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Diagnostics only.

# Kynt<sup>®</sup>

## Kynt<sup>®</sup> Streptococcus suis epf, mrp, sly

### Real-Time PCR Detection

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



## Kylt® *Streptococcus suis* epf, mrp, sly




### Real-Time PCR Detection

#### A. General

- Kylt® *Streptococcus suis* epf, mrp, sly kits are intended for the specific detection of bacterial DNA of *Streptococcus suis* and the simultaneous detection of the virulence factors “extracellular protein factor – epf”, “muramidase released protein– mrp” and “sullysin – sly”. The kits are suitable for the analysis of samples from swine, such as swab samples (e.g. from tonsils, brain), tissues and organs and pure or mixed colony material / isolates derived from cultural processes of the aforementioned samples.
- The qualitative testing with Kylt® *Streptococcus suis* epf, mrp, sly kits is based on a multiplex Real-Time PCR: In one reaction setting, the target genes for sly, epf and mrp as well as for a *Streptococcus suis* house keeping gene (Internal Control (IC)) 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 genes of sly, epf and mrp and the IC target genes are labeled with fluorescent dyes Cy5, FAM, TXR and HEX, respectively, and their emitted fluorescence is separately optically measured by the Real-Time PCR thermal cycler. By means of the individual analyses in one reaction vessel per sample and the Negative Control and Positive Control per run the sly-, epf- and mrp-specific status of a sample can be evaluated in the end. This way, results can be achieved within a few hours after sample receipt.
- It is recommended to initially screen samples with Kylt® *Streptococcus suis* Real-Time PCR Detection to rule out negative samples and to assess potential PCR-inhibitors in the sample (see chapter F “Related and Accessory Products”).
- 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® Streptococcus suis epf, mrp, sly kits are available and comprise the following reagents:

Reagent	Colour of Lid	100 Reactions	25 Reactions	Store at
		Article No 31541	Article No 31542	
Reaction-Mix	 blue-white	4 x 450 µl	1 x 450 µl	≤ -18 °C
Positive Control	 red	4 x lyophilizate (final 50 µl each)	2 x lyophilizate (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 Cy5, FAM, TXR and HEX (emission 670, 520, 620 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® Streptococcus suis epf, mrp, sly kits:
  - DNA preparation kit / protocol (e.g. 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 a *Streptococcus suis* house keeping gene (channel HEX) to detect possible inhibitory effects of the DNA preparation and 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. 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.
- 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® RNA/DNA Purification products

- All kinds of sample matrices, including swabs, tissues and organs 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.

### b) Alternative methods

- All kinds of sample matrices, including swabs, tissues and organs 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 Cy5, FAM, TXR 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 Cy5, FAM, TXR 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 Cy5-, FAM-, TXR- and HEX-curve in the linear increase of their slope (log scaling of the y-axis). By setting the threshold, the crossing points with the Cy5-, FAM-, TXR- and HEX-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 sly-, epf- and mrp-specific status of each sample is analyzed (Cy5, FAM and TXR).

##### 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	Cy5	FAM	TXR
Negative Control	negative	negative	negative	negative
Positive Control	<b>positive</b>	<b>positive</b>	<b>positive</b>	<b>positive</b>

- For a valid test the Ct-values (Cy5, FAM and TXR) of the Positive Control have to be > 15 and ≤ 35 and the HEX-Ct-value of the Positive Control has to be ≤ 40.
- *Streptococcus suis* virulence factors can only be analyzed in *Streptococcus suis* positive samples or isolates.

## Test Evaluation - Samples

Target	Channel	Signal				
Internal Control / <i>Streptococcus suis</i>	HEX	<b>positive</b>	positive / negative	positive / negative	positive / negative	negative
Suilysin (sly)	Cy5	negative	<b>positive</b>	negative	negative	negative
Extracellular protein factor (epf)	FAM	negative	negative	<b>positive</b>	negative	negative
Muramidase released protein (mrp)	TXR	negative	negative	negative	<b>positive</b>	negative
<b>The sample is <i>Streptococcus suis</i></b>		<b>positive</b>	<b>positive / negative</b>	<b>positive / negative</b>	<b>positive / negative</b>	<b>inhibited / <i>Streptococcus suis</i> not detectable *</b>
<b>The sample is suilysin (sly)</b>		<b>negative</b>	<b>positive</b>	<b>negative</b>	<b>negative</b>	
<b>The sample is extracellular protein factor (epf)</b>		<b>negative</b>	<b>negative</b>	<b>positive</b>	<b>negative</b>	
<b>The sample is muramidase released protein (mrp)</b>		<b>negative</b>	<b>negative</b>	<b>negative</b>	<b>positive</b>	

\* Inhibited results may also occur if samples which are not *Streptococcus suis* positive are analyzed.

- A **sample** is **negative for the virulence factors sly, epf and mrp**, but **positive for *Streptococcus suis*** if its HEX-curve is positive (Ct ≤ 40), but its Cy5-, FAM- and TXR-curve are negative.
- A **sample** is **positive for suilysin (sly)**, if its Cy5- and HEX-curve is positive (Ct ≤ 42), independent of the FAM- and TXR-curve.
- A **sample** is **positive for extracellular protein factor (epf)**, if its FAM- and HEX-curve is positive (Ct ≤ 42), independent of the Cy5- and TXR-curve.
- A **sample** is **positive for muramidase released protein (mrp)**, if its TXR- and HEX-curve is positive (Ct ≤ 42), independent of the Cy5- and FAM-curve.
- A **sample** is **inhibited** if neither the HEX-curve nor the Cy5-, FAM- and TXR-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.
- Alternatively, an inhibited sample may also indicate that there is no *Streptococcus suis* present in the sample to be analyzed for virulence factors. In this case it is recommended to analyze the sample for the presence of *Streptococcus suis* using Kylt® *Streptococcus suis* (Art. 31380/31381). Only *Streptococcus suis* positive samples can be analyzed for virulence factors using Kylt® *Streptococcus suis* epf, mrp, sly.
- 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	Content / Reactions	Description
Kylt® Streptococcus suis	31380 / 31381	100 / 25	Real-Time PCR detection of <i>Streptococcus suis</i> .
Kylt® Streptococcus suis 1	31382 / 31383	100 / 25	Real-Time PCR detection of <i>Streptococcus suis</i> Serotype 1.
Kylt® Streptococcus suis 2	31384 / 31385	100 / 25	Real-Time PCR detection of <i>Streptococcus suis</i> Serotype 2.
Kylt® Streptococcus suis 7	31386 / 31387	100 / 25	Real-Time PCR detection of <i>Streptococcus suis</i> Serotype 7.
Kylt® Streptococcus suis 9	31388 / 31389	100 / 25	Real-Time PCR detection of <i>Streptococcus suis</i> Serotype 9.
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	31436	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.eu](mailto:orders@kylt.eu) 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:

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.

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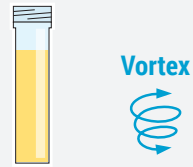


# PROTOCOL AT A GLANCE

## Real-Time PCR Setup

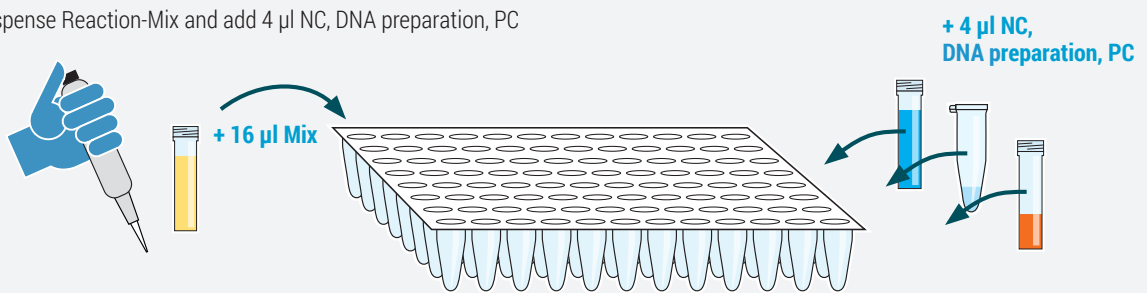
1

Pulse-vortex and spin down



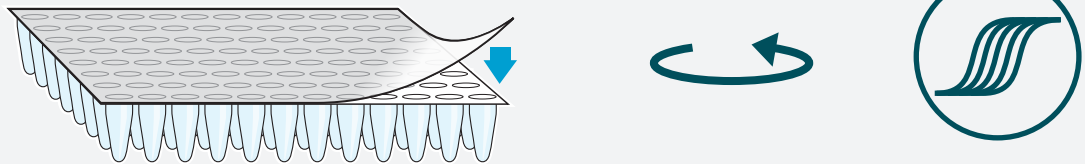
2

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



3

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



4

Analysis

