For *in vitro* Veterinary Diagnostics only.

Kylt®

Kylt® Salmonella Virchow

Real-Time PCR Detection

www.kylt.eu

Kylt® Salmonella Virchow

Real-Time PCR Detection

A. General

- Kylt® Salmonella Virchow kits are intended for the specific detection of bacterial DNA of *Salmonella* enterica subsp. enterica serovar Virchow. The serovar-specific detection is based on the detection of the genes responsible for O and H antigen biosynthesis, more specifically for group O:7 (C1), H-phase 1(r) and H-phase 2 (1.2).
- The kits are suitable for the analysis of pre-enrichment samples positively tested for *Salmonella* spp. (Ct value < 35) as well as *Salmonella* spp. positive colony material from cultural processes (e.g. DIN EN ISO 6579). For conduction of the protocol described in this manual, it is obligatory to combine the test kit with the Kylt® Salmonella spp. 2.0 Real-Time PCR Detection kit (Art. No. 31302).
- The qualitative testing with Kylt® Salmonella Virchow kits is based on a triplex Real-Time PCR: In one reaction setting, the target genes responsible for antigen biosynthesis of group O:7 (C1), H-phase 1 (r) as well as for H-phase 2 (1.2) 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 the amplified target genes are labeled with fluorescent dyes FAM, Cy5 and TXR, respectively, and their emitted fluorescence is separately optically measured by the Real-Time PCR thermal cycler. By means of all individual analyses in one reaction vessel per sample and the Negative Control and Positive Control per run the *Salmonella* Virchow-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® Salmonella Virchow kits are available and comprise the following reagents:

Reagent	Colour of Lid	100 Reactions Article No 31523	25 Reactions Article No 31524	Store at
2x qPCR-Mix	○ transparent	4 x 280 µl	1 x 280 µl	≤ -18 °C
Primer-Probe-Mix	green	4 x lyophilizate (final 150 µl each)	1 x lyophilizate (final 150 µl each)	≤ -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 Primer-Probe-Mix needs to be stored protected from abundant light. Do not expose to direct (sun)light. Before first use, rehydrate the lyophilized Primer-Probe-Mix: add 150 µl of the Negative Control per vial of Primer-Probe-Mix, briefly incubate at room temperature and mix by pulse-vortexing. Generate aliquots of suitable volumes and store them at ≤ -18 °C.

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, Cy5 and TXR (emission 520, 670 and 620 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® Salmonella Virchow kits:
  - 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.

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

- Kylt® Salmonella Virchow should only be used on *Salmonella* spp. positive samples. 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 & 2. Cultural Pre-enrichment & DNA Extraction

- Before using Kylt® Salmonella Virchow screen the samples with regard to the *Salmonella* spp. status, please refer to chapter F "Accessory products" and the according Directions For Use. Only confirmed *Salmonella* spp.-positive samples can be analyzed by Kylt® Salmonella Virchow. Thus, cultural pre-enrichment and DNA extraction is to be done according to the Direction for Use for the *Salmonella* spp.-confirmatory PCR method applied. By this, the DNA extract to be applied for Kylt® Salmonella Virchow is also already proven to be of appropriate quality by the preceeding PCR methods.

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

- Before its first use, rehydrate the Positive Control (add 50 µl of Negative Control) and the Primer-Probe-Mix (add 150 µl of Negtive Control).
- Before each use, briefly vortex and spin down the 2x qPCR-Mix, rehydrated Primer-Probe-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.
- Prepare the Master-Mix using the components listed below. A larger volume of a ready to use Master-Mix can be prepared and stored at ≤ -18 °C for convenient use over a longer period of time up to the expiry date given on the label. In case of frozen storage the Master-Mix should be aliquoted in such a way that freeze-thaw-cycles are reduced to a maximum of three.
- Vortex, spin down and add 16 µl of the finalized Master-Mix to each of the PCR tubes or plate wells ("cavities").

Reagent	Volume (µl)	
	per Reaction	e.g. n=7
2x qPCR-Mix	10 µl	70 µl
Primer-Probe-Mix	6 µl	42 µl
Total Master-Mix	**16 µl**	**112 µl** dispense 16 µl per reaction
DNA (Negative Control / sample DNA / Positive Control)	4.0 µl	
Total Reaction	**20.0 µl**	

- Keep exposure of the 2x qPCR-Mix, Primer-Probe-Mix and prepared Master-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 the Master-Mix, samples and controls.
- Add 4 µl of the Negative Control to the corresponding cavity and seal it 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, Cy5 and TXR		

- 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, Cy5 and TXR		

- 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-, Cy5- and TXR-curves in the linear increase of their slope (log scaling of the y-axis). By setting the threshold, the crossing points with the FAM-, Cy5- and TXR-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. Finally, the *Salmonella* Virchow-specific status of each sample is analyzed (FAM, Cy5 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		
	FAM	Cy5	TXR
Negative Control	negative	negative	negative
Positive Control	**positive**	**positive**	**positive**

- The Ct-values (FAM, Cy5 and TXR) of the Positive Control have to be > 15 and ≤ 35.
- *Salmonella* Virchow can only be analyzed in *Salmonella* spp. positive samples (Ct value < 35) or isolates.

Test Evaluation - Samples

Target	Channel	Signal				
Salmonella group O:7 (C1)	FAM	**positive**	**positive**	negative	negative	negative
Salmonella H-phase 1 (r)	Cy5	**positive**	negative	**positive**	negative	negative
Salmonella H-phase 2 (1.2)	TXR	**positive**	negative	negative	**positive**	negative
The sample is *Salmonella* Virchow		**positive**	**negative**	**negative**	**negative**	**negative**

- A **sample** is **negative for *Salmonella* Virchow,** if none or only one or two channels are positive.
- A **sample** is only **positive for *Salmonella* Virchow,** if all channels are positive (Ct ≤ 42).
- Due to possible cross-reactions in the TXR channel with *Salmonella* H-phase 2 (1.5) and as double positive samples (samples containing more than one *Salmonella* serovar) can not be excluded it is strongly recommended to confirm PCR positive results by cultural, immunological or biochemical methods according to your local regulations.
- 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® Salmonella spp. 2.0	31302	100	Detection of *Salmonella* spp. in veterinary and in food and feed samples.
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® Purifer 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 384 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 cataloge number
- Quantity and size of products
- Indicate if your account is VAT exempt

Production:

SAN Group Biotech Germany GmbH | Muehlenstr. 13 | 49685 Hoeltinghausen | Germany
www.kylt.eu | kylt-de@san-group.com

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

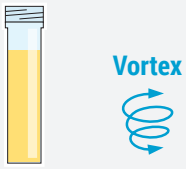
For veterinary use only. For *in vitro* use only. Regulatory requirements vary by country, not all of the products described herein may be available in your geographic area.

qPCR.Salm.Virchow.02, Rev001, September 2023

PROTOCOL AT A GLANCE
Real-Time PCR Setup

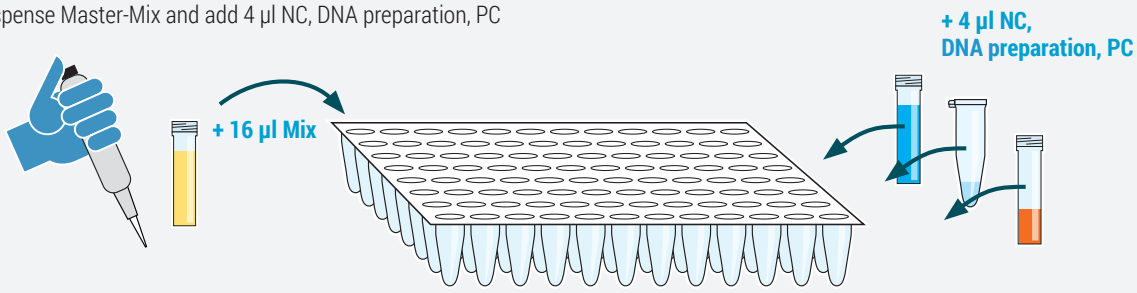
1

Pulse-vortex and spin down



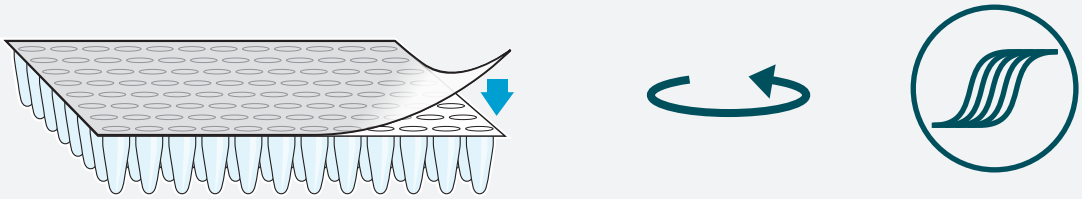
2

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



3

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



4

Analysis