



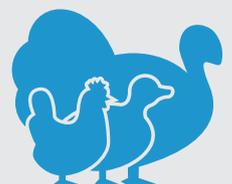
**V** For *in vitro* Veterinary  
Diagnostics only.

# Kylt<sup>®</sup>

## Kylt<sup>®</sup> SE DIVA 2

### DNA-Extraction and Real-Time PCR Detection

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



## Kylt® SE DIVA 2

### DNA-Extraction and Real-Time PCR Detection for differentiation of *Salmonella* Enteritidis vaccine strain Sm24/Rif12/Ssq from field strains

Revision No.	Amendments
009	general revision of layout

#### A. General

- Kylt® SE DIVA 2 DNA Extraction and Real-Time PCR kits are intended for the differentiation of *Salmonella* Enteritidis (SE) live vaccine strain Sm24/Rif12/Ssq (SEV2) from field strains (SEf). The live vaccine strain Sm24/Rif12/Ssq is present in the commercially available vaccine AviPro SALMONELLA VAC E. Kylt® SE DIVA 2 can be used for *Salmonella* spp.-positive pre-enrichment and *Salmonella* spp.-positive material out of cultural processes.
- With limitations the kit can also be used for *Salmonella* spp. positive pre-enrichments in Buffered Peptone Water. In this case only the detection of the vaccine strain is significant, any indication for an SE field strain has to be confirmed by cultural isolation methods e.g. ISO 6579 and Kauffmann-White Agglutination.
- If the kit is to be used for examination of flocks which have been vaccinated with the AviPro SALMONELLA DUO vaccine, the kit can only be used on *Salmonella* spp. positive colony material from cultural processes.
- Kylt® SE DIVA 2 comprises all reagents and controls for DNA extraction, detection and differentiation of bacterial DNA of SE. After bacterial pre-enrichment and DNA extraction the qualitative testing of Kylt® SE DIVA 2 is based on a triplex Real-Time PCR system: the target genes specific for serovar SE, SEf and live vaccine strain Sm24/Rif12/Ssq (SEV2) are amplified in parallel by respective primer pairs in the Polymerase Chain Reaction (PCR) in one reaction vessel. 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 target genes SE, SEf and SEV2 are labeled with fluorescent dyes FAM, TXR and Cy5, 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 Controls per run the SE field strain- or SE vaccine strain Sm24/Rif12/Ssq-specific status of a sample can be evaluated in the end. This way, results can be achieved within few hours after sample receipt.

- Only samples which were positive *Salmonella* spp. with a Ct value <25 in the Kylt® *Salmonella* spp. kit (Art. No. 31019) are suitable for analysis with Kylt® SE DIVA 2. This restriction applies regardless of the origin of the sample (e.g. colony material derived from cultural process or pre-enrichments in Buffered Peptone Water). In case of positive samples with Ct >25 in *Salmonella* spp. screening a second enrichment step should be performed. For detailed information see chapters E. 1 & 2. »Cultural Pre-enrichment & DNA Extraction«.
- This kit was 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® SE DIVA 2 products are available and comprise the following reagents:

Reagent	Colour of Lid	100 Reactions Article No 31161	25 Reactions Article No 31162	Store at
DNA Extraction Mix II	○ white	1 x 20 ml	1 x 7 ml	+2 °C to +8°C
Reaction-Mix	● violet	4 x 450 µl	1 x 450 µl	≤ -18 °C
SEf-Positive Control (SEf PC)	● red	2 x lyophilizate (final 50 µl each)	1 x lyophilizate (final 50 µl each)	≤ -18 °C
SEV2-Positive Control (SE Sm24/Rif12/Ssq PC)	● red	2 x lyophilizate (final 50 µl each)	1 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 except for the DNA-Extraction Mix II 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 DNA-Extraction Mix II is 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 SEf and SEV2: 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 FAM, TXR and Cy5 (emission 520, 620 and 670 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® SE DIVA 2 kits:
  - Incubators for sample pre-enrichment ( $+37^{\circ}\text{C} \pm 1^{\circ}\text{C}$  and  $+41.5^{\circ}\text{C} \pm 1^{\circ}\text{C}$ )
  - Table top microcentrifuge
  - Dry heating block ( $+100^{\circ}\text{C} \pm 3^{\circ}\text{C}$ )
  - Magnetic stirrer
  - Vortex
  - Micropipettes covering volumes of 1  $\mu\text{l}$  to 1000  $\mu\text{l}$
  - 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 included in this kit provide the ability to monitor the specificity and efficiency of the reagents used, i.e. the performance of Real-Time PCR itself and of the Real-Time PCR thermal cycler, respectively. The kit contains separate Positive Controls with SEf- or SE Sm24/Rif12/Ssq-specific DNA standards (channels FAM and TXR positive or channels FAM and Cy5 positive, respectively). The sample testing is only valid if both, the Negative Control and the Positive Controls, are included in every Real-Time PCR run and used to verify the validity of such run.
- 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.
- This kit does not include a primer-probe-setup/ channel for detection of the Internal Amplification Control (IAC). During initially screening of the DNA extracts with Kylt® *Samonella* spp. it is possible to exclude potential inhibition of Real-Time PCR amplification and detection.

## 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  $\leq -18^{\circ}\text{C}$  until further processing. Avoid repeated freezing and thawing of the DNA preparations.

## 1 & 2. Cultural Pre-enrichment & DNA Extraction

- Before using Kylt® SE DIVA 2 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® SE DIVA 2. 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® SE DIVA 2 is also already proven to be of appropriate quality by the preceding 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, once all sample cavities are sealed, 4 µl of the SEf-Positive Control is added to the corresponding cavity and sealed. Afterwards, 4 µl of the SEV2-Positive Control is added to the corresponding cavity and sealed. 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 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, TXR and Cy5		

- 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

- For evaluating the amplification data the threshold has to be set manually, preferably in linear scale of x- and y-axis of the amplification plot. For the amplification plot of the **SEf**-Positive Control reaction a strong increase in fluorescence in the **FAM- and TXR-channel** for SE field strain is expected. For the amplification plot of the **SEV2**-Positive Control reaction a strong increase in fluorescence in the **FAM- and Cy5-channel** is expected.
- The thresholds should cross the FAM-curve, TXR-curve and Cy5-curve in the linear increase of their slope (log scaling of the y-axis). By setting the thresholds, the crossing points with the FAM-, TXR- and Cy5-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. For detailed information about the threshold setting please refer to the manufacturer's Direction For Use of the respective Real-Time PCR thermal cycler.
- 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 logarithmic scaling), should be considered as positive.
- The actual test analysis starts with the validity check of the entire Real-Time PCR run. Finally, the SEf- or SE Sm24/Rif12/Ssq-specific status of each sample is analyzed (channels FAM and TXR or FAM and Cy5, respectively).

## Test Evaluation

- In order to correctly interpret the obtained results it is mandatory to know which vaccine has been applied in the flock.
- The **Real-Time PCR test run** is only **valid** if the following conditions are met:
  - The Negative Control is negative in the FAM-, TXR- and Cy5-channel,
  - The SEf-Positive Control is positive in the FAM- and TXR-channel,
  - The SEV2-Positive Control is positive in the FAM- and Cy5-channel.
- **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 Sm24/Rif12/Ssq could be detected. However, another *Salmonella* serovar might be present in the sample.
- If the conditions mentioned above are met the following results are to be expected:

Target	Channel	Signal			
<i>Salmonella</i> Enteritidis	FAM	positive	positive	positive	negative
SEf*	TXR	positive	negative	negative	positive/negative
SE Sm24/Rif12/Ssq	Cy5	negative	positive	negative	positive/negative
<b>The sample is <i>Salmonella</i> Enteritidis</b>		<b>positive</b>	<b>positive</b>	<b>positive/no further typing possible</b>	<b>negative</b>
<b>The sample is SEf</b>		<b>positive</b>	<b>negative</b>	<b>negative</b>	<b>negative</b>
<b>The sample is SE Sm24/Rif12/Ssq</b>		<b>negative</b>	<b>positive</b>	<b>negative</b>	<b>negative</b>

\* An indication for a SEf strain also includes detection of live vaccine strains which are not Sm24/Rif12/Ssq. In case of direct use of Buffered Peptone Water enrichments only the detection of the vaccine strain Sm24/Rif12/Ssq is a valid result. Any indication of a field strain has to be confirmed by cultural and biochemical methods.

- A **sample is negative for SE, SEf and SE Sm24/Rif12/Ssq** if its FAM-, Cy5- and TXR-curve are negative.
- A **sample is positive for Serovar *Salmonella* Enteritidis** 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 TXR- and Cy5-channel, the SE strain present in the sample could not be further differentiated. In this case the analysis should be repeated using fresh colony material from ISO 6579 derived processes.
- A **sample is positive for SE Sm24/Rif12/Ssq** if its FAM- and Cy5-curves are positive.
- A **sample is positive for SEf** if its FAM- and TXR-curves are positive.  
**Attention:** An indication for a SEf strain also includes detection of live vaccine strains which are not Sm24/Rif12/Ssq.
- In case Kylt® SE DIVA 2 has been used directly on Buffered Peptone Water enrichments only the detection of a vaccine strain represents a valid result. Every indication of a SEf infection has to be confirmed by cultural and biochemical methods (e.g. ISO 6579 and Kauffmann White scheme).
- A potential **double infection with SEV2 and SEf or with SEV2 and another *Salmonella* serovar** is possible if its FAM-, TXR- and Cy5-curves are positive. 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.

## F. Related and Accessory Products

Product	Article No	Reactions	Description
Kylt® <i>Salmonella</i> spp.	31019	100	Detection of <i>Salmonella</i> spp.
Kylt® DNA Extraction-Mix II	31398	100	Simplified and economic DNA extraction
Kylt® RNA / DNA Purification	31314 / 31315	250 / 50	Combined RNA and DNA purification from all relevant sample matrices
Kylt® RNA / DNA Purification HTP	31826	4x96	Combined, magnetic beads-based purification of RNA and DNA from all relevant sample matrices, suitable for automated high throughput processing
Kylt® <i>Salmonella</i> Purification HTP	31433	4x96	Magnetic beads-based purification of <i>Salmonella</i> spp. DNA from cultural pre-enrichments or colony material, suitable for automated high throughput processing
Kylt® Purifier	31436	--	Purification system for magnetic beads. Up to 96 samples in under 30 minutes.

## 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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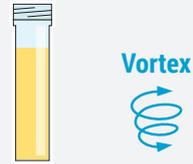


# PROTOCOL AT A GLANCE

## Real-Time PCR Setup

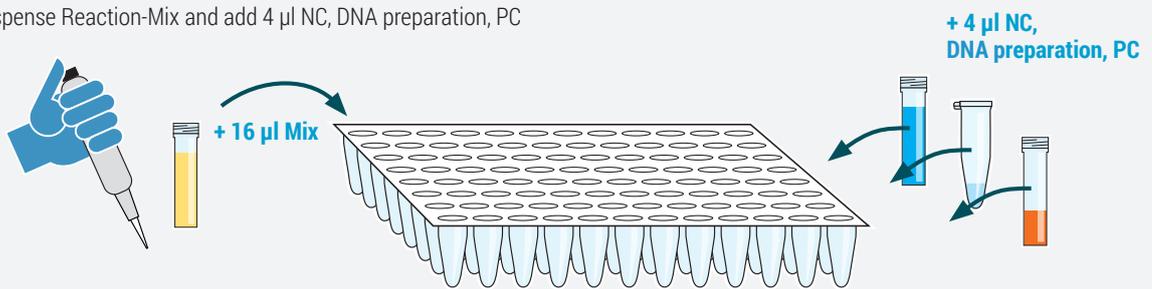
1

Pulse-vortex and spin down



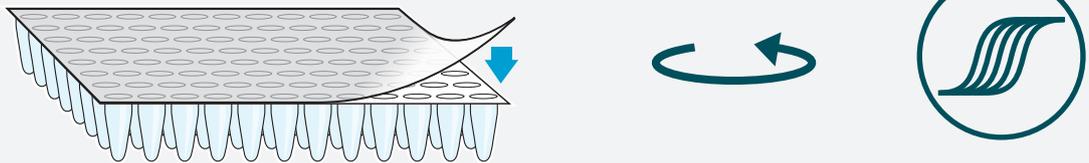
2

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



3

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



4

Analysis

