KYLT®

SAN VET

KYLT® *SGP & 9R DIVA*

PCR DETECTION

ℹ For in vitro Veterinary Diagnostics only.

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Passion for Innovation

KYLT®
SGP & 9R DIVA

PCR Detection

1. GENERAL

Characteristic	Description
Article name	Kylt® SGP & 9R DIVA LD 100, Kylt® SGP & 9R DIVA LD 25
Name of pathogen	Salmonella Gallinarum, Salmonella Gallinarum vaccine strain 9R, Salmonella Pullorum
Organism	Bacteria
Target molecule	DNA
Technology	PCR
Host-group	Poultry (Chicken)
Sample types	▪ isolates derived from cultural processes

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 PCR the target genes are amplified by respective primer pairs in the Polymerase Chain Reaction (PCR). The amplified target sequences (PCR products) are then separated by size using agarose gel or capillary electrophoresis. The pathogen-specific status of a sample can be evaluated by considering all present PCR products per sample and the negative and positive controls per run.

3. GENERAL

Characteristic	Lid color	100 Reactions Article no. 31420	25 Reactions Article no. 31421	Storage temperature
PCR-Mix	White	4× 280 µl	1× 280 µl	<= -18°C
Loading Dye	Transparent	4× 60 µl	1× 60 µl	<= -18°C
Primer-Mix	White	4× Lyoph. a final 160 µl	1× Lyoph. a final 160 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

- Components from different batches may not be mixed or interchanged.
- 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).

5. REAGENT PREPARATION

- Before first use, rehydrate the lyophilized **Primer-Mix**: add 160 µl of the Negative Control per vial, briefly incubate at room temperature and mix by pulse-vortexing.
- 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

- PCR cycler.
- Equipment, media and disposables for agarose gel or capillary electrophoresis.
- Purification Kit yielding sufficiently high concentration of inhibtor-free DNA/RNA (e.g. Kylt RNA / DNA Purification products)."
- Compatible PCR-plates strips or single tubes.
- Vortex mixer.
- Table top microcentrifuge.
- 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

- 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.
- Prepare the Master-Mix using the components listed below.
- Vortex, spin down and add 18 µl of the finalized Master-Mix to each of the PCR tubes or plate wells ("cavities").

	Volume (µl)	
Reagent	**per Reaction**	**e.g. n= 7**
2x PCR-Mix	10 µl	70 µl
10x Loading Dye	2 µl	14 µl
Primer-Mix	6 µl	42 µl
Total Volume Master-Mix	**18 µl**	**126 µl (dispense 18 µl per reaction)**
DNA (Negative Control / sample / Positive Control)	2 µl	
Total Volume per Reaction	**20 µl**	

- Avoid the formation of bubbles when pipetting the mix, samples and controls.
- Return the 2x PCR-Mix, Primer-Probe-Mix back to appropriate storage temperature right after application.
- Add 2 µl of the **Negative Control** to the corresponding cavity and seal it individually, if possible.
- Add 2 µl of each **sample** to the corresponding cavities and seal them individually, if possible.
- Add 2 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 PCR thermal cycler and run the test with the settings indicated below.
- Please follow the specified instructions of your Real-Time PCR thermal cycler as recommended by the manufacturer.

Step No	Description	Temperature	Duration	
1	Activation of Polymerase	94 °C	3 min	
2	Denaturation	94 °C	30 sec	35 cycles
3	Annealing	64 °C	30 sec	
4	Extension	72 °C	30 sec	
5	Final Extension	72 °C	7 min	
6	Post-PCR cooling (optionally)	7 °C	hold	

9. AGAROSE GEL ELECTROPHORESIS

- You may run any standard agarose gel electrophoresis method appropriate for this product size. An appropriate method is described briefly below:
- Prepare a 2% agarose gel for separation of the DNA sample after PCR amplification.
- Load the wells of the agarose gel with 5 µl of the PCR reactions from the DNA sample(s), Positive Control and Negative Control, respectively. Load at least an additional well of agarose gel with sufficient volume of e.g. an 100 bp reference DNA ladder. Make notes of the position of sample(s), controls and ladder.
- Run the electrophoresis at a voltage of approximately 15 V/cm (the distance in cm refers to the distance between electrodes) for 45 min to 60 min.
- Following electrophoresis, stain the gel with appropriate amount of nucleotide / intercalating dye (e.g. Ethidium bromide, GelRed Nucleic Acid stain or SYBR green) and visualize by using the corresponding technique. For more details, please refer to the respective Direction For Use of the dye.

10. GENERAL PCR-RUN EVALUATION AND QUALITY CONTROL

- The readily stained agarose gel must give discrete bands of expected sizes for control reactions and the reference DNA ladder.
- Check the results of Positive Control and Negative Control for presence / absence of expected product sizes of appr. 120 bp, 175 bp and 255 bp.

11. TEST EVALUATION - CONTROL REACTIONS

- The PCR run and subsequent elektrophoresis is only valid if the product sizes of the control reactions can be evaluated as follows:

Control reactions	Product size		
	120 bp	**175 bp**	**255 bp**
Negative Control	-	-	-
Positive Control	**present**	**present**	**present**

12. TEST EVALUATION - SAMPLES

Target	120 bp	175 bp	255 bp
Salmonella Pullorum positive	-	-	**present**
Salmonella Gallinarum positive	-	**present**	**present**
Salmonella Gallinarum vaccine strain 9R positive	**present**	**present**	**present**

13. 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

14. REVISION HISTORY

Revision	Status	Amendments
Rev002	December 2024	New layout/design, changed volume of Positive Control, changed temperature profile for amplification

Production: SAN Group Biotech Germany GmbH · Muehlenstrasse 13 · 49685 Hoeltinghausen · Germany
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