Product Instructions

Porcine Circovirus Type 2 DNA Test Kit

vetproof® Porcine Circovirus Type 2 qPCR Kit

Product No. KIT252002

100 reactions

Store at 2°C - 8°C

For veterinary in vitro use only

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1 Product Description

The **vet**proof® Porcine Circovirus Type 2 qPCR Kit (PCV 2 qPCR) will detect the presence of DNA from Porcine Circovirus Type 2 in extracts from swine samples (Whole blood, serum and tissues, oral fluid and environmental samples). Primers and probes are specific for Porcine Circovirus Type 2. The kit contains all reagents and controls required for the detection of Porcine Circovirus Type 2. Amplified products are detected in real-time via a specific labelled hydrolysis probe, which is detected in a designated channel on the qPCR thermocycler. Porcine Circovirus Type 2 is detected in the FAM channel, and an Internal Control (IC) is detected in the HEX channel for identification of PCR inhibition to safeguard against false negative results.

In summary, the **vet**proof® Porcine Circovirus Type 2 qPCR Kit enables the simultaneous detection of:

- Porcine Circovirus Type 2 (PCV 2; detected in FAM channel)
- Internal control (IC; detected in HEX channel)

1.1 Number of Tests

The kit is designed for 100 reactions with a final reaction volume of 15 µl each.

1.2 Storage and Stability

- Store the kit at a temperature below 2°C - 8 °C after reception.
- Store away from sunlight and keep dry.
- After reconstitution, prepare aliquots and store at a temperature below -15 °C until the expiration date.
- Do not thaw more than 3 times.

1.3 Kit Contents

Item No.	Component	Format	Contents / Function / Storage
1	PCR Mix (PCR MIX)	1 x vial with clear cap	• Lyophilized • Needs to be reconstituted/rehydrated in Rehydration Buffer • For the amplification and detection of Porcine Circovirus Type 2 specific sequences • 10 µl per reaction
2	Rehydration Buffer (RB)	1 x 6ml vial with clear cap	• Ready to use • Used for reconstituting the PCR Mix
3	Control Template PCV 2 (PCV2 CTL+)	1 x vial with purple cap	• Dried • Needs to be reconstituted/rehydrated in H2O PCR • For use as a PCR run positive control for PCV 2
4	H2O PCR-grade (H2O PCR)	2x 1000 µL vial with clear cap	• Ready-to-use nuclease-free, PCR-grade H2O • For use as a PCR run negative control • For reconstitution of PCV2 CTR+

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1.4 Applicability Statement

This kit is compatible with all real-time PCR instruments suitable for the detection of FAM and HEX fluorophores, including the following: LightCycler® 96 and LightCycler® 480 (Roche Diagnostics), AriaMx® (Agilent Technologies), Applied Biosystems™ 7500 and QuantStudio™ 5 (Thermo Fisher Scientific) and CFX96™ (Bio-Rad Laboratories) Rotor-gene Q (Qiagen) Chromo 4 (Biorad) and MIC (Biomolecular Systems)

1.5 Additional Equipment and Reagents Required

- Real-time PCR cycler suitable for the detection of FAM, HEX
- DNA extraction method (see: Preparation of Samples > DNA extraction)
- Vortex mixer (x2)
- PCR plate-spinner
- Pipettes & disposable filter-tips for volumes of 1 – 1000 µl
- Nuclease-free microtubes of 1.5 ml and 2 ml
- Plates for PCR reaction or PCR tubes (suitable for use with your real-time PCR cycler)
- Heat resistant plate sealers or cap strips
- Disposable powder-free gloves
- Refrigerator and freezer

1.6 Preparation of Samples

Sample material

Swine samples – Whole Blood, Serum, Tissue Samples, Oral Fluids, Environmental Swabs.

DNA extraction

Before running the PCR, DNA must be extracted from the sample.

Extract DNA from the sample using an appropriate manual or automated extraction. Recommended are spin column methods or magnetic bead extraction methods (e.g. **vet**proof® MagBead Extraction Kit I Product no. KIT230342). Extracted DNA can be stored at -20°C prior to running the PCR.

It is recommended to validate the chosen extraction method and the **vet**proof® Porcine Circovirus Type 2 qPCR Kit combined, internally, prior to generating results.

2 How to Use This Product

2.1 Good Laboratory Practices for PCR

- Assays must be performed by qualified laboratory personnel only.
- Wear disposable powder-free gloves at any stage of running the assay and/or sample preparation. Change gloves when changing work areas or if you suspect that they are contaminated.
- Handle all reagents with care.
- Do not thaw the reagents more than 3 times.
- Treat all biological materials as potentially biohazardous, including all field samples.
- Never pipette anything by mouth. There must be no eating, drinking or smoking in areas designated for using kit reagents and handling field samples.
- Avoid prolonged exposure of the PCR Mix to direct light.

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- Keep the kit components separate from other reagents in the laboratory.
- Use nuclease-free lab ware (e.g., pipettes, pipette tips, reaction vials).
- To avoid cross-contamination of samples and reagents, use aerosol-preventive pipette tips.
- Strict adherence to the test protocol will lead to achieving the best results.
- Dedicate one airspace for kit storage/reagent preparation (Room 1, Clean Room) and another airspace for running the assay and sample preparation/extraction (Room 2). A third airspace is optional (Room 3) for dedicated amplification/running the assay.
- Never move any materials and equipment from Room 2 or 3 to Room 1.
- Include positive and negative control in every run.
- Decontaminate PCR laboratories with bleach or an alternative nucleic acid decontaminant and UV light (optional) after testing.
- Do not use reagents after the expiration date.
- Do not use reagents if the packaging is damaged.
- Do not mix reagents from different kit batches.
- Do not open the PCR wells after amplification and discard the plates/tubes safely avoiding breaking or leaking of the plates/tubes.
- Used material must be disposed of in compliance with the legislation in force regarding environmental protection and biological waste management.

2.2 Procedure

Recommended workflow protocol

When running complete assay including DNA extraction in 1 day

1. Start in Room 1 with reagent preparation.
2. Go to Room 2/3 for DNA extraction and running assay.
3. Never go from Room 2/3 to Room 1 during the same day.

When first performing DNA extraction:

Day 1:

1. Start in Room 2 with DNA extraction.

Day 2:

1. Start in Room 1 with reagent preparation.
2. Go to Room 2/3 to run the assay.
3. Never go from Room 2/3 to Room 1 during the same day.

Reagent preparation (Room 1 – Clean Room)

1. Add 1000 µl of Rehydration Buffer per PCR Mix vial
2. Vortex reagents thoroughly for at least 20 seconds and briefly spin to remove any residues from the lid
3. Calculate total volume of PCR MIX required for all reactions. Do not forget to include reactions for controls (minimum one positive and one negative), and to compensate for dead volume (+1 reaction for instance).
4. This mixture will now be referred to as Reaction Mix. Of this mixture (Reaction Mix) 10 µl per PCR reaction will be used.
5. Put the vials with Reaction Mix back in the freezer immediately after usage (at temperature below -15°C). Do not thaw more than three times.

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Assay preparation (Room 1 – Clean Room)

1. Take a suitable qPCR plate and record location of samples on template.
2. Add 10 µl of Reaction Mix as prepared above for every sample plus controls.
3. Add 5 µl of H2O PCR-grade (negative control) into control well. This is a reagent and environment control (Optional control for Room 1).
4. Cover plate and take into Room 2.

Positive Control preparation (Room 2/3 – Extraction/Amplification Room)

1. Control Template PCV 2 (PCV2 CTL+) Add 100µl of H2O PCR-grade (H2O PCR) to the PCV2 CTL+ tube and vortex at least 20 seconds until the blue pellet is dissolved.
2. Aliquot this solution and store them at temperature below -15°C until the expiration date. Do not thaw more than 3 times.
3. If quantification is needed, prepare the following range in Nuclease-free water as below. 5 µL of each dilution will be used in the dedicated wells.

Dilution	PCV 2 CTL+ concentration (Copies/PCR)
Undiluted	10^6
1/10	10^5
1/100	10^4
1/1000	10^3

DNA amplification (Room 2/3 – Extraction/Amplification Room)

1. Add 10 µl of Reaction mix in each well
2. Add 5 µl of the prepared Control Template (Positive Control) into the dedicated control well.
3. Add 5 of H2O PCR-grade (H2O PCR; Negative Control) into dedicated control well. This is an environment control.
4. Add 5 µl of sample (DNA extract) into the appropriate wells.
5. Cover plate with heat resistant sealer or caps. Place plate in qPCR thermocycler and start one of the specified thermal cycler programs as soon as possible.

DNA/RNA Standard Program	
10 min. 45 °C	
2 min. 95 °C	
5 sec. 95 °C	40 cycles
30 sec. 60 °C*	
Data collection (@ 60 °C): FAM = PCV 2 HEX = IC	

Note: Program the PCR instrument before preparing the PCR samples. For details on how to program the protocol, see the instrument operator's manual of your real-time PCR cycler.

Note: For some real-time PCR instruments, the type of probe quencher as well as passive reference dye must be specified. For the AB7500, ROX should be specified as passive reference dye.

Note: Alternative channel names for reporter dyes: - FAM: no alternative name; HEX: VIC

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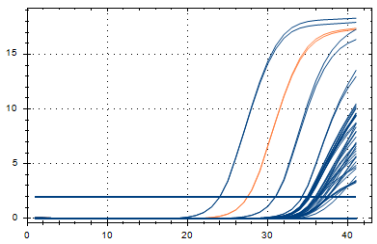
2.3 Validation and Interpretation

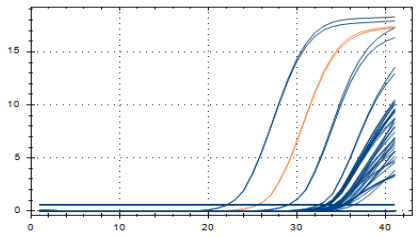
Setting thresholds in the PCR cycler software

In the analysis section of your PCR instrument software, open the graphical display of the amplification curves. Select all sample-containing wells on the plate, select linear view, and select the FAM channel. Turn off other channels

Picture 1



Picture 2



Depending on the number of positive samples, the linear curves should look like the ones in Picture 1. To set the threshold, identify the Positive Control reaction (highlighted orange). Look at which cycle the curve transitions into a straight line; in this case, around cycle 27. The threshold is first placed at the point where the curve becomes a straight line. The threshold should then be set halfway between this point and the baseline; see Picture 2.

Repeat this process for the HEX channel.
Note: After setting the thresholds for all channels, keep using those thresholds for all future PCR runs.

2.3.1 Validation of the PCR run

The following must apply for the PCR run to be valid:

	PCV 2 (FAM) Cq values	IC (HEX) Cq values	Interpretation
Negative Control (H2O PCR-grade)	N/A*	N/A*	Valid Control
Control Template/ Positive Control PCV 2 (PCV2 CTL+)	<40	N/A*	Valid Control

**No Cq value*

Note: Repeat the PCR plate in the event of control failure.

2.3.2 Validation and interpretation of sample results

The amplification of Porcine Circovirus Type 2 DNA is analysed in the FAM channel, and the Internal Control is analysed in the HEX channel. Nucleic acids amplification is valid for each sample if at least one typical amplification curve is observed in FAM or HEX or equivalent.

Note: Always check the validity of the amplification curves.

PCV 2 (FAM)	IC (HEX)	Interpretation
N/A*	<40	Negative sample
<40	Not considered	Positive sample
N/A*	N/A*	Invalid well**

**No Cq value*

***The assay is invalid for this particular sample and should be repeated with a 1/10 dilution of the extract or a new DNA extract.*

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2.3.3 Validation and Interpretation of Quantitative results.

To interpret quantitative results, set up a calibration curve (number of cycles = f (Log concentration). Determine the curve equation ($y = ax + b$) and check PCR efficiency ($Eff\% = (10^{(\frac{-1}{a})} - 1) \times 100$). The calibration curve is valid if:

- The 4 points of the range are amplified.
- The coefficient of correlation R^2 is higher than 0,9.
- Efficiency lies between 75 and 125 %.
- Points of the range are spread homogenously.

Quantification interpretation

Quantification of a positive sample is only possible in the quantification domain of the method use (see validation data).

PCV 2 Amplification (FAM)	Sample Status for PCV 2
No Signal	**Undetected** Nucleic Acid undetected
Ct sample > Ct PCV2 CTL+ (1/1000)	**Detected** Nucleic acid detected with a quantity under the range
Ct PCV2 CTL+ < Ct sample < Ct PCV2 CTL+ (1/1000)	**Detected** Quantifiable nucleic acid
Ct sample < Ct PCV2 CTL+	**Detected** Nucleic acid detected with a quantity over the range

In the case of a “quantifiable” sample (Ct value comprised in between the extreme points of the CTL+ range), the concentration is determined using the calibration curve equation:

$$x = 10^{\left(\frac{y-b}{a}\right)} \text{ x F}$$

Where: x: Concentration PCV2 (Copies/PCR)
y: Cq value in FAM
b: Intercept
a: Slope
F: Multiplying coefficient (optional)

To calculate the quantity per sample, include a multiplying coefficient. It is determined according to the volume of sample extracted and the volume of elution in extraction step.

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3 Supplementary Information

Symbols Glossary

REF	Product Reference Number		Expiry (Expiration) Date
	Kit Size/Reactions		Protect from Moisture
	Store at		Protect from Heat and Direct Sunlight
	Batch		Manufacturer

Quality Control

All products are monitored by our quality control on a batch-to-batch basis. A certificate of analysis (CoA) is available from BioChek.

Ordering Information

This kit and associated products are available from BioChek. For a complete overview and for more information, please visit our website at www.biochek.com.

Trademarks

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Warranty and Disclaimer of Liability

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2. BioChek B.V. does not warrant its product against any and all defects when: the defect is a result of material or workmanship not provided by BioChek B.V.; defects caused by misuse or use contrary to the instructions supplied, or improper storage or handling of the product;
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6. BioChek B.V. reserves the right to replace or allow credit for any modules returned under this warranty.

Regulatory Disclaimers

For veterinary in vitro use only.

Regulatory requirements vary by country; this product may not be available in your geographic area. For information on availability, please contact BioChek.

4 Revision Index

Revision A: New document.

5 Supplier Information

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