Instruction manual
ADL67Y1-PCV2_NO_(EN)_V03
06/2024

PCV2

Reference : ADL67Y1-100

Test for the detection of Porcine CircoVirus 2 (PCV2) by real time enzymatic amplification
PCR Test – 100 reactions

For veterinary *in vitro* use only

Sample	Individual analysis	Pool of sample possible*, up to:
Whole blood / Serum	✓	5
Tissue (lung, heart, runt)	✓	5

* Depending on the epidemiological case and on the quality of samples.

Kit composition

Content		ADL67Y1-100 Kit 100 reactions
A6	Amplification solution	1 lyophilized vial with blank cap (To reconstitute)
Rehydration buffer	Rehydration solution	1 x 6 mL vial (Ready to use)
PCV2 CTL+	Porcine CircoVirus 2 (PCV2) positive control	1 tube with purple cap (To reconstitute)
NF-Water	Nuclease Free water	2 x 1000 µL tubes with blank caps (Ready to use)

Revision history

Date	Version	Modifications
08/04/2022	V01	First version
15/11/2022	V02	Adaptation of packaging in 100 reactions
06/2024	V03	Version harmonization with other languages

Note: minor typographical, grammar and formatting changes are not included in the revision history.

A. Introduction

The agents involved in piglet wasting disease and dermatonephropathy syndrome are DNA viruses of the Circoviridae family. They cause one of the major diseases affecting pigs with a significant economic impact worldwide.

B. Test principle

ADIALYO™ PCV2 test is based on the specific amplification of PCV2 DNA.

This test is intended to detect simultaneously, in one well:

- Porcine Circovirus 2 (FAM labeled probe)
- Internal control of extraction and amplification specific from an endogenous nucleic acid (HEX labeled probe or its equivalent)

Included in the kit, the positive control PCV2 CTL+ enables the quantification of PCV2.

C. Storage conditions

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

D. Material required but not provided

- Real-time Thermal cycler and device for Real Time PCR.
- Instrument for homogenous mixing of tubes.
- Pipettes of 1 - 10 µL, 20 - 200 µL and 200 - 1000 µL.
- Nuclease-free filtered pipette tips.
- Nuclease-free microtubes of 1,5 mL and 2 mL.
- Powdered-free latex or nitrile gloves.
- Nuclease-free Water.
- Kit for nucleic acids extraction.

E. Warnings and precautions

- For veterinary *in vitro* use only.
- For animal use only.
- For professionnal use only.
- All instructions must be read before performing the test and strictly respected.
- Do not use reagents after the expiration date.
- Do not use reagents if the package is damaged.
- Do not mix reagents from different batches.
- Do not open PCR wells or tubes after amplification.
- Used material must be disposed of in compliance with the legislation in force regarding environmental protection and biological waste management.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (do not ingest or inhale).

F. Nucleic acids extraction

1. Extraction kits

Nucleic acids must be extracted from the samples before using the kit. The RNA/DNA purification kits listed below are recommended by Bio-X Diagnostics:

Product name	Extraction system	Number of tests and reference
ADIAMAG	Magnetic beads	200 tests : ref. NADI003 800 tests : ref. NADI003-XL
QIAamp® DNA Mini Kit	Silica column	50 tests : ref. 51304 250 tests : ref. 51306

For the extraction, consult the user manual version, available on the website, indicated on the certificate of analysis included in the PCR kit of interest.

Other purification kits can be used if they have been validated by the user.

After extraction, nucleic acid extracts can be kept on ice or at +2/8 °C for 24 hours or until use. For long term storage, they must be kept at a temperature below -15 °C or -65 °C.

2. Controls to include

Using controls allow to verify the reliability of the results. Controls are included according to the recommendations defined by current standards (*cf.* AFNOR U47-600...).

Controls	Validation of	Usage
No Template Control (NTC)	Absence of amplification contamination	5 µL NF-Water in a well per run
PCV2 CTL+ (Dilution pure to 1/10 000)	PCV2 target amplification and range	5 µL CTL+ in a well per run

G. Procedure

1. Amplification solution (A6) preparation

- Add **1000 µL** of **« Rehydration buffer »** per A6 tube.
- Homogenize tube contents using a mixer, such as vortex, at least 20 seconds.
- After reconstitution, aliquot the solution and store at a temperature below -15 °C until the expiration date. Do not thaw more than 3 times.
- To use the A6, please refer to §« Amplification », Step 1.

2. Preparation of controls

a. Preparation of CTL+ control

- Add **200 µL** of **« NF-Water »** to each CTL+ tube.
- Homogenize the tubes using a mixer, such as vortex, at least 20 seconds and until dissolution of the blue pellet.
- After reconstitution, aliquot the solution and store at a temperature below -15 °C until the expiration date. Do not thaw more than 3 times.
- To use PCV2 CTL+ :
  - Please refer to § « Amplification », Step 1.
  - In case of a quantitative test, prepare a standard range with Nuclease-free water, just before experiment:

Dilution	PCV2 CTL+ concentration (copies/PCR)
Pure	5.10^5
1/10	5.10^4
1/100	5.10^3
1/1000	5.10^2
1/10000	5.10^1

- Use **5 µL** of each dilution in the dedicated wells (see § « Amplification », Step 2).

3. Amplification

Warning :

- Before starting, rehydrate or thaw reagents at room temperature in the dark.
- Homogenize all reagents and samples before use.
- Store reagents at a temperature below -15 °C after use.

Step 1: Dispense **10 µL** of amplification solution (A6) per well.
Step 2: Dispense **5 µL** of nucleic acids extracts and **5 µL** of controls in each dedicated well.
Use NF-Water for the No Template Control (NTC).
Step 3: Cover the wells with appropriate optical film or caps.
Step 4: Set up the amplification program.

The following program is defined for ABI Prism thermocyclers (like 7500, QuantiStudio5, Step-one…) from Applied Biosystems, for Mx3000, Mx3005P and AriaMx from Agilent, for LightCycler from Roche Diagnostics, for Rotor-Gene Q from Qiagen, for CFX96 and Chromo 4 from Biorad and for MIC from BioMolecular System.

DNA/RNA Program	
10 min. 45 °C	
2 min. 95 °C	
5 sec. 95 °C	40 cycles
30 sec. 60 °C*	

*Reading and parameters for fluorescence acquisition :

Fluorochrome	Absorbance (nm)	Emission (nm)
FAM	494	520
HEX or equivalent	538	554
ROX	575	602

Note: The quencher is non-fluorescent. The A6 solution contains a passive reference read in the same spectra as ROX for ABI machines.

For other thermal cycler instruments**,** please contact your sales representative or the customer relations department.

H. Interpretation of results

1. Validation and interpretation of qualitative results

Set threshold line separately for each dye.

a. Test validation

Amplification is valid if the following results are obtained.
Expected Ct (Threshold Cycle) values for CTL+ are indicated on the certificate of analysis of the kit.

Controls	Amplification		Validation of
	FAM	HEX or equivalent	
No Template Control (NTC)	No	No	Absence of amplification contamination
PCV2 CTL+	Yes	No	Amplification of PCV2 target

b. Interpretation of results

Nucleic acids extraction and amplification are **valid** for each sample if at least one typical amplification curve is observed in FAM and/or HEX or equivalent.

Amplification		Interpretation
FAM	HEX or equivalent	PCV2
No	No	Not detected
Yes	Yes / No	Detected
No	No	Undetermined

« Undetermined » : no characteristic amplification curve.
Possible causes :
Defective PCR due to inhibitors, set up error, absence of samples, degraded samples and/or issue with nucleic acids extraction (loss or destruction of nucleic acids).

Recommendations :
Set up a new PCR assay using pure nucleic acids extracts and 10x dilutions in Nuclease-free water ;
If assay is inconclusive, perform a new nucleic acids extraction.

2. Validation and interpretation of quantitative results

a. CTL+ range

PCV2 CTL+ Dilution	Concentration (copies/PCR)	Amplification		Validation of
		FAM	HEX or equivalent	
Pure	5.10^5	Yes	No	PCV2 target amplification and calibration curve set up
1/10	5.10^4	Yes	No	
1/100	5.10^3	Yes	No	
1/1000	5.10^2	Yes	No	
1/10000	5.10^1	Yes	No	

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 ($Eff\% = \left(10^{\left(\frac{-1}{a}\right)} - 1\right) \times 100$).
The calibration curve is valid if:

- The 5 points of the range are amplified. However, one point of the range can be omitted if that point is not one of the extreme points.
- The coefficient of correlation R^2 is higher than 0,9.
- Efficiency lies between 75 et 125 %.
- Points of the range are spread homogenously.

b. Quantification interpretation

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

PCV2 amplification	Sample status for PCV2
No signal	Undetected Nucleic acid undetected
Signal < LQ_{METHOD}	Detected Nucleic acid detected with a quantity under the $LQ_{METHODE}$
LQ_{METHOD} < signal < LQ_{max}	Detected Nucleic acid quantifiable
Signal > LQ_{max}	Detected Nucleic acid detected with a quantity over the $LQ_{METHODE}$

In the case of a « quantifiable » sample, PCV2 concentration is determined using the calibration curve equation:

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

where x : concentration in PCV2 (copies/PCR)
y : Ct value in FAM for the sample to quantify
b : intercept
a : slope
F : multiplying coefficient (optional)

The multiplying coefficient is determined according to the sample matrix and extraction method and allows conversion of quantification from copies / PCR into copies / mL or copies / mg.

Multiplying coefficient examples with ADIAMAG extraction kit according to the NEKF user manual:

Matrix	Multiplying coefficient (F)	Unit
Serum/Blood	200	copies / mL
Tissue	10	copies / mg

Symbols

Symbole	Signification
	Catalog number
	Manufacturer
	Temperature limitation
	Use by
	Batch code
	Consult Instructions for Use
	Contain sufficient for "n" tests
	For veterinary *in vitro* use only – For animal use only
	Keep away from sunlight
	Keep dry

Notes

1 Extract nucleic acids with

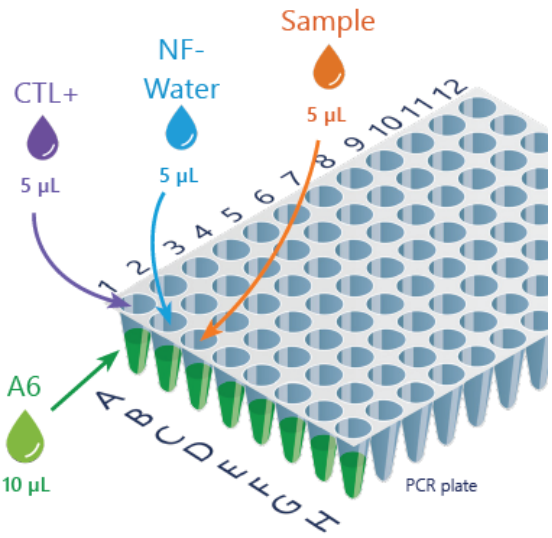
Adia X Mag

Scan me to discover Adiamag™

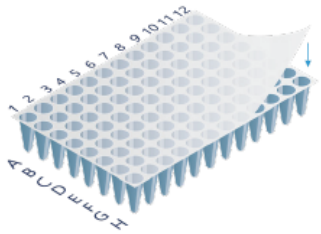
2 Add **1000 µL** of Rehydration buffer to the **A6** amplification solution

3 Distribute **10 µL** of **A6** amplification solution

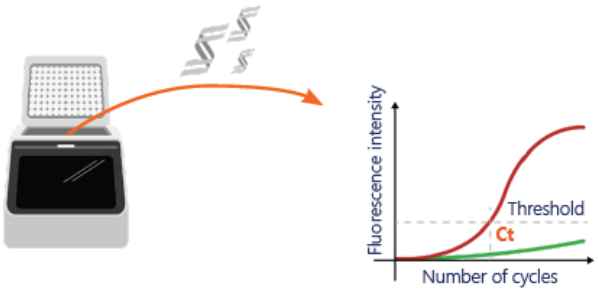
4 Distribute **5 µL** of nucleic acids, **CTL+** and **NF-Water**



5 Seal the wells



6 Start PCR analysis



*The notes do not replace the instructions for use of which they are a summary.

Contact us
info@biox.com
+32 (0) 84 32 23 77
www.biox.com