



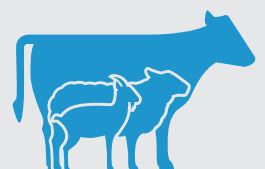
**V** For *in vitro* Veterinary  
Diagnostics only.

# Kylt<sup>®</sup>

## Kylt<sup>®</sup> *Mannheimia haemolytica*

### Real-Time PCR Detection

[www.kylt.eu](http://www.kylt.eu)



## Kylt® *Mannheimia haemolytica*

### Real-Time PCR Detection

#### A. General

- Kylt® *Mannheimia haemolytica* kits are intended for the specific detection of bacterial DNA of *Mannheimia haemolytica*. The kits are suitable for the analysis of samples from ruminants, such as swab samples (e.g. nasal, lungs, pleura), tissues and organs (e.g. trachea, lungs, pleura), BAL (bronchoalveolar) fluid samples, milk samples and pure or mixed colony material / isolates derived from cultural processes of the aforementioned samples.
- The qualitative testing with Kylt® *Mannheimia haemolytica* kits is based on a duplex Real-Time PCR: In one reaction setting, the target genes for *Mannheimia haemolytica* as well as for the endogenous control (beta-Actin) are amplified in parallel by respective primer pairs in the Polymerase Chain Reaction (PCR). Amplified target gene fragments are detected via fluorescently labeled probes during the PCR reaction in real-time (Real-Time PCR). The probes specific for detection of amplified *Mannheimia haemolytica* and the endogenous control target genes are labeled with fluorescent dyes FAM and HEX, respectively, and their emitted fluorescence is separately optically measured by the Real-Time PCR thermal cycler. By means of both individual analyses in one reaction vessel per sample and the Negative Control and Positive Control per run the *Mannheimia haemolytica*-specific status of a sample can be evaluated in the end. This way, results can be achieved within a few hours after sample receipt.
- These kits were developed for use by trained laboratory personnel following standardized procedures. This Direction For Use must be followed strictly.

## B. Reagents and Materials

- The following Kylt® *Mannheimia haemolytica* kits are available and comprise the following reagents:

| Reagent          | Colour of Lid | 100 Reactions                          | 25 Reactions                           | Store at |
|------------------|---------------|--|--|----------|
|                  |               | Article No 31445                       | Article No 31446                       |          |
| Reaction-Mix     | ● brown       | 4 x 450 µl                             | 1 x 450 µl                             | ≤ -18 °C |
| Positive Control | ● red         | 4 x lyophilizate<br>(final 50 µl each) | 2 x lyophilizate<br>(final 50 µl each) | ≤ -18 °C |
| Negative Control | ● blue        | 1 x 1 ml                               | 1 x 1 ml                               | ≤ -18 °C |

- After receipt, the components are immediately stored at ≤ -18 °C. Avoid repeated freezing and thawing of all the reagents and keep them thawed as short as possible. If occasional processing of few samples only is expected you may prepare appropriate aliquots of reagents before storage at ≤ -18 °C. Prepare aliquots in such a way that freeze-thaw-cycles are reduced to a maximum of three. The Negative Control can alternatively be stored at +2°C to +8°C.
- The components are to be used within the indicated shelf life (see box label). The components of different batches may not be mixed.
- Before its first use, rehydrate the Positive Control: add 50 µl of Negative Control per vial, briefly incubate at room temperature and mix thoroughly by repeated vortexing. It is recommended to generate aliquots of suitable volumes and store them at ≤ -18 °C.
- The Reaction-Mix needs to be stored protected from abundant light. Do not expose to direct (sun)light.

## C. Equipment and Reagents not included

- This detection method can be used on all commercially available Real-Time PCR thermal cyclers that detect the emitted fluorescence of the fluorescent dyes FAM and HEX (emission 520 and 550 nm, respectively). Note that default normalization option against ROX (e.g. using ABI cyclers) must be deactivated.
- Apart from the disposables, the following further devices are needed and are not included in the Kylt® *Mannheimia haemolytica* kits:
  - DNA preparation kit / protocol (e.g. Kylt® DNA Extraction-Mix II or Kylt® RNA / DNA Purification products)
  - Table top microcentrifuge
  - Vortex
  - Micropipettes covering volumes of 1 µl to 1000 µl
  - Centrifuge for PCR tubes or plates
- Accessory Kylt® products: see chapter F “Related and Accessory Products”.
- We recommend the exclusive use of certified Nuclease-free disposables as well as powder-free protective gloves. Please wear gloves during the entire experimental procedure. Gloves need to be changed frequently, especially after spillage or suspected contaminations.

## D. Control Reactions

- The Positive Control allows for control of 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.
- The Negative Control allows for exclusion of contaminations. The sample testing is only valid if both, Positive and Negative Controls, are used and verified for validity in every Real-Time PCR run.
- The Internal Control is based on detection of beta-Actin DNA, which is ubiquitous in the cells of the host that the sample is derived from. The beta-Actin DNA target gene is co-amplified (channel HEX) with every single reaction and allows for evaluation of sufficient sampling, sample storage and shipment, sample preparation and the Real-Time PCR run itself.

## E. Protocol *(see also „Protocol At A Glance“ at the end of this Direction For Use)*

- The overall protocol of the analysis consists of the following main workflow:
  1. Sample Preparation
  2. DNA Preparation
  3. Reaction Setup and Amplification (Real-Time PCR)
  4. Data Analysis – Validity and Qualitative Result
- We recommend proceeding through the protocol without interruption to avoid potential degradation of the processed samples and reagents. If necessary, you may store the final DNA preparation at  $\leq -18$  °C until further processing. Avoid repeated freezing and thawing of the DNA preparations.

### 1. Sample Preparation

- We recommend pooling of at most five samples or samples from five individuals, respectively, per DNA preparation.
- Pool swabs in a sufficient volume of sterile buffer (e.g. 1 ml of Normal Saline or 0.1 x TE), let the swabs soak for an adequate period of time and finally wash out the swabs by thorough pulse-vortexing.
- The supernatant is used for DNA preparation.
- For Kylt® DNA Extraction the supernatant is (fully) transferred to a conical screw cap tube (please refer to 2 "DNA Preparation").
- Small swabs may directly be immersed in Kylt® DNA Extraction-Mix II or lysis buffer, if applicable.
- Tissue and organ samples are homogenized thoroughly in sterile buffer (see above) and a suitable volume is used for the DNA preparation.
- Material derived from cultural processes, i.e. colony material, is directly transferred into respective tubes for Kylt® DNA Extraction (please refer to 2 "DNA Preparation"), such as conical screw cap tube; therefore a little amount of a single colony is picked with a sterile loop wire or sterile pipette tip and transferred to the tube.
- Material derived from cultural processes, i.e. cell culture supernatant or allantoic fluid, can be used directly for DNA preparation.

## 2. DNA Preparation

### a) Kylt® DNA Extraction (requires Kylt® DNA Extraction-Mix II)

- For detailed information, please refer to the Direction For Use of Kylt® DNA Extraction-Mix II.

### b) Kylt® RNA/DNA Purification products

- All kinds of sample matrices, including pure isolates, swabs, tissues, organs, sputum, feces and environmental samples may be processed with Kylt® RNA/DNA Purification products (please refer to chapter F “Related Products”).
- For detailed information on the DNA preparation process, please refer to the respective Direction For Use.

### c) Alternative methods

- All kinds of sample matrices, including pure isolates, swabs, tissues, organs, sputum, feces and environmental samples may be processed with appropriate DNA preparation kits 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 specific kit or in-house method, respectively.

## 3. Reaction Setup and Amplification (Real-Time PCR)

- Before each use, briefly vortex and spin down the Reaction-Mix and Negative Control.
- To determine the total number of reactions needed, count the number of samples and add **two** more for the Negative Control and the Positive Control.
- The Reaction-Mix is ready-to-use, add 16 µl to each of the PCR tubes or plate wells (“cavities”).
- Keep exposure of the Reaction-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 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 DNA preparation to the corresponding cavities and seal them individually, if possible.
- To minimize risk of potential cross-contaminations, 4 µl of the Positive Control 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 Positive Control (see also chapter B “Reagents and Materials”).
- If not already done, finally seal the cavities. It is recommended to briefly spin them down before the start of the Real-Time PCR run.
- Place the cavities in the Real-Time PCR thermal cycler and run the test with Kylt® Profile II as given below.

| Kylt® Profile 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 FAM and HEX |          |             |

- Kylt® Profile II allows for combined run of this and most other Kylt® qPCR detection methods.
- Alternatively, the [Kylt® Profile I](#) given below can be applied. Kylt® Profile I allows for combined run of this and most other Kylt® qPCR detection methods as well as Kylt® RT-qPCR detection products that need Reverse Transcription, such as those for detection of viral RNA.

| Kylt® Profile 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 FAM and HEX |          |             |

- 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.

#### [4. Data Analysis – Validity and Qualitative 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 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.
- The actual test analysis starts with the validity check of the entire Real-Time PCR run. Afterwards, by means of the Internal Control the validity of each sample reaction and its true test result can be verified according to the Ct-value of the Internal Control channel (HEX). Finally, the *Mannheimia haemolytica*-specific status of each sample is analyzed (FAM).

## Test Evaluation

- The **Real-Time PCR test run** is only **valid** if the FAM-curve and HEX-curve of the Negative Control are negative ( $Ct > 35$ ) and the FAM-curve and HEX-curve of the Positive Control are positive. For a valid test the FAM-Ct-value and HEX-Ct-value of the Positive Control have to be  $> 15$  and  $\leq 35$ .

| Target   | Channel | Signal          |                     |                  |
|--|---------|-----------------|---------------------|------------------|
| Internal Control                                   | HEX     | positive        | positive / negative | negative         |
| <i>Mannheimia haemolytica</i>                      | FAM     | negative        | positive            | negative         |
| <b>The sample is <i>Mannheimia haemolytica</i></b> |         | <b>negative</b> | <b>positive</b>     | <b>inhibited</b> |

- A **sample** is **negative for *Mannheimia haemolytica***, if its HEX-curve is positive ( $Ct \leq 35$ ), but its FAM-curve is negative.
- A **sample** is **positive for *Mannheimia haemolytica***, if its FAM-curve is positive ( $Ct \leq 42$ ), independent of the HEX-curve.
- A **sample** is **inhibited** if neither the FAM-curve nor the HEX-curve are positive.
- **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 (9 volumes Negative Control + 1 volume DNA Extract or eluted DNA). 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.

## F. Related and Accessory Products

| Product                          | Article No | Reactions | Description  |
|----------------------------------|------------|-----------|--|
| Kylt® DNA Extraction-Mix II      | 31398      | 100       | Simplified and economic DNA extraction   |
| Kylt® RNA / DNA Purification     | 31315      | 50        | Combined RNA and DNA purification from veterinary samples.   |
| Kylt® RNA / DNA Purification HTP | 31826      | 4 x 96    | Combined, magnetic beads-based purification of RNA and DNA from veterinary samples, suitable for automated high throughput processing. |
| Kylt® Purifier                   | 31436      | 1         | Purification system for magnetic beads. Up to 96 samples in under 45 minutes.  |
| Kylt® Purifier Spin Tips         | 31434      | 5         | Plate with 96 separate spin tips, used by the Kylt® Purifier to mix the well contents by stirring. One set used per run.               |
| Kylt® Purifier Plates            | 31435      | 20        | Plates to be used for the several reactions and reagents in a nucleic acid purification kit. 4 - 5 plates used per run.                |

## G. Ordering information

For a fast and efficient service please send your order to [orders@kylt.eu](mailto:orders@kylt.eu) and please provide the following information:

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

Production:

AniCon Labor GmbH | Muehlenstr. 13 | D-49685 Hoeltinghausen | Germany | [www.kylt.eu](http://www.kylt.eu) | [info@kylt.eu](mailto:info@kylt.eu)

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

Kylt® is a registered trademark.

For veterinary use only. For *in vitro* use only. Regulatory requirements vary by country, not all of the products described herein may be available in your geographic area.

© 2022 AniCon Labor GmbH. All rights reserved. The trademark mentioned herein is the property of AniCon Labor GmbH or their respective owners.



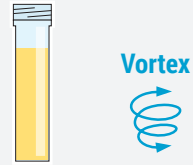


# PROTOCOL AT A GLANCE

## Real-Time PCR Setup

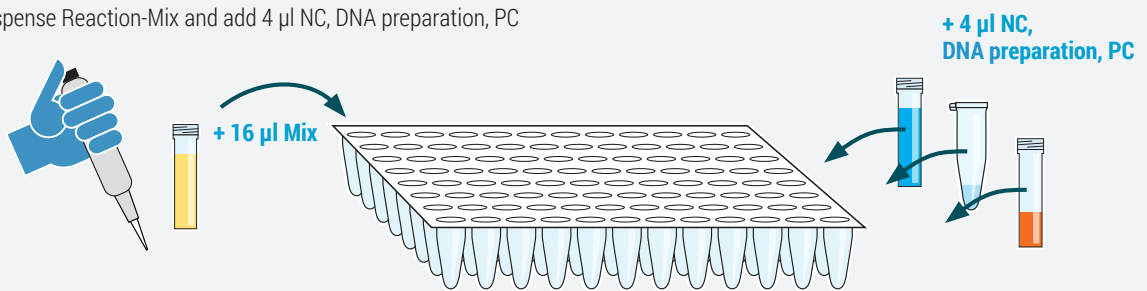
1

Pulse-vortex and spin down



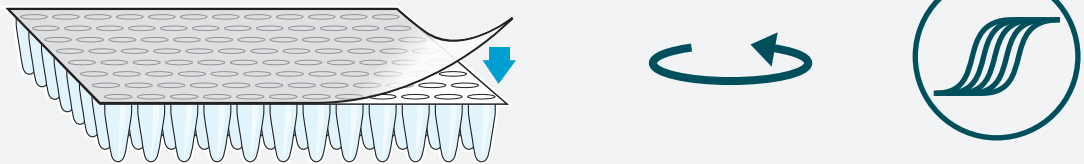
2

Dispense Reaction-Mix and add 4 µl NC, DNA preparation, PC



3

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



4

Analysis

