Instruction manual
ADL13Y1_PRRS_NO_(EN)_V02
25/10/2022

EU/NA PRRSV

Reference: ADL13Y1-100

Test for the detection, EU/NA differentiation and quantification of *Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)* 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,...)	✓	3
Saliva	✓	3
Oral fluids, bronchoalveolar lavages (BALs)	✓	✗

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

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Kit composition

Content		ADL13Y1-100 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)
EU PRRSV CTL+	PRRSV EU positive control	1 tube with purple cap (To reconstitute)
NA PRRS CTL+	PRRSV NA 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
02/05/2022	V01	First version
04/08/2022	V01	Correction of "DNA/RNA program" table
25/10/2022	V02	Adaptation of packaging in 100 reactions

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

A. Introduction

The Porcine Reproductive and Respiratory Syndrome is caused by a small ribonucleic acid virus belonging to the *Arteviridea* family. PRRS is one of the major porcine diseases and leads to important economic loss worldwide. The virus can be divided in 2 groups based on genetic differences: PRRSV I (so called European strains or EU) and PRRSV II (so called North American strains or NA) (Adam *et al.*, 2016; Kuhn *et al.*, 2016).

Two main symptoms can be observed after infection: reproductive issues (late abortion, abnormaly long pregnancy, high rate of piglets' death, fertility decrease) and respiratory issues associated with hyperthermia and loss of appetite.

PRRSV can be found in Europe and North America, but has also been identified in China since 1995. It's also present in Japan, Vietnam, the Philippines, Malaysia and Korea among other countries in Asia. Australia, New-Zealand, India, some european countries and some parts of Africa are as of today free of the disease (OIE website).

Since different PRRSV genotypes can be found in a herd, the fight against the virus is difficult. A fast, efficient and easy detection of different PRRSV genotypes becomes crucial in order to manage infections. Hence, RT-qPCR is the recommended method for PRRSV identification or infections control.

B. Test principle

ADIALYO EU/NA PRRSV test is based on the reverse transcription of RNA into complementary DNA (cDNA). The cDNA is then amplified by Polymerase Chain Reaction (PCR) thanks to specific primers and a DNA polymerase. Both enzymatic reactions occur in the same tube (One-Step RT-PCR):

- PRRSV EU genotype (probe labeled in FAM)
- PRRSV NA genotype (probe labeled in Cy5)
- RNAse P: an internal control of extraction and amplification specific from endogenous DNA (probe labeled with a fluorochrome read in the same spectra as VIC and HEX).

EU PRRSV CTL+ et NA PRRSV CTL+ allow PRRSV quantification.

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.
- 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 professional 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 packaging is damaged.
- Do not open PCR wells or tubes after amplification.
- Do not mix reagents from different batches.
- 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

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…).

Control	Validation of	Usage
No Template Control (NTC)	Absence of amplification contamination	5 µL NF-Water in a well per run
EU PRRSV CTL+ (Dilution pure to 1/10 000)	EU PRRSV target amplification and range	5 µL CTL+ in a well per run
NA PRRSV CTL+ (Dilution pure to 1/10 000)	NA PRRSV target amplification and range	5 µL CTL+ in a well per run
Negative extraction control	Absence of contamination for the extraction and amplification	1 extraction (water or lysis buffer) per run
Positive extraction control	Extraction and amplification	1 extraction (Positive sample between 1 et 100X LOD_{Method}) 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

- 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.
- If quantification is needed, prepare the following ranges in Nuclease-free water:

Dilution	EU PRRSV CTL+ concentration (copies/PCR)
Pure	4.10^6
1/10	4.10^5
1/100	4.10^4
1/1000	4.10^3
1/10000	4.10^2

Dilution	NA PRRSV CTL+ concentration (copies/PCR)
Pure	2.10^6
1/10	2.10^5
1/100	2.10^4
1/1000	2.10^3
1/10000	2.10^2

- 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	530	549
Cy5	646	662
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. Reading and interpretation

1. Qualitative results

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	Cy5	HEX or equivalent	
No Template Control (NTC)	No	No	No	Absence of amplification contamination
EU PRRSV CTL+	Yes	No	No	Amplification of EU PRRSV target
NA PRRSV CTL+	No	Yes	No	Amplification of NA PRRSV target
Extraction negative control	No	No	No	Absence of extraction contamination
Extraction PRRSV EU positive control	Yes	No	Yes	PRRSV EU extraction and amplification
Extraction PRRSV NA positive control	No	Yes	Yes	PRRSV NA extraction and amplification

b. Results interpretation

Nucleic acids extraction and amplification are **valid** for each sample if at least one typical amplification curve is observed in FAM, Cy5 and/or HEX or equivalent.

Amplification			Interpretation	
FAM	Cy5	HEX or equivalent	EU PRRSV	NA PRRSV
No	No	Yes	Not detected	Not detected
Yes	Yes	Yes	Detected	Detected
Yes	No	Yes	Detected	Not detected
No	Yes	Yes	Not detected	Detected
No	No	No	Undetermined	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. Quantitative results

a. CTL+ range

EU PRRSV CTL+ dilution	Concentration (copies/PCR)	FAM amplification	Validation of
Pure	4.10^6	Yes	EU PRRSV target amplification and calibration curve set up
1/10	4.10^5	Yes	
1/100	4.10^4	Yes	
1/1000	4.10^3	Yes	
1/10000	4.10^2	Yes	

NA PRRSV CTL+ dilution	Concentration (copies/PCR)	Cy5 Amplification	Validation of
Pure	2.10^6	Yes	NA PRRSV target amplification and calibration curve set up
1/10	2.10^5	Yes	
1/100	2.10^4	Yes	
1/1000	2.10^3	Yes	
1/10000	2.10^2	Yes	

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 isn't one of the extreme points.
- The coefficient of correlation R^2 is higher than 0,9.
- Efficiency 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).

EU PRRSV or NA PRRSV amplification	Sample status for EU PRRSV or NA PRRSV
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, PRRSV concentration is determined using the calibration curve equation:

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

Where x : concentration (in copies / PCR if F is omitted)
y : Ct value in FAM or Cy5 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

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Symbols

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

Notes

1 Extract nucleic acids with

AdiaX Mag

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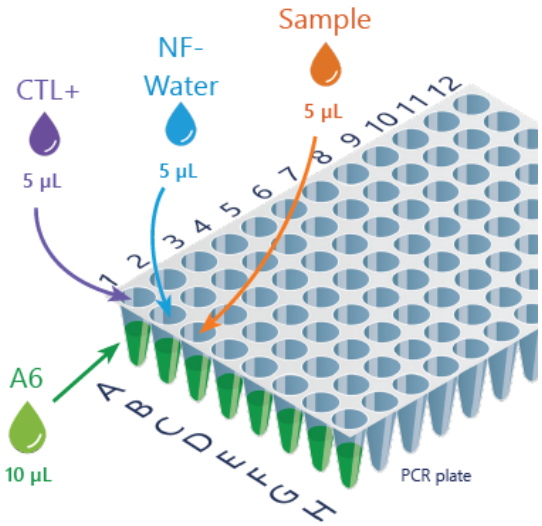
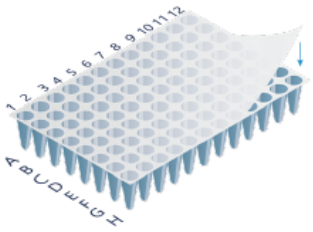
2 Add **1000 µL** of Rehydration buffer to the **A6** amplification solution

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

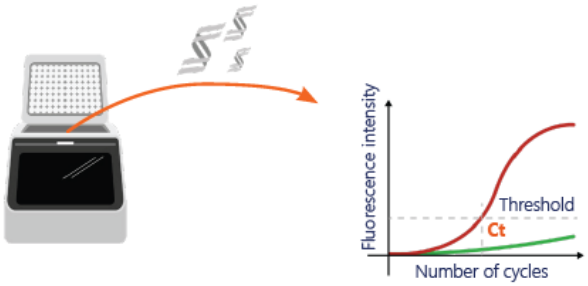
4 Distribute **5 µL** of nucleic acids, CTL+ and NF-Water



5 Seal the wells



6 Start PCR analysis



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

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