MEBEP BIOSCIENCE

MEBEP TECH(HK) Co., Limited

Email: sales@mebep.com Website: www.mebep.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

Hantavirus (HFRS) Universal Nucleic Acid Real-Time Fluorescent PCR Detection Kit

Product Number: DTK562

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	25T
Nucleic acid amplification reaction mixture	450μL
Enzyme mixture	50μL
Positive control of Hantavirus in hemorrhagic fever with renal syndrome	100μL
Negative 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 Hantavirus extracted from serum samples, environmental samples, and virus isolates. Each reaction system contains specific primers and fluorescent probes for detecting the Hantavirus gene. By collecting the fluorescent signal generated by PCR amplification, qualitative detection of Hantavirus nucleic acid can be performed.

Application

This kit is suitable for qualitative detection of Hantavirus nucleic acid extracted from serum samples, environmental samples, and virus isolates. The test results are for scientific research purposes only and cannot be used alone as a basis for clinical diagnosis or exclusion of cases.

Hantavirus can cause Hantavirus pulmonary syndrome (HPS) and Hantavirus hemorrhagic fever with renal syndrome (HFRS). Hantavirus hemorrhagic fever with renal syndrome (HFRS) is a natural focus disease caused by Hantavirus. It is one of the viral diseases that seriously endanger the health of our people, and is a Class B infectious disease specified in the Law of the People's Republic of China on the Prevention and Treatment of Infectious Diseases.

Applicable instruments

The fully automatic real-time fluorescence PCR detector that has undergone channel calibration needs to include a FAM detection channel.

Specimen collection

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

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

Protocol

1. **Sample preparation**

Email: sales@mebep.com Website: www.mebep.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

Extract serum 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. Fluorescence PCR cycle condition setting

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, corresponding to the detection of Hantavirus nucleic acid, and "Quencher Dye" is set to 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, it is determined to be positive for Hantavirus nucleic acid;

5.2. If the sample has S-type amplification in the FAM channel 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 in the FAM channel and Ct value ≤ 40, it is determined as positive for Hantavirus nucleic acid, otherwise it is determined as negative;

5.3. If there is no obvious S-type amplification curve in the FAM channel of the sample, but Ct values are reported, it is still considered negative for Hantavirus nucleic acid.

Quality control standards

The negative control had no amplification curve, and the positive control FAM channel had an S-shaped amplification curve, 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 Hantavirus 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.

Product performance indicators

1. Minimum detection limit: 5×10^2 copies/mL.
2. Linear range: $5 \times 10^2 \sim 2 \times 10^{10}$ copies/mL.
3. Cross reaction: no cross reaction was found for other pathogens (dengue virus, chikungunya virus, Zika virus, yellow fever virus, Rift Valley fever virus, Xinjiang hemorrhagic fever virus, new Bunia virus, Ebola hemorrhagic fever virus, Yersinia pestis, hemolytic streptococcus suis, Rickettsia, Leptospira) that may cross with Hantavirus.
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.
3. 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.
4. 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.