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Enterovirus 71 (EV-71) Nucleic Acid Detection Kit

(Fluorescent PCR Method)

Product Number:DTK326

Shipping and Storage

-20℃ ± 5℃, avoid repeated freezing and thawing (no more than 5 cycles). Valid for 12 months.

For short-term storage, samples can be kept at -20℃. For long-term storage, use -70℃, but do not exceed 6 months. During transportation, samples should be shipped with ice packs, and repeated freezing and thawing must be avoided.

Component

Component	50T
EV-71 reaction solution	500μL×2
Enzyme solution	50μL
EV-71 positive quality control product	50μL
Negative quality control product	250μL

Note: Different batches of reagents cannot be mixed.

Description

Enterovirus Type 71 (EV-71) belongs to the small RNA virus species and is one of the main pathogens causing hand, foot, and mouth disease (HFMD) in infants and young children. EV-71 infection clinically manifests as herpes on the hands, feet, mouth, and other areas, and can also lead to various neurological diseases such as aseptic meningitis, brainstem encephalitis, and paralysis of the spinal cord and skin[1]. EV-71 is primarily transmitted through oral routes, with a high proportion of asymptomatic carriers, making outbreaks more likely. Rapid, simple, and efficient detection methods are crucial for effective disease prevention and control[1-3]

This kit is suitable for detecting Enterovirus Type 71 RNA in fecal or diarrheal samples and serves as an auxiliary diagnostic tool for EV-71 infections.

Application

This kit is designed based on the conserved region of the Enterovirus Type 71 gene and utilizes specific probes for biological species. It employs a one-step fluorescent RT-PCR technology to amplify and detect EV-71 RNA in vitro, thereby improving the etiological diagnosis of patients in clinical settings.

Applicable instruments

ABI 7500, Agilent MX3000/3005P, LightCycler®, Bio-Rad, Eppendorf, and other fluorescent quantitative PCR instruments.

Specimen collection

This kit is suitable for serum and urine samples. For serum collection, draw 2mL of venous blood into a sterile centrifuge tube and let it stand at room temperature for no more than 4 hours. Centrifuge at 1500 rpm for 5 minutes, then transfer the serum (avoid red blood cells) to another sterile centrifuge tube. For urine collection, take approximately 10mL of the first-morning urine, mix it with the preservative, and seal it to avoid heat.

Protocol

1. Sample processing (sample processing area)

1.1. Sample Preparation

Take an appropriate amount of soybean particle sized feces or water sample abdomen and place it in a 1.5mL centrifuge

tube. Extract according to the instructions of the RNA extraction kit.

1.2. Nucleic acid extraction

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

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 portions of reaction tubes, an additional 1 portion is prepared. The preparation of each test reaction system is shown in the following table:

reagent	EV-71 Reaction solution	Enzyme solution
Dosage (sample size N)	20μL	1μL

Transfer the mixed test reaction solution into a PCR reaction tube at a concentration of 21uL per tube.

3. Sample addition (sample processing area)

Take 4μL 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, Quencher Dye NONE, please do not select ROX reference fluorescence for ABI series instruments, select None.

4.3. Recommended loop parameter settings:

step	Cycles	Temperature	Time	Collect fluorescence signals
1	1 cycle	42℃	20min	No
2	1 cycle	95℃	10min	No
3	40 cycles	94℃	15sec	No
		55℃	30sec	Yes

5. Result analysis and judgment

5.1. Result Analysis Condition Setting

Set Baseline and Threshold: Generally, the analysis is based on the results automatically analyzed by the machine. When the curve shows an overall tilt, adjust the start value (usually within the range of 3-15) and stop value (usually within the range of 5-20) of the Baseline and the Value value of the Threshold (drag the threshold line up and down to be higher than the negative control) based on the analyzed image, and reanalyze the results.

5.2. Result judgment

Positive: The Ct value of the detection channel is ≤ 35, and the curve shows a significant exponential growth curve;

Suspicious: Detection channel 35<Ct value ≤ 38, it is recommended to repeat the detection. If the detection channel is still 35<Ct

If the value is ≤ 38 and the curve has a significant growth curve, it is judged as positive, otherwise it is negative;

Negative: The Ct value of the sample test result is greater than 38 or there is no Ct value.

Quality control standards

Negative quality control product: Ct>38 or no Ct value displayed;

Positive quality control product: The amplification curve has 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. During the epidemic, genetic mutations and recombination of pathogens 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 inaccurate 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.

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"