



ADIALYO™ MS/MG

VET

INTENDED USE

ADIALYO™ MS/MG kit is intended to detect *Mycoplasma synoviae* and *Mycoplasma gallisepticum* using real-time Polymerase Chain Reaction (PCR) technology from swab of poultry, environmental specimen, bacterial culture and FTA card.

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 labeled probe)
- *Mycoplasma gallisepticum* (CY5 labeled probe)
- Amplification exogenous internal control (HEX labeled probe or its equivalent)

CONTENT OF THE KIT

Kits	
REF ADL01Y1-200	Pack of 200 tests
REF ADL01Y1-1000	Pack of 1000 tests

COMPOSITION

REF ADL01Y1-200		
A6	amplification solution	2 lyophilized vials with blank cap (to reconstitute)
MS CTL+	positive control <i>Mycoplasma synoviae</i>	1 tube with purple cap (to reconstitute)
MG CTL+	positive control <i>Mycoplasma gallisepticum</i>	1 tube with purple cap (to reconstitute)
Rehydration buffer	rehydration solution	1 x 6 mL vial (Ready to use)
NF-Water	nuclease-free Water	1 x 1000 µL tube with blank cap (Ready to use)
REF ADL01Y1-1000		
A6	amplification solution	10 lyophilized vials with blank cap (to reconstitute)
MS CTL+	positive control <i>Mycoplasma synoviae</i>	2 tubes with purple cap (to reconstitute)
MG CTL+	positive control <i>Mycoplasma gallisepticum</i>	2 tubes with purple cap (to reconstitute)
Rehydration buffer	rehydration solution	2 x 6 mL vials (Ready to use)
NF-Water	nuclease-free Water	2 x 1000 µL tubes with blank cap (Ready to use)

STORAGE CONDITIONS

- Store the kit at +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.

MATERIAL REQUIRED BUT NOT PROVIDED

- Real-time PCR 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
- Powder-free latex or nitrile gloves
- Kit for nucleic acids extraction

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 expiry date.
- Do not use reagents if the packaging is damaged.
- Do not open PCR wells or tubes after amplification process.
- Do not mix reagents from different batches.
- 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).

NUCLEIC ACIDS EXTRACTION

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

Product Name	Supplier	Extraction system	Number of test & Reference
ADIAMAG	Bio-X Diagnostics	Magnetic beads	200 tests: ref. NADI003 800 tests: ref. NADI003-XL
ADIAPURE™ LYSIS FLEX	Bio-X Diagnostics	Direct lysis	500 mL: ref. ADPLF1-500
QIAamp® DNA Mini Kit	Qiagen	Silica column	50 tests: ref. 51304 250 tests: ref. 51306
NucleoSpin® Tissue	Macherey-Nagel	Silica column	50 tests: ref. 740952.50 250 tests: ref. 740952.250

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

Analysis options according to the specimen:

Specimen	Individual analysis	Pool of sample is possible*, up to
Swab on live animals (palate slit, tracheal...)	<input checked="" type="checkbox"/>	6
Swab on dead animals (joint, injured organ...)	<input checked="" type="checkbox"/>	6
Environmental specimen	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Bacterial culture (solid, liquid)	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
FTA card	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

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

PREPARATION OF WORKING SOLUTION

1. PREPARATION OF A6 REAGENT

1. Add 1000 µL of "Rehydration solution" per A6 tube.
2. Homogenize tube contents using a mixer such as vortex, at least 20 seconds.
3. After reconstitution - Aliquot the solution. Store the obtained solution at a temperature below -15°C until the expiration date. Do not thaw more than 3 times.
4. To use the A6, please refer to the "AMPLIFICATION" section.

2. PREPARATION OF CONTROLS (CTL+)

1. Add 200 µL of "NF-water" per tube.
2. Homogenize tube contents using a mixer such as vortex, at least 20 seconds until complete dissolution of the blue pellet.
3. After reconstitution, aliquot the solution. Store the obtained solution at a temperature below -15°C until the expiration date. Do not thaw more than 3 times.
4. To use the CTL+, please refer to the "AMPLIFICATION" section.

AMPLIFICATION PROTOCOL

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.
Keep DNA extracts at +2/8°C for 24 hours and then store at a temperature below – 15°C.

STEP 1: DISPENSE 10 µL OF AMPLIFICATION SOLUTIONS A6 PER WELLS

STEP 2: DISPENSE 5 µL OF NUCLEIC ACIDS EXTRACTS AND CONTROLS

Dispense nucleic acids extracted from sample and controls in each dedicated well.
Dispense NF-Water for the reagent control (Negative Control - NTC)

STEP 3: COVER THE WELLS WITH APPROPRIATE OPTICAL FILM OR CAPS

STEP 4: SET UP THE APPROPRIATE AMPLIFICATION PROGRAM

The following program is defined for ABI Prism thermocyclers (like 7500, StepOne, QS5...) from Applied Biosystems (check the "emulation 9600" option if available), for the MX3005P and ARIAMX of Agilent, for CFX96 of BioRad and MIC of Bio Molecular System:

RNA/DNA 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:

Dyes	Absorbance (nm)	Emission (nm)
FAM	494	520
HEX (or its equivalents)	538	554
CY5	646	662
ROX	575	602

Note: The Quencher is non-fluorescent. The A6 solution contains a passive reference ROX for the ABI machines. For other thermal cycler instruments, please contact your sales representative or the customer relations department.

READING AND INTERPRETATION

Set Threshold line separately for each dye.

1. VALIDATION OF TEST

The real-time PCR run is validated if the following results are met.

The indicative Ct (Threshold Cycle) values expected from CTL+ are notified on the kit's analysis certificate.

Controls	Amplification			Validation
	FAM	Cy5	HEX or equivalent	
Negative control	No	No	Yes	No contamination for amplification
MS CTL+	Yes	No	Yes	Amplification of the <i>M. synoviae</i> target
MG CTL+	No	Yes	Yes	Amplification of the <i>M. gallisepticum</i> target

2. RESULTS INTERPRETATION

Nucleic acids extraction and amplification are valid if at least one characteristic amplification curve is observed per sample in FAM, Cy5 and/or HEX or its equivalent.

Amplification	Sample			
	FAM	Cy5	HEX or equivalent	<i>M. synoviae</i>
No	No	Yes	-	-
Yes	No	Yes / No	+	-
No	Yes	Yes / No	-	+
Yes	Yes	Yes / No	+	+
No	No	No	Undetermined	Undetermined

«**Undetermined**»: no characteristic amplification curve.

Possible causes:

- Defective PCR due to inhibitors, set up error, absence of sample or degraded sample and/or
- Issue with nucleic acids extraction (loss or destruction of nucleic acids).

Recommendations:

1. Set up a new PCR assay using pure nucleic acids extracts and 10x dilution in Nuclease-free water.
2. If assay is inconclusive, perform a new nucleic acids extraction.

QUALITY CONTROL

ADIALYO™ MS/MG kit is planned and designed to ensure that the toughest quality requirements are met. The certificate of analysis is supplied with the kit.

WASTE DISPOSAL

Unused reagents may be considered as non-hazardous waste and disposed of accordingly. Dispose of all used reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.

It is the responsibility of each laboratory to handle waste and effluents produced according to their type and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

INDEX OF SYMBOLS

Symbol	Meaning
	GB: Catalogue number US: Catalog number
	Manufacturer
	Temperature limitation
	Use by
	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
	For veterinary <i>in vitro</i> use only – For animal use only
	Keep away from sunlight
	Keep dry

WARRANTY

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REVISION HISTORYChanges type categories:

N/A	Not Applicable (first publication)
Correction	Correction of document anomalies
Technical change	Addition, revision and/or removal of information related to the product
Administrative	Implementation of non-technical changes noticeable to the user

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

Release date	Part number	Change type	Change summary
2020-05	NE01Y1-01	N/A	First publication
2021-05	NE01Y1-02	Technical change	Modification of the volume of Rehydration buffer added to the A6 reagent (1000 µL instead of 1040 µL)
2021-05	NE01Y1-02	Administrative	Trade mark ADILYO replaced by ADIALYO™

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