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Influenza Virus H1N1/H3 Subtypes Nucleic Acid Detection Kit (Dual Fluorescent PCR Method)

Product Number:DTK536

Shipping and Storage

1. Store at -20 ℃ away from light, with a shelf life of 12 months.
2. Low temperature transportation cannot exceed 4 days; After opening, store in the dark at -20 ℃ without affecting the expiration date. Avoid repeated freeze-thaw cycles, as six freeze-thaw cycles will not affect the detection results.

Component

Component	25T	50T	Main components
qRT-PCR reaction solution	300μL	600μL	Tris, KCl, $MgCl_2$, dNTPs, etc.
qRT-PCR enzyme mixture	100μL	200μL	Reverse transcriptase, RNAse inhibitor, Taq enzyme, UNG enzyme, etc.
Primer probe pdmH1N1/H3	100μL	200μL	Primer probe.
Positive control pdmH1N1/H3	500μL	500μL	Plasmids containing target detection gene fragments.
Negative control	500μL	500μL	Normal saline.

Description

This reagent kit is designed based on the principle of fluorescence PCR technology, with specific primers and Taqman probes designed for the pdmH1N1/H3 subtype influenza virus. It is detected by a fluorescence PCR detector to achieve the detection of pdmH1N1/H3 subtype influenza virus nucleic acid.

Application

This kit is used for qualitative detection of pdmH1N1/H3 subtype influenza virus (pdmH1N1/H3) nucleic acid, and for auxiliary diagnosis and epidemiological monitoring of pdmH1N1/H3 subtype influenza virus infection.

Applicable instruments

Suitable for ABI 7500 Bio-Rad CFX96,Roche480,Hongshi SLAN-96S,SLAN-96P and other fully automatic fluorescence PCR detectors.

Sample requirements

1. Sample types: nasal/pharyngeal swabs, sputum, bronchoalveolar lavage fluid; Cell and chicken embryo cultures; Poultry throat swabs and feces; Environmental specimens and other samples.
2. Storage conditions: The collected specimens should be sent for testing in a timely manner. If tested within 24 hours, they should be stored at 4℃. If stored for more than 24 hours, it is best to store them at -70℃ (if there is no -70℃ storage condition, the test sample can be stored in a -20℃ refrigerator for 10 days), and repeated freezing and thawing should be avoided.

Protocol

1. Reagent Preparation (Reagent Preparation Area)

Melt the components of the reagent kit at room temperature, shake and mix thoroughly, and centrifuge immediately. Calculate the number of reagents used N (N=number of samples+1<positive control>+1<negative control>), configure the reaction system per person according to the table below, add each component to an appropriate volume centrifuge tube, mix thoroughly, centrifuge immediately, divide into 20μL PCR reaction tubes/plates, and transfer to the sample processing area.

Component	Volume (μL)

qRT-PCR reaction solution	12
qRT-PCR enzyme mixture	4
Primer probe pdmH1N1/H3	4
Total volume (reaction system mix)	20

2. Sample processing (sample processing area)

2.1. Nucleic acid extraction

Select the appropriate nucleic acid extraction kit to extract viral nucleic acid, and follow the instructions of the corresponding kit for specific operations.

2.2. Add sample

Add 5μL of processed nucleic acid, negative control, and positive control to the PCR reaction tube/plate that has been added to the reaction system mix, resulting in a final volume of 25μL. Cover the tube tightly or seal the membrane, centrifuge at low speed instantly, and amplify with a fluorescence PCR detector.

3. Amplification testing (nucleic acid amplification area)

Step	Temperature	Time	Cycles
Reverse transcription	50℃	10min	1cycle
Pre denaturation	95℃	5min	1cycle
Denaturation	95℃	10s	40cycles
Annealing/extension/fluorescence detection*	55℃	40s	

Note:1)*Fluorescence detection at 55℃ in step Denaturation, using FAM as the detection channel HEX/VIC。

2)*Roche480 series instruments need to complete color compensation experiments before starting the experiment (when using VIC/HEX and ROX/TEXS RED simultaneously)

3)*The ABI series fluorescence PCR instrument does not select ROX calibration, and the quenching group is selected as None.

4. Result analysis

According to the analysis of the image, adjust the start and end values (it is recommended to start from 3-15 and end from 5-20, and adjust the amplification curve of the negative control to be flat or below the threshold line). Click on the analysis button and view the results on the report interface.

Quality control

1. Negative control: Ct value>38 or not detected.
2. Positive control: The amplification curve is S-shaped and the Ct value is ≤ 35.
3. The above requirements must be met simultaneously for the same experiment, otherwise this experiment will be considered invalid.
4. Each detection target requires a positive and negative control, and the baseline threshold is adjusted for different targets based on their corresponding negative results.

Result interpretation

1. FAM channel detects pdmH1N1, HEX/VIC channel detects H3.
2. Negative: Ct value>38 or not detected.
3. Positive: The amplification curve is S-shaped and the Ct value is ≤ 35.
4. Suspected positive: The amplification curve shows an 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 protocol

1. Improper sample collection, transportation, and storage, as well as improper transportation, storage, and configuration of

reagents, can all affect experimental results and even lead to false negative results.

2. If there is laboratory contamination, reagent contamination, or sample cross contamination, false positive results may occur.

Performance indicators of reagent kit

1. Minimum detection limit: 1×10^3 copies/mL.
2. Specificity: No cross reactivity with other pathogenic pathogens.

Note

1. Each stage of PCR operation should be strictly partitioned to avoid cross contamination.
2. The components of the reagent kit should be thoroughly melted and mixed before use, and centrifuged for a few seconds before use.
3. Each component shall not be interchanged with other products or corresponding ingredients of different batch numbers.
4. If the specimen to be tested is not tested in a timely manner, it should be stored at -70 ℃.
5. The processing of samples should strictly follow biosafety regulations.
6. PCR operators should have experience and receive professional training.
7. This kit is only used for scientific research purposes and is not intended for clinical diagnosis.