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Ebola Virus (EBOV) Nucleic Acid Detection Kit (Fluorescent PCR Method)

Product Number: DTK324

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

Component

Component	50T
EBOV reaction solution	500μL×2
Enzyme solution	50μL
EBOV positive control	50μL
Negative control	250μL

Note: Different batches of reagents cannot be mixed.

Description

This kit utilizes TaqMan probe–based real-time fluorescent PCR technology. A pair of specific primers and one specific probe are designed targeting Ebola virus RNA. Fluorescent PCR is used for in vitro amplification and detection of Ebola virus nucleic acid, supporting etiological diagnosis of clinically suspected infections.

Application

This kit is intended for the detection of Ebola virus in blood samples and for the auxiliary diagnosis of Ebola virus infection. Test results are for reference only.

Applicable instruments

ABI 7500, Agilent MX3000P, MX3005P, LightCycler, Bio-Rad, Eppendorf, and other real-time fluorescent quantitative PCR detectors.

Sample requirements

Collect 2mL of venous blood from suspected patients into an EDTA-2Na anticoagulant tube.

Protocol

1. Sample processing (sample processing area)

1.1. Sample pre-processing

Centrifuge at 2000 rpm for 5 min. Transfer 100μL of supernatant into a 1.5mL sterile 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 7 samples, an additional 1 sample is prepared. The preparation of each

test reaction system is shown in the following table:

Reagent	EBOV reaction solution	Enzyme solution
Usage	19μL	1μL

Mix thoroughly and dispense 20μL of the mixture into each PCR tube.

3. Sample addition (sample processing area)

Add 5μL of extracted nucleic acid, positive control, and negative control into corresponding PCR tubes. Cap tightly, mix briefly, and centrifuge briefly.

4. **Result analysis**

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	1cycle	52℃	10min	No
2	1cycle	95℃	2min	No
3	45cycles	95℃	15sec	No
		60℃	30sec	Yes

5. Result analysis and judgment

5.1. Result Analysis Condition Setting

(Follow the instrument manual; take ABI 7500 as example) Results are saved automatically. Adjust Baseline Start (3–15), End (5–20), and Threshold so that the threshold line lies in the exponential phase of amplification and the negative control curve is flat or below threshold. Click Analyze.

5.2. Result judgment

Positive: Ct value ≤ 40 with obvious exponential amplification curve.

Negative: No Ct value and no specific amplification curve.

Suspected: 40 < Ct value ≤ 45. Retest is recommended. If retest still shows 40 ≤ Ct ≤ 45 with obvious amplification curve, report as positive; otherwise negative.

Quality control

Negative Control: No specific amplification curve and no Ct value.

Positive Control: Clear exponential amplification with Ct ≤ 32.

Both criteria must be met; otherwise the run is invalid.

Limitations of protocol

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 shall be strictly carried out in accordance with the instructions;

2. Before use, all components in the reagent kit should be naturally melted, completely mixed, and briefly centrifuged;
3. The reaction solution should be stored away from light;
4. Try to avoid the presence of bubbles during the reaction and tightly cover the tube cap;
5. Use disposable suction heads, 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".