KYLT®

SAN® VET

KYLT® *MYCOPLASMA IOWAE*

REAL-TIME PCR DETECTION

ℹ For in vitro Veterinary Diagnostics only.

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Passion for Innovation

DIRECTION FOR USE

qPCR.MI.02, Rev004, November 2024

KYLT®
MYCOPLASMA IOWAE

Real-Time PCR Detection

1. GENERAL

Characteristic	Description
Article name	Kylt® MI LD 100, Kylt® MI LD 25
Name of pathogen	Mycoplasma iowae
Pathogen also known as	MI
Caused disease	Mycoplasmoses
Organism	Bacteria
Target molecule	DNA
Technology	Real-Time PCR
Internal Control	Internal Amplification Control (IAC; exogenous)
Host-group	Poultry (Chicken, Turkey)
Sample types	▪ tissues and organs (e.g. lung, trachea) ▪ swab samples (e.g. nasal, cloacal, choanal, tracheal, lung, conjunctiva, air sacs) ▪ isolates derived from cultural processes
Target HEX channel (550 nm)	Internal Amplification Control (IAC)
Target FAM channel (520 nm)	Mycoplasma iowae
Temperature profile	Kylt® Profile I or Kylt® Profile II

These kits were developed for use by trained laboratory personnel following standardized procedures. This Direction For Use must be followed strictly.

2. REAL-TIME PCR

During the Real-Time PCR the target genes are amplified by respective primer pairs in the Polymerase Chain Reaction (PCR). Amplified target sequences are detected via fluorescently labeled probes during the PCR reaction in real-time (Real-Time PCR). Their emitted fluorescence is separately optically measured by the Real-Time PCR thermal cycler. The pathogen-specific status of a sample can be evaluated by considering all amplified targets per sample and the negative and positive controls per run.

3. KIT CONTENTS

Reagent	Lid color	100 Reactions Article no. 31026	25 Reactions Article no. 31027	Storage temperature
2x qPCR-MIX	Transparent	4× 280 µl	1× 280 µl	<= -18°C
Primer-Probe-Mix	Orange	4× Lyoph. a final 150 µl	1× Lyoph. a final 150 µl	<= -18°C
Positive Control	Red	4× Lyoph. a final 50 µl	2× Lyoph. a final 50 µl	<= -18°C
Negative Control	Blue	1× 1 ml	1× 1 ml	<= -18°C

4. STORAGE REQUIREMENTS

- If necessary, prepare aliquots of the reagents to limit freeze-thaw cycles to 3.
- The components are to be used within the indicated shelf life (see box label).
- Components from different batches may not be mixed or interchanged.
- The **Primer-Probe-Mix** needs to be stored and handled protected from light.

5. REAGENT PREPARATION

- Before its first use, rehydrate each vial of **Positive Control**: add 50 µl of Negative Control per vial, briefly incubate at room temperature and mix thoroughly by repeated vortexing.
- Before first use, rehydrate the lyophilized **Primer-Probe-Mix**: add 150 µl of the Negative Control per vial, briefly incubate at room temperature and mix by pulse-vortexing.

6. NECESSARY EQUIPMENT, DEVICES AND CONSUMABLES

- Real-time PCR cycler capable of detecting the appropriate fluorescence wavelength. (Note that the default normalization option against ROX (e.g. using ABI cyclers) must be disabled).
- Purification Kit yielding sufficiently high concentration of inhibtor-free DNA/RNA (e.g. Kylt RNA / DNA Purification products)."
- Table top microcentrifuge.
- Vortex mixer.
- Adjustable Micropipettes covering the appropriate volume range.
- Certified Nuclease-free (PCR-clean) consumables.
- Compatible PCR-plates strips or single tubes.
- Matching PCR-clean pipette tips with filters.
- Powder-free gloves to be worn during the entire setup and changed in case of contamination.

7. CONTROL REACTIONS

- Each PCR run must include a **Positive Control** to monitor the specificity and efficiency of the reagents and the reaction itself, including the performance of the Real-Time PCR and of the Real-Time PCR thermal cycler.
- Each PCR run must include a **Negative Control** to ensure the absence of contaminations.
- The **Internal Amplification Control** is included in the Reaction-Mix. It is co-amplified with every single reaction to detect possible inhibitory effects of the DNA preparation on the Real-Time PCR itself and thus to verify true-negative results.
- If appropriate sampling is unsure, we recommend to analyze the samples in parallel with **Kylt Host Cells** Real-Time RT-PCR Detection for presence of amplifiable nucleic acids derived from host cell material.

8. REACTION SETUP

- Before each use, briefly vortex and spin down the used reagents.
- The total number of reactions is the number of samples plus one Positive Control and one Negative Control per run. In case of combination this setup with a Kylt Standard, please add appropriate numbers of reactions.
- Prepare the Master-Mix using the components listed below.
- Vortex, spin down and add 16 µl of the finalized Master-Mix to each of the PCR tubes or plate wells ("cavities").

	Volume (µl)	
Reagent	**per Reaction**	**e.g. n= 7**
2x qPCR-Mix	10 µl	70 µl
Primer-Probe-Mix	6 µl	42 µl
Total Volume Master-Mix	**16 µl**	**112 µl (dispense 16 µl per reaction)**
DNA (Negative Control / sample DNA / Positive Control)	4µl	
Total Volume per Reaction	**20 µl**	

- Keep exposure of the 2x RT-qPCR-Mix, Primer-Probe-Mix and prepared Master-Mix to (sun)light as short as possible and return it back to appropriate storage temperature right after application.
- Avoid the formation of bubbles when pipetting the mix, samples and controls.
- Add 4 µl of the **Negative Control** to the corresponding cavity and seal it individually, if possible.
- Add 4 µl of each **sample** to the corresponding cavities and seal them individually, if possible.
- Add 4 µl of the **Positive Control** to the corresponding cavity.
- Once all reactions are set up, seal the cavities and briefly spin down.
- Place the cavities in the Real-Time PCR thermal cycler and run the test with either Kylt Profile II or Kylt Profile I.
- With Kylt Profile II this and most other Kylt qPCR detection methods can be carried out simultaneously in a single PCR run.
- Kylt Profile I allows for combined run of this and most other Kylt RT-qPCR detection methods as well as Kylt PCR detection products.
- In the event of a combined Real-Time (RT-)PCR run, make sure all necessary channels are detected.
- Please follow the specified instructions of your Real-Time PCR thermal cycler as recommended by the manufacturer.

Kylt® Profil II

Step No	Description	Temperature	Duration	
1	Activation of Polymerase	95 °C	10 min	
2	Denaturation	95 °C	15 sec	42 cycles
3	Annealing & Extension	60 °C	1 min	
4	Fluorescence Detection	channels / wave lenghts see page 2		

Kylt® Profil I

Step No	Description	Temperature	Duration	
1	Reverse Transcription	50 °C	10 min	
2	Activation of Polymerase	95 °C	1 min	
3	Denaturation	95 °C	10 sec	42 cycles
4	Annealing & Extension	60 °C	1 min	
5	Fluorescence Detection	channels / wave lenghts see page 2		

9. GENERAL PCR-RUN EVALUATION AND QUALITY CONTROL

- An ideal positive PCR curve starts with a linear phase which then increases exponentially and tapers off to a plateau phase.
- The baseline is the mean value of a large section of the beginning of the experiment which is subtracted from all fluorescence values to show only the net increase of fluorescence per well and channel.
- Automated evaluation of the respective Cycler Software may be used. Please take care to identify possible artifacts.
- The threshold, if adjusted manually, should be set close enough to the baseline to include all curves that show a clear exponential phase but to exclude all unspecific fluorescence increases.
- The intersection between the curve and the threshold is the Ct-value. The lower the Ct-value, the higher the concentration of the target molecule in the sample at the beginning of the test.

10. TEST EVALUATION - CONTROL REACTIONS

- The Real-Time PCR run is only valid if the curves of the control reactions can be evaluated as follows:

Control Reactions	Channel			
	HEX		FAM	
	Internal Amplification Control (IAC)		Pathogen-Specific Channel	
Negative Control	**positiv**	**Ct ≤ 40**	negative	Ct > 42
Positive Control	**positiv**	**Ct ≤ 40**	**positive**	**Ct > 15 and ≤ 35**

11. TEST EVALUATION - SAMPLES

Target	Channel	Signal		
Interal Amplification Control (IAC)	HEX	**positive**	positive / negative	negative
Mycoplasma iowae	FAM	negative	**positive**	negative
The sample is ***Mycoplasma iowae***		negative	**positive**	inhibited

- The **Interal Amplification Control (IAC)** is positive: Ct-value ≤ 40.
- The **pathogen-specific channel** is positive: Ct-value ≤ 42.
- **Recommendation:** In the case of an inhibited sample the test may be repeated with a dilution of the DNA preparation at e.g. 1:10. The Negative Control is used as the diluting agent. Preferably, the entire DNA preparation process is repeated using Kylt RNA/DNA Purification products or appropriate alternative.
- Convenient and reliable sample data entry, Real-Time PCR start, final qualitative analysis and documentation can be conducted with the Kylt Software, please inquire.

12. ORDERING INFORMATION

For a fast and efficient service please send your order to orders.kylt-de@san-group.com and please provide the following information:

- Delivery address
- Invoice address
- Purchaser contact telephone number
- End user name and telephone number (if different)
- Purchase order number
- Product name and cataloge number
- Quantity and size of products
- Indicate if your account is VAT exempt

13. REVISION HISTORY

Revision	Status	Amendments
Rev003	June 2019	Layout
Rev004	November 2024	New Layout/Design, changed kit content (2x qPCR-Mix and Primer-Probe-Mix instead of Reaction-Mix), changed Ct-value in HEX-channel for negative result

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The development, manufacturing and distribution of Kylt® In-Vitro Diagnostics is certified to ISO 9001:2015.

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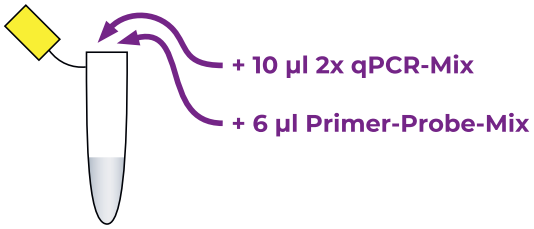
PROTOCOL AT A GLANCE
REAL-TIME PCR SETUP

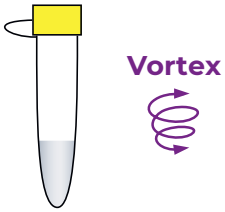
1

Prepare a Master-Mix



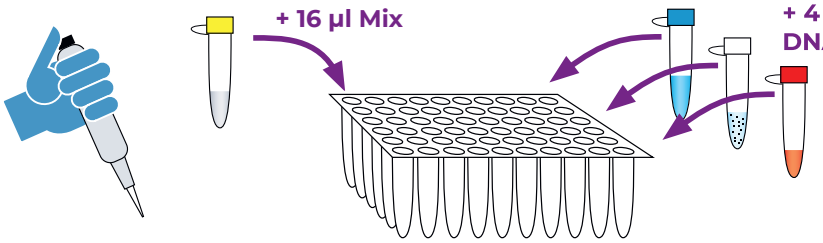
2

Pulse-vortex and spin down



3

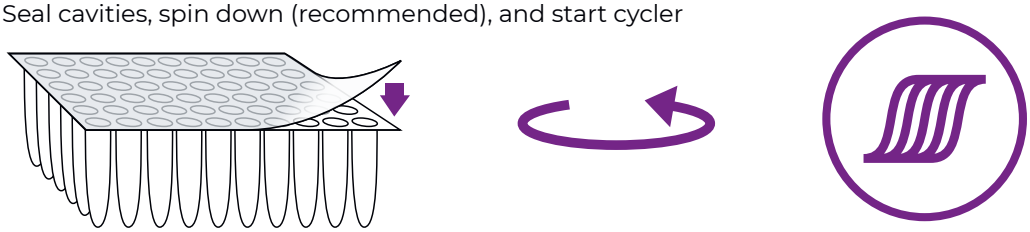
Dispense Master-Mix and add 4 µl NC, sample DNA/RNA, PC



NC = Negative Control
PC = Positive Control

4

Seal cavities, spin down (recommended), and start cycler



5

Analysis