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Mumps virus nucleic acid detection kit (fluorescent PCR method)

Product Number: DTK803

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 reaction solution	300μL	600μL	Tris, KCl, $MgCl_2$, dNTPs, Taq enzyme, UNG enzyme, etc.
qRT-PCR enzyme mixture	100μL	200μL	Reverse transcriptase, RNAse preparation, Taq enzyme UNG Enzymes, etc.
Primer probe MuV	100μL	200μL	Primer probe
Positive control MuV	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 for mumps virus. It is detected by a fluorescence PCR amplifier to achieve the detection of mumps virus nucleic acid.

Application

This kit is used for qualitative detection of mumps virus (MuV) nucleic acid, as well as for auxiliary diagnosis and epidemiological monitoring of mumps virus infection.

Applicable instruments

Apply to ABI 7500, Bio-Rad CFX96, Roche Lightcycler480I, Lightcycler480II, cobas Z480, Real time fluorescence quantitative PCR instruments such as Hongshi SLAN-96S and SLAN-96P.

Specimen collection

1. Sample types: Samples of oral and respiratory secretions, blood, urine, breast milk, cerebrospinal fluid, and other tissues.
2. Storage conditions: The collected samples 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, the test samples 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 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 reaction solution	12

qRT-PCR enzyme mixture	4
Primer probe MuV	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
Reverse transcription	1 cycle	50℃	10min
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 ≤ 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. Detect targets in each channel.

Detection target	Detection channel
MARV	FAM

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 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. 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: 500 copies/mL.

2. Specificity: No cross reactivity with other pathogens that may cross the detection target.

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 -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.