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15 Types of Hemorrhagic Fever Pathogens Multiplex Nucleic Acid Detection Kit (Real-Time PCR Method)

Product Number:DTK534

Shipping and Storage

1. The reagent kit should be stored in a cold and dark place below -15 ℃; Valid for 12 months; Sealed transportation with foam box and ice bag, the temperature shall not exceed 8 ℃; The production date and expiration date are detailed in the outer packaging box.
2. The reagent kit should avoid repeated freeze-thaw cycles, with no more than 2 freeze-thaw cycles.
3. After opening, store in a cold and dark place below -15 ℃ without affecting the use within the expiration date.

Component

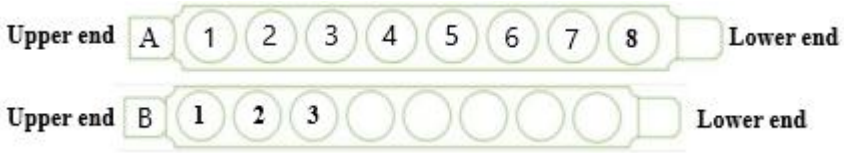
Component	24T
15 types of hemorrhagic fever pathogen RT-PCR reaction solution	24×8-tube in bag A and 24×8-tube in bag B (take one 8-tube each from bag A and bag B to make 1T)
Positive controls for 15 hemorrhagic fever pathogens (universal)	240μL×2
15 types of hemorrhagic fever pathogen negative controls (universal)	480μL

Required but not provided: Nucleic acid extraction or purification kits produced by our company

Note: 1. Different batch numbers of reagents cannot be mixed.

2. Each reagent component in the kit is sufficient for the number of tests indicated by the packaging specifications.

3.There are bags A and B of reaction solution in the reagent kit; After taking out the 8-tube from bag A and bag B, mark the protruding position of the 8-tube cover near the 1st end, as shown in the following figure:



Specifications	End 1 marking	Schematic diagram of 8-tube	8-end marking
0.1 tube	Sharp is one end		The circle has 8 ends
0.2 tube	The lid has a hole on one end		Non porous is 8-terminal

A bag with 8 tubes and B bag with 8 tubes (actual 3 holes), a total of 11 reaction solutions, were used to detect 15 pathogens. The corresponding pathogens detected are as follows:

Number	Bag A	Number	Bag B
1	chikungunya virus	1	Ebola virus
2	Yellow fever virus	2	Lassa fever virus
3	Dengue virus types I, II, III, IV	3	New Bunyavirus
4	Hantan virus, Seoul virus		
5	Pumala virus		
6	Rift Valley fever virus		
7	Congo Crimean hemorrhagic fever virus		
8	Marburg virus		

Description

This reagent kit utilizes real-time fluorescence PCR principle to qualitatively detect specific genes of 15 hemorrhagic fever pathogens.

This kit uses real-time fluorescence PCR technology, and each tube of reaction solution contains specific amplification primers and detection probes for each target gene. The probes are oligonucleotide sequences containing 5 'labeled luminescent groups and 3' labeled quenching groups. If the probes remain intact, the fluorescence signal emitted by the luminescent groups will be absorbed by the quenching groups and no signal will be detected. During the PCR amplification process, probes that specifically bind to the template are cleaved by Taq enzyme (with 5 '-3' exonuclease activity), and the luminescent and quenching groups are separated, producing a fluorescent signal. Real time monitoring and output of signal strength of corresponding channels during PCR process using instruments to achieve qualitative analysis of detection results.

Application

This kit is suitable for qualitative detection of 15 hemorrhagic fever pathogens in blood samples, cerebrospinal fluid, tissue specimens (including brain tissue, liver, and spleen), and mosquito specimens. The test results are for scientific research purposes only and cannot be used alone as a basis for clinical diagnosis or exclusion of cases.

The main pathogens detected are as follows: chikungunya virus, yellow fever virus, dengue virus types I, II, III, IV, Hantaan virus, Seoul virus, Pumala virus, Rift Valley fever virus, Congo Crimean hemorrhagic fever virus, Marburg virus, Ebola virus, Lassa fever virus, and New Bunyavirus, which are 15 common hemorrhagic fever pathogens.

Applicable instruments

This kit is suitable for ABI7500, Hongshi SLAN-96P, Bole CFX96, Agilent AriaMX real-time fluorescence quantitative PCR instrument.

Note: Please use transparent 0.1ml consumables for Bole CFX96 and Agilent AriaMX. ABI7500、 Please use transparent 0.2ml consumables for Hongshi SLAN-96P.

Sample requirements

1. The collection method and requirements mainly refer to the guiding principles of biological sample analysis, and the specific methods are as follows:
   - 1.1. Blood samples: Use sterile syringes to extract whole blood from patients in the acute phase, recovery phase, and monitoring points with fever or viral encephalitis. Place the blood in centrifuge tubes and collect serum for testing.
   - 1.2. Cerebrospinal fluid: Use a sterile syringe to extract the patient's cerebrospinal fluid and place it in a centrifuge tube for testing.
   - 1.3. Tissue specimen: Use sterile scissors and tweezers to take about 1.0g of the sample to be tested and transfer it to a frozen mortar or homogenizer. Add 2mL of diluent, grind the tissue into a slurry, centrifuge at 4 ℃ 5000g for 15 minutes, and collect the supernatant for testing.
   - 1.4. Mosquito specimens: Mosquitoes captured at the monitoring point are placed in sterile and low-temperature resistant outer spiral centrifuge tubes, frozen to death, and washed thoroughly with physiological saline containing 25% alcohol. The washing solution is discarded and washed twice with sterile physiological saline before discarding the washing solution. Add 1mL of diluent, grind the mosquitoes evenly, centrifuge 5000g at 4℃ for 10 minutes, and take the supernatant for testing.
2. Cross contamination between samples should be avoided.
3. After sample collection, it should be tested in a timely manner and can also be stored at -20±5 ℃ for testing. Long term storage should be kept below -70 ℃.

Protocol

1. **Reagent Preparation: (Reagent Preparation Area)**

1.1. Calculate the number of reactions required for the experiment (number of samples+1 positive control+1 negative control), take an equal number of people (8 wells in bag A and 3 wells in bag B as a set) in 8-connected tubes (containing PCR reaction solution), and store the remaining reagents in a -15℃ freezer.

1.2. Take out the reagent kit from the refrigerator below -15℃ and equilibrate it to room temperature (20-25℃). Shake gently and mix thoroughly, then centrifuge immediately to the bottom of the tube.

1.3. Transfer the required 8 tubes for the experiment to the sample processing area.

2. Sample Processing: (Sample Processing Area)

We recommend using our nucleic acid extraction or purification reagents to extract sample nucleic acids for PCR testing. Extract according to the instructions of the extraction kit. Due to the total amount of 60μL added to the reagent kit at once (calculated based on 12 wells to reduce errors), if the amount of nucleic acid after elution is less than 60μL, it is necessary to dilute with appropriate water before adding the sample.

Sample addition:

2.1. Take out the prepared reagents from the reagent preparation area and centrifuge at low speed for 10 seconds.

2.2. Open the PCR reaction tube cap and add 5μL of each sample (including positive and negative controls) into 11 reaction systems, including A and B eight tubes.

2.3. Cover the PCR reaction tube cap, record the order of template sample addition, and make corresponding markings (please mark the protruding parts at both ends of the eight piece PCR reaction tube cap, do not mark it in the middle of the eight piece PCR reaction tube cap to avoid affecting signal collection). Shake and mix thoroughly for more than 10 seconds, then centrifuge at low speed above 2000rmp for 10 seconds to ensure that there are no bubbles in the tube.

2.4. Transfer the PCR reaction tube to the nucleic acid amplification area for loading.

Note: During the process of extracting and adding nucleic acid samples, contamination should be avoided. If the extracted nucleic acid template cannot be detected immediately, it is recommended to store it below -70 ℃.

3. PCR：(Nucleic acid amplification zone)

3.1. Preheat the machine and test its performance.

3.2. Take the PCR reaction tubes prepared in the sample processing area and place them in the corresponding position of the instrument sample slot (before starting the machine, pay attention to checking whether each reaction tube is tightly covered to avoid the leakage of PCR products and aerosol pollution of the instrument and environment). And record the placement order.

3.3. Set the parameters related to instrument nucleic acid amplification and perform PCR amplification.

Instrument nucleic acid amplification related parameters

System	The reaction system is set to 25μL		
Signal acquisition	Select four fluorescence channels, FAM, HEX/VIC/JOE, CY5, and ROX, to collect fluorescence from the reaction solution separately		
PCR reaction conditions	**Step**	**Condition**	**Cycles**
	Reverse transcription	50℃：15 min	1
	Pre denaturation	95℃：5 min	1
	PCR	95℃：3s	45
		55℃：45s（Collect fluorescence signals at the end of this stage）	

Note: The ABI series fluorescence quantitative PCR instrument does not select ROX calibration, and the quenching group is selected as None.

4. Result analysis

After the reaction is complete, the results will be automatically saved. Please make sure to select the "absolute fluorescence value method" for Hongshi SLAN-96P ABI7500、 Please use the "absolute fluorescence value method" or the same analytical method for equipment from brands such as Bole CFX96. Adjust the Start and End threshold values of the baseline based on the analyzed curve (adjust the Start value within the range of 3-15 and the End value within the range of 5-20 according to the

actual situation, adjust the curve of the negative control to be straight or below the threshold line), click on Analysis to automatically obtain the analysis results, and view the results on the report interface.

Reference value (reference range)

1. **Validity assessment of reagent kit:**
   (1) Positive control: CT value ≤ 32, with significant exponential growth. In order to avoid contamination of the laboratory by high concentration positive controls, the reagent kit uses medium to low concentration positive controls, and attention should be paid to preventing contamination during operation.
   (2) Negative control: Ct value>40 or no Ct value, linear or slightly sloping, without significant exponential growth or plateau periods.
2. **Sample result determination:**
   (1) Positive: The Ct value of the sample test result is ≤ 37, with a significant exponential increase.
   (2) Suspicious: The Ct value of the sample test result is in the range of 37-40. At this point, the sample should be subjected to repeated testing. If the Ct value of the repeated experiment is still within the range of 37-40, there should be a significant exponential increase. If it is judged as positive, otherwise it is judged as negative.
   (3) Negative: The Ct value of the sample test result is greater than 40 or there is no Ct value.

Interpretation of Inspection Results

Hole	Pathogen	Detection channel	Interpretation of channel detection results
A1	Chikungunya virus	FAM	Detection of Chikungunya virus
A2	Yellow fever virus	FAM	Detected yellow fever virus
A3	Dengue virus type I	FAM	Dengue virus type I detected
	Dengue virus type II	HEX/VIC/JOE	Dengue virus type II detected
	Dengue virus type III	CY5	Dengue virus type III detected
	Dengue virus type IV	ROX	Detected dengue virus type IV
A4	Hantan virus	FAM	Detected Hantan virus
	Seoul virus	HEX/VIC/JOE	Detected Seoul virus
A5	Pumala virus	FAM	Detected Pumala virus
A6	Rift Valley fever virus	FAM	Detection of Rift Valley Fever Virus
A7	Congo Crimean hemorrhagic fever virus	FAM	Detected Congo Crimean hemorrhagic fever virus
A8	Marburg virus	FAM	Detected Marburg virus
B1	Ebola virus	FAM	Detected Ebola virus
B2	Lassa fever virus	FAM	Detected Lassa fever virus
B3	New Bunyavirus	FAM	New Bunyavirus detected

Product performance indicators

Minimum detection limit: 500 copies/mL.

Limitations of detection methods

1. The test results of this kit are only for scientific research purposes and cannot be used for clinical diagnosis.
2. Improper handling of the collection, transportation, storage, and nucleic acid extraction process of the tested sample can easily lead to nucleic acid degradation and false negative results.
3. When the concentration of the tested nucleic acid in the sample is below the minimum detection limit, false negative results may occur.
4. If cross contamination occurs during the sample collection and preparation process, false positive results can easily be obtained.

5. Some infected individuals may have a large amount of dead virus in the sample due to taking antiviral drugs. In this case, the test method of this kit may result in a strong positive result but the culture method may result in a negative result. When such results occur, the recent medication history of the tested individual should be inquired.
6. 6. Mutations in the target sequence of the virus or other reasons that cause sequence changes may lead to false negative results.
7. For sudden outbreaks of novel viruses, the optimal sample type for detection and the optimal sampling time after infection may not have been confirmed. Therefore, collecting samples in multiple locations from the same patient in multiple batches can reduce the possibility of false negative results.

Note

1. This kit is only used for scientific research. Operators should receive professional training and have certain experience.
2. To ensure the accuracy and reliability of the experimental results, please use a calibrated pipette and select qualified disposable PCR reaction tubes, centrifuge tubes, gun heads, etc. for sample processing and liquid preparation operations. All tools should be free of DNA enzymes and RNA enzymes.
3. Please strictly partition the experiment; All items and work clothes in each district are for exclusive use and must not be used interchangeably to avoid contamination. Please clean the workbench immediately after the experiment.
4. This product should be fully melted at room temperature before use, mixed well, and centrifuged at low speed in an instant.
5. Sample processing should be carried out in a biosafety cabinet to protect the safety of operators and prevent environmental pollution.
6. Negative and positive controls should be set up for each experiment. Do not mix reagents of different batches. Use the reagent kit within its expiration date.
7. The test sample should be kept as fresh as possible, and the extraction process should strictly prevent RNA enzyme contamination and RNA degradation caused by improper operation.
8. RNA samples stored at 8-70 ℃ should be thoroughly melted, mixed, and centrifuged at low speed at room temperature before use.
9. Reaction tubes containing reaction solution should be capped or packed in a compact bag before being transferred to the sample processing area.
10. When adding the sample, it should be completely added to the reaction solution, and there should be no sample adhering to the tube wall. After adding the sample, the tube cover should be tightly closed as soon as possible.
11. When packaging the reaction solution, try to avoid generating bubbles as much as possible. Before starting the machine, pay attention to checking whether each reaction tube is tightly covered to avoid leaking and contaminating the instrument.
12. After amplification, remove the reaction tube, seal it in a special plastic bag, and discard it at the designated location.
13. The gun head used in the experiment should be directly injected into the waste tank containing 10% sodium hypochlorite and discarded together with other waste items.
14. Workbenches and various experimental items are often disinfected with 10% sodium hypochlorite, 75% alcohol, and ultraviolet lamps.
15. The real-time fluorescence PCR instrument needs to be regularly calibrated and cleaned for the loading plate holes.
16. The test samples involved in this kit should be considered as infectious substances, and the operation and handling must comply with the relevant requirements of the Ministry of Health's "General Guidelines for Biosafety of Microbial Biomedical Laboratories" and "Regulations on the Management of Medical Waste".