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PCR test kit Rift Valley Fever

Product Number:RVF01

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. The freeze-thaw cycle of the reagent kit shall not exceed 7 times.
3. Validity period: 12 months, please use within the validity period.

Component

Component	RVF01 50T
qRT-PCR reaction solution,mix	900μl
qRT-PCR enzyme mixture	100μl
Positive control RVFV	100μl
negative control	1ml

Description

This reagent uses real-time fluorescence PCR technology and is suitable for detecting Rift Valley fever virus nucleic acid extracted from whole blood, serum, and their cultures. Each reaction system contains specific primers and fluorescent probes for detecting the genes of Rift Valley fever virus. By collecting the fluorescent signals generated by PCR amplification, qualitative detection of Rift Valley fever virus nucleic acid can be performed.

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 Rift Valley fever virus nucleic acid extracted from whole blood or serum and their cultures. The experimental results only provide reference for basic research and are not used as clinical diagnostic basis.

Rift Valley fever is an acute febrile animal disease caused by the Rift Valley fever virus. The virus is mainly transmitted by mosquitoes and can infect humans and animals. After infection, people usually experience symptoms such as fever, headache, bleeding, and shock.

Applicable instruments

A 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 LightCycler480 II, and other fully automatic fluorescence PCR detectors.

Sample requirements

RNA samples extracted from whole blood, serum, and their cultures can be detected using this kit.

Serum: After natural coagulation of blood without anticoagulants, extract the supernatant.

Protocol

1. Sample preparation

Extract whole blood or serum and their cultures according to the corresponding requirements and steps in the virus RNA

extraction kit. 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 system
qRT-PCR reaction solution,mix	18μL	18μL× (n+1)
qRT-PCR 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. Fluorescence PCR cycle condition setting

Step	Cycle	Temperature	Time	
1	1	50℃	10min	
2	1	95℃	30min	
3	45	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. 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.

6. Result analysis and judgment

6.1. 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 Rift Valley Fever virus nucleic acid;

6.2. 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 judged as positive for Rift Valley Fever virus nucleic acid, otherwise it is judged as negative;

6.3. 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 Rift Valley Fever virus nucleic acid.

6.4. 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.

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 results.

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: No cross reaction was found against other pathogens that may cross with RVF virus (human cytomegalovirus, human herpesvirus, polyoma virus, influenza A virus, influenza B virus, measles virus, rubella virus, coxsackie virus, enterovirus, hantavirus, chikungunya virus, zika virus, yellow 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.