



## ADILYO MS/MG

VET

## INTENDED USE

ADILYO™ 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

ADILYO™ 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 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.

## 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<br>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<br>250 tests: ref. 51306          |
| NucleoSpin® Tissue   | Macherey-Nagel    | Silica column     | 50 tests: ref. 740952.50<br>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 1040 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.
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 |              |
| 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:

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

## QUALITY CONTROL

ADILYO™ 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<br>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 HISTORY

Changes 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 |

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