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Hepatitis A/E Virus Dual Real-Time Fluorescent PCR Detection Kit

Product Number: DTK554

Shipping and Storage

1. Transportation: The reagent kit must be transported under frozen conditions.
2. Storage: Store at -20℃ and avoid repeated freezing and thawing.
3. Validity period: 12 months, please use within the validity period.

Component

Component	50T
Nuclease-Free Water	1000μL
2×nucleic acid amplification reaction solution	500μL
20×reverse transcriptase	50μL
10×Primer Probe Reaction Solution (MP351Z)	100μL
MP351Z - Positive control	100μL
Negative control	1000μL

Note: Different batches of reagents cannot be mixed.

Description

This kit uses real-time fluorescence PCR technology and is suitable for detecting hepatitis A virus and hepatitis E virus nucleic acids extracted from feces. Each reaction system contains specific primers and fluorescent probes for detecting hepatitis A virus and hepatitis E virus. By collecting the fluorescent signals generated by PCR amplification, qualitative detection of the nucleic acid of hepatitis A virus and hepatitis E virus can be performed.

Application

This kit is suitable for qualitative detection of nucleic acids of Human Hepatitis A Virus (HAV) and Human Hepatitis E Virus (HEV) extracted from feces. The experimental results only provide reference for basic research and are not used as clinical diagnostic basis.

Human Hepatitis A virus (HAV) is a small RNA virus belonging to the Hepadnavirus family. HAV has a stable structure, high titer secretion, and can be effectively transmitted between humans and water sources through the fecal oral route. After infection, the virus can manifest as acute viral hepatitis. Hepatitis E virus (HEV) belongs to the hepatitis virus genus of the hepatitis virus family, and is an unencapsulated spherical particle. It has been found that four genotypes can infect humans. HEV is mainly transmitted through fecal oral route in developing countries, while in regions such as Western Europe and Japan, it is transmitted through direct ingestion of meat or organs from infected animals. HEV infection often presents as self limiting, but chronic infection may occur in patients treated with immunosuppressive agents, which has become a serious clinical problem in developed countries with a high incidence of organ transplantation cases.

Applicable instruments

The fully automatic fluorescence PCR detector that has undergone multi-channel calibration needs to include FAM and VIC (HEX) detection channels, such as ABI7500, 7500FAST, ABIQ5, Bio-Rad CFX96, Roche LightCycler 480 II, Shanghai Hongshi SLAN-96P and other fully automatic fluorescence PCR detectors.

Sample requirements

RNA samples extracted from feces can be tested using this kit.

Protocol

1. Sample preparation

Extract feces according to the corresponding requirements and steps in the virus RNA extraction kit. We recommend using our company's extraction kit and following the instructions for nucleic acid extraction. The extracted RNA can be directly used for detection. If the sample is not detected immediately after extraction, it can also be stored at -70 ℃ for future use, and repeated freezing and thawing should be avoided.

2. Preparation of reaction system

2.1. System preparation:

Take out the reagent from the kit and melt it at room temperature. Wait for the reagent to completely thaw, invert and mix well, and then centrifuge immediately. If the number of samples to be tested is n (n=number of samples+positive control+negative control), prepare the system according to n+1 reactions. The reaction system is prepared as shown in the table below:

Reagent	Quantity of 1 reaction system	Quantity of n+1 reaction systems
Nuclease-Free Water	2μL	2μL×(n+1)
2×nucleic acid amplification reaction solution	10μL	10μL×(n+1)
20×reverse transcriptase	1μL	10μL×(n+1)
10×Primer Probe Reaction Solution (MP351Z)	2μL	2μL×(n+1)

2.2. System packaging:

After mixing and centrifuging the above reaction solution, package 15μL per tube into PCR tubes suitable for fluorescence PCR equipment.

2.3. Sample addition:

Take 5μL of RNA samples extracted in step 1 and add them to the packaged PCR tubes, with a total reaction volume of 20uL. Add 5μL of negative control to the negative control reaction tube. Add 5μL of the corresponding template to the positive control reaction tube.

3. Fluorescence PCR cycle condition setting

Step	Cycle	Temperature	Time
1	1cycle	45℃	20min
2	1cycle	95℃	5min
3	45cycle	95℃	15sec
		55℃	45sec Collect fluorescence

Detection settings: "Reporter Dye" is set to FAM and VIC (HEX), corresponding to nucleic acid testing for hepatitis E virus and hepatitis A virus, respectively. Quencher Dyes are all None. For ABI series instruments, please note to set "Passive Reference" to None.

4. Threshold setting

The threshold setting principle is to use the highest point of the fluorescence signal that just exceeds the normal negative control as the threshold line, or adjust it according to the instrument noise situation.

5. Quality control standards

The negative control had no amplification curve, and the positive control had S-shaped amplification curves in both FAM and VIC (HEX) detection channels, confirming the validity of the experiment. Otherwise, the experimental results will be deemed invalid.

6. Result analysis and judgment

a) If the sample has S-type amplification and Ct value ≤ 38, determine according to the fluorescence channel corresponding to the detection target according to the following table; b) If there is S-type amplification and 38<Ct value ≤ 40, it is determined as an uncertain sample and requires re extraction of nucleic acid for testing; If the retested sample still has S-type amplification and Ct value ≤ 40, determine according to the fluorescence channel corresponding to the detection target in the following table.

Otherwise, it is judged as negative; c) If there is no obvious S-type amplification curve in the sample, but Ct value is reported, it is judged as negative.

Fluorescence channel (detection target)	FAM channel (hepatitis E virus)	VIC channel (hepatitis A virus)
Hepatitis E virus	+	-
Hepatitis A virus	-	+

Limitations of protocol

The target sequence detected by this kit is the conserved region of the hepatitis A virus and hepatitis E virus genes, which are highly conserved and stable. But if the virus undergoes genetic mutations at the target sequence, false negative results may occur, that is, missed detection; Meanwhile, the quality of sample collection, processing, transportation, and preservation all have an impact on the test results.

Performance indicators of reagent kit

1. Minimum detection limit: 1.0×10^3copies/mL.
2. Linear range: 1000~$2\times10_{10}$ copies/mL.
3. Cross reactivity: There is no cross reactivity with other pathogens that may cross with hepatitis A virus and hepatitis E virus, such as rotavirus, norovirus, enteroadenovirus, zarovirus, astrovirus, Salmonella, Shigella, Escherichia coli, Campylobacter jejuni, Yersinia enterotoxigenic, and Aeromonas hydrophila.
4. Precision: The coefficient of variation of the reference standard for detecting precision is less than 5%.

Note

1. Please read the instructions of this reagent kit carefully before the experiment and strictly follow the operating steps.
2. The components in the reagent kit should be thoroughly melted and mixed before use, and then subjected to high-speed and brief centrifugation before use.
3. The reagent kit must be stored away from light to prevent the decay of fluorescent substances. The centrifuge tubes and Tip heads used should be sterilized under high pressure and free of DNase and RNase.
4. The entire operation process and the software and hardware facilities of the PCR laboratory should comply with the requirements of regulations such as the "Management Measures for Clinical Gene Amplification Testing Laboratories in Medical Institutions" and the "Guidelines for the Work of Clinical Gene Amplification Testing Laboratories in Medical Institutions" issued by the Ministry of Health. Properly handle the waste and amplification products generated during the experimental process to prevent cross contamination.
5. This product is for scientific research only, and the test results are for reference only. If a diagnosis is required, please combine clinical symptoms and other testing methods.