Product Instructions

Mycoplasma Gallisepticum-Synoviae DNA Test Kit

vetproof® Mycoplasma Gallisepticum-Synoviae qPCR LyoKit

Product No. KIT230345/KIT230346

96 reactions

Store at 2 to 8°C

For veterinary in vitro use only

Contents

1 Product Description 3
1.1 Number of Tests 3
1.2 Storage and Stability 3
1.3 Kit Contents 4
1.4 Applicability Statement 4
1.5 Additional Equipment and Reagents Required 4
1.6 Preparation of Samples 5
2 How to Use This Product 5
2.1 Good Laboratory Practices for PCR 5
2.2 Procedure 6
2.3 Validation and Interpretation 8
3 Supplementary Information 11
4 Revision Index 12
5 Supplier Information 12

1 Product Description

The **vet**proof® Mycoplasma Gallisepticum-Synoviae qPCR LyoKit (MgMs qPCR) will detect the presence of DNA from *Mycoplasma gallisepticum* and *Mycoplasma synoviae* strains in extracts from avian samples (e.g. tracheal, oropharyngeal, choanal or cloacal swabs, tissue samples, and FTA cards). The kit contains all reagents and controls required for the detection of *Mycoplasma gallisepticum* (Mg) and *Mycoplasma synoviae* (Ms). Primers and probes are specific for Mg and Ms; each probe is labelled with a specific fluorophore which is detected in a designated channel on the qPCR thermocycler. Mg is detected in the FAM channel and Ms is detected in the HEX channel. An Internal Control (IC) is detected in the ROX channel for identification of PCR inhibition to safeguard against false negative results. An Endogenous Control (EnC) is detected in the CY-5 channel to verify the presence of avian test matrix and successful DNA extraction. After extraction of the DNA, samples are added to the PCR tubes prefilled with lyophilized reaction mix. The prepared PCR tubes are placed in the real-time PCR cycler for amplification and detection.

In summary, the MgMs qPCR assay enables simultaneous detection of:

- Mycoplasma gallisepticum (Mg; detected in FAM channel)
- Mycoplasma synoviae (Ms; detected in HEX channel)
- Internal Control (IC; detected in ROX channel)
- Endogenous Control (EnC; detected in CY-5 channel)

1.1 Number of Tests

The kit is designed for 96 reactions with a final reaction volume of 25 µl each.

1.2 Storage and Stability

Store the kit at 2 to 8°C throughout the expiration date printed on the label. Store the 8-tube PCR strips with the lyophilized reagents in the aluminum bag to protect them from light and moisture. Close the bag tightly after each use.

1.3 Kit Contents

Item No.	Component	Format	Contents / Function / Storage
1	PCR Plate (96 reactions), separable into 8-tube strips, prefilled with lyophilized reaction mix	Aluminum bag containing a 96 well plate with 8-tube PCR strips • KIT230345 (LP) with white "low profile" tubes* • KIT230346 (RP) with clear "regular profile" tubes*	• Ready-to-use PCR reaction mix, containing primers and hydrolysis probes, Internal Control, and Taq DNA Polymerase • For the amplification and detection of *Mycoplasma gallisepticum and M. synoviae*-specific sequences • 25 µl per reaction • **Protect from light and moisture!**
2	Control Template	One (1) vial with purple cap	• 1 x 350 µl • Contains a stabilized solution of plasmid DNA carrying the Mg, Ms, and Endogenous Control target sequences • For use as a PCR run positive control
3	H2O PCR-grade	Two (2) vials with clear cap	• 2 x 1 ml • Nuclease-free, PCR-grade H2O • For use as a PCR run negative control
4	Cap Strips	Plastic bag containing 8-cap strips	• 12 x 8-cap strip • For sealing the PCR strips after addition of samples

** Compatibility of PCR-tubes with qPCR thermocyclers must be checked prior to the assay set-up. For more information regarding compatibility, check www.biochek.com. In case the provided PCR tubes do not fit the cycler, samples can be transferred after resuspension of lyophilized PCR mix to appropriate PCR vessels. Take necessary precautions to avoid cross-contamination during this pipetting step.*

1.4 Applicability Statement

This kit is compatible with all real-time PCR instruments suitable for the detection of FAM, HEX, ROX and CY-5 fluorophores, including the following: LightCycler® 96 and LightCycler® 480 (Roche Diagnostics), AriaMx® and Mx3005P® (Agilent Technologies), Applied Biosystems™ 7500 Fast and QuantStudio™ 5 (Thermo Fisher Scientific) and CFX96™ (Bio-Rad Laboratories).
Note: A Color Compensation is necessary and can be supplied by BioChek for users of the LightCycler® 480 instrument (Color Compensation Set 3, Product no. KIT230005).

1.5 Additional Equipment and Reagents Required

- Real-time PCR cycler suitable for the detection of FAM, HEX, ROX and CY-5-labeled probes
- DNA extraction method (see: Preparation of Samples > DNA extraction)
- Pipettes suitable for volumes of 5 - 25 µl
- Nuclease-free disposable filter-tips for volumes of 5 - 25 µl
- Disposable powder-free gloves
- Centrifuge for 8-strip PCR tubes (150 - 1000 x g)
- Real-time PCR instrument adapter for 8-strip tubes (optional)
- Scissors (optional)

1.6 Preparation of Samples

Sample material

Avian samples – Tracheal, oropharyngeal, choanal or cloacal swabs, tissue samples, and FTA cards.

DNA extraction

Before running the PCR, DNA must be extracted from the sample.

Heat boil method, lysis buffer extraction methods, spin column extraction methods, or magnetic bead extraction methods (e.g. **vet**proof® MagBead Extraction Kit I (Product no. KIT230342)) can be used for DNA extraction. Extracted DNA can be stored at -20°C prior to running the PCR.

It is recommended to validate the chosen extraction method and the MgMs qPCR combined, internally, prior to generating results.

2 How to Use This Product

2.1 Good Laboratory Practices for PCR

- Assays must be performed by qualified laboratory personnel only.
- Wear disposable powder-free gloves at any stage of running the assay and/or sample preparation. Change gloves when changing work areas or if you suspect that they are contaminated.
- Treat all biological materials as potentially biohazardous, including all field samples.
- Avoid prolonged exposure of the lyophilized reaction mix to direct light and moisture.
- Keep the kit components separate from other reagents in the laboratory.
- Use nuclease-free lab ware (e.g., pipettes, pipette tips, reaction vials).
- To avoid cross-contamination of samples and reagents, use aerosol-preventive pipette tips.
- Physically separate the work areas for DNA/RNA extraction, PCR setup (work area 2) and PCR amplification (work area 3) to minimize the risk of carry-over contamination. Use a PCR hood for all pipetting steps. As the kit is provided as ready-to-use lyophilized mix, a dedicated work area for reagent set up (work area 1) is not required.
- Never move any materials and equipment from work area 3 to work area 2 or from work area 2 or 3 to work area 1.
- Strict adherence to the test protocol will lead to achieving the best results.
- Decontaminate PCR laboratories with bleach or an alternative nucleic acid decontaminant and UV light (optional) after testing.

2.2 Procedure

Real-time PCR protocol
Program the PCR instrument before preparing the PCR samples. For details on how to program the protocol, see the instrument operator's manual of your real-time PCR cycler.

Temperature	Time	No. of Cycles
95°C	3 min	1
95°C	15 sec	40
60°C	60 sec	
Data collection (@ 60°C): FAM = Mycoplasma gallisepticum HEX = Mycoplasma synoviae ROX = Internal Control CY-5 = Endogenous Control		

Note: A pre-incubation step of one cycle at 48°C for 10 minutes can be added prior to the first step of the above PCR protocol to run this assay simultaneously with other BioChek RNA assays.

Note: For some real-time PCR instruments, the type of probe quencher as well as passive reference dye has to be specified. The **vet**proof® Mycoplasma Gallisepticum-Synoviae qPCR LyoKit contains probes with a non-fluorescent ("dark") quencher and no passive reference dye.

Note: Alternative channel names for reporter dyes:

- FAM: no alternative name
- HEX: VIC
- ROX: Red 610, TXR
- CY-5: Quasar 670

Preparation of the PCR reactions
Proceed as described below to prepare a 25 µl standard reaction.
Always wear gloves when handling PCR strips or caps. Sample material should be suitable for PCR concerning purity, concentration, and presence of inhibiting substances.

1. Take the needed number of PCR tube strips (number of samples plus minimum one positive and one negative control) out of the aluminum bag. Use scissors to cut the required amount of reaction tubes. Put unused tubes back in the provided aluminum bag. Tightly seal the bag and make sure the silica gel is included.

2. Place the 8-strip PCR tubes containing the lyophilized reagents in a suitable PCR tube rack. Check that the reagent pellets are at the bottom of the tubes. If not, briefly centrifuge or tap the pellets to the bottom before proceeding.

3. Carefully uncap the tube strips and discard the clear cap strips.
   Note: Do not leave strips open for extended periods of time. To avoid unwanted liquid absorption, open strips just before filling.

4. Pipette samples, positive and negative control into the PCR tube. Carefully resuspend the pellets by pipetting up and down (3 - 5 times).
   a. Depending on the extraction method used, up to 25 µl of sample can be added to the lyophilized reaction mix.
      i. For purified extracts, add 25 µl of the sample extract to the lyophilized extraction mix.
      ii. For non-purified extracts, first add 20 µl H_2O PCR-grade (component [3], vial with clear cap) to the lyophilized mix and then 5 µl of sample extract (total reaction volume of 25 µl).
   b. For the negative control, add 25 µl of H_2O PCR-grade (component [3], vial with clear cap).
   c. For the positive control, add 25 µl of Control Template (component [2], vial with purple cap).
   Note: Depending on the nucleic acid extraction procedure and after internal verification/ validation, up to 25 µl sample DNA may be added to the lyophilized reaction mix.
   Note: Include a positive and negative control in every run.

5. Seal the tubes tightly with new cap strips (component [4]).

6. Briefly (5 seconds) spin the PCR strips in a suitable centrifuge at 150 - 1000 x g. Do not exceed 1000 x g.

7. Put the samples in the real-time PCR cycler and start the program (see Procedure > Real-time PCR protocol).
   Note: The PCR strips should be placed evenly distributed into the cycler block (e.g., in case of 2 strips, place strips in columns 1 and 12). For some PCR instruments, a suitable adapter may be required to hold the 8-strip PCR tubes.

2.3 Validation and Interpretation

Analysis settings

After the run has finished, create a new analysis in your real-time PCR instrument software with the below settings:

Bio-Rad CFX96™	Applied Biosystems® 7500 Fast	Mx3005P®	AriaMx®
Fluorescence drift correction: Yes	Passive reference: No	Background based threshold Cycle range: 3 - 10 Sigma multiplier: 10	Background based threshold Cycle range: 3 - 10 Sigma multiplier: 10
Cycles to analyze: 5 - 40	Auto baseline	Adaptive baseline	
	Or alternative: Baseline cycles: 6 -10	Gain factors: CY-5 x1, ROX x1, HEX/JOE x4, FAM x4	

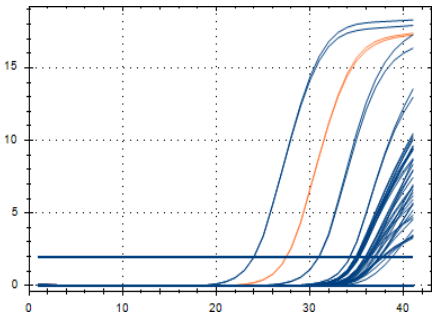
QuantStudio™ 5	LightCycler® 96	LightCycler® 480
Auto baseline	Abs quant settings:	Abs quant/Fit points
Or alternative: Baseline cycles: 6 - 10	Minimal Slope: 0.01	First cycle: 5 Last cycle: 40
	Minimal EPF: 0.100	Background: 6 - 10

Note: Other PCR machines can be used. Contact BioChek for further information regarding the use of the kit on other qPCR thermocyclers.

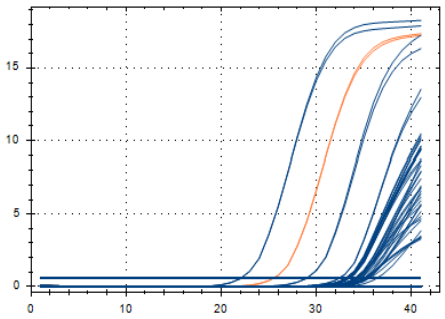
Setting thresholds in the PCR cycler software

This section is only applicable to PCR software analysis using a threshold-based determination of Cq values. Some instruments use a regression-based analysis (e.g. LightCycler® 96), which does not require manual user settings.

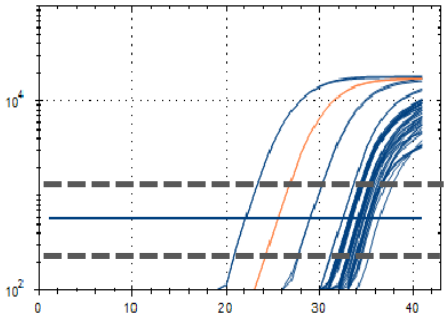
In the analysis section of your PCR instrument software, open the graphical display of the amplification curves. Select all sample-containing wells on the plate, select linear view and select the FAM channel. Turn off other channels.



Picture 1



Picture 2



Picture 3

Depending on the number of positive samples, the linear curves should look like the ones in Picture 1. To set the threshold, identify the positive control reaction (highlighted in orange). Look at which cycle the curve transitions into a straight line, in this case around cycle 27. The threshold is first placed at the point where the curves become a straight line, see Picture 1. The threshold should then be set halfway between this point and the baseline, see Picture 2.

In case highly precise Cq values are required, the threshold setting can be refined in an optional step. Switch to logarithmic view while keeping the threshold applied. Determine the part of the curve chart where the increase of all curves appears linear, see dashed lines in Picture 3. Move the threshold towards the center of this area.

Repeat this process for the HEX, ROX and CY-5 channel.

Note: After setting the thresholds for all channels, keep using those thresholds for all future PCR runs.

Validation of the PCR run

The following must apply for the PCR run to be valid:

	Mg (FAM) Cq values	Ms (HEX) Cq values	IC (ROX) Cq values	EnC (CY-5) Cq values	Interpretation
Positive Control (Control Template)	23 - 29	23 - 29	<40	23 - 29	Valid Control
Negative Control (H_2O PCR-grade)	N/A*	N/A*	27 - 34	>33	Valid Control

**No Cq value*

Note: Repeat the PCR plate in the event of control failure.

Validation and interpretation of sample results

The amplification of *Mycoplasma gallisepticum* DNA is analyzed in the FAM channel, *Mycoplasma synoviae* in the HEX channel, the Internal Control is analyzed in the ROX channel, and the Endogenous Control in the CY-5 detection channel.
To verify true negative results and exclude PCR inhibition as cause of a negative signal in the FAM and HEX channel, check for amplification of the Internal Control in the ROX detection channel. An avian matrix check and successful DNA extraction and amplification must be observed in the CY-5 channel to confirm the presence of an avian matrix.

Note: Always check validity of the amplification curves.

Mg (FAM) Cq values	Ms (HEX) Cq values	IC (ROX) Cq values	EnC (CY-5) Cq values	Interpretation
<40	<40	Not considered	Not considered	Positive for Mg and Ms
<40	N/A*	Not considered	Not considered	Positive for Mg
N/A*	<40	Not considered	Not considered	Positive for Ms
N/A*	N/A*	Not considered	<33	Negative for Mg and Ms, Positive for Avian DNA
N/A*	N/A*	Within acceptance range**	>33	Negative Sample for Mg, Ms and Avian DNA
N/A*	N/A*	Out of acceptance range**	>33	Invalid well***

**No Cq value*

***The general acceptance threshold of a negative sample is the Cq value of the IC of the Negative Control (H_2O PCR-grade) ± 2 Cq (e.g. If the Negative Control of a run yields a Cq of 30 for the IC, only samples with an IC between 28 - 32 (acceptance range) are valid.*

****The assay is invalid for this particular sample and should be repeated with a 1/5 dilution of the extract or a new DNA extract.*

Note: For sample results with a Cq between 38 and 40 (in FAM and/or HEX channel), it is recommended to check the amplification curve. If the curve is good, report the sample as positive. Otherwise repeat the sample.

3 Supplementary Information

Symbols Glossary

REF	Product Reference Number		Expiry (Expiration) Date
	Kit Size/Reactions		Protect from Moisture
	Store at		Protect from Heat and Direct Sunlight
	Batch		Manufacturer

Quality Control

All products are monitored by our quality control on a batch-to-batch basis. A certificate of analysis (CoA) is available from BioChek.

Ordering Information

This kit and associated products are available from BioChek. For a complete overview and for more information, please visit our website at www.biochek.com.

Trademarks

vetproof®, and LyoKit® are trademarks of Hygiena Diagnostics GmbH.

Warranty and Disclaimer of Liability

"Limited Warranty" and "Disclaimer of Liability": BioChek B.V. warrants that this product is free from defects in materials and workmanship through the expiration date printed on the label and only if the following are complied with:

1. The product is used according to the guidelines and instructions set forth in the product literature;
2. BioChek B.V. does not warrant its product against any and all defects when: the defect is a result of material or workmanship not provided by BioChek B.V.; defects caused by misuse or use contrary to the instructions supplied, or improper storage or handling of the product;
3. All warranties of merchantability and fitness for a particular purpose, written, oral, expressed or implied, shall extend until the expiry date of the product. There are no other warranties that extend beyond those described on the face of this warranty;
4. BioChek B.V. does not undertake responsibility to any purchaser of its product for any undertaking, representation or warranty made by any dealers or distributors selling its products beyond those herein expressly expressed unless expressed in writing by an officer of BioChek B.V.;
5. BioChek B.V. does not assume responsibility for incidental or consequential damages, including, but not limited to responsibility for loss of use of this product, removal or replacement labor, loss of time, inconvenience, expense for telephone calls, shipping expenses, loss or damage to property or loss of revenue, personal injuries or wrongful death;
6. BioChek B.V. reserves the right to replace or allow credit for any modules returned under this warranty.

Regulatory Disclaimers

For veterinary in vitro use only.

Regulatory requirements vary by country; this product may not be available in your geographic area. For information on availability, please contact BioChek.

4 Revision Index

Revision A: New document.

5 Supplier Information

AUTHORIZED REPRESENTATIVE

BioChek B.V.
Fokkerstraat 14
2811ER Reeuwijk - Netherlands
Phone +31 (0)182 582 592
Website: www.biochek.com
E-mail: info@biochek.com

Hygiena Diagnostics GmbH
Hermannswerder 17
14473 Potsdam - Germany

