

# ViroReal<sup>®</sup> Kit Lumpy Skin Disease Virus

## Manual

For use with the

- ABI PRISM<sup>®</sup> 7500 (Fast)
- Mx3005P<sup>®</sup>
- LightCycler<sup>®</sup> 480



For veterinary use only



DVEV03311, DVEV03313



100



DVEV03351, DVEV03353



50



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## 1. Product description

ViroReal® Kit Lumpy Skin Disease Virus is a real-time PCR assay for detection of DNA of the lumpy skin disease virus (LSDV). This kit might detect also the sheeppox virus and the goatpox virus. This test allows the rapid and sensitive detection of DNA of LSDV from samples purified from serum, blood, skin biopsies and swabs (saliva, eye, nose) (e.g. with the QIAamp DNA Mini Kit or QIAamp DSP Virus Kit).

ViroReal® Kit Lumpy Skin Disease Virus detects the GPCR gene of the lumpy skin disease virus. A probe-specific amplification-curve at 530 nm (FAM channel) indicates the amplification of LSDV specific DNA.

An internal positive control system for detection in VIC/HEX channel, (554 nm, order no. DVEV03311 or DVEV03351) or Cy5 channel (667 nm; order no. DVEV03313 or DVEV03353) excludes false-negative interpretation of results due to inhibition of real-time PCR (see 8. Interpretation of PCR-data).

This test was developed and validated for the ABI PRISM® 7500 (Fast) instrument (Thermo Fisher Scientific), LightCycler® 480 (Roche) and Mx3005P® (Agilent), but is also suitable for other real-time PCR instruments. When using PCR-platforms not validated by ingenetix, an evaluation of the multiplex-PCR is recommended. Please be aware that some PCR-platforms have to be calibrated with the corresponding dye before performing multiplex-PCR.

BactoReal®, MycoReal, ParoReal and ViroReal® Kits are optimized to run under the same thermal cycling conditions. RNA and DNA material can be analysed in one run.

## 2. Pathogen information

Lumpy skin disease virus (LSDV) is a dsDNA virus within the genus Capripoxvirus. It is genetically very similar to the other two Capripoxvirus species sheeppox virus and goatpox virus. There is one serotype of LSDV. LSDV causes lumpy skin disease in cattle and buffalo. It is transmitted by arthropod vector. Traditionally it has been found in southern Africa but has been steadily spreading north to the Middle East and into eastern Europe and Russia.

### References:

K. A. Al-Salihi. 2014. Lumpy Skin disease: Review of literature. MRSVA. 3, 6-23.

## 3. Principle of real-time PCR

A specific DNA sequence of the pathogen genome is amplified and the generated PCR-product is detected by an oligonucleotide-probe labelled with a fluorescent dye. This technology allows for a sequence-specific detection of PCR amplicates.

## 4. General Precautions

The user should always pay attention to the following:

- Always include a negative control per PCR-run (water instead of sample).
- Optional: for valid interpretation of results, a negative control should be included during DNA-extraction (for example extraction of water instead of sample material), in order to exclude false-positive results due to contamination with LSDV DNA during extraction.
- Be careful when handling the positive control.
- Store and extract positive material (specimens, controls and amplicons) separately from all other reagents and add it to the reaction mix in a spatially separated workspace.
- Periodically decontaminate benches and devices.
- Use sterile pipette tips with filters.
- Thaw all components thoroughly at room temperature before starting an assay. When thawed, gently mix the components and centrifuge briefly.
- For MSDS, see [www.ingenetix.com](http://www.ingenetix.com).

## 5. Contents of the Kit

### 5.1. ViroReal® Kit Lumpy Skin Disease Virus order no. DVEV03311 or DVEV03351

Labelling	Content	Amount		Storage
		DVEV03311	DVEV03351	
LSDV Assay Mix (green cap)	Primer and probe (FAM) for detection of LSDV	2 x 50 µl	1 x 50 µl	-20°C
CR-1 Assay Mix (yellow cap)	Primer, probe (VIC/HEX) and target for detection of IPC	2 x 50 µl	1 x 50 µl	-20°C
LSDV Positive Control (red cap)	Control-DNA (approx. 10,000 target copies/µl)	1 x 25 µl	1 x 25 µl	-20°C
DNA Reaction Mix (white cap) #	Reaction Mix	2 x 500 µl	1 x 500 µl	<b>-20°C until first use, then at +4°C</b>
Water (blue cap)	Water	1 x 1000 µl	1 x 1000 µl	-20°C to +4°C

#DNA Reaction Mix contains uracil-N glycosylase (UNG)

### 5.2. ViroReal® Kit Lumpy Skin Disease Virus order no. DVEV03313 or DVEV03353

Labelling	Content	Amount		Storage
		DVEV03313	DVEV03353	
LSDV Assay Mix (green cap)	Primer and probe (FAM) for detection of LSDV	2 x 50 µl	1 x 50 µl	-20°C
CR-3 Assay Mix (yellow cap)	Primer, probe (Cy5) and target for detection of IPC	2 x 50 µl	1 x 50 µl	-20°C
LSDV Positive Control (red cap)	Control-DNA (approx. 10,000 target copies/µl)	1 x 25 µl	1 x 25 µl	-20°C
DNA Reaction Mix (white cap) #	Reaction Mix	2 x 500 µl	1 x 500 µl	<b>-20°C until first use, then at +4°C</b>
Water (blue cap)	Water	1 x 1000 µl	1 x 1000 µl	-20°C to +4°C

#DNA Reaction Mix contains uracil-N glycosylase (UNG)

The components of ViroReal® Kit Lumpy Skin Disease Virus are stable until the expiry date stated on the label. Repeated thawing and freezing should be avoided. Please protect kit components from light.

## 6. Additionally required materials and devices

- Reagents and devices for DNA-extraction
- PCR-grade water
- Disposable powder-free gloves
- Pipettes (adjustable)
- Sterile pipette tips with filters
- Vortex mixer
- Desktop centrifuge with rotor for 2 ml reaction tubes
- Real-time PCR instrument which is able to detect and differentiate fluorescence in FAM and VIC/HEX or Cy5 channel
- Appropriate 96 well reaction plates or reaction tubes with corresponding (optical) closing material

## 7. Preparation of real-time PCR

Please make sure that at least one negative control (water, blue cap), as well as one positive control (red cap) and one extraction negative control (optional, recommended) are included per PCR run.

Ingenetix highly recommends performing PCR analyses in duplicates, which increases the probability of detection of the pathogen and facilitates interpretation of results.

### 7.1. Pipetting scheme

		Per sample
Preparation of Master Mix (mix well)	Water*	3.0 µl
	DNA Reaction Mix (2x)	10.0 µl
	LSDV Assay Mix	1.0 µl
	CR Assay Mix	1.0 µl
	<b>Total volume Master Mix</b>	<b>15.0 µl</b>
Preparation of PCR	Master Mix	15.0 µl
	Sample*	5.0 µl
	<b>Total volume</b>	<b>20.0 µl</b>

\*1-8 µl of the sample can be used. When using an amount ≠ 5 µl of the sample, the amount of H<sub>2</sub>O has to be changed accordingly.

**Positive Control:** As positive control please use 1 µl of the LSDV Positive Control + 4 µl H<sub>2</sub>O.

Optional: a 1:10 dilution of the positive control can be used and defined as second standard value (approx. 1000 target copies/µl).

### 7.2. Programming of the temperature profile

Please find further information on programming the real-time PCR instrument in the respective operator's manual. Please be aware that some PCR-platforms have to be calibrated with the corresponding dye before performing multiplex-PCR.

**Select dyes:** FAM-TAMRA for detection of LSDV

Cy5-NONE (CR-3 Assay Mix) or VIC-TAMRA (CR-1 Assay Mix) for detection of IPC

**Select reference dye (passive reference):** ROX

**Sample Volume:** 20 µl

**Temperature Profile:**

Program 1	Program 2	Program 3
Cycles: 1 Analysis: None	Cycles: 1 Analysis: None	Cycles: 45 Analysis: Quantification Acquisition at 60°
50°C 2 min*	95°C 20 sec	95°C 5 sec 60°C 1 min

For ABI PRISM® 7500:

Ramp speed: Without "fast cycling" parameter

For LightCycler® 480 instrument:

Detection format: 2 Color Hydrolysis Probe  
(dyes see above)

**\*Note:** If viral RNA should be also detected in the same PCR run, program 1 has to be prolonged to 15 min at 50°C. This temperature profile can be used for all BactoReal®, MycoReal®, ParoReal®, and ViroReal® kits for the detection of DNA or RNA.

## 8. Interpretation of PCR-data

Examples for interpretation of positive reactions are shown in the amplification plots below.

**For a valid interpretation, the following criteria must be fulfilled:**

	<b>Ct/Cp (FAM channel) LSDV target</b>	<b>Ct/Cp IPC target</b>
Negative control	Negative	Positive
Positive control (undiluted, 1 µl/PCR)	28.0-31.0	Positive
Or: positive control (1:10 diluted, 1 µl/PCR)	31.0-34.0	Positive
Extraction negative control (optional)	Negative	Positive
Negative sample	Negative	Positive
Positive sample	Positive	Positive/Negative

**For analysis of PCR data please proceed as follows:**

For analysis of PCR results gained with ViroReal® Kit Lumpy Skin Disease Virus please select fluorescence display options FAM channel for the LSDV target and VIC/HEX channel (order no. DVEV03311, DVEV03351) or Cy5 channel (order no. DVEV03313, DVEV03353) for the internal positive control target. Samples with a positive Ct or Cp-value are considered positive. Please also check the presence of amplification-curves manually.

### 8.1. Signal in FAM channel:

→ DNA of LSDV was amplified. The sample has to be interpreted as positive.

LSDV DNA can lead to a reduced or absent fluorescence signal of the internal positive control (competition of PCR).

### 8.2. No signal in FAM channel but signal of the internal positive control:

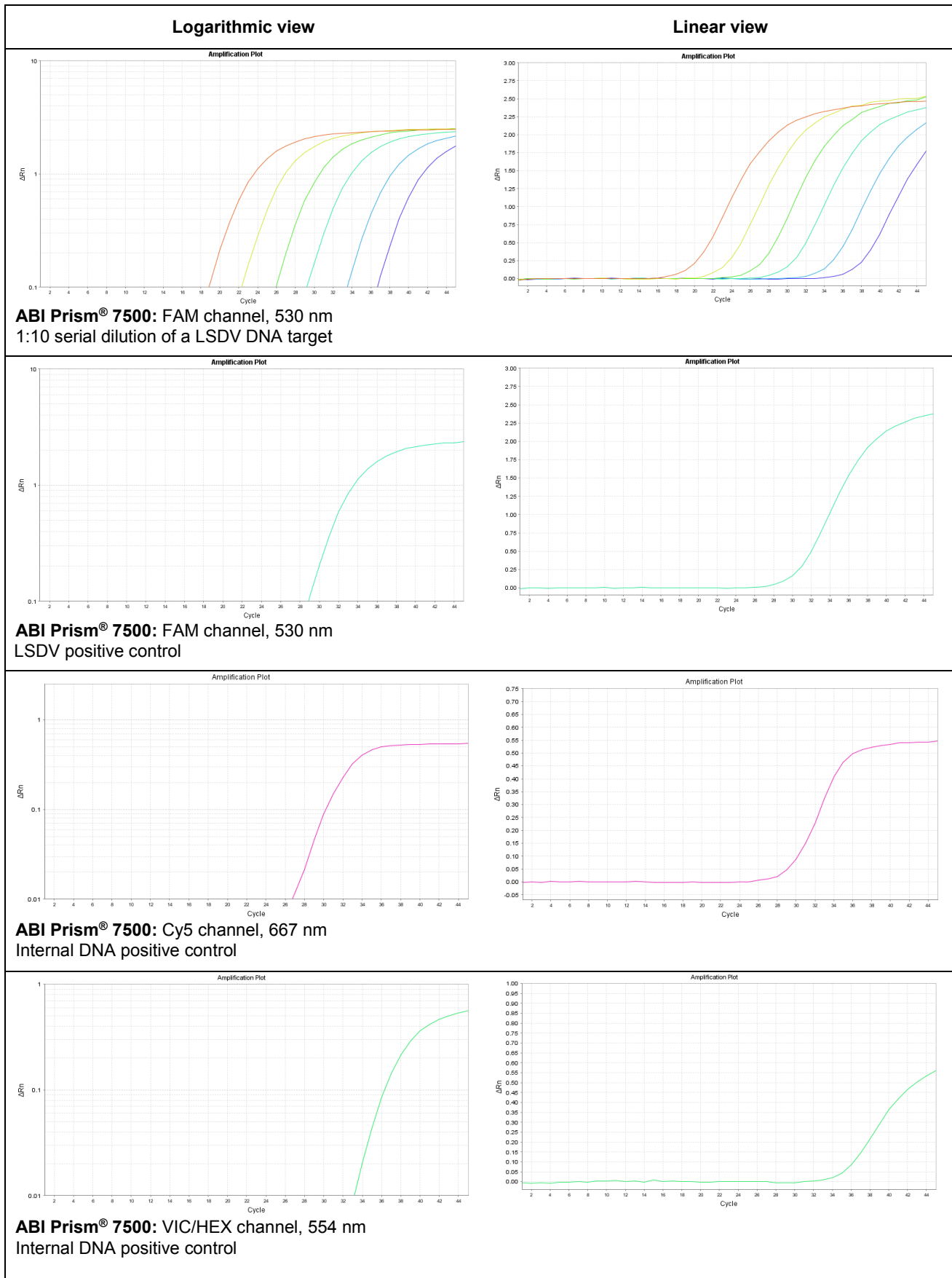
→ No LSDV DNA is detectable in the sample. The sample has to be interpreted as negative.

The positive signal of the internal positive control assay excludes a putative PCR inhibition.

### 8.3. No signals in FAM channel and no signal with internal positive control:

→ No interpretation statement can be made.

Information about possible sources of error and their solution can be found in 9. Troubleshooting.



**Figure 2** Performance of ViroReal® Kit Lumpy Skin Disease Virus

## 9. Troubleshooting

### 9.1. No LSDV specific signal with positive control

- Incorrect programming of the temperature profile of the real-time PCR instrument.  
→ Compare the temperature profile with the protocol (see 7. Preparation of real-time PCR).
- Incorrect configuration of the PCR reaction.  
→ Check your work steps (see 7. Preparation of real-time PCR) and repeat the PCR, if necessary.

### 9.2. No signal with the internal positive control and no LSDV specific signal with sample

- The PCR reaction was inhibited. No interpretation can be made.  
→ Make sure that you use a recommended method for DNA isolation and stick closely to the manufacturer's instructions.  
→ If no operating mistakes during extractions can be retraced, it is recommended to repeat the PCR with lower amounts of DNA-eluate (1/5 or 1/10 of sample volume + the adequate amount of H<sub>2</sub>O).
- Incorrect PCR conditions.  
→ Check the PCR conditions and repeat the PCR, if necessary.

### 9.3. LSDV specific signal with negative control

- A contamination occurred during preparation of the PCR.  
→ Repeat the PCR with new reagents in replicates.  
→ Strictly pipette the positive controls at last.  
→ Make sure that work space and instruments are decontaminated at regular intervals.

### 9.4. LSDV specific signal with negative control of DNA-extraction

- A contamination occurred during extraction.  
→ Repeat the extraction and PCR using new reagents.  
→ Make sure that work space and instruments are decontaminated at regular intervals.

## 10. Specifications

ViroReal® Kit Lumpy Skin Disease Virus was evaluated with the ABI PRISM® 7500 (Fast) instrument (Thermo Fisher Scientific), with the LightCycler® 480 (Roche) and the Mx3005P® (Agilent). For further validation data please contact ingenetix.

### 10.1. Analytical sensitivity

The analytical sensitivity is about 10 template copies/PCR.

### 10.2. Analytical specificity

The specificity is ensured by the selection of highly specific primers and probes. The primers and probes were checked for possible homologies to currently published sequences by sequence comparison analyses. This also validated the detection of so far known LSDV strains.

ViroReal® Kit Lumpy Skin Disease Virus detects the lumpy skin disease virus and might detect also the sheeppox virus and the goatpox virus

## 11. Annex – symbols



Batch code



Catalogue number



Contains sufficient for <n> tests



Use by



Manufactured by



Store at