



M.HYOP

Reference: ADL05Y1-100

Test for the detection of *Mycoplasma hyopneumoniae* 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 (lung)	✓	✗
Tracheobronchial washing	✓	✗
Swab	✓	3
Oral fluid	✓	✗
FTA card	✓	✗
Bacterial culture	✓	✗

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

Kit composition

Content		ADL05Y1-100 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)
M.HYOP CTL+	<i>Mycoplasma hyopneumoniae</i> 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

Mycoplasma hyopneumoniae is the primary agent of enzootic porcine pneumonia (EPP), one of the most important direct or indirect causes of respiratory infectious diseases. The disease has a worldwide distribution and causes considerable economic losses in swine production due to reduce growth rate and feed conversion efficiency. *M. hyopneumoniae* has a specific pathogenicity and leads to secondary infection by other pathogenic bacteria such as *Pasteurella multocida* or *Actinobacillus pleuropneumoniae*. The contamination occurs at each stage of piglet production, from sows to piglets but also from pigs themselves by direct contact.

The PCR test allows the specific and rapid detection of mycoplasma directly on live pigs (Mattsson *et al.*, 1995; Verdin *et al.*, 1996; Baumeister *et al.*, 1998) and allows the detection of *M. hyopneumoniae* on seropositive pigs (infected or vaccinated pigs).

B. Test principle

ADIALYO™ M.HYOP test is based on the amplification of specific *Mycoplasma hyopneumoniae* DNA. This test is intended to detect simultaneously, in one well:

- *Mycoplasma hyopneumoniae* (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 – M.HYOP (Ref.: ADC05LD)**
Confirmation of performances – LOD_{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 validation data. 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
M.HYOP CTL+	M.HYOP 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
M.HYOP 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	<i>Mycoplasma hyopneumoniae</i>
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

- Baumeister A. K., Runge M., Ganter M., Feenstra A. A., Delbeck F. and Kirchoff H. (1998). Detection of *M. hyopneumoniae* in bronchoalveolar lavage fluids of pigs by PCR. J. Clin. Microbiol. 36: 1984-1988.
- Mattsson J. G., Bergstöm K., Wallgren P. and Johansson K. E. (1995). Detection of *Mycoplasma hyopneumoniae* in nose swabs from pigs by in vitro amplification of the 16S RNA gene. J.Clin.Microbiol. 33: 893-897.
- Verdin E., Blanchard B., Kobisch M., Bove J. M. and Saillard C. (1996) Use of a nested PCR diagnosis test to detect *M. hyopneumoniae* under field conditions. IOM lett. 4: 101-102.

Symbols

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

- 1 Extract nucleic acids with

Adia^X
Pure



Scan me to
discover Adiapure™

or

Adia^X
Mag



Scan me to
discover Adiamag™

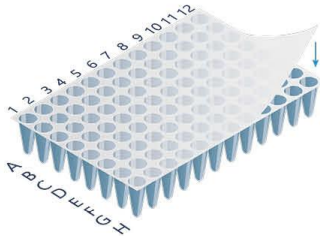
- 2 Add **1000 µL** of Rehydration buffer to the **A6** amplification solution



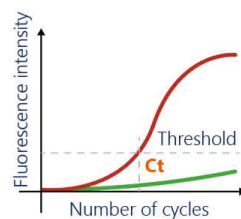
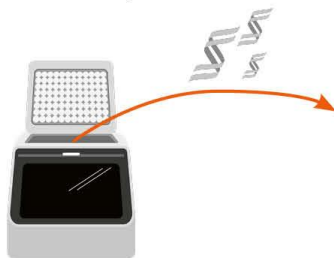
- 3 Distribute **10 µL** of **A6** amplification solution

- 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.