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Monkeypox virus nucleic acid detection kit/with internal standard (fluorescence PCR method)

Product Number: DTK806

Shipping and Storage

1. Store in the dark at -20℃± 5℃, with a shelf life of 12 months.
2. The transportation mode of foam box and ice bag shall be adopted, and the transportation shall not exceed 4 days; Store in the dark at -20 ± 5℃ after opening; Repeated freezing and thawing should not exceed 6 times.
3. Production date and expiration date: see outer packaging box.

Component

Component	25T	50T	Main components
qRT-PCR Pre mixed solution (containing enzymes)	400μL	800μL	Tris, KCl, $MgCl_2$, dNTPs, Taq enzyme, UNG enzyme, etc.
Primer probe MPV (including IC)	100μL	200μL	Primer probe
Positive control MPV (including IC)	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 monkeypox virus (MPXV). It is detected by a fluorescence PCR detector to achieve the detection of monkeypox virus (MPXV) nucleic acid. The target gene in the reagent is a conserved gene in humans.

Application

This kit is used for qualitative detection of monkeypox virus (MPV) nucleic acid and for auxiliary diagnosis and epidemiological monitoring of monkeypox virus (MPV) infection.

Applicable instruments

Suitable for real-time fluorescence quantitative PCR instruments such as ABI 7500, Bio Rad CFX96, Roche480, etc.

Specimen collection

1. Sample types: Blood (collected fresh anticoagulant) (non heparin), serum, vesicles or pustules, rash exudate.
2. Storage conditions: The collected specimens should be sent for testing in a timely manner. Those tested within 24 hours should be stored at 4℃, and those that exceed 24 hours should be stored at -70℃ (if there is no -70℃ storage condition, they should be temporarily stored at -20 ± 5℃), 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 thoroughly and mix well, then centrifuge immediately. Calculate the number of reagents used N (N=number of samples+1<positive control>+1<negative control>), configure the reaction system according to the table below, add each component to the same appropriate volume centrifuge tube, mix thoroughly, and centrifuge immediately to prepare the reaction system mixture. Transfer it to the PCR reaction tube/plate at a rate of 20μL/well and transfer it to the sample processing area.

Component	Volume (μL)

qRT-PCR Pre mixed solution (containing enzymes)	16
Primer probe MPV (including IC)	4
Total volume (reaction system mixture)	20

2. Sample processing (sample processing area)

2.1. nucleic acid extraction

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

2.2. Sampling

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, resulting in a final volume of 25μL. Cover the tube tightly or seal it with a membrane, and perform transient centrifugation followed by detection on a fluorescence PCR amplification instrument.

3. Amplification testing (nucleic acid amplification area)

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

Note: In step 2, fluorescence detection is performed at 55 ℃ using FAM as the detection channel.

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

4. Result analysis

Adjust the start and end values based on the analyzed image (it is recommended to start at 3-15 and end at 5-20, while adjusting 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 standards

1. Negative control: Ct value>38 or not detected.
2. Positive control: The amplification curve shows a typical S-shape, and the Ct value is ≤ 30.
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 monkeypox virus (MPV), and ROX channel detects internal reference genes.
2. Negative: Ct value>38 or not detected.
3. Positive: The amplification curve is S-shaped and the Ct value is ≤ 35.
4. Suspicious: The amplification curve shows an S-shaped pattern and 35<Ct value ≤ 38, requiring retesting; If the retest results are consistent, the judgment result is positive.

Limitations of detection methods

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: 5×10^2 copies/mL.
2. Specificity: No cross reactivity with other pathogens that may cross the detection target.

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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 test specimen is not tested in a timely manner, it should be stored at -20℃ or -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 for scientific research use and is not intended for clinical diagnosis.