KYLT®

SAN VET



KYLT® *E. COLI STX1, STX2, EAE*

REAL-TIME PCR DETECTION

ℹ For Feed and Food Testing Applications only.

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Passion for Innovation

KYLT®

E. COLI STX1, STX2, EAE

Real-Time PCR Detection

1. GENERAL

Characteristic	Description
Article name	Kylt® Stx1, Stx2, eae FS 100, Kylt® Stx1, Stx2, eae FS 25
Name of pathogen	E. coli virulence factor Stx1 (Shigatoxin 1), E. coli virulence factor Stx2 ((Shiga-toxin 2, incl. Stx2f), E. coli virulence factor eae (E. coli intimin)
Caused disease	E. coli Infection
Organism	Bacteria
Target molecule	DNA
Technology	Real-Time PCR
Internal Control	Internal Amplification Control (IAC; exogenous)
Sample type	▪ pre-enrichment samples ▪ isolates derived from cultural processes
Target HEX channel (550 nm)	Internal Amplification Control (IAC)
Target FAM channel (520 nm)	Stx1
Target Cy5 channel (670 nm)	eae
Target TXR channel (620 nm)	Stx2
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

During the Real-Time PCR the target genes are amplified by respective primer pairs in the Polymerase Chain Reaction (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.

3. KIT CONTENTS

Reagent	Lid color	100 Reactions Article no. 31734	25 Reactions Article no. 31735	Storage temperature
Reaction-Mix	Green	4× 450 µl	1× 450 µl	<= -18°C
Positive Control	Red	4× Lyoph. a final 50 µl	2× 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 inhibtor-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.

- The **Internal Amplification Control** is included in the Reaction-Mix. It is co-amplified 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.

8. REACTION SETUP

- 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 the **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 PCR 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	42 cycles
3	Annealing & Extension	60 °C	1 min	
4	Fluorescence Detection	channels / wave lenghts see page 2		

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 / wave lenghts see page 2		

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:

Control Reactions	Channel			
	HEX		Cy5 / FAM / TXR	
	Internal Amplification Control (IAC)		Pathogen-Specific Channels	
Negative Control	**positive**	**Ct ≤ 40**	negative	Ct > 42
Positive Control	**positive**	**Ct ≤ 40**	**positive**	**Ct > 15 and ≤ 35**

11. TEST EVALUATION - SAMPLES

Target	Channel	Signal					
Internal Amplification Control (IAC)	HEX	**positive**	positive / negative	positive / negative	positive / negative	positive / negative	negative
E. coli Stx1	FAM	negative	**positive**	negative	negative	**positive**	negative
E. coli Stx2	TXR	negative	negative	**positive**	negative	**positive**	negative
E. coli eae	Cy5	negative	negative	negative	**positive**	**positive**	negative
The sample is ***E. coli Stx1***		negative	**positive**	negative	negative	**positive**	inhibited
The sample is ***E. coli Stx2***		negative	negative	**positive**	negative	**positive**	
The sample is ***E. coli eae***		negative	negative	negative	**positive**	**positive**	

- The **Interal Amplification Control (IAC)** is positive: Ct-value ≤ 40.
- The **pathogen-specific channel** is positive: Ct-value ≤ 42.
- When testing isolates the following applies: The **pathogen-specific channel** is positive Ct-value ≤ 30.
- **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. 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.

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 cataloge number
- Quantity and size of products
- Indicate if your account is VAT exempt

13. REVISION HISTORY

Revision	Status	Amendments
Rev002	April 2021	New Layout, Color of lid of Reaction-Mix changed to green (from Batch 20STEC:01 on), Storage temperature of reagents ≤ -18 °C
Rev003	April 2025	New Layout/Design; changed volume of Reaction-Mix and Positive Control; changed reaction setup (changed from 18 µl (Reaction-Mix) + 2 µl (e.g. sample) to 16 µl + 4 µl), cut-off-value for the evaluation of isolates added; changed TÜV logo

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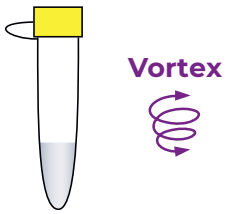
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PROTOCOL AT A GLANCE
REAL-TIME PCR SETUP

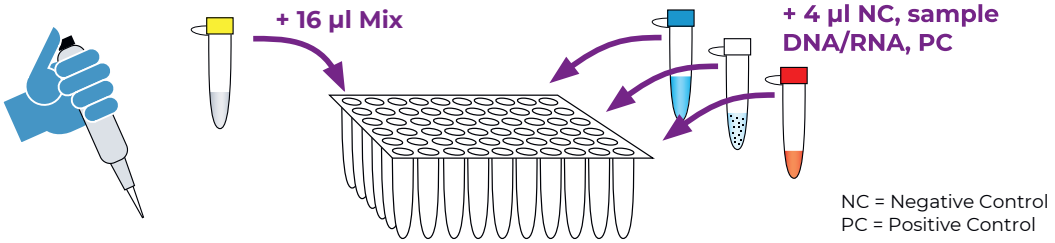
1

Pulse-vortex and spin down



2

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



NC = Negative Control
PC = Positive Control

3

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



4

Analysis

