Sheeppox Virus (SPPV), Goatpox Virus (GTPV) and Lumpy Skin Disease Visus (LSDV) Nucleic Acid Multiplex Identification Detection Kit (fluorescent PCR method, exogenous internal standard)

Please read the manual carefully before use.

Cat. No. DV232Y-2

Version No. Version 1.0

Specification: 48 tests/ box

Description

Using Taqman probe technology, specific primers and probes were designed based on the specific conservative genes of Sheeppox Virus (SPPV), Goatpox Virus (GTPV) and Lumpy Skin Disease Visus (LSDV) as the target sequences for the auxiliary detection of SPPV/ GTPV/ LDSV 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 CY5 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	Specification
SPPV/ GTPV/ LDSV PreMix-2	qPCR reaction buffers, dNTPs, UDG enzymes, Taq polymerases, primers, probes, etc.	960 µl/ tube
SPPV/ GTPV/ LDSV Negative Control-2	Nuclease-free Water	400 µl/ tube
SPPV/ GTPV/ LDSV Internal Positive Control-2	Nucleic acid containing amplified fragment of internal positive control	480 µl/ tube
SPPV/ GTPV/ LDSV 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.

Applicable Instruments

ABI7500, Hongshi 96P, Bio-RadCFX96 real-time PCR instrument, etc.

Sample Requirements

1. Applicable Sample Types

Lung, lymph nodes, blood, 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 qPCR working solution, but only for quality control the amplification process and monitoring whether the qPCR system contains PCR inhibitors, cannot be used for quality control the extraction process of the nucleic acid.

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) Recommended Method (Internal Positive Control Quality Control Nucleic Acid Extraction and Nucleic Acid Amplification)

Take out the components in the kit and self-prepared reagents . Place them at room temperature. Wait for the temperature to equilibrate to room temperature. And mix well for later use;

Depending on the number of testing samples, it is recommended to set a negative control and positive control for each test. Distribute the required amount (N) of SPPV/ GTPV/ LDSV PreMix-2 to each microcentrifuge tube (e.g., eight-tube strips), 20 μl/ tube. 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).

*Table: qPCR working solution preparation (other method)

Component	Volume per reaction (μl)
SPPV/ GTPV/ LDSV PreMix-2	20 μl×N
SPPV/ GTPV/ LDSV 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.

Mix the prepared qPCR working solution thoroughly and centrifuge briefly before use. When the working solution is ready to use, dispense an equal volume of 20 μl into each microcentrifuge tube (eg, eight-tube strips) 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. qPCR Amplification (in the Amplification and Analysis Area)

(1) Fluorescence Channel Selection

Selects the reporter group to the **FAM** channel to detect **LDSV**, the **VIC** channel to detect the **SPPV**, the **ROX** channel to detect the **GTPV**; the **CY5** 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
UDG Digestion		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 (LDSV)	Ct≦32	No Ct value or Ct>40
VIC Channel (SPPV)	Ct≦32	No Ct value or Ct>40
ROX Channel (GTPV)	Ct≦32	No Ct value or Ct>40
CY5 Channel (Internal Positive Control)	No Ct value or Ct>40	a typical S-shaped curve, Ct≦35

Refer to Table: qPCR working solution preparation (other method), the CY5 channel should show an S-shaped curve, and the Ct value should be≦35.

Interpretation of Test Results

(1) If the CY5 channel of the sample shows a typical S-shaped curve and Ct≦35,

Channel\Ct Value	Positive Control	Negative Control	Marginal Negative
FAM Channel (LDSV)	Ct≦35	35<Ct≦40	No Ct value or Ct>40
VIC Channel (SPPV)	Ct≦35	35<Ct≦40	No Ct value or Ct>40
ROX Channel (GTPV)	Ct≦35	35<Ct≦40	No Ct value or Ct>40

Marginal Positive samples are recommended to re-test. After the re-test, if the Ct value is ≦40 and has a typical S-shaped curve, the result is judged as positive, otherwise, it is judged as negative.

(2) If the Ct value of the CY5 channel is higher than 35 without showing a clear sigmoid amplification curve, the reasons are as follow:

1) PCR inhibitors are present in the sample, it is recommended to dilute the template 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 blood samples will significantly reduce the height of the amplification curve. Therefore, when using this kit to detect the blood sample stock solution and the sample diluted by normal 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.

8) 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.

This product is for scientific research use only, and the test results cannot be used as the sole basis for clinical diagnosis and judgment.