Foot-and-mouth Disease Virus Type O(FMDV-O) PCR Nucleic Acid Detection Kit

Please read the manual carefully before use.
Cat. No. DV212-2
Version No. Version 1.0
Specification: 48 tests/ box

Description

Using Taqman probe technology, specific primers and probes were designed based on the *VP2* gene of Foot-and-mouth Disease Virus Type O (FMDV-O) as the target sequence for the auxiliary detection of FMDV-O nucleic acid in samples. This product introduces a dUTP/UDG mix that degrades U-containing ssDNA and dsDNA, eliminating cross-contamination caused by PCR products. At the same time, a positive internal control (an internal standard, using a VIC reporter group) is set to monitor whether the qPCR reaction contains PCR inhibitors by detecting whether the internal standard is normal to avoid false-negative results.

Kit Contents

Name	Component	DV212-21-V1
2×FMDV-O One-Step Reaction Mix	qPCR reaction buffers, dNTPs, PCR enhancers, etc.	600 µl/ tube
FMDV-O One-Step Enzyme Mix	UDG enzymes, reverse transcriptase, Taq polymerases	48 µl/ tube
FMDV-O Primer Probe Mix-2	Primers, probes	312 µl/ tube
FMDV-O Negative Control-2	Nuclease-free Water	400 µl/ tube
FMDV-O Internal Positive Control-2	Nucleic acid containing amplified fragment of internal positive control	480 µl/ tube
FMDV-O Positive Control-2	Nucleic acid containing target amplified fragment	50 µl/ tube

Note: The components of different batches of products cannot be mixed or interchanged.

Storage and Shelf Life

Store in the dark at -20±5°C. Valid for 12 months.
Shipping with dry ice.
Repeat freeze-thaw less than 5 times.
Please refer to the outer box for the production date and expiration date.

Sample Requirements

1. Applicable Sample Types
Serum, blister skin, blister fluid, or living O/P fluid, etc.
Fresh samples are required to be sent for inspection, and repeated freezing and thawing are prohibited!
2. Sample processing (nucleic acid extraction area)
Sample pretreatment is carried out following "NY/T 541-2016 Technical Specifications for Collection, Preservation and Transportation of Veterinary Diagnostic Samples" and other standards, with a commercial extraction kit used to extract nucleic acids in the samples. During extraction, add 10 µl of internal positive control to each 200 µL of negative control and processed sample solution. The extracted nucleic acid should be detected immediately, otherwise, it should be frozen at -20±5°C, and the storage time should not exceed 6 months. Do not repeat freezing and thawing!
*Other methods of using the internal positive control: Mix the internal positive control into the qRT-PCR working solution, but only for quality control of the amplification process and to monitor whether the qRT-PCR system contains PCR inhibitors. It cannot be used for quality control of the nucleic acid extraction.

Detection Method

To avoid contamination, the experiment needs to be performed in separate areas!
Note: Nucleic acid extraction is performed in the nucleic acid extraction area or the sample processing area according to the sample requirements.
1. Reagent preparation (in the reagent preparation area)
(1) Take out the components and self-prepared reagents in the kit. Place them at room temperature. Wait for the temperature to equilibrate to room temperature. And mix well for later use;
(2) qPCR working solution preparation (avoid direct light during the whole process)
Prepare the reaction solution according to the following table and the number of samples to be tested. It is recommended to set a negative control and positive control for each test. When the number of samples to be tested is n, it is necessary to prepare the

number of reactions N = number of samples to be tested (n) + positive control (1) + negative control (1) + 1.

Table 1: qRT-PCR working solution preparation (recommended)
(Internal positive control is used for quality control of nucleic acid extraction and nucleic acid amplification)

Component	Volume per reaction (μl)
2×FMDV-O One-Step Reaction Mix	12.5 μl×N
FMDV-O One-Step Enzyme Mix	1 μl×N
FMDV-O Primer Probe Mix-2	6.5 μl×N

*Table 2: qRT-PCR working solution preparation (other methods)
(Internal standard control only for quality control of nucleic acid amplification)

Component	Volume per reaction (μl)
2×FMDV-O One-Step Reaction Mix	12.5 μl×N
FMDV-O One-Step Enzyme Mix	1 μl×N
FMDV-O Primer Probe Mix-2	6.5 μl×N
FMDV-O Internal positive Control-2	0.5 μl×N

*Note: This preparation method is an alternative method when the sample nucleic acid is extracted without adding an internal positive control, and it is not recommended.

(3) Mix the prepared qRT-PCR working solution thoroughly and centrifuge briefly before use.

(4) When the working solution in (3) is ready to use, dispense an equal volume of 20 μl into each microcentrifuge tube (eg, eight-tube) and transfer it to the sample processing area.

2. Sample Loading (in the sample processing area)

Add 5 μl of the extracted sample to be tested, negative control and positive control to each PCR tube, cover the tube and centrifuge briefly to make the liquid attach to the bottom of the tube.

3. qRT-PCR amplification (in the amplification and analysis area)

(1) Fluorescence Channel Selection

Selects the **FAM** channel to detect **FMDV-O**, the VIC channel to detect the **internal positive control**, and the quencher to none. As for the ABI qPCR instrument, it also needs to set Passive Reference to none. For other instruments, refer to the instrument manual.

(2) qPCR Amplification Program Settings

Set the reaction volume to 25 μl

Procedure		Temperature	Reaction Time	Cycle
Reverse Transcription		50°C	5 min	1
Predenaturation		95°C	30 sec	1
Amplify	Denaturation	95°C	5 sec	45
	Annealing and Extending	60°C	35 sec，data collection	

4.Result Analysis (taking the ABI7500 qPCR instrument as an example)

After the reaction is complete, the qPCR instrument will automatically generate results. If the amplification curve or threshold line automatically generated by the instrument is abnormal, the user can adjust it according to the actual situation. The baseline Start value can be set at 3~10, and the baseline End value can be set at 5~20 level. Click Analyze to analyze.

5. Quality Control

Each control in the kit should meet the following requirements, otherwise, the experiment will be invalid.

	Positive Control	Negative Control
FAM channel (FMDV-O)	Ct≦32	No Ct value or Ct>40
VIC channel (internal positive control)	No Ct value or Ct>40	Typical S-shaped curve, Ct ≦35

Refer to Table 2: qRT-PCR working solution preparation (other methods), the VIC channel should show an S-shaped curve, and the Ct value should be≦35.

Interpretation of Test Results

(1) If the VIC channel of the sample shows a typical S-shaped curve and Ct≦35,

A. When the FAM channel Ct≦35, FMDV-O is determined as positive;

B. When the FAM channel Ct value is between 35 and 40, the amplification curve of the target gene needs to be observed whether it is S-shaped or not. If the curve is S-shaped, the sample should be regarded as suspected FMDV-O positive and need to be re-tested. If the Ct value is ≦40 and has a typical S-shaped curve in the retest results, it is determined as FMDV-O positive; otherwise, if there is no Ct display or no typical S-shaped curve, it is determined as FMDV-O negative.

C. When the FAM channel Ct>40, FMDV-O is determined as negative.

(2) If the Ct value of the VIC channel is higher than 35 without showing a clear sigmoid amplification curve, the reasons are as follows:

1) PCR inhibitors are present in the sample. It is recommended to dilute the sample before testing.

2) Nucleic acid extraction operation is defective. It is recommended to repeat nucleic acid extraction for detection.

3) If a qualified sample is not obtained during processing, or the sample has degraded during transportation and storage, re-sampling is recommended.

Limitations of Test Methods

(1) Negative results may be due to the low quality of nucleic acid extracted from the sample, improper storage conditions, inappropriate storage period, presence of inhibitors in the sample, nucleic acid degradation and poor extraction efficiency of the nucleic acid extraction method.

(2) Unreasonable sample collection, transportation and storage conditions, too low virus content in the sample, or unreasonable experimental operation and environment are likely to cause false-negative or false-positive results. Other clinical observations and related data should be combined with the determination, and the test should be performed again if necessary.

(3) Sequence changes in the target sequence, due to mutation or other reasons, may generate false-negative results.

Notes

(1) This product is only for scientific research use. Please read this manual carefully before use.

(2) Before the experiment, please acquaint with and master the operation methods and precautions of various instruments to be used, and conduct quality control of each experiment.

(3) Laboratory management must strictly follow the management specifications of PCR gene amplification laboratories. The experimental personnel must undergo professional training. The experimental process is strictly carried out by separate areas. All consumables are only for one-time use. Supplies in different areas and stages cannot be used interchangeably.

(4) All testing samples are regarded as infectious substances. During the experiment, wear work clothes and change gloves frequently to avoid cross-contamination between samples;

(5) Sample handling and waste disposal should comply with local relevant regulations: "General Biosafety Standard for Microbiological and Biomedical Laboratories" and "Medical Waste Management Regulations"

(6) All reagents (including the components in this product and customer-supplied reagents) must be thawed and mixed thoroughly before use.

(7) Severe hemolysis of serum samples will significantly reduce the height of the amplification curve. Therefore, when using this kit to detect the serum sample stock solution and the normal sample diluted by saline at the same time, it is recommended to set different threshold lines for the two types of templates to ensure the accuracy of the results.

When the Ct value of multiple samples is greater than 40, it may be caused by the high threshold line automatically generated by the qPCR instrument. It is recommended to set the threshold line height at about 1/15 of the maximum fluorescence value of the amplification curve before re-analysis.

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