Adia X Lyo

Instruction manual
ADL01Y1-MSMG_NO_(EN)_V01
03/2023

MS/MG

Reference: ADL01Y1-200 & ADL01Y1-1000

Test for the detection of *Mycoplasma synoviae* et *Mycoplasma gallisepticum* by real time enzymatic amplification

PCR Test – 200 reactions & 1000 reactions

For veterinary *in vitro* use only

Sample	Individual analysis	Pool of sample possible*, up to:
Swab on live animals (palate slit, tracheal…)	✓	6
Swab on dead animals (joint, injured organ…)	✓	6
Environmental specimen	✓	✗
Bacterial culture (solid, liquid)	✓	✗
FTA card	✓	✗

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

Kit composition

Content		ADL01Y1-200 Kit	ADL01Y1-1000 Kit
		200 reactions	1000 reactions
A6	Amplification solution	2 lyophilized vials with blank caps (To reconstitute)	10 lyophilized vials with blank caps (To reconstitute)
Rehydration buffer	Rehydration solution	1 x 6 mL vial (Ready to use)	2 x 6 mL vial (Ready to use)
MS CTL+	*Mycoplasma synoviae* positive control	1 tube with purple cap (To reconstitute)	2 tubes with purple cap (To reconstitute)
MG CTL+	*Mycoplasma gallisepticum* positive control	1 tube with purple cap (To reconstitute)	2 tubes with purple cap (To reconstitute)
NF-Water	Nuclease-Free Water	1 x 1000 µL tube with white cap (Ready to use)	2 x 1000 µL tubes with white cap (Ready to use)

Revision history

Date	Version	Modifications
05/2021	NE01Y-02	Last version
03/2023	V01	Switch to simplified format

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

A. Introduction

Mycoplasma are known to cause economic losses in commercial poultry production. *M. gallisepticum* causes chronic respiratory disease in chickens and infection sinusitis in turkeys. *M. synoviae* most frequently occurs as subclinical upper respiratory infection and synovitis in chickens and turkeys.

Prevention through surveillance and vaccination has become an effective control method. PCR diagnosis is a rapid and specific method for monitoring farms.

B. Test principle

ADIALYO™ MS/MG test is based on the amplification of specific *Mycoplasma synoviae* and *Mycoplasma gallisepticum* DNA. This test is intended to detect simultaneously, in one well:

- *Mycoplasma synoviae* (FAM labelled probe)
- *Mycoplasma gallisepticum* (CY5 labelled probe)
- Internal control of amplification specific from an exogenous DNA (HEX labelled probe or its equivalent).

The test detects the MG TS-11 and MS-H vaccine strains but not the MG 6/85 and MG-F vaccine strains.

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 for method adoption and PCR

- **LD_{PCR} Positive Control – MS/MG (Ref.: ADC01YLD)**

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 from swab	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.

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.

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
MS CTL+	MS target amplification	5 µL CTL+ in a well per run
MG CTL+	MG 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 controls

- Add **200 µL** of **« NF-Water »** per tube.
- Homogenize the tubes using 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.
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	538	554
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

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	Cy5	HEX or equivalent	
No Template Control (NTC)	No	No	Yes	Absence of amplification contamination
MS CTL+	Yes	No	Yes	Amplification of MS target
MG CTL+	No	Yes	Yes	Amplification of MG target
Extraction negative control	No	No	Yes	Absence of extraction contamination
Extraction positive control	Yes	Yes	Yes	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, Cy5 and/or HEX or equivalent.

Amplification			Interpretation	
FAM	Cy5	HEX or equivalent	*M. synoviae*	*M. gallisepticum*
No	No	Yes	Undetected	Undetected
Yes	Yes	Yes/No	Detected	Detected
Yes	No	Yes/No	Detected	Undetected
No	Yes	Yes/No	Undetected	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 the assay is inconclusive, perform a new nucleic acids extraction.

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

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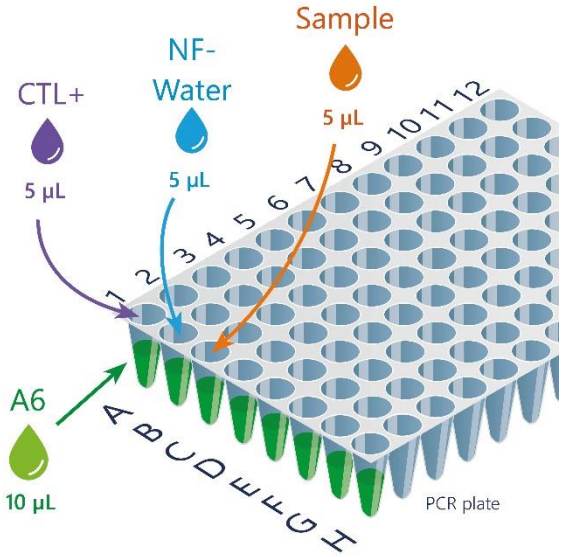
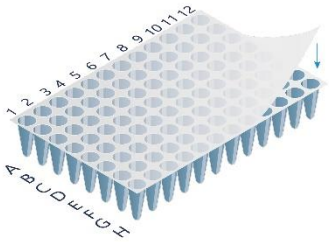
2 Add **1000 µL** of Rehydration buffer to the **A6** amplification solution

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

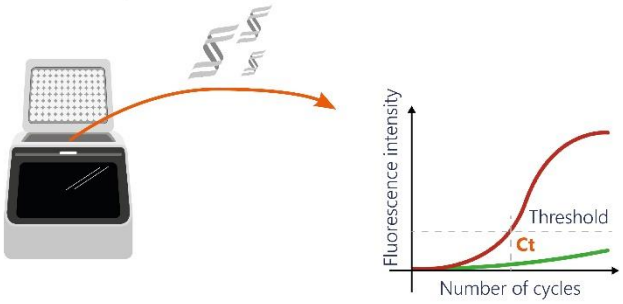
4 Distribute **5 µL** of nucleic acids, **CTL+** and **NF-Water**



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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