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Hepatitis A Virus (HAV) Nucleic Acid Detection Kit (Fluorescent PCR Method)

Product Number: DTK325

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
HAV reaction solution	500μL x 2
Enzyme solution	50μL
HAV positive quality control product	50μL
Negative quality control product	250μL

Note: Different batches of reagents cannot be mixed.

Description

This kit uses a pair of hepatitis A virus specific primers, combined with a specific fluorescent probe, to perform in vitro amplification and detection of hepatitis A virus RNA using one-step fluorescent RT-PCR technology, for clinical pathogen diagnosis of suspected infectious materials.

Application

Hepatitis A is an intestinal infectious disease caused by the hepatitis A virus (HAV). Distributed worldwide, China is a high-risk area with the highest incidence of various types of hepatitis. The traditional detection of hepatitis A virus is through cell culture. Due to the long culture cycle and low proliferation ability of HAV cells, they generally do not cause cell damage and are difficult to meet the detection requirements. PCR technology has improved sensitivity and specificity, and greatly shortened detection time, bringing convenience to hepatitis A detection [1-3].

This kit is suitable for detecting hepatitis A virus RNA in blood and other samples of suspected infected individuals, and is suitable for auxiliary diagnosis of hepatitis A virus infection.

Applicable instruments

ABI7500, Agilent MX3000P/3005P, LightCycler, Bio-Rad, Eppendorf and other series of fluorescence quantitative PCR detectors.

Sample requirements

2mL of venous blood from suspected infected individuals is transferred to EDTA-2Na anticoagulant tubes.

Protocol

1. Sample processing (sample processing area)

1.1. Sample pre-processing

Extract according to the instructions of the RNA extraction kit.

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	HAV reaction solution	Enzyme solution
Usage	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. 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	50℃	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

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 the Start value to 3-15 and End value to 5-20 according to their actual situation, 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 lower than 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.

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 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".