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

Bluetongue Virus (BTV) Nucleic Acid Detection Kit (Fluorescent PCR Method)

Product Number:DTK183

Shipping and Storage

1. -20 ℃± 5 ℃, stored in the dark, transported, and subjected to repeated freeze-thaw cycles no more than 5 times, with a validity period of 12 months.
2. The collected or processed samples should be stored at 2 ℃~8 ℃ for no more than 24 hours; If long-term storage is required, it should be stored at -70 ℃ or below, with no more than 3 freeze-thaw cycles.

Component

Component	50T
BTV reaction solution	500μL×2
Enzyme solution	50μL
BTV positive quality control product	50μL
Negative quality control product	250μL

Note: Different batches of reagents cannot be mixed.

Description

This kit uses TaqMan probe method for real-time fluorescence PCR technology, designs a pair of bluetongue virus specific primers, combines with a specific probe, and uses fluorescence PCR technology to amplify and detect the RNA of bluetongue virus in vitro, which is used for pathogen diagnosis of suspected infected individuals in clinical practice.

Application

Bluetongue disease is a non-contact infectious disease mainly affecting ruminant animals such as sheep or cattle caused by the bluetongue virus (BTV) of the family Enterotoxidae. It is mainly transmitted by bites from midges on their ruminant hosts. Sheep, goats, and cattle are the main susceptible animals to this disease, while camels and wild ruminant animals can all be infected. Its symptoms are severe catarrhal inflammation of the cheek mucosa and gastrointestinal mucosa. Infected animals are mentally depressed, lose appetite, often experience bloody diarrhea, and often limp due to the shedding of the hoof crest epithelium. At present, there is no highly effective prevention and treatment method for bluetongue disease. The rapid diagnosis and treatment of the pathogen of bluetongue disease are the main methods for controlling this disease.

This kit is suitable for detecting bluetongue virus RNA in animal spleen, lymph node, liver, kidney tissue, semen samples and other specimens, and is suitable for auxiliary diagnosis of bluetongue virus infection.

Applicable instruments

ABI7500, Agilent MX3000P/3005P, LightCycler, Bio Rad, Eppendorf and other series of fluorescence quantitative PCR detectors.

Specimen collection

Animal spleen, lymph nodes, liver, and kidney tissues; Semen sample

Protocol

1. **Sample processing (sample processing area)**
   1.1. **Sample Preparation**

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Organizational samples: Weigh approximately 1g of each tissue from 3 different positions, cut and mix it with surgical scissors, and then take 0.5g and grind it in a grinder. Add 1.5mL of physiological saline and continue grinding. After homogenization, transfer it to a 1.5mL sterile centrifuge tube and centrifuge at 8000rpm for 2 minutes. Take 100% of the supernatant μL in a 1.5mL sterilized centrifuge tube; Take 100 μL of throat swab sample directly into a 1.5mL sterilized centrifuge tube.

1.2. Nucleic acid extraction

We recommend using our company's nucleic acid extraction or purification reagents (magnetic bead method or centrifugal column method) for nucleic acid extraction. Please follow the reagent instructions for operation.

2. Reagent preparation (reagent preparation area)

Based on the total number of samples to be tested, the required number of PCR reaction tubes is N (N=number of samples+1 negative control tube+1 positive control tube); For every 10 samples, an additional 1 sample is prepared. The preparation of each test reaction system is shown in the table below:

reagent	BTV reaction solution	Enzyme solution
usage	19μL	1μL

Transfer the mixed test reaction solution into a PCR reaction tube at a concentration of 20uL per tube.

3. Sample addition (sample processing area)

Take 5μL of the nucleic acid, positive control sample, and negative control sample extracted in step 1, and add them to the corresponding reaction tubes. Cover the tubes, mix well, and briefly centrifuge.

4. PCR amplification (nucleic acid amplification zone)

4.1. Place the reaction tube to be tested in the reaction tank of the fluorescence quantitative PCR instrument;

4.2. Set the channel and sample information, and set the reaction system to 25μL;

Fluorescence channel selection: Detection channel (Reporter Dye) FAM, Quencher Dye NONE, please do not select ROX reference fluorescence for ABI series instruments, select None.

4.3. Recommended loop parameter settings:

step	Cycles	Temperature	Time	Collect fluorescence signals
1	1 cycle	50°C	10min	No
2	1 cycle	95°C	2min	No
3	45 cycles	95°C	15sec	No
		60°C	30sec	Yes

5. Result analysis and judgment

5.1. Result Analysis Condition Setting

(Please refer to the user manuals of each instrument for setting up, taking the ABI7500 instrument as an example)

After the reaction is complete, the results will be automatically saved. Based on the analyzed image, adjust the Start value, End value, and Threshold value of the baseline (users can adjust them according to their actual situation, with Start value set between 3-15 and End value set between 5-20, so that the threshold line is in the exponential period of the amplification curve, and the amplification curve of negative quality control products is flat or below the threshold line). Click Analyze to automatically obtain the analysis results.

5.2. Result judgment

Positive: The Ct value of the detection channel is ≤ 40, and the curve shows a significant exponential growth curve;

Negative: The Ct value of the sample test result is greater than 40 or there is no Ct value.

Quality control standards

Negative quality control product: no specific amplification curve or Ct value display;

Positive quality control product: The amplification curve shows a significant exponential growth period, and the Ct value is ≤32;

The above conditions should be met simultaneously, otherwise the experiment will be considered invalid.

Limitations of detection methods

1. The results of sample testing are related to the quality of sample collection, processing, transportation, and preservation;

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2. Failure to control cross contamination during sample extraction can result in false positive results;
3. Leakage of positive controls and amplification products can lead to false positive results;
4. During the epidemic, genetic mutations and recombination of pathogens can lead to false negative results;
5. Different extraction methods have differences in extraction efficiency, which can lead to false negative results;
6. Improper transportation, storage, or inaccurate preparation of reagents can lead to a decrease in reagent detection efficiency, resulting in false negatives or inaccurate quantitative testing results;
7. The test results are for reference only. If a diagnosis is required, please combine clinical symptoms and other testing methods.

Note

1. All operations must be strictly carried out in accordance with the instructions;
2. The various components in the reagent kit should be naturally melted, completely mixed, and briefly centrifuged before use;
3. The reaction solution should be stored away from light;
4. Try to avoid the presence of bubbles during the reaction, and cover the tube tightly;
5. Use disposable suction tips, disposable gloves, and specialized work clothes for each area;
6. Sample processing, reagent preparation, and sample addition should be carried out in different areas to avoid cross contamination;
7. After the experiment is completed, treat the workbench and pipette with 10% hypochlorous acid, 75% alcohol, or a UV lamp;
8. All items in the reagent kit should be treated as contaminants and handled in accordance with the "Biosafety Guidelines for Microbial Biomedical Laboratories".