MGMS Triplex Real-time PCR detection Kit

Mycoplasma gallisepticum (MG) and *Mycoplasma synoviae* (MS)

Catalogue number: MD03181, 150 reactions

Application

MGMS Triplex Real-time PCR detection Kit is designed for the simultaneous detection of *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) genomes by multiplex real-time PCR. This multiplex kit was developed to detect MG and MS target genes in a single-tube reaction, as well as an Internal control. The primers and probes sequences used in this kit are highly specific for each pathogen and present no homology with other *Mycoplasma* species. The usual hosts for MG and MS infections are chickens and turkeys. Biological samples, such as swabs, tissues and organs are suitable for analysis using this detection kit.

Description

Mycoplasmas are small bacteria of the *Mycoplasma* genus devoid of cell walls. Two of them, *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) are important avian respiratory pathogens, which cause severe economic losses to the poultry industry. Reduced egg production and downgrading of carcass quality are some of the consequences of the infection. MG infection causes chronic respiratory disease (CRD), as well as infectius synovitis of chickens and turkeys. MS infection occurs most commonly as a subclinical infection of the upper respiratory tract, especially in multi-age layer flocks. Infectious sinusitis and airsacculitis can be also present in commercial infected poultry.
MG and MS transmissions between birds occurs readily by direct contact with an infected bird, via dust or by drinking water and food. MS infection occurs most commonly in chickens with 4 to 16 weeks-old. MS infection rates are extremily high when the bacteria is introduced into a flock of birds.

Regarding MG infection, symptoms usually last for several weeks with resultant high mortality rates. In addition, birds that recover from infection remain carriers.
Real-time PCR is the fastest and most reliable method to perform an accurate detection of MG and MS, allowing regular monitoring of the presence of both pathogens in commercial poultry.

Kit composition

The kit provides a set of reagents sufficient to perform 150 in vitro Real-time PCR reactions. The MGMS qPCR Master Mix is ready-to-use and does not require any previous preparation.

Component	Tubes	Volume
MGMS qPCR Master Mix	3	800 µL
RNase/DNase free water	1	1000 µL
MGMS Positive control	3	50 µL

Storage Conditions and Kit Stability

This MGMS Triplex Real-time PCR detection Kit is shipped at -20°C. All components should immediately be stored at -20°C upon arrival. Alternatively, the RNase/DNase free water can be stored at 2°C to 8°C.
Minimize the number of freeze-thaw cycles by storing both MGMS qPCR Master Mix and MGMS positive control template in working aliquots of suitable volumes.
The MGMS qPCR Master Mix should be stored protected from abundant light. Particularly, do not expose this qPCR Master Mix to direct sun light.
NZYTech does not recommend using the kit after the expiry date.

Required Reagents and Equipment

- Real-time PCR Instrument that detect the emitted fluorescence of FAM, Cy5 and HEX fluorescent dyes (emission 520, 550 and 670 nm, respectively).
- DNA extraction kit: we recommend using NZYTech's DNA extraction kits
- RNase/DNase free qPCR plasticware: PCR tubes, strips, caps, 96-well plates, adhesive films
- Pipettors and filter tips
- Vortex and centrifuge

Sample Material

All nucleic acid samples that are suitable for PCR amplification can be used with this kit. However, sample collection of biologic material, transport, storage and processing time are critical to achieve optimal results. Please ensure the samples are suitable in terms of purity, concentration and DNA integrity. We recommend running at least one negative control with the samples (see below). To prepare a negative control, replace the template DNA sample by RNase/DNase free water.

Dynamic range of test

Under optimal PCR conditions NZYTech's MGMS Triplex Real-time PCR detection Kit display very high priming efficiencies of >95%, and it can detect very low amounts of pathogen target genes from different samples.

Rational for the test

Multiplex Real-time PCR

MGMS Triplex Real-time PCR detection Kit is based on the amplification and detection of bacterial DNA of *Mycoplasma gallisepticum* and *Mycoplasma synoviae*, using multiplex real-time PCR. In a single reaction, this kit enables to co-amplify the MG and MS genes, as well as an Internal control gene, using three specific sets of primers and fluorogenic probes that specifically hybridizes in target regions. The selected probes for detection of amplified MG, MS and Internal control targets genes are labelled with fluorescent dyes allowing the detection of MG through the FAM channel, MS in the Cy5 channel, and the detection of the Internal control through the HEX/VIC channel. During PCR amplification, the probe is cleaved, resulting in the reporter dye and quencher separation. The resulting increase in fluorescence can be detected on a wide range of real-time PCR platforms.

Positive control

The kit includes a positive control template that allows controlling the PCR set-up, as well as the specificity and efficiency of the reagents. Each time the kit is used, at least one positive control reaction must be included in the run. A positive result indicates that the primers and probes for detecting the MG and MS target genes worked properly in that particular experimental scenario. If a negative result is obtained, the test results are invalid and must be repeated. Care should be taken to ensure that the positive control does not contaminate any other kit component which would lead to false-positive results. This can be achieved by handling this component in a post-PCR environment. Care should also be taken to avoid cross-contamination of other samples when adding the positive control to the run. This can be avoided by sealing all other samples and negative controls before pipetting the positive control into the positive control well. MG and MS positive controls are detected through the FAM and Cy5 channels respectively, and both give a Ct value between 16 and 35.

Negative control

To validate any positive findings, a negative control reaction should be included every time the kit is used. For this reaction the RNase/DNase free water should be used instead of template. A negative result indicates that the reagents have not become contaminated while setting up the run.

Internal control

This Internal control DNA is included in MGMS qPCR Master Mix, in a defined copy number, as well as a mix of primers and probe to detect the Internal Control gene. Successful co-amplification indicates that PCR inhibitors are not present. These primers are present at PCR

limiting concentrations, which allows multiplexing with the targets sequence primers. Co-amplification of the Internal control DNA does not interfere with detection of the MG and MS targets DNAs even when present at low-copy number. The Internal control is detected through the HEX/VIC channel and gives a Ct value between 20 and 35.

Nucleic Acids Extraction

Usually, DNA is isolated from tracheal/oropharyngeal swabs of birds or from culture medium. However, tissues and organs samples are also suitable as sources of DNA for real-time analysis. We recommend pooling samples from five individuals, per DNA preparation, in order to increase the representability of the analysed population.

Real-time PCR Detection Protocol

To help preventing any carry-over DNA contamination, we recommend assigning independent areas for reaction set-up, PCR amplification and any post-PCR gel analysis. It is essential that any tubes containing amplified PCR product are not opened in the PCR set-up area. We also recommend the use of RNase and DNase-free plasticware/reagents, filter tips (eventually of low-retention) for all pipetting steps and a clean area to work. Before start, spin down each tube in a centrifuge before opening. Proceed as explained below:

1. Pipette **16 µL** of the ready-to-use **MGMS qPCR Master Mix** into individual wells according to your real-time PCR experimental plate set-up.
2. Pipette **4 µL** of **sample DNA** into each well, according to your experimental plate set-up. The final volume in each well should be 20 µL (see table below).

Component	**Volume (µL) / reaction**
MGMS qPCR Master Mix	16
DNA (sample DNA or RNase/DNase free water or Positive Control♦)	4
Final Volume	**20**

♦For controls, use 4 µL of RNase/DNase free water (Negative control) or 4 µL of Positive Control (Positive control) instead 4 µL of DNA sample.

Note: *Care should also be taken to avoid cross-contamination of other samples when adding the positive control to the run. This can be avoided by sealing all other samples and negative controls before pipetting the positive control into the positive control well.*

3. Close the reaction wells and briefly spin down the plate before to proceed with the amplification and detection steps.
4. Place the reaction plate in the real-time PCR instrument and run the real-time protocol according to table below.

Suggested thermal cycling conditions

MGMS qPCR Master Mix is an optimized and highly efficient reaction mixture developed for real-time PCR. The table below displays a standard protocol optimized on a number of platforms. However, these conditions may be adapted to suit different machine-specific protocols.

Cycles	Temperature	Time	Notes
1	95 °C	10 min	Polymerase activation
40	95 °C	15 s	Denaturation
	60 °C	30 s	Annealing/Extension*

*Fluorogenic data should be collected during this step through FAM (MG target), Cy5 (MS target) and HEX/VIC (Internal control) channels.

Data analysis

The qualitative real-time PCR test is only valid if:

- The curves of FAM, Cy5 and HEX of **Positive control** are positives. Positive control is expected to amplify between Ct 16 and 35. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised.
- The **Negative control** is positive for HEX channel (Ct ≤ 35) and negative for FAM and Cy5 channels.

Interpretation of the results:

The **sample is negative for MG and MS** if the HEX curve is positive but the FAM and Cy5 are negatives.

The **sample is positive for MG** if the FAM curve is positive (Ct ≤ 35, independent of the HEX¥ curve.

The **sample is positive for MS** if the Cy5 curve is positive (Ct ≤ 35), independent of the HEX¥ curve.

The **sample is positive for both MG and MS** if the FAM and Cy5 (Ct ≤ 35) curves are positives, independent of the HEX¥ curve.

The **sample is inhibited** if neither the HEX curve nor the FAM and Cy5 curves are positives.

¥A positive HEX curve means that amplification was successful indicating that PCR inhibitors are not present. However, a reduced or no HEX signal can also be observed, it may indicate that a very high concentration of Mycoplasma DNA in present in the sample, which competes with Internal control amplification.

The follow table summarize the interpretation of principal results.

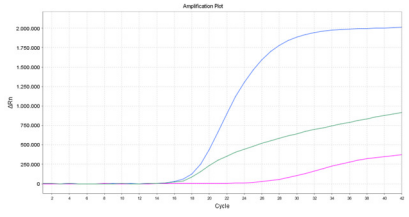
Internal control (HEX)	*Mycoplasma gallisepticum*,(FAM)	*Mycoplasma synoviae*,(Cy5)	Result
positive	negative	negative	Negative for **MG** and **MS**
positive / negative[¥]	positive	negative	Positive for **MG** and negative for **MS**
positive / negative[¥]	negative	positive	Negative for **MG** and positive for **MS**
positive / negative[¥]	positive	positive	Positive for **MG** and positive for **MS**
negative	negative	negative	Experiment failed due to inhibited sample[♣]

[¥] A reduced or no HEX signal can also be observed, it may indicate that a very high concentration of Mycoplasma DNA in present in the sample, which competes with Internal control amplification.

[♣] Inhibited sample: If the sample is not positive for FAM, Cy5 and HEX channels, the test should be repeated. We recommend using a 1:10 dilution of the DNA sample.

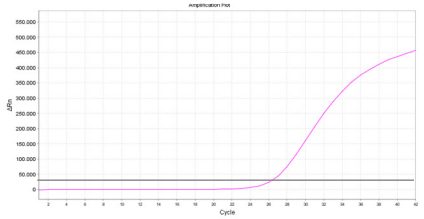
Typical amplification observed for the Positive control sample:

Detection of MG and MS present in Positive control sample



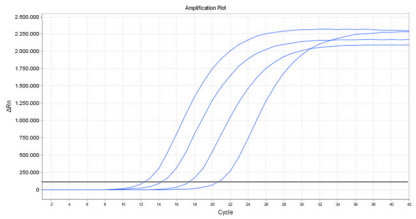
Detection of MG through FAM channel (blue), MS through Cy5 channel (green) and the Internal control is detected through HEX/VIC channel (pink).

Detection of Internal control



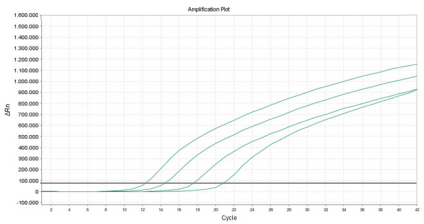
Detection of Internal control through HEX/VIC channel.

Detection of *Mycoplasma gallisepticum*



1:10 dilutions of *Mycoplasma gallisepticum* through FAM channel.

Detection of *Mycoplasma synoviae*



1:10 dilutions of *Mycoplasma synoviae* through Cy5 channel.

Notes:

Quality control assay

All components of MGMS Triplex qPCR Real-time detection Kit are tested following the protocol described above. The triplex real-time PCR system allows the detection of both pathogens, as well as the Internal control in the Positive control sample. Positive amplifications are observed for MG, MS and Internal control through FAM, CY5 and HEX channels, respectively.

V2102

Certificate of Analysis

Test	Result
Functional assay	Pass

Approved by:

Patrícia Ponte
Senior Manager, Quality Systems

For research use only

nzytech
genes & enzymes

Estrada do Paço do Lumiar, Campus do Lumiar - Edifício E, R/C, 1649-038 Lisboa, Portugal
Tel.:+351.213643514 Fax: +351.217151168
www.nzytech.com