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Yellow Fever Virus (YFV) Nucleic Acid Detection Kit (Fluorescent PCR Method)

Product Number: DTK340

Shipping and Storage

1. The reagent kit must be transported under frozen conditions.
2. Stored at -20℃, repeated freezing and thawing should be avoided. The freezing and thawing times of the reagent kit shall not exceed 7 times.
3. Valid for 12 months, please use within the validity period.

Component

Component	50T
Nucleic acid amplification reaction mixture	900μL
Enzyme mixture	100μL
Yellow fever virus positive control	100μL
Negative quality control	1000μL

Note: Different batches of reagents cannot be mixed.

Description

This reagent uses real-time fluorescence PCR technology and is suitable for nucleic acid detection of yellow fever virus (YFV) extracted from clinical samples, environmental samples, and virus isolates. Each reaction system contains specific primers and fluorescent probes for detecting the yellow fever virus (YFV) gene. By collecting the fluorescent signal generated by PCR amplification, qualitative detection of YFV nucleic acid can be quickly completed.

In addition, specific primers and fluorescent probes for internal standard control of human clinical specimens were added to each reaction system to monitor the collection, transportation, and extraction process of the test samples, indicating false negatives in the detection results.

Application

This kit is suitable for qualitative detection of yellow fever virus nucleic acid extracted from clinical samples, environmental samples, and virus isolates. The experimental results only provide reference for basic research and are not used as clinical diagnostic basis.

Yellow fever is an acute infectious disease caused by the yellow fever virus, mainly transmitted through mosquito bites. The disease has a high mortality rate and strong infectivity, and has been included as one of the quarantine infectious diseases regulated by the World Health Organization.

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, Bio Rad CFX96, Roche LightCycler 480 II, Shanghai Hongshi SLAN-96P and other fully automatic fluorescence PCR detectors.

Specimen collection

RNA samples extracted from clinical samples, environmental samples, and virus isolates can all be tested using this kit.

Protocol

1. Sample Preparation

Extract clinical samples, environmental samples, and virus isolates according to the corresponding requirements and steps in the virus RNA extraction kit. We recommend using our company's extraction kit for nucleic acid extraction. The extracted RNA can be directly used for detection. If the sample is not tested immediately after extraction, it can also be stored at -70℃ for future use. 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 following table:

reagent	Quantity of 1 reaction system	Quantity of n+1 reaction systems
Nucleic acid amplification reaction mixture	18μL	18μL× (n+1)
Enzyme mixture	2μL	2μL× (n+1)

2.2. System packaging:

After mixing and centrifuging the above reaction solution, package 20μL 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 pre packaged PCR reaction tubes. Tighten the tube cap, gently mix, and centrifuge immediately before moving to the amplification zone. The total reaction volume is 25μL. Add 5μL of negative control to the negative control reaction tube and 5μL of corresponding template to the positive control reaction tube.

3. Sample addition (sample processing area)

step	Cycles	Temperature	Time	
1	1 cycle	50℃	10min	
2	1 cycle	95℃	30min	
3	45 cycles	95℃	5sec	
		60℃	30sec	Collect fluorescence

Other instruments, such as ABI7500, set the fluorescence collection time to 31 seconds and have no effect on the results. Detection settings: "Reporter Dye" is set to FAM and VIC (HEX) respectively, corresponding to the detection of yellow fever virus nucleic acid and internal standard control. 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. Result analysis and judgment

5.1. If the sample has S-type amplification in the FAM channel and the Ct value is ≤ 38, and there is no requirement in the VIC (HEX) channel, it is determined to be positive for yellow fever virus nucleic acid;

5.2. If the sample has S-type amplification in the FAM channel, with a Ct value of 38<Ct ≤ 40, and a Ct value in the VIC (HEX) channel ≤ 38, it is considered an uncertain sample and requires re extraction of nucleic acid for testing; If the retested sample still has S-type amplification in the FAM channel and the Ct value is ≤ 40, it is determined to be positive for yellow fever virus nucleic acid

5.3. Sex, otherwise it will be judged as negative;

5.4. If the sample has no obvious S-type amplification curve in the FAM channel, but a Ct value is reported and the Ct value in the VIC (HEX) channel is ≤ 38, it is still considered negative for yellow fever virus nucleic acid.

5.5. If there is no signal in the FAM detection channel and the Ct value of the VIC (HEX) channel is greater than 38 for

clinical samples, please collect samples again for testing.

Quality control standards

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

Limitations of detection methods

The target sequence detected by this kit is the conserved region of the yellow fever virus gene, which is highly conserved. 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 result.

Product performance indicators

1. Minimum detection limit: 5×10^2 copies/mL.
2. Linear range: 5×10^2~2×10^{10} copies/mL.
3. Cross reaction: there is no cross reaction against other pathogens that may cross with yellow fever virus (Hantavirus, dengue virus, chikungunya virus, Zika virus, Rift Valley fever virus, Xinjiang hemorrhagic fever virus, new Bunia virus, Ebola hemorrhagic fever virus, Yersinia pestis, hemolytic streptococcus suis, Rickettsia, Leptospira).
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.