Instruction manual
ADL03Y1_ABPP_NO_(EN)_V01
01/2025

APP

Reference: ADL03Y1-100

Test for the detection of *Actinobacillus pleuropneumoniae* by real time enzymatic amplification

PCR Test – 100 reactions

For veterinary *in vitro* use only

Sample	Individual analysis	Pool of sample possible*, up to:
Tissue / Biospy	✓	3
Brush / Swab	✓	3
Oral fluid	✓	✗
Bacterial culture	✓	✗

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

Kit composition

Content		ADL03Y1-100 Kit 100 reactions
A6	Amplification solution	1 lyophilized vial with blank caps (To reconstitute)
Rehydration buffer	Rehydration solution	1 x 6 mL vial (Ready to use)
APP CTL+	*Actinobacillus pleuropneumoniae* positive control	1 tube with purple cap (To reconstitute)
NF-Water	Nuclease-Free Water	1 x 1000 µL tube with white cap (Ready to use)

Revision history

Date	Version	Modifications
01/2025	V01	First version

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

A. Introduction

Actinobacillus pleuropneumoniae is the etiological agent of pig hemorrhagic pleuropneumonia, or actinobacillosis. This disease is the cause of economic losses in a large number of industrial farms.
Serotyping of *A. pleuropneumoniae* strains is based upon the organism's capsular polysaccharide antigens (Mittal *et al.*, 1983). In this manner, 15 serotypes have been distinguished, serotypes 1 and 5 being subdivided into 1a, 1b and 5a, 5b respectively (Jolie et al., 1994 – Nielsen, 1986). There are 4 cytotoxins proteins (apxI, apxII, apxIII, apxIV). The combination of toxins makes the serotype more or less virulent. Serovar prevalence varies from country to country and with time (Gottschalk, 2015). *A. pleuropneumoniae* is frequently isolated from the nasal cavities, from tonsils and from lungs.
Diagnosis by PCR identifies the presence of the bacterium regardless of serotype.

B. Test principle

ADIALYO™ APP test is based on the amplification of specific *Actinobacillus pleuropneumoniae* DNA. This test is intended to detect simultaneously, in one well:

- *Actinobacillus pleuropneumoniae* – Serotypes 1 to 15 (FAM labelled probe).
- RNAse P: internal control of extraction and amplification specific from an endogenous nucleic acid (HEX labelled probe or its equivalent).

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.

Additional kits (on request) for method adoption and PCR

- **LD_{PCR} Positive Control – APP (Ref.: ADC03LD)**

Confirmation of performances – LD_{PCR} of kit.

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
ADIAPURE Lysis Flex	Direct lysis	500 mL: ref. ADPLF1-500

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.

Extraction protocols are described in the validation data file. 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 a few hours or until use. For long term storage, they must be kept at a temperature below -15 °C or -65 °C.

2. Controls

Using controls allow to verify the reliability of the results. Controls can be included.

Control	Validation of	Usage
No Template Control (NTC)	Absence of amplification contamination	5 µL NF-Water in a well per run
APP CTL+	APP target amplification	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 CTL+ control

- Add **200 µL** of **« NF-Water »** per tube.
- Homogenize the tubes by suction and pressure, then use a mixer, such as vortex, > 20 seconds and until dissolution of the blue pellet.
- After reconstitution, aliquot and store the solution at a temperature below -15 °C until the kit expiration date. Do not thaw more than 3 times.
- For each assay, use **5 µL** of CTL+ in one of 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 an appropriate optical film or caps. Centrifuge briefly (recommended).
Step 4: Set up the amplification program.

The following program is defined for ABI Prism thermocyclers (like 7500, QuantStudio5, 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
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

Display all curves and position the threshold line for each fluorochrome.

1. Test validation

Amplification is valid if the following results are obtained.
Expected Ct (Threshold Cycle*)* values for the 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
APP CTL+	Yes	Yes/No	Target amplification
Extraction negative control	No	No	Absence of extraction contamination
Extraction positive control	Yes	Yes/No	Extraction and amplification steps

2. Results interpretation

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	*Actinobacillus pleuropneumoniae*
No	Yes	Undetected
Yes	Yes	Detected
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 the assay is inconclusive, perform a new nucleic acids extraction.

Bibliography

- Gottschalk, M. (2015) The challenge of detecting herds sub-clinically infected with Actinobacillus pleuropneumoniae. The Veterinary Journal 206,30–38.
- Jolie R. A., Mulks M. H. and Thacker B. J. (1994) Antigenic differences within Actinobacillus pleuropneumoniae serotype 1. Vet. Microbiol. 38 (4): 329-349.
- Mittal K. R., Higgins R. and Larivière S. (1983) Detection of type-specific antigens in the lungs of Haemophilus pleuropneumoniae-infected pigs by coagglutination test. J. Clin. Microbiol. 18 (6): 1355-1357.
- Nielsen R. (1986) Serological characterization of Actinobacillus pleuropneumoniae strains and proposal of a new serotype: serotype 12. Acta Vet. Scand. 27: 453-455.

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 Pure → Scan me to discover Adiapure™

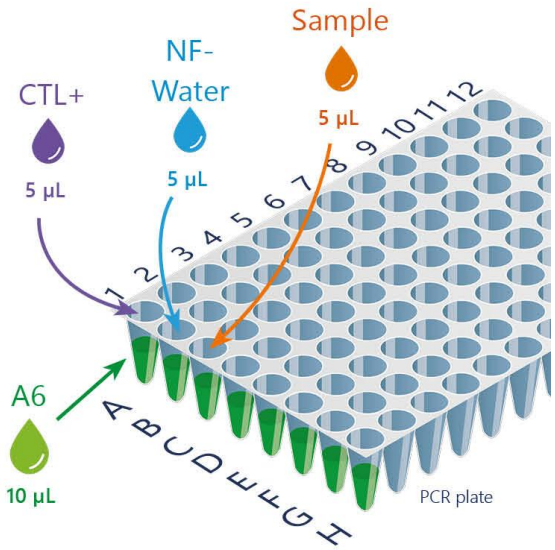
or

AdiaX Mag → Scan me to discover Adiamag™

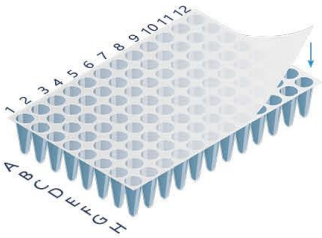
2 Add **1000 µL** of Rehydration buffer to the A6 amplification solution

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

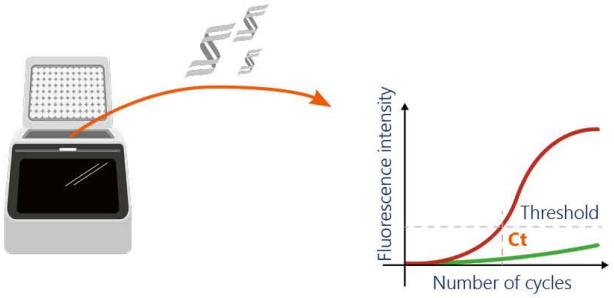
4 Distribute **5 µL of nucleic acids**, CTL+ and NF-Water



5 Seal the wells



6 Start PCR analysis



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

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