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Kylt®

Kylt® Standard for Mycoplasma hyopneumoniae

Quantitative Standard for Real-Time PCR Detection

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A. General

- Kylt® Standard for Mycoplasma hyopneumoniae is used for quantification of *Mycoplasma hyopneumoniae* positive samples by Real-Time PCR.
- Kylt® Standard for Mycoplasma hyopneumoniae comprises a set of reference controls solely for the purpose of quantification of bacterial DNA of *Mycoplasma hyopneumoniae*. These controls are to be combined with the Kylt® MHP Real-Time PCR Detection products, for further information please refer to the respective Direction For Use.
- For a quantitative application the sample of interest is analyzed in a PCR run including the set of seven Quantitative Standards; this set consist of a dilution series with defined Genome Equivalents (GE)/µl. The generated standard curve is then used to determine the concentration of *Mycoplasma hyopneumoniae* in the sample (GE/ml or g).
- This kit was developed for use by trained laboratory personnel following standardized procedures. This Direction For Use must be followed strictly.

B. Reagents and Materials

- Kylt® Standard for Mycoplasma hyopneumoniae contains the following reagents:

Reagent	Concentration (GE = Genome Equivalent)	Quantity in Kit (10 assays)	Reconstitution	Store at
Quantitative Standard 1	1x 10^5 GE/µl	each Quantitative Standard 1 x lyophilisate (final 60 µl)	add 60 µl of Negative Control	≤ -18 °C
Quantitative Standard 2	1x 10^4 GE/µl			
Quantitative Standard 3	1x 10^3 GE/µl			
Quantitative Standard 4	1x 10^2 GE/µl			
Quantitative Standard 5	1x 10^1 GE/µl			
Negative Control	-	1 x 1 ml	-	≤ -18 °C

- The Quantitative Standards are stored at ≤ -18 °C. Avoid repeated freezing and thawing of the components.
- Before the first use the lyophilized Quantitative Standards are rehydrated: 60 µl of the Negative Control (Nuclease-free water) are added per vial of Quantitative Standard, briefly incubated at room temperature and mixed thoroughly by repeated vortexing. Make sure that the pellet is completely solved; insufficient rehydration and/or mixing will lead to an invalid standard curve derived from the Quantitative Standards (see also chapter C.3 "Data Analysis").
- This Quantitative Standard is to be used together with Kylt® MHP Real-Time PCR Detection products and can be used on all commercially available Real-Time PCR thermal cyclers that detect the emitted fluorescence of the fluorescent dyes FAM (emission 520 nm) and HEX (emission 550 nm).
- Apart from the disposables, the following further devices are needed:
  - Kylt® MHP Real-Time PCR Detection
  - Table top microcentrifuge
  - Vortex
  - Micropipettes covering volumes of 1 µl to 1000 µl
  - Centrifuge for PCR tubes or plates
  - Real-Time PCR thermal cycler
- Accessory Kylt® products: see chapter D "Related and Accessory Products".
- We recommend the exclusive use of certified Nuclease-free disposables as well as powder-free protective glove. Please wear gloves during the entire experimental procedure. Gloves need to be changed frequently, especially after spillage or suspected contaminations.

C. Protocol

- The overall protocol for Quantification of *Mycoplasma hyopneumoniae* consists of the following steps:

1. Sample Processing and DNA Preparation
2. Reaction Setup and Amplification (Real-Time PCR)
3. Data Analysis - Validity and Quantitative Result

1. Sample Processing and DNA Preparation

- Please take respective dilution factors into consideration when calculating the concentration.
- For quantitative applications pooling of samples is not recommended.
- Ground samples may be processed with appropriate DNA preparation kits, such as Kylt® RNA/DNA Purification (please refer to chapter B. "Reagents and Materials") or appropriate in-house methods.
- For detailed information on the DNA preparation process, please refer to the Direction For Use or Standard Operating Procedure of the respective DNA preparation kit or in-house method, respectively, and to the Direction For Use of Kylt® MHP Real-Time PCR Detection Kit.

2. Reaction Setup and Amplification (Real-Time PCR)

- Follow the Direction For Use of the Kylt® MHP Real-Time PCR Detection kit and add the reactions for all seven Quantitative Standards for *Mycoplasma hyopneumoniae* to the assay.
- To minimize risk of potential cross-contaminations, 4 µl of each Quantitative Standard are added to the corresponding cavity after all previous samples and control reactions are set up. Before each use, briefly vortex and spin down the rehydrated Quantitative Standards (see also chapter B "Reagents and Materials").
- Avoid formation of bubbles when pipetting Reaction-Mix, samples, Controls and Standards. It is recommended to always centrifuge cavities before the PCR run.
- Place the cavities in the Real-Time PCR thermal cycler and amplify using the following parameters:

Kylt® Profile I				
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 FAM and HEX		

- Please follow the specified instructions of your Real-Time PCR thermal cycler as recommended by the manufacturer.

4. Data Analysis – Validity and Quantitative Result

General

- The amplification data can be processed automatically using the specific software tool of your Real-Time PCR thermal cycler. Alternatively, the threshold can be set manually considering the following directions: The threshold should cross the FAM-curve and the HEX-curve in the linear increase of their slope (log scaling of the y-axis). By setting the threshold, the crossing points with the HEX- and FAM-curves determine the respective cycle threshold (Ct), which is negatively correlated with the initial concentration of copies of the target genes in the Real-Time RT-PCR reaction.
- Only curves with the typical exponential amplification, meaning the curve of the raw data shows a flat baseline at the beginning, followed by a clear (exponential) slope in fluorescence and possibly reaching a plateau-phase (y-axis in log scaling), should be regarded as positive.
- If available, use specific quantitation tool in the software of your Real-Time PCR thermal cycler and define concentration of applied Quantitation Standard 1 to 5 as given in tabella in chapter B "Reagents and Materials". By this, regression analysis, i.e. quantitation of GE/ µl of sample, can automatically be conducted.

Test Evaluation

- Based on the PCR-derived data the concentration of *Mycoplasma hyopneumoniae* in the initial sample material can be calculated with the following formula:
  - GE/ml = PCR quantification (GE/µl) * [EV(µl)/SV(µl)] * 1000

    GE = genome equivalents
    EV = elution volume in µl
    SV = sample volume used for DNA preparation in µl
- Please take potential dilution factors during sample preparation into consideration.

D. Related and Accessory Products

Product	Article No	Reactions	Description
Kylt® MHP	31378 / 31379	100 / 25	Real-Time PCR detection of *Mycoplasma hyopneumoniae*
Kylt® RNA / DNA Purification	31314 / 31315	250 / 50	Combined RNA and DNA purification from veterinary samples

Production:

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Development, manufacturing and distribution of Kylt® *In-Vitro* Diagnostica is certified according to ISO 9001:2015.

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