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Hantaan, Seoul, Andes and American Cardiopulmonary Syndrome Hantavirus Multiplex Nucleic Acid Detection Kit(Fluorescence PCR Method)

Product Number: DTK588

Shipping and Storage

1. Reagents should be kept away from light and sealed. Reagent components should be stored at -20 ± 5℃. The validity period of the reagent kit is 12 months.
2. Please refer to the outer packaging box for the production date and expiration date.
3. Curling with ice or foam box with blue ice seal (2-8℃) shall be used for transportation.
4. Store in the dark at -20 ± 5℃ after opening; Freeze repeatedly no more than 7 times.

Component

Component	25T	50T	Main components
PCR reaction solution 1	475μL	950μL	dNTPs, RNasin, PCR buffer, probes, primers, etc.
PCR reaction solution 2	475μL	950μL	dNTPs, RNasin, PCR buffer, probes, primers, etc.
Eenzyme mixture	25μL	50μL	UNG enzyme, Reverse transcriptase, Taq enzyme
Positive control	0.2mL	0.2mL	Plasmids containing target genes of Hantaan virus, Seoul virus, Andes virus, American HCPS-associated hantavirus and internal control gene
Negative control	0.2mL	0.2mL	Buffer

1. The ingredients of products with different batch numbers cannot be mixed or exchanged.
2. Required but not provided reagents: nucleic acid extraction or purification reagents.

Description

This kit designs target-specific primers and probes targeting the conserved gene sequences specific to Hantaan virus, Seoul virus, Andes virus and American HCPS-associated hantavirus respectively. It applies fluorescent PCR technology and realizes the differentiation of the above hantaviruses through multi-channel detection design.

The PCR detection system simultaneously contains specific primers and probes for endogenous internal control nucleic acid (human housekeeping gene). The normality of the internal control detection signal is used to monitor the entire process of sample collection, extraction and amplification, avoiding false negative results.

Application

This kit is intended for the qualitative detection and differentiation of nucleic acids of Hantaan virus, Seoul virus, Andes virus and American hantavirus cardiopulmonary syndrome (HCPS)-associated hantavirus.

The experimental results are only for reference in basic research and shall not be used as a basis for clinical diagnosis.

Applicable instruments

Suitable for multi-channel fluorescence quantitative PCR instruments such as SLAN-96P, Gentier 96R, AGS 4800, ABI7500, Roche LightCycler480/480 II, Bio Rad CFX96, ABI QuantStudio 5/6/7, QuantStudio Dx, etc.

Distribution Chart of Multiplex Fluorescent PCR Detection Reagents

Please refer to the following table for distribution:

Reaction Tube	FAM-labeled Target	VIC-labeled Target	ROX-labeled Target	CY5-labeled Target
1	Hantaan virus	Seoul virus	/	Internal Control
2	American HCPS-associated hantavirus	/	Andes virus	/

Note: Positive control and negative control must be set for each test to monitor the detection system and operating environment.

Specimen collection

1. Applicable sample types: various clinical specimens and pathogen isolates, etc.
2. Sample preservation and transportation: The samples to be tested should be processed as soon as possible, and specimens that can be tested within 24 hours can be stored at 4℃; Specimens that cannot be detected within 24 hours can be stored at -70℃ or below for a long time (if there is no -70℃ storage condition, the test sample can be stored at -20±5℃ for 1 month). Repeated freeze-thaw cycles should not exceed 5 times. The samples shall be transported by means of curling bottle with ice or foam box with ice seal.

Protocol

1. Reagent preparation (conducted in the reagent preparation area)

1.1. Take out each component from the box and let it stand at room temperature. After the temperature reaches room temperature, mix well and centrifuge immediately for later use.

1.2. According to the number of samples to be tested, positive controls and negative controls, take the corresponding amounts of components as shown in the table below:

Component	Volume per Reaction
PCR Reaction Solution 1/2	19μL
Enzyme Mixture	1μL
Total Volume	20μL

1.3. Mix thoroughly to prepare the PCR master mix, mix well and centrifuge briefly. Aliquot 20μL into PCR reaction tubes/plates and transfer to the Sample Processing Area for later use.

2. Sample Processing and Sample Loading (Carried out in the Sample Processing Area)

2.1. Nucleic acid extraction

Use nucleic acid extraction or purification reagents (applicable to the type of sample to be tested) to extract nucleic acid from the test sample, and follow the instructions of the corresponding reagent kit for specific operations.

Positive and negative controls can be used directly without the need for nucleic acid extraction.

2.2. Sample addition

Add 5μL of processed nucleic acid, negative control, and positive control to the PCR reaction tube/plate that has been mixed with the reaction system mixture, with a final volume of 25μL. Cover the tube tightly, mix well, and centrifuge at low speed immediately. Transfer to the amplification area for later use.

3. PCR amplification (performed in the amplification and analysis area) (please refer to the instrument manual for setup)

3.1. Place the PCR reaction tube into the sample slot of the amplifier, set the positive control, negative control, and test sample according to the corresponding positions, and set the sample name.

3.2. Fluorescence detection channel selection:

Set the corresponding fluorescence channels according to the above distribution chart of multiplex fluorescent PCR detection reagents.

3.3. Recommended loop parameter settings:

Step	Cycles	Temperature	Time
Reverse transcription	1 cycle	50℃	20min
Pre denaturation	1 cycle	95℃	3min
Denaturation	40 cycles	95℃	15sec

Annealing/extension/fluorescence detection*		60℃	30sec

After the settings are completed, save the file and run the reaction program.

4. **Result analysis (please refer to the instrument manual for settings)**

The results are automatically saved after the reaction is completed, and the amplification curves are analyzed. Adjust the baseline start point, baseline end point and threshold according to the analyzed images (users can adjust according to the actual situation; the baseline start point can be set between 3 and 15, and the baseline end point can be set between 5 and 20. Adjust the threshold line to be located in the exponential phase of the amplification curve and make the detection result of the negative control amplification curve be NoCt when it is flat). Click "Analyze" and record the sample test results.

Quality control

1. Negative Control*: No Ct value or Ct > 38 in all detection channels (except the internal control channel);
2. Positive Control: Ct≤35 in all detection channels;
3. Internal Control: Ct≤35 in the internal control channel for human samples;
4. The above requirements must be met simultaneously in the same experiment.

Note: The signal value of Ct<38 may appear in the negative control internal standard channel due to the presence of human derived nucleic acids in the environment, which is a normal situation.

Result judgment

1. The targets detected by the fluorescence channels of each well are shown in the table under the "Distribution Chart of Multiplex Fluorescent PCR Detection Reagents" section of this manual.
2. Negative: Ct value > 38 or not detected.
3. Positive: The amplification curve shows a typical S-shape and the Ct value≤35.
4. Suspected Positive: The amplification curve shows a typical S-shape and 35 < Ct value≤38, which requires retesting; if the retest result is consistent, the result is judged as positive; if the Ct value > 38 or not detected, the result is judged as negative.

Note:

1. The results of Hantaan virus and Seoul virus are independently interpreted directly according to the amplification signals of their corresponding detection channels.
2. Andes virus and American HCPS-associated hantavirus require combined interpretation: If the test result of American HCPS-associated hantavirus is positive and the test result of Andes virus is positive, it is judged that Andes virus is detected; If the test result of American HCPS-associated hantavirus is positive and the test result of Andes virus is negative, it is judged as positive for American HCPS-associated hantavirus (such as Sin Nombre virus, etc.), and it is noted that Andes virus is not detected.
3. If there is no amplification signal in all the four target detection channels and the amplification signal of the internal control channel is normal, it is judged that the nucleic acid of the virus to be tested is not detected.

Limitations of detection methods

1. Unreasonable sample collection, processing, transportation, and low sample concentration can all lead to false negative results.
2. Sequence changes caused by variations or other reasons in the target sequence of the pathogen may result in false negative results.
3. Unreasonable storage of reagents may lead to false negative results.
4. Unverified interference or PCR inhibitors may lead to false negative results.
5. Cross contamination during sample processing can result in false positive results.

Performance indicators of reagent kit

1. Accuracy: Positive reference products were tested by the enterprise, and the results were all positive.
2. Specificity: It can detect the target in all specimens and does not cross with other types.

3. Minimum detection limit: The minimum detection limit of this kit is 500 copies/mL.
4. Precision: The coefficient of variation (CV) of Ct values detected within/between batches and within/between days is less than 5%.

Note

1. This product is only used for in vitro testing. Please read this manual carefully before use.
2. Before the experiment, please familiarize yourself with and master the operation methods and precautions of various instruments to be used, and conduct quality control for each experiment.
3. Laboratory management should strictly follow the management standards of PCR gene amplification laboratories. Experimental personnel must receive professional training, and the experimental process should be strictly divided into zones. Consumables used should be RNase Free and DNase Free, and specialized instruments and equipment should be used for each stage of experimental operations. Supplies for each zone and stage cannot be used interchangeably.
4. All test samples should be considered as having infectious substances. During the experiment, work clothes should be worn, disposable gloves should be worn, and gloves should be replaced frequently to avoid cross contamination between samples; Sample handling and waste disposal must comply with relevant regulatory requirements.
5. Reminder: Improper storage, transportation, and use of reagents may affect their detection results, such as improper storage and transportation, improper sample collection, sample processing, and testing procedures. Please strictly follow the instructions for operation.
6. To ensure the accuracy and reliability of the test results, it is recommended to conduct PCR testing experiments immediately after sample nucleic acid extraction.