MEBEP TECH(HK) Co., Limited
Email: sales@mebep.com Website: www.mebep.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

Influenza B Virus Victoria/Yamagata Lineages (Bv/By) Nucleic Acid Detection Kit (Dual Fluorescent PCR Method)

Product Number: DTK518

Shipping and Storage

1. Store in dark at -20℃, with a shelf life of 12 months.
2. Low temperature transportation cannot exceed 4 days; After opening, store in the dark at -20℃. The expiration date has no impact. Avoid repeated freezing and thawing, freezing and thawing 6 times will not affect the detection effect.

Component

Component	25 T	50 T	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 enzymes, etc
Primer probe BV/BY	100μL	200μL	Primer probe
Positive control BV/BY	50μL	50μL	Plasmids containing target detection gene fragments
Negative control	50μL	50μL	Water treated with diethyl carbonate

Description

This kit is designed based on the principle of fluorescence PCR technology, with specific primers and Taqman probes designed for the Victoria/Yamagata strain of influenza B virus. Amplification is performed using a fluorescence quantitative PCR instrument to achieve detection of influenza B virus Victoria/Yamagata strain nucleic acid.

Application

This kit is used for qualitative detection of Victoria/Yamagata strain (BV/BY) nucleic acid of influenza B virus, and for auxiliary diagnosis and epidemiological monitoring of Victoria/Yamagata strain infection of influenza B.

Applicable instruments

Suitable for fluorescence quantitative PCR instruments such as ABI 7500 and Bio Rad CFX96.

Specimen collection

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, they should be stored in a -20±5℃ freezer for no more than one month). During specimen transportation, repeated freezing and thawing should be avoided for more than 6 times.

Protocol

1. **Reagent Preparation (Reagent Preparation Area)**

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

Component	Volume (μL)
qRT-PCR reaction solution	16μL
qRT-PCR enzyme mixture	4μL
Primer probe BV/BY	4μL
Total Volume	20μL

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. Sampling:

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, with a final volume of 25μL. Cover the tube tightly or seal the membrane, centrifuge at low speed instantly, and then amplify and detect with a fluorescence PCR detector.

3. Amplification testing (nucleic acid amplification area)

step	Temperature	Time	cycles
Reverse transcription	50℃	10min	1 cycle
Pre denaturation	95℃	5min	1 cycle
Denaturation	95℃	10sec	40 cycles
Annealing/extension/fluorescence detection	55℃	40sec	

***Fluorescence detection at 55 ℃ in step 3, using FAM as the detection channel ROX 。**

***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 standards

1. Negative control: Ct value>38 or not detected.
2. Positive control: The amplification curve is S-shaped 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 BV, ROX channel detects BY.
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

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1. Minimum detection limit: 1×10^3 copies/mL.
2. Specificity: It can detect all specimens of the Victoria/Yamagata strain of influenza B virus and does not cross with other types.

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.