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7 Types of Arthropod-Borne Pathogens Multiplex Nucleic Acid Detection Kit (Fluorescent PCR Method)

Product Number:DTK535

Shipping and Storage

1. The reagent kit should be stored in a cold and dark place below -15℃; Valid for 12 months; Use foam box to add sealed transportation in ice packs, with a temperature not exceeding 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 5 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	DTK535	50T
7 types of Arthropod-Borne Pathogens RT-PCR reaction solution	A-tube reaction solution	425 μL×2
	B-tube reaction solution	425 μL×2
	C-tube reaction solution	425 μL×2
	D-tube reaction solution	425 μL×2
	E-tube reaction solution	425 μL×2
	F-tube reaction solution	425 μL×2
	G-tube reaction solution	425 μL×2
7 types of Arthropod-Borne Pathogens primer probe mixture	A-tube primer probe	50 μL×2
	B-tube primer probe	50 μL×2
	C-tube primer probe	50 μL×2
	D-tube primer probe	50 μL×2
	E-tube primer probe	50 μL×2
	F-tube primer probe	50 μL×2
	G-tube primer probe	50 μL×2
7 types of Arthropod-Borne Pathogens Mixed enzyme solution		175 μL×2
Negative control (universal)		60 μL
Positive control (universal)		30 μL×2

Note:1)Required but not provided: Nucleic acid extraction or purification kits produced by our company

2)Different batch numbers of reagents cannot be mixed.

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

4)The corresponding detected pathogens are as follows:

Number	Detecting pathogens
A	Dengue virus
B	Chikungunya fever virus virus
C	Japanese encephalitis virus
D	Forest encephalitis virus
E	Przewalski's Rickettsia
F	Rickettsia Mori
G	Rickettsia scrub typhus

Description

For Research Use Only

This kit utilizes real-time fluorescence PCR principle to qualitatively detect specific genes of 7 insect borne 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 7 insect borne pathogens in blood samples, cerebrospinal fluid, tissue specimens (including brain tissue, liver, and spleen), and mosquito specimens. The test results are for clinical reference and 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: dengue virus, chikungunya fever virus, Japanese encephalitis virus, rickettsia pneumoniae, rickettsia pneumoniae, forest encephalitis virus, and rickettsia tsutsugamushi.

Applicable instruments and consumables

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. This kit contains seven different PCR reaction solutions, namely A, B, C, D, E, F, and G, for each person. The data needs to be amplified and collected in seven different PCR reaction tubes.

1.2. Take out the reagent kit from the refrigerator below -15 ℃ and let it equilibrate to room temperature (20-25 ℃). Shake

gently and mix thoroughly, then centrifuge immediately to the bottom of the tube.

1.3. According to the sample size of the experiment, it is necessary to configure the number of reactions n (n=number of negative controls (1T)+number of positive controls (1T)+error margin+number of samples). Take 7 sterile centrifuge tubes of appropriate volume, labeled as A.B.C., D, E, F, G, and take the corresponding components of each reaction solution according to the proportions in the reaction solution configuration table.

Reaction solution preparation table (per person)		
Components of reaction solution		Dosage(μl)per person
A	A-tube reaction solution	17μL
	A-tube primer probe	2μL
	7 types of Arthropod-Borne Pathogens primer probe mixture	1μL
B	B-tube reaction solution	17μL
	B-tube primer probe	2μL
	7 types of Arthropod-Borne Pathogens primer probe mixture	1μL
C	C-tube reaction solution	17μL
	C-tube primer probe	2μL
	7 types of Arthropod-Borne Pathogens primer probe mixture	1μL
D	D-tube reaction solution	17μL
	D-tube primer probe	2μL
	7 types of Arthropod-Borne Pathogens primer probe mixture	1μL
E	E-tube reaction solution	17μL
	E-tube primer probe	2μL
	7 types of Arthropod-Borne Pathogens primer probe mixture	1μL
F	F-tube reaction solution	17μL
	F-tube primer probe	2μL
	7 types of Arthropod-Borne Pathogens primer probe mixture	1μL
G	G-tube reaction solution	17μL
	G-tube primer probe	2μL
	7 types of Arthropod-Borne Pathogens primer probe mixture	1μL

1.4. Shake and mix the seven prepared reagents A, B, C, D, E, F, and G thoroughly, and centrifuge at low speed of 2000rpm for 10 seconds. Divide the reagents into the corresponding wells of the eight PCR reaction tubes at a rate of 20 μ L per tube (one well for each sample containing the seven reaction solutions A, B, C, D, E, F, and G).

1.5. Tighten the cover of the eight link PCR reaction tube and pay attention to the corresponding labeling (please mark the protruding parts at both ends of the eight link PCR reaction tube cover, do not mark it in the middle of the eight link PCR reaction tube cover to avoid affecting signal acquisition). Transfer the PCR reaction tube to the sample processing area. Put the remaining reagents back into the freezer below -15 ℃ for freezing storage.

2. PCR：(Nucleic acid amplification zone)

2.1. Preheat the machine and test its performance.

2.2. 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.

2.3. 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 FAM fluorescence channels 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 signal 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.

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:

1.1. 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.

1.2. Negative control: Ct value>40 or no Ct value, linear or slightly sloping, without significant exponential growth or plateau periods.

2. Sample result determination:

2.1. Positive: The Ct value of the sample test result is≤37, with a significant exponential increase.

2.2. 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.

2.3. Negative: The Ct value of the sample test result is greater than 40 or there is no Ct value.

Interpretation of Inspection Results

Tube number and test results		**Explanation of Sample Testing Results**
A tube	Positive (+)	Dengue virus detected in the sample
	Negative (-)	Dengue virus not detected in the sample
B tube	Positive (+)	Chikungunya fever virus detected in the sample
	Negative (-)	No chikungunya virus was detected in the sample
C tube	Positive (+)	Japanese encephalitis virus detected in the sample
	Negative (-)	No detection of Japanese encephalitis virus in the sample
D tube	Positive (+)	Forest encephalitis virus detected in the sample
	Negative (-)	Forest encephalitis virus not detected in the sample
E tube	Positive (+)	Brucella was detected in the sample
	Negative (-)	No Rickettsia pneumoniae was detected in the sample

F tube	Positive (+)	Rickettsia aeruginosa was detected in the sample
	Negative (-)	No Rickettsia Mo was detected in the sample
G tube	Positive (+)	Rickettsia tsutsugamushi was detected in the sample
	Negative (-)	No Rickettsia tsutsugamushi was detected in the sample

Product performance indicators

Minimum detection limit: 500 copies/mL.

Limitations of protocol

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 sample collection and preparation, false positive results can easily be obtained.
5. Some infected individuals may have a large number of dead viruses in their samples 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 test subject should be inquired.
6. Mutations in the target sequence of the virus or other reasons leading to sequence changes may result in 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".