



For Feed and Food
Testing Applications only.

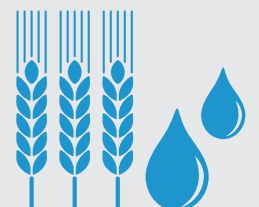


Kylt[®]

Kylt[®] Staphylococcus aureus

Real-Time PCR Detection

www.kylt.eu



Kylt® *Staphylococcus aureus*

Real-Time PCR Detection

Revision No.	Amendments
002	valid from 01 August 2023: exclusion of Kylt® DNA Extraction-Mix II and Lysis beads, changed storage temperatures, new layout, changed lid colour (Reaction-Mix).

A. General

- Kylt® *Staphylococcus aureus* kits are intended for the detection of bacterial DNA of *Staphylococcus aureus*. The kits are suitable for the analysis of feed and food samples as well as hygienic environmental samples.
- The qualitative testing with Kylt® *Staphylococcus aureus* kits is based on a duplex Real-Time PCR: In one reaction setting, the target genes for *Staphylococcus aureus* as well as for the exogenous control (Internal Amplification Control (IAC)) 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 *Staphylococcus aureus* and the exogenous 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 *Staphylococcus aureus*-specific status of a sample can be evaluated in the end. This way, results can be achieved within a 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. 31007/31008).
- 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® Staphylococcus aureus kits are available and comprise the following reagents:

Reagent	Colour of Lid	100 Reactions Article No 31009	25 Reactions Article No 31010	Store at
Reaction-Mix	● orange	4 x 500 µl	1 x 500 µl	≤ -18 °C
Positive Control	● red	4 x lyophilizate (final 20 µl each)	2x lyophilizate (final 20 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 20 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® Staphylococcus aureus kits:
 - DNA preparation kit / protocol (e.g. Kylt® DNA Extractionmix III)
 - 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 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 on the Real-Time PCR itself and thus to verify true-negative results.

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. Cultural Pre-Enrichment
 2. DNA Extraction
 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 ≤ -18 °C until further processing. Avoid repeated freezing and thawing of the DNA preparations.

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. DNA Extraction

a) Kylt® DNA Extraction (requires Kylt® DNA Extractionmix III)

- For detailed information, please refer to the Direction For Use of Kylt® DNA Extractionmix III.

b) Alternative methods

- 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 three more for the Negative Control and the Positive Control.
- The Reaction-Mix is ready-to-use, add 18 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 2 µl of the Negative Control to the corresponding cavity and seal it individually, if possible.
- Add 2 µl of each DNA preparation to the corresponding cavities and seal them individually, if possible. Solely use clear supernatant of DNA extract, avoid transferring debris.
- To minimize risk of potential cross-contaminations, 2 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 Controls (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 KyIt® Profile II as given below.

KyIt® 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		

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

KyIt® 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 *Staphylococcus aureus*-specific status of each sample is analyzed (FAM).

Test Evaluation - Control Reactions

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

Control Reactions	Channel	
	HEX	FAM
Negative Control	positive	negative
Positive Control	positive	positive

- The HEX-Ct-values of the Negative and Positive Control have to be ≤ 40 .
- The FAM-Ct-value of the Positive Control has to be > 15 and ≤ 35 .

Test Evaluation - Samples

Target	Channel	Signal		
Internal Control	HEX	positive	positive / negative	negative
<i>Staphylococcus aureus</i>	FAM	negative	positive	negative
The sample is <i>Staphylococcus aureus</i>		negative	positive	inhibited

- A **sample is negative for *Staphylococcus aureus***, if its HEX-curve is positive ($Ct \leq 40$), but its FAM-curve is negative.
- A **sample is positive for *Staphylococcus aureus***, 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 III	31404	100	Simplified and economic DNA extraction from pure bacterial isolates or enrichments from gram-positive bacteria (e.g. <i>Listeria monocytogenes</i>).
Kylt® RNA / DNA Purification	31315	50	Combined RNA and DNA purification from veterinary samples (spin-column based).
Kylt® RNA / DNA Purification HTP	31826	4 x 96	Magnetic bead based combined RNA and DNA purification kit for veterinary diagnostic samples. Suitable for Kylt® Purifier and Kylt® Purifier 48.
Kylt® Purifier	31436	1 unit	Purification system for magnetic bead based kits. Up to 96 samples are processed in under 30 minutes. Intended for high-throughput laboratories.
Kylt® Purifier 48	31748	1 unit	Purification system for magnetic bead based kits. Up to 48 samples are processed in under 30 minutes. Intended for low to medium throughput laboratories.
Kylt® Purifier Spin Tips	31434	5 Sets	Plate with 96 separate spin tips, used by the Kylt® Purifier to mix the well contents by stirring. Sufficient for 480 samples.
Kylt® Purifier Plates	31435	20 Plates	Plates to be used for the several reactions and reagents during automated nucleic acid purification. Sufficient for 320 to 480 samples (depending on device and protocol) .

G. 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 catalogue number
- Quantity and size of products
- Indicate if your account is VAT exempt

Production:

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is certified according to ISO 9001:2015.

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For feed and food applications 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.

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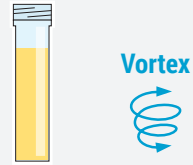


PROTOCOL AT A GLANCE

Real-Time PCR Setup

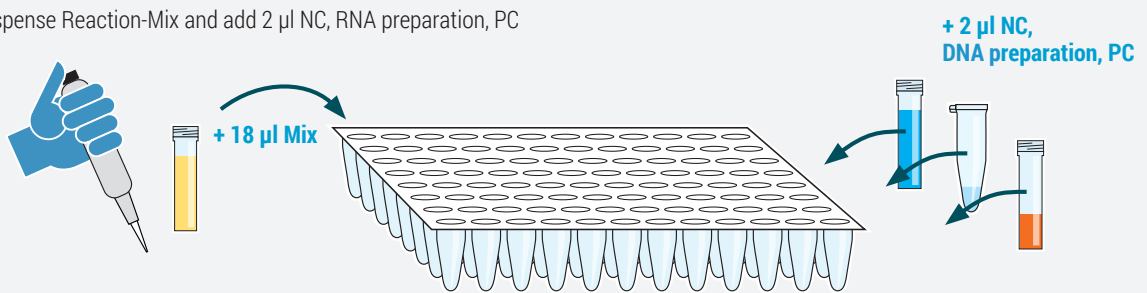
1

Pulse-vortex and spin down



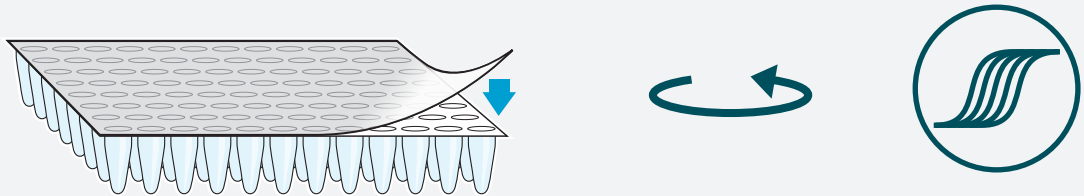
2

Dispense Reaction-Mix and add 2 µl NC, RNA preparation, PC



3

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



4

Analysis

