct value ≤ 45 and the curve has a clear growth curve, it is judged as positive. Otherwise, it is considered negative.

Quality control standards

1. Negative quality control product: no specific amplification curve or Ct value display;
2. Positive quality control product: The amplification curve shows a significant exponential growth period, and the Ct value is ≤ 32;
3. 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;
7. The test results are for reference only. If a diagnosis is required, please combine clinical symptoms and other testing methods.

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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".