Kylt®

For feed and food applications only.

Kylt® *Staphylococcus aureus*

Real-Time PCR Detection Kit
for detection of *Staphylococcus aureus*

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100 / 25 reactions
For feed and food applications

A. General

- Kylt® *Staphylococcus aureus* Real-Time PCR Detection Kit is for detection of *Staphylococcus aureus* from feed and food samples as well as from hygienic environmental samples.
- Kylt® *Staphylococcus aureus* comprises all reagents and controls for detection of bacterial DNA of *Staphylococcus aureus*. The qualitative testing of Kylt® *Staphylococcus aureus* is based on a duplex Real-Time PCR system: the target genes specific for *Staphylococcus aureus* as well as for the Internal Amplification Control are amplified in parallel by respective primer pairs in the subsequent Polymerase Chain Reaction (PCR) in one reaction vessel. 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 target genes of amplified *Staphylococcus aureus* and Internal Amplification Control 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 two individual analyses in one reaction vessel per sample and the Negative Control and Positive Control per run the *Staphylococcus aureus*-specific status of a sample can be evaluated in the end. This way, results can be achieved within few hours after sample receipt.
- *Staphylococcus aureus* positive strains can be tested for the presence of the Methicillin resistance gene by using Kylt® MRS (Art. No. 31008).
- 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® *Staphylococcus aureus* contains the following reagents:

Reagent	Colour code of lid	Quantity in kit with 25 / 100 reactions	Storage conditions
DNA Extraction-Mix II	○ white	1 x 7 ml / 1 x 20 ml	+2 °C to +8 °C
Reaction-Mix	○ transparent	1 x / 4 x 500 µl	+2 °C to +8 °C
Positive Control	● red	2 x / 4 x lyophilizate (final 20 µl each)	+2 °C to +8 °C lyophilized -18 °C to -20 °C rehydrated
Negative Control	● blue	1 x 1 ml	+2 °C to +8 °C

- After receipt the components of the kit are immediately stored at the respective temperature listed in in the table above. Avoid repeated freezing and thawing of the kit or its components and keep them thawed as short as possible. If occasional processing of few samples is expected, you may prepare aliquots of the Reaction-Mix before storage at -18 °C to -20 °C. Reaction-Mix has to be stored dark, do not expose to direct sun(light). The kit and its components are to be used within the indicated shelf life (see label on the outer packing), if stored properly. The components of different batches may not be mixed.

- Before its first application rehydrate both lyophilized Positive Control by adding 20 µl of Negative Control per vial, briefly incubate at room temperature and mix by repeated vortexing. Storage of aliquots with 5 µl to 10 µl (depending on the expected number of Positive Control reactions per kit) at -18 °C to -20 °C is recommended.

- This kit 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 nm and 550 nm).

- 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. Pipette tips must be changed between each sample to avoid cross-contaminations.

- Apart from the disposables, the following equipment is needed (not included in this kit):
  - Table top microcentrifuge
  - Dry heating block (+100 °C ± 3 °C)
  - Magnetic stirrer
  - Micropipettes covering volumes of 1 µl to 1000 µl
  - Centrifuge for PCR tubes or plates
  - Real-Time PCR thermal cycler

C. Control Reactions

- The Negative Control included in this kit provides the ability to exclude possible contaminations of the reagents. The Positive Control included in this kit provides the ability to monitor the specificity and efficiency of the reagents used, i.e. the performance of Real-Time PCR itself and of the Real-Time PCR thermal cycler, respectively. The sample testing is only valid if both, the Negative Control and the Positive Control, are included in every Real-Time PCR run and used to verify the validity of such run.

- The Internal Amplification Control is included in the Reaction-Mix in a defined copy number; it is co-amplified (channel HEX) with every single reaction to detect possible inhibitory effects of the DNA preparation and to verify true-negative results.

D. Protocol

- The overall protocol of the analysis consists of the following main workflow:
  1. Cultural pre-enrichment
  2. Sample preparation and DNA extraction
  3. reaction setup and amplification (Real-Time PCR)
  4. data analysis – validity and qualitative result

1.Cultural pre-enrichment

- All samples are homogenized with nine times the volume of Mueller-Hinton-Bouillon with 6,5% NaCl in a stomacher bag (e.g. 25 ml or 25 g sample in 225 ml Mueller-Hinton-Bouillon). The pre-enrichment is incubated at 37±1 °C for 16-20 h.

2.Sample preparation and DNA Extraction

- Pre-heat heating block to set temperature of +100 °C, the block must have an actual temperature of +100 °C ± 3 °C at first use.

- Transfer 1,5 ml of the pre-enrichment into a conical screw cap tube and centrifuge the sample at 10.000 g to 12.000 g for 5 min.

- Remove the supernatant using a 1000 µl pipette tip (not by decantation) and discard it.

- The DNA Extraction-Mix II is stirred on a magnet stirrer, it must be used as a homogenous suspension. The pellet is resuspended by repeated up-and-down pipetting with 200 µl of DNA Extraction-Mix II. Avoid formation of bubbles and aerosols.

- Screw cap tight, vortex thoroughly and incubate for 10 min to 15 min at +100 °C ± 3 °C.

- Vortex sample thoroughly and centrifuge at 10.000 g to 12.000 g for 5 min; the supernatant in the DNA extract and can be used for Real-Time PCR immediately. Short-term storage (few hours) of the DNA extract at +2 °C to + 8°C is possible. For long-term storage of the DNA extract at -18 °C to -20 °C, take supernatant and transfer to new (screw cap) tube. Before next use in Real-Time PCR incubate DNA extracts that were stored at -18 °C to -20 °C for few minutes at +100 °C ± 3 °C, vortex and spin down.

- For sample preparation and DNA extraction of samples known to lead to PCR-inhibition use a commercially available DNA extraction kit (e.g. Kylt® RNA/DNA Purification) suitable for the sample matrix to be analyzed. Please carry out the DNA preparation according to the manufacturer's instructions of the DNA extraction kit.

3. Reaction Setup, Reverse Transcription and Amplification

- Before each use, briefly vortex and spin down the Reaction-Mix and Negative Control.
- Determine the number of needed PCR-reactions: number of samples + Positive Control and Negative Control.
- Pipette 18 µl of Reaction-Mix to each PCR reaction tube / each PCR plate well (»cavity«). Keep exposure of Reaction-Mix to light as short as possible!
- Add 2 µl of Negative Control to corresponding cavity and seal.
- Add 2 µl of the prepared DNA (DNA extract or eluted DNA) of sample to corresponding cavity and seal. Solely use clear supernatant of DNA extract, avoid transferring debris.
- Once all sample cavities are sealed, 2 µl of the Positive Control is added to the corresponding cavity and sealed. The Positive Control is vortexed and spun down before each use.
- Avoid formation of bubbles when pipetting Reaction-Mix, samples and controls. It is recommended to consistently spin down cavities before the PCR run.
- Place the cavities in the Real-Time PCR thermal cycler and run the test using the following parameters:

Step	Temperature	Duration	
Activation of Polymerase	95 °C	10 min	
Denaturation	95 °C	15 sec	42 cycles
Annealing & Extension	60 °C	1 min	
Fluorescence Detection	channels FAM and HEX		

- Please follow the specified instructions of your Real-Time PCR thermal cycler as recommended by the manufacturer.
- The amplification parameters above allow for combination of this Kylt® *Staphylococcus aureus* Real-Time PCR with other Kylt® Real-Time PCRs, e.g. for detection of the Methicilin resistance gene. In the event of a combined Real-Time PCR run, make sure all necessary channels are used!

4.Data Analysis – Validity and Qualitative Result

General

- The data of the amplification reactions can automatically be processed using specific software for the Real-Time PCR thermal cycler. Alternatively a threshold can be set manually, with following considerations: The threshold should be at least a ten-fold standard deviation above the average fluorescence value of cycles 3 to at least 15. The threshold should cross the HEX-curve of the Negative Control and the FAM-curve of the Positive Control, respectively, in the linear area of its slope. By setting the thresholds, 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 logarithmic scaling), should be considered as positive.

- The actual test analysis starts with the validity check of the entire Real-Time PCR run. Afterwards, by means of the Internal Amplification 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 *Staphylococcus aureus*-specific status of each sample is analyzed (channels FAM).

Test Evaluation

- The **Real-Time PCR test run** is only **valid** if the FAM-curve of the Negative Control is negative (Ct > 42), if the HEX-curve of the Negative Control is positive and if the FAM-curve of the Positive Control is positive (Ct ≤ 35).

HEX-curve is positive	yes	yes / no	no
FAM-curve is positive	no	yes	no
The sample is *Staphylococcus aureus*	**negative**	**positive**	**inhibited**

- A **sample** is **negative for *Staphylococcus aureus*** if its FAM-curve is negative and the HEX-curve is positive.
- A **sample** is **positive for *Staphylococcus aureus*** if its FAM-curve is positive, independent of the HEX-curve.
- A **sample** is **inhibited** if neither the FAM-curve nor the HEX-curve is positive.
- **Recommendation**: In the case of an inhibited sample the test should be repeated with a dilution of the DNA preparation at 1:10 (9 volumes Negative Control + 1 volume eluted DNA). The Negative Control serves as the diluting agent. Alternatively, the original sample or the eluted DNA can be utilized for repeated DNA preparation using appropriate alternative systems, such as the Kylt® RNA/DNA Purification Kit.
- Convenient and reliable sample data input, start of the Real-Time PCR run as well as final qualitative test result analysis and documentation can be managed by the Kylt® Software package. Please feel free to contact us.

Production:

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