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Foot-and-mouth Disease Virus Detection Kit(Real-Time PCR Method)

Product Number:DTK105

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 sample can be stored at -20℃ for a short period of time, and at -70℃ for a long period of time, but cannot exceed 6 months. The sample should be transported in ice packs at 2-8℃, and repeated freezing and thawing are strictly prohibited.

Component

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

Note: Different batches of reagents cannot be mixed.

Description

This kit designs primers and probes for the universal type specific nucleic acid sequence of foot-and-mouth disease virus, and uses one-step fluorescence RT-PCR technology to amplify and detect the universal type RNA of foot-and-mouth disease virus in vitro, which is used for pathogen diagnosis of suspected infectious materials in clinical practice.

Application

Foot and mouth disease (FMD) is an acute, severe, and contagious disease in ungulates caused by the foot-and-mouth disease virus (FMDV) infection. The World Organization for Animal Health (OIE) has listed foot-and-mouth disease as an animal infectious disease that must be reported, and China has designated foot-and-mouth disease as a Class I animal disease.

The main sources of foot-and-mouth disease infection are latent infection and clinical onset animals. Vulnerable animals can be infected with viruses through respiratory, digestive, reproductive, and wound pathways, usually transmitted through direct or indirect contact (droplets, etc.), or through animal vectors such as humans or dogs, flies, cicadas, etc. Infected animals' exhaled breath, saliva, feces, urine, milk, semen, meat, and by-products can all carry toxins. After infection in ruminant animals such as cattle and sheep, the virus can continue to carry the virus in the esophagus throat region.

This kit is suitable for detecting foot-and-mouth disease virus universal RNA in blister skin, blister fluid, tissue samples, ruminant O-P fluid samples, and serum samples of animals susceptible to foot-and-mouth disease. It is suitable for auxiliary diagnosis of foot-and-mouth disease virus universal infection.

Applicable instruments

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

Specimen collection

Collection of blister fluid: The blister fluid from typical clinically affected animals is aspirated using a sterilized syringe and placed in a sample storage tube. Penicillin 1000 IU/mL and streptomycin 500 IU/mL are added, sealed, and frozen for preservation.
For other sample collection, refer to GB/T 18935-2018 "Diagnostic Techniques for Foot and Mouth Disease" [1] for standardized collection of blister skin samples (Chapter 5.3.2), tissue samples (Chapter 5.3.3), ruminant O-P liquid samples (Chapter 5.3.4), and serum samples (Chapter 5.3.5).

Protocol

1. Sample processing (sample processing area)

1.1. Sample Preparation

Bubble liquid sample: Centrifuge the bubble liquid at 3000 r/min for 10 minutes, and take the supernatant for later use. The processing of blister skin samples, tissue samples, ruminant O-P liquid samples, and serum samples shall be standardized according to GB/T 18935-2018 "Diagnostic Techniques for Foot and Mouth Disease" [1].

1.2. Nucleic acid extraction

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

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 following table:

reagent	FMDV-U Reaction solution	Enzyme solution
Dosage (sample size N)	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℃	10min	No
2	1 cycle	95℃	2min	No
3	45 cycles	95℃	15sec	No
		60℃	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 >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;
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"