For *in vitro* Veterinary Diagnostics only.

Kylt®

Kylt® ST DIVA 1

Real-Time PCR Detection

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Real-Time PCR Detection for the differentiation of *Salmonella* Typhimurium field strains and vaccine strain *Salmonella* Typhimurium-Mutant (Histidin-Adenin-auxotroph) (present in Zoosaloral H, Zoosaloral R, Zoosal T and Salmoporc from IDT)

Revision No.	Amendments
002	valid from 01 August 2023: exclusion of Kylt® DNA Extraction-Mix II, new layout for test evaluation.

A. General

- Kylt® ST DIVA 1 kits are intended for the specific detection and differentiation of bacterial DNA of *Salmonella* Typhimurium field strains (STf) and vaccine strain *Salmonella* Typhimurium-Mutant (Histidin-Adenin auxotroph) (present in Zoosaloral H, Zoosaloral R, Zoosal T and Salmoporc from IDT) (STvac). The kits are suitable for the analysis of **confirmed ST-positive** samples from birds, swine and ruminants, such as swab samples (e.g. cloacal or rectal), tissues and organs (e.g. gut mucosa, lymph nodes, cecal tonsils), feces, environmental samples (e.g. dust, feathers, sock swabs, swipes) and pure or mixed colony material / isolates derived from cultural processes of the aforementioned samples. Before analysis by Kylt® ST DIVA 1 kits the samples have to be tested positive for *Salmonella* Typhimurium as results are otherwise not valid.

- The qualitative testing with Kylt® ST DIVA 1 kits is based on a triplex Real-Time PCR: In one reaction setting, the target genes for *Salmonella* Typhimurium field strains (STf) and *Salmonella* Typhimurium IDT vaccine strain (STvac) 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 *Salmonella* Typhimurium field strains (STf), *Salmonella* Typhimurium IDT vaccine strain (STvac) and the exogenous control target genes are labeled with fluorescent dyes Texas Red (TXR), Cy5 and HEX, respectively, and their emitted fluorescence is separately optically measured by the Real-Time PCR thermal cycler. By means of three individual analyses in one reaction vessel per sample and the Negative Control and Positive Control per run the ST field- and vaccine strain-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® ST DIVA 1 kits are available and comprise the following reagents:

Reagent	Colour of Lid	100 Reactions Article No 31855	25 Reactions Article No 31856	Store at
Reaction-Mix	green	4 x 450 µl	1 x 450 µl	≤ -18 °C
Positive Control (STf)	red	4 x lyophilizate (final 50 µl each)	2 x lyophilizate (final 50 µl each)	≤ -18 °C
Positive Control (STvac)	red-white	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 Controls STf and STvac: 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 HEX, Cy5 and TXR (emission 550, 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® ST DIVA 1 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 Controls allow 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 & 2. Cultural Pre-enrichment & DNA Extraction

- Before using Kylt® ST DIVA 1 screen the samples with regard to the *Salmonella* Typhimurium (ST) status, please refer to chapter F "Accessory products" and the according Directions For Use. Only confirmed ST-positive samples can be analyzed by Kylt® ST DIVA 1. Thus, cultural pre-enrichment and DNA extraction is to be done according to the Direction for Use for the ST-confirmatory PCR method applied. By this, the DNA extract to be applied for Kylt® ST DIVA 1 is also already proven to be of appropriate quality by the preceeding PCR methods.

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 Controls.
- 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 **each Positive Control (STf and STvac)** are added to the corresponding cavities after all previous samples and control reactions are set up. Before each use, briefly vortex and spin down the rehydrated Positive Controls STf and STvac (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 TXR, Cy5 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 TXR, Cy5 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-curve, the TXR-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-, 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. 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 ST field- and ST vaccine strain-specific status of each sample is analyzed (TXR and Cy5).

Test Evaluation - Control Reactions

- The **Real-Time PCR test run** is only **valid** if the samples were initially analyzed and tested positive for *Salmonella* Typhimurium and the curves of the control reactions can be evaluated as follows:

Control Reactions	Channel		
	FAM	TXR	Cy5
Negative Control	**positive** Ct ≤ 40	negative Ct > 35	negative Ct > 35
Positive Control (STf)	**positive** Ct ≤ 35	**positive** Ct ≤ 35	negative Ct > 35
Positive Control (STvac)	**positive** Ct ≤ 35	negative Ct > 35	**positive** Ct ≤ 35

Test Evaluation - Samples

Target	Channel	Signal			
Internal Control	HEX	positive / negative	positive / negative	positive / negative	positive / negative
STf	TXR	**positive**	**positive**	negative	negative
STvac	Cy5	**positive**	negative	**positive**	negative
The sample is ST field strain		**positive**	**positive**	**negative**	**No further typing possible**
The sample is ST vaccine strain		**positive**	**negative**	**positive**	

- A **sample** is **positive for *Salmonella* Typhimurium field strain** if its TXR-curve is positive (Ct ≤ 42), independent of the HEX-curve.
- A **sample** is **positive for *Salmonella* Typhimurium vaccine strain** if its Cy5-curve is positive (Ct ≤ 42), independent of the HEX-curve.
- A **sample** is **inhibited** if neither the TXR- and Cy5-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® *Salmonella* spp. 2.0	31302	100	Detection of *Salmonella* spp. in veterinary and in food and feed samples.
Kylt® ST FS	31207 / 31208	100 / 25	Kit to detect *Salmonella* Typhimurium
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 (spin-column based).
Kylt® RNA / DNA Purification HTP	31575	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 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 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
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Development, manufacturing and distribution of Kylt® *In-Vitro* Diagnostica is certified according to ISO 9001:2015.

Kylt® is a registered trademark.

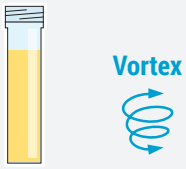
For veterinary use only. For *in vitro* use only. Regulatory requirements vary by country, not all of the products described herein may be available in your geographic area.

qPCR.ST DIVA 1.02, Rev002, August 2023

PROTOCOL AT A GLANCE
Real-Time PCR Setup

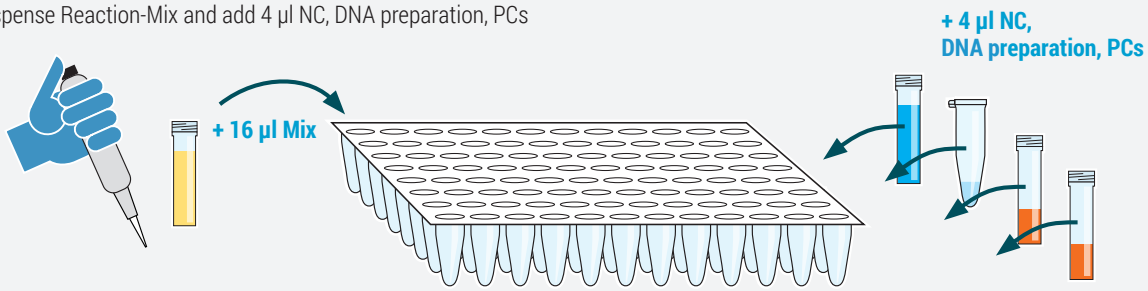
1

Pulse-vortex and spin down



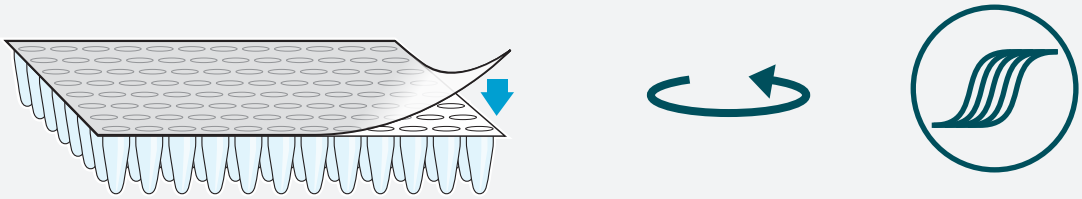
2

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



3

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



4

Analysis