

ViroReal[®] Kit PCV3


Manual

For use with

- Applied Biosystems[®] 7500 (Fast)
- Mx3005P[®]
- LightCycler[®] 480



For veterinary use only

REF	DVEV03611, DVEV03613		100
REF	DVEV03651, DVEV03653		50



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1. Product description

ViroReal[®] Kit PCV3 is a real-time PCR assay for detection of DNA of the porcine circovirus type 3 (PCV3). This test was developed for the Applied Biosystems[®] 7500 (Fast) instrument (Thermo Fisher Scientific), but is also suitable for other real-time PCR instruments. This test allows the rapid and sensitive detection of DNA of PCV3 from samples purified from serum, oral fluid, swab specimens from respiratory tract and biopsies (e.g. with the QIAamp DNA Mini Kit or QIAamp DSP Virus Kit).

ViroReal[®] Kit PCV3 detects the capsid gene of the porcine circovirus type 3. A probe-specific amplification-curve at 530 nm (FAM channel) indicates the amplification of PCV3 specific DNA.

An internal positive control system for detection in VIC/HEX channel, (554 nm, order no. DVEV03611 or DVEV03651) or Cy5 channel (667 nm; order no. DVEV03613 or DVEV03653) excludes false-negative interpretation of results due to inhibition of real-time PCR (see 8. Interpretation of PCR-data).

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

Porcine circovirus type 3 (PCV3) is a novel circovirus species identified in pigs with porcine dermatitis and nephropathy syndrome (PDNS), reproductive failure, respiratory disease and multi-systemic inflammation. Porcine circovirus is a small, non-enveloped DNA virus that contains a single strand of circular DNA. It belongs to the genus *Circovirus* in the family *Circoviridae*.

References:

T. G. Phan et al. Detection of a novel circovirus PCV3 in pigs with cardiac and multi-systemic inflammation. *Virology Journal* (2016) 13:184.

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 PCV3 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, mix the components and centrifuge briefly.
- For MSDS, see www.ingenetix.com.

5. Contents of the Kit

5.1. ViroReal® Kit PCV3 order no. DVEV03611 or DVEV03651

Labelling	Content	Amount		Storage
		DVEV03611	DVEV03651	
PCV3 Assay Mix (green cap)	Primer and probe (FAM) for detection of PCV3	2 x 50 µl	1 x 50 µl	-15°C to -25°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	-15°C to -25°C
PCV3 Positive Control (red cap)	Control-DNA (approx. 10,000 target copies/µl)	1 x 25 µl	1 x 25 µl	-15°C to -25°C
DNA Reaction Mix (white cap) #	Reaction Mix	2 x 500 µl	1 x 500 µl	-15°C to -25°C until first use, then at +4°C
Water (blue cap)	Water	1 x 1000 µl	1 x 1000 µl	-25°C to +4°C

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

5.2. ViroReal® Kit PCV3 order no. DVEV03613 or DVEV03653

Labelling	Content	Amount		Storage
		DVEV03613	DVEV03653	
PCV3 Assay Mix (green cap)	Primer and probe (FAM) for detection of PCV3	2 x 50 µl	1 x 50 µl	-15°C to -25°C
CR-3 Assay Mix (yellow cap)	Primer, probe (Cy5) and target for detection of IPC	2 x 50 µl	1 x 50 µl	-15°C to -25°C
PCV3 Positive Control (red cap)	Control-DNA (approx. 10,000 target copies/µl)	1 x 25 µl	1 x 25 µl	-15°C to -25°C
DNA Reaction Mix (white cap) #	Reaction Mix	2 x 500 µl	1 x 500 µl	-15°C to -25°C until first use, then at +4°C
Water (blue cap)	Water	1 x 1000 µl	1 x 1000 µl	-25°C to +4°C

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

The components of ViroReal® Kit PCV3 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
- Nuclease-free 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
	PCV3 Assay Mix	1.0 µl
	CR Assay Mix	1.0 µl
	Total volume Master Mix	15.0 µl
Preparation of PCR	Master Mix	15.0 µl
	Sample*	5.0 µl
	Total volume	20.0 µl

*1-8 µl of the sample can be used. When using an amount < 5 µl of the sample, the amount of H₂O has to be changed accordingly.

Positive Control: As positive control please use 1 µl of the PCV3 Positive Control + 4 µl H₂O.

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 PCV3

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 Applied Biosystems® 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:

	Ct/Cp (FAM channel) PCV3 target	Ct/Cp IPC target	Interpretation
Negative control	Negative	33.0 ± 2	Valid
Positive control (undiluted, 1 µl/PCR)	28.0-31.0	33.0 ± 2	Valid
Or: positive control (1:10 diluted, 1 µl/PCR)	31.0-34.0	33.0 ± 2	Valid
Extraction negative control (optional)	Negative	33.0 ± 2	Valid
Negative sample	Negative	33.0 ± 2	Valid
Positive sample	Positive	Positive/Negative	Valid

For analysis of PCR data please proceed as follows:

For analysis of PCR results gained with ViroReal[®] Kit PCV3 please select fluorescence display options FAM channel for the PCV3 target and VIC/HEX channel (order no. DVEV03611, DVEV03651) or Cy5 channel (order no. DVEV03613, DVEV03653) for the internal positive control target. Samples with a positive Ct or Cp-value are considered positive. Please also check amplification-curves and if necessary adjust threshold manually.

8.1. Signal in FAM channel

→ DNA of PCV3 was amplified. The sample has to be interpreted as positive.

PCV3 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 PCV3 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.

9. Troubleshooting

9.1. No PCV3 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 internal positive control and no PCV3 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 H₂O).
- Incorrect PCR conditions.
→ Check the PCR conditions and repeat the PCR, if necessary.

9.3. PCV3 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 workspace and instruments are decontaminated at regular intervals.

9.4. PCV3 specific signal with negative control of DNA-extraction

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

10. Specifications

ViroReal[®] Kit PCV3 was evaluated with the Applied Biosystems[®] 7500 (Fast) instrument (Thermo Fisher Scientific). For further validation data, please contact ingenetix.

10.1. Analytical sensitivity and linearity

The analytical sensitivity is 10 template copies/PCR. The assay shows linearity over the range of 10 to 10,000,000 target copies/reaction with a slope of -3.1 and a R2 of > 0.99 as shown in Figure 1.

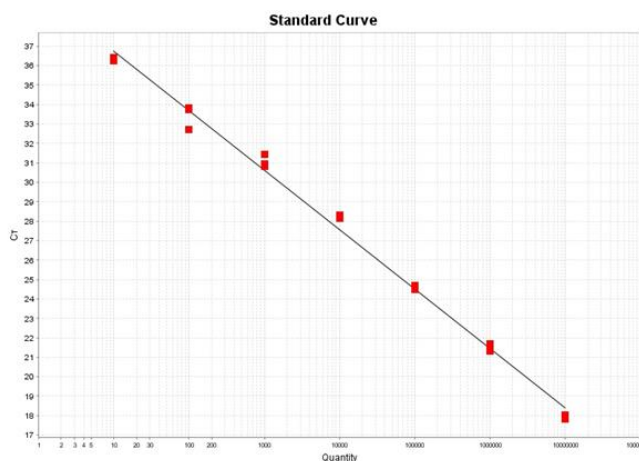


Figure 1 Ten-fold dilution series of a PCV3 DNA standard plotted against CT

10.2. Analytical specificity

The specificity is ensured by the selection of highly specific primers and probe. The primers and probe were checked for possible homologies to currently published sequences by sequence comparison analyses. This also validated the detection of so far known PCV3 strains.

ViroReal[®] Kit PCV3 is specific for porcine circovirus type 3 (no cross-reaction with PCV1 or PCV2).

10.3. Kit performance

Performance of ViroReal[®] Kit PCV3 with an Applied Biosystems[®] 7500 (Fast) instrument (Thermo Fisher Scientific) is shown in Figure 2.

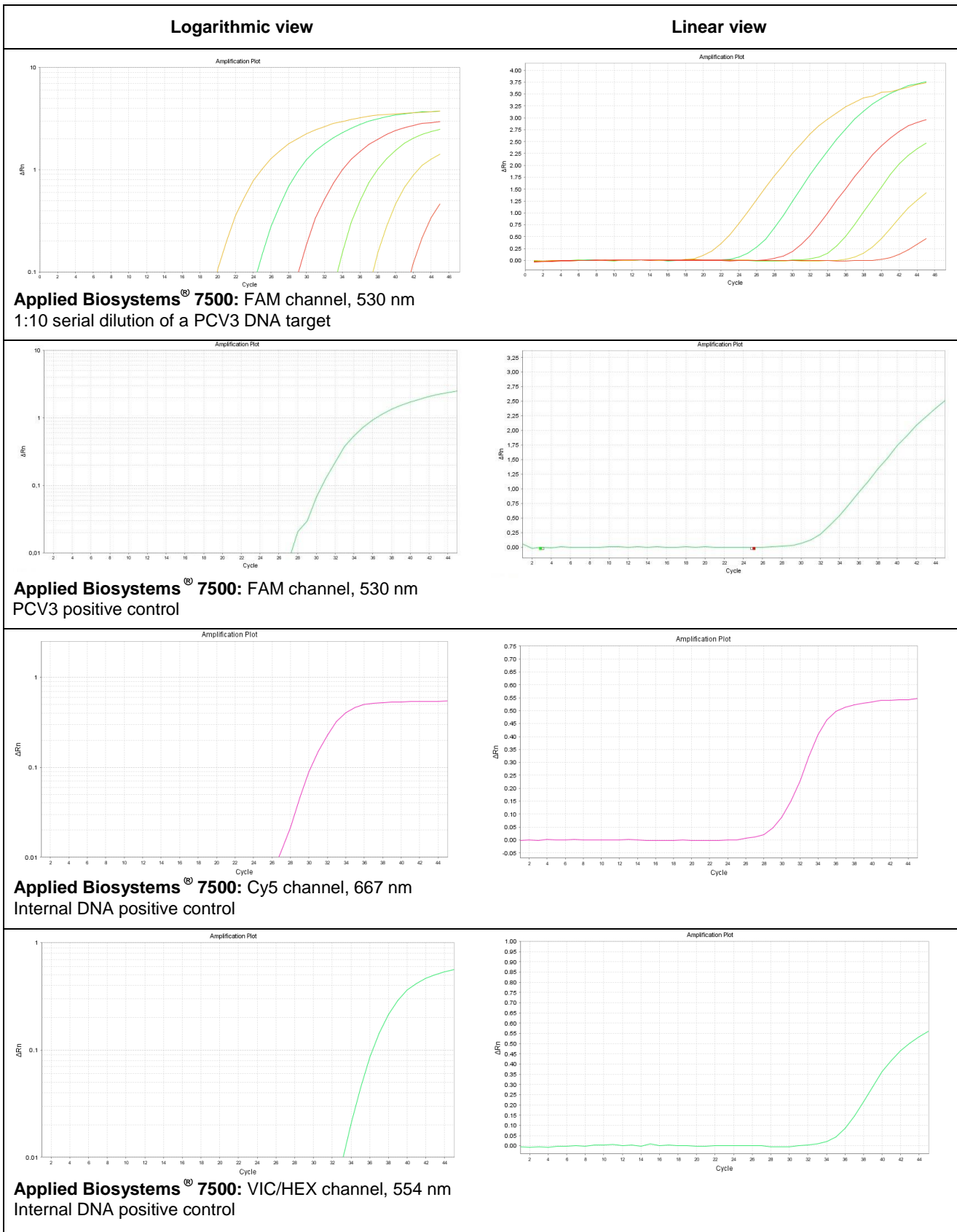


Figure 2 Performance of ViroReal® Kit PCV3

11. Annex – symbols



Batch code



Catalogue number



Contains sufficient for <n> tests



Use by



Manufactured by



Store at