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Hantavirus Cardiopulmonary Syndrome (HCPS) PCR Kit

Product Number: DTK587

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	475μL	950μL	dNTPs, $MgCl_2$, RNasin, PCR buffer, Probes, primers, etc
Eenzyme mixture	25μL	50μL	UNG enzyme, reverse transcriptase, Taq enzyme
Positive control	0.2mL	0.2mL	Plasmid containing Hantavirus Heart Lung Syndrome (HCPS) target gene and internal standard 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 reagent kit is designed for the conserved gene sequences of Hantavirus Cardiopulmonary Syndrome (HCPS) related viruses (including Andean Hantavirus). Specific primers and probes are designed, and fluorescence PCR technology is applied to qualitatively detect the nucleic acid of Hantavirus HCPS related viruses through changes in fluorescence signal.

The PCR detection system also includes specific primer probes for endogenous internal standard nucleic acid substances (human housekeeping genes), which monitor the entire process of sample collection, extraction, and amplification by checking whether the internal standard detection signal is normal, avoiding false negative results.

Application

This kit is used for qualitative detection of Hantavirus Cardiopulmonary Syndrome (HCPS) related viral nucleic acids and can specifically detect Hantavirus Cardiopulmonary Syndrome (HCPS) containing Andean Hantavirus.

The experimental results only provide reference for basic research and are not used as clinical diagnostic basis.

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.

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, calculate the number of reagents used N (N=number of samples to be tested+positive control+negative control+1), and take the corresponding amount of components in proportion (PCR reaction solution 19μL/person+enzyme mixture 1μL/person).

1.3. Mix thoroughly to form a PCR mixture, centrifuge immediately after thorough mixing, and transfer 20μL into PCR reaction tubes/plates, then transfer to the sample processing area for later use.

2. Sample processing and sample addition (conducted 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:

FAM channel	CY5 channel
HCPS related Hantavirus	Internal standard

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

Once set up, save the file and run the reaction program.

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

After the reaction is completed, the results will be automatically saved and the amplification curve will be analyzed. Adjust the baseline starting point, baseline endpoint, and threshold based on the analyzed image (users can adjust them according to their actual situation. The baseline starting point can be set between 3-15 and the baseline endpoint can be set between 5-20. Adjust the threshold line to be in the exponential phase of the amplification curve and make the negative control amplification curve flat. The detection result will be NoCt). Click on analyze and record the sample testing results.

Quality control

1. Negative control *: All detection channels (except for the internal standard channel) have no Ct values or Ct>38;
2. Positive control: Ct ≤ 35 for all detection channels;
3. Internal standard: Human sample internal standard channel Ct ≤ 35;
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. Negative: Ct value>38 or not detected.
2. Positive: The amplification curve shows a typical S-shape, and the Ct value is ≤ 35.
3. Suspected positive: The amplification curve shows a typical S-shaped pattern, and the Ct value is between 35 and 38, requiring retesting; If the retest results are consistent, the judgment result is positive. If the Ct value is greater than 38 or not detected, the judgment result is negative.

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.