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KYLT[®] SE DIVA 1

REAL-TIME PCR DETECTION

i For in vitro Veterinary Diagnostics only.

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Passion for Innovation



DIRECTION FOR USE

qPCR.SE DIVA 1.02, Rev006, June 2025

KYLT® SE DIVA 1

Real-Time PCR Detection

1. GENERAL

The kit is intended for the differentiation of the following *Salmonella Enteritidis* (SE) live vaccine strains (SEV, SEV1 respectively) from field strains (SEf):

- Name of the strain: 441/014(ade-/his-)
- Available in the commercially vaccines: SE, Salmovac 440, Gallivac SE and Zoosal 440





Characteristic	Description
Article name	Kylt® SE DIVA 1 LD 100, Kylt® SE DIVA 1 LD 25
Organism	Bacteria
Target molecule	DNA
Technology	Real-Time PCR
Host-group	Poultry (Chicken)
Sample prerequisite	<i>Salmonella</i> spp. 2.0
Sample type	<ul style="list-style-type: none">■ pre-enrichment samples positively tested for <i>Salmonella</i> spp. (Ct < 30), <i>Salmonella</i> spp. positive colony material from cultural processes (e.g. DIN EN ISO 6579), In case of samples with a positive result Ct > 30 in <i>Salmonella</i> spp. screening either a second enrichment step should be performed or the analysis should be repeated using colony material from microbiological culture
Target FAM channel (520 nm)	<i>Salmonella Enteritidis</i>
Target Cy5 channel (670 nm)	SE 441/014
Target TXR channel (620 nm)	SE field strain
Temperature profile	Kylt® Profile I or Kylt® Profile II

- These kits were developed for use by trained laboratory personnel following standardized procedures. This Direction For Use must be followed strictly.

2. REAL-TIME PCR

Amplified target sequences are detected via fluorescently labeled probes during the PCR reaction in real-time (Real-Time PCR). Their emitted fluorescence is separately optically measured by the Real-Time PCR thermal cycler. The pathogen-specific status of a sample can be evaluated by considering all amplified targets per sample and the negative and positive controls per run. During the Real-Time PCR the target genes are amplified by respective primer pairs in the Polymerase Chain Reaction (PCR).

3. KIT CONTENTS

Reagent	Lid color	100 Reactions Article no. 31159	25 Reactions Article no. 31160	Storage temperature
Reaction-Mix	 Violet	4× 450 µl	1× 450 µl	<= -18°C
Positive Control SEF	 Red-white	2× Lyoph. a final 50 µl	1× Lyoph. a final 50 µl	<= -18°C
Positive Control SEVI	 Red	2× Lyoph. a final 50 µl	1× Lyoph. a final 50 µl	<= -18°C
Negative Control	 Blue	1× 1 ml	1× 1 ml	<= -18°C

4. STORAGE REQUIREMENTS

- If necessary, prepare aliquots of the reagents to limit freeze-thaw cycles to 3.
- The components are to be used within the indicated shelf life (see box label).
- Components from different batches may not be mixed or interchanged.
- The **Reaction-Mix** needs to be stored and handled protected from light.

5. REAGENT PREPARATION

- Before its first use, rehydrate each vial of **Positive Control**: add 50 µl of Negative Control per vial, briefly incubate at room temperature and mix thoroughly by repeated vortexing.

6. NECESSARY EQUIPMENT, DEVICES AND CONSUMABLES

- Real-time PCR cycler capable of detecting the appropriate fluorescence wavelength. (Note that the default normalization option against ROX (e.g. using ABI cyclers) must be disabled).
- Compatible PCR-plates strips or single tubes.
- Purification Kit yielding sufficiently high concentration of inhibitor-free DNA/RNA (e.g. Kylt RNA / DNA Purification products).
- Table top microcentrifuge.
- Vortex mixer.
- Adjustable Micropipettes covering the appropriate volume range.
- Matching PCR-clean pipette tips with filters.
- Certified Nuclease-free (PCR-clean) consumables.
- Powder-free gloves to be worn during the entire setup and changed in case of contamination.

7. CONTROL REACTIONS

- Each PCR run must include a **Positive Control** to monitor 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.

- Each PCR run must include a **Negative Control** to ensure the absence of contaminations.

8. REACTION SETUP

- Note:** Before using this method screen the samples with regard to the *Salmonella* spp. status, please refer to the according Directions For Use. Only confirmed *Salmonella* spp.-positive samples can be analyzed by this method. Thus, cultural pre-enrichment and DNA extraction is to be done according to the Direction for Use for the *Salmonella* spp.-confirmatory PCR method. By this, the DNA extract to be applied for this method is also already proven to be of appropriate quality.
- Before each use, briefly vortex and spin down the used reagents.
- The total number of reactions is the number of samples plus one Positive Control and one Negative Control per run. In case of combination this setup with a Kylt Standard, please add appropriate numbers of reactions.
- Pipet 16 µl of the ready-to-use **Reaction-Mix** to each of the used well or PCR tube.
- 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 the mix, 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 **sample** to the corresponding cavities and seal them individually, if possible.
- Add 4 µl of each **SEf-Positive Control** to the corresponding cavity. Afterwards, add 4 µl of the **SEV-Positive Control** to the corresponding cavity.
- Once all reactions are set up, seal the cavities and briefly spin down.
- Place the cavities in the Real-Time PCR thermal cycler and run the test with either Kylt Profile II or Kylt Profile I.
- With Kylt Profile II this and most other Kylt qPCR detection methods can be carried out simultaneously in a single PCR run.
- Kylt Profile I allows for combined run of this and most other Kylt RT-qPCR detection methods as well as Kylt qPCR detection products.
- 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.

Kylt® Profile II

Step No	Description	Temperature	Duration
1	Activation of Polymerase	95 °C	10 min
2	Denaturation	95 °C	15 sec
3	Annealing & Extension	60 °C	1 min
4	Fluorescence Detection	channels / wavelengths see page 2	

} 42 cycles

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
4	Annealing & Extension	60 °C	1 min
5	Fluorescence Detection	channels / wavelengths see page 2	

} 42 cycles

9. GENERAL PCR-RUN EVALUATION AND QUALITY CONTROL

- An ideal positive PCR curve starts with a linear phase which then increases exponentially and tapers off to a plateau phase.
- The baseline is the mean value of a large section of the beginning of the experiment which is subtracted from all fluorescence values to show only the net increase of fluorescence per well and channel.
- Automated evaluation of the respective Cycler Software may be used. Please take care to identify possible artifacts.
- The threshold, if adjusted manually, should be set close enough to the baseline to include all curves that show a clear exponential phase but to exclude all unspecific fluorescence increases.
- The intersection between the curve and the threshold is the Ct-value. The lower the Ct-value, the higher the concentration of the target molecule in the sample at the beginning of the test.

10. TEST EVALUATION - CONTROL REACTIONS

- The Real-Time PCR run is only valid if the curves of the control reactions can be evaluated as follows:
- In order to correctly interpret the obtained results it is mandatory to know which vaccine has been applied to the flock.
- Attention: In samples with a positive TXR- and / or Cy5-channel and a negative FAM-channel, respectively, neither the Serovar *Salmonella Enteritidis* nor the SE vaccine strain could be detected. However, another *Salmonella* serovar might be present in the sample.

Control Reactions	Pathogen-Specific Channels					
	FAM		TXR		Cy5	
Negative Control	negative	Ct > 35	negative	Ct > 35	negative	Ct > 35
SEf-Positive Control (non-SE 441/014 PC)	positive	Ct ≤ 35	positive	Ct ≤ 35	negative	Ct > 35
SEVI-Positive Control (SE 441/014 PC)	positive	Ct ≤ 35	negative	Ct > 35	positive	Ct ≤ 35

11. TEST EVALUATION - SAMPLES

Target	Channel	Signal			
<i>Salmonella Enteritidis</i> (SE)	FAM	positive	positive	positive	negative
SE field strain (SEf; non-SE 441/014)*	TXR	negative	positive	negative	positive / negative
SEVI (SE vaccine strain SE 441/014)	Cy5	positive	negative	negative	positive / negative
The sample is <i>Salmonella Enteritidis</i>		positive	positive	positive no further typing possible	negative
The sample is <i>SEf (non-SE 441/014)*</i>		negative	positive	negative	negative
The sample is <i>SE 441/014</i>		positive	negative	negative	negative

* An indication for a SEf strain also includes detection of live vaccine strains other than SE 441/014.

- A **sample** is **negative for SE, SEf and SEV** if its FAM-, Cy5- and TXR-curve are negative.
- A **sample** is **positive for Serovar *Salmonella Enteritidis* (SE)** if its FAM-curve is positive, independent of the Cy5- and TXR-curves. In case the sample is positive in the FAM channel and negative in the Cy5- and TXR-channel, the SE strain present in the sample could not be further differentiated. In this case the analysis should be repeated using colony material from ISO 6579 derived processes.
- A **sample** is **positive for SEV (vaccine strain 441/014)** if its FAM- and Cy5-curves are positive (Ct < 30).

- A **sample** is **positive for SEf** if its FAM- and TXR-curves are positive (Ct < 30).
- **Attention:** This result also includes the detection of live vaccine strains other than SE 441/014-.
- A potential **double infection with SEV and SEf or with SEV and another *Salmonella* serovar** is possible if its FAM-, TXR- and Cy5-curves are positive (CT < 30). Double infections are to be confirmed by cultural and biochemical methods (e.g. ISO 6579 and Kaufmann-White scheme).
- Convenient and reliable sample data entry, Real-Time PCR start, final qualitative analysis and documentation can be conducted with the Kylt Software, please inquire.

12. ORDERING INFORMATION

General terms and conditions of SAN Group Biotech Germany GmbH apply (www.anicon.eu). 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

13. REVISION HISTORY

Revision	Status	Amendments
Rev005	July 2023	valid from 01 August 2023: exclusion of Kylt® DNA Extraction-Mix II, new layout for test evaluation.
Rev006	June 2025	New layout/design; Revision status and content of all available languages aligned to english version, changed TÜV logo

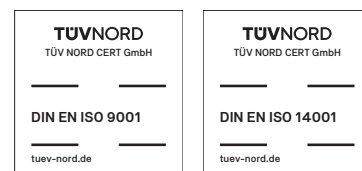
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Development, production and distribution of in vitro diagnostics is certified to ISO 14001:2015 and ISO 9001:2015.

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.

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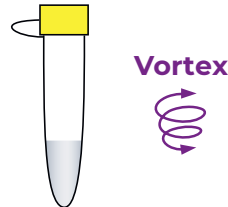




PROTOCOL AT A GLANCE REAL-TIME PCR SETUP

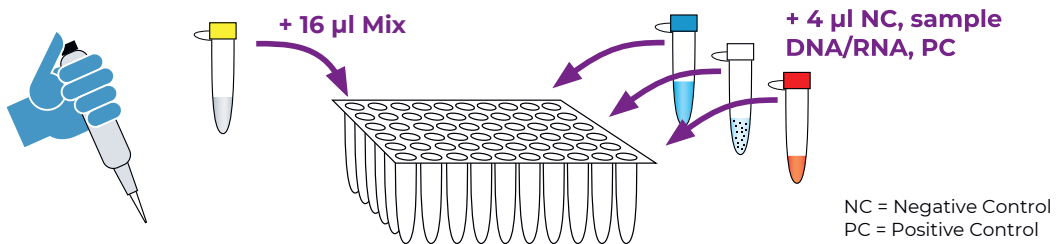
1

Pulse-vortex and spin down



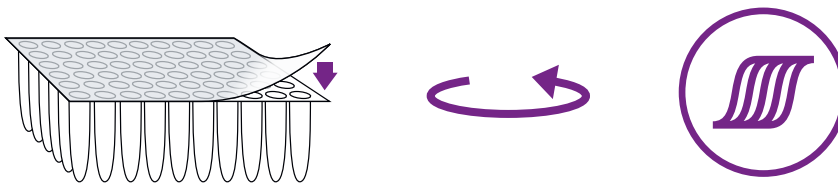
2

Dispense Reaction-Mix (or RTU-Mix) and add 4 μ l NC, sample DNA/RNA, PC



3

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



4

Analysis

