KYLT®

SAN® VET

KYLT® *INFLUENZA A - H9*

REAL-TIME RT-PCR DETECTION

ℹ For in vitro Veterinary Diagnostics only.

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Passion for Innovation

DIRECTION FOR USE

RT-qPCR.AIV.H9.02, Rev008, March 2025

KYLT®

INFLUENZA A - H9

Real-Time RT-PCR Detection

1. GENERAL

Characteristic	Description
Article name	Kylt® Infl. A H9 LD 100, Kylt® Infl. A H9 LD 25
Name of pathogen	Influenza Virus A - H9
Caused disease	Avian Influenza
Organism	Virus
Target molecule	RNA
Technology	Real-Time RT-PCR
Internal Control	beta-actin (endogenous)
Host-group	Poultry
Sample types	▪ feces ▪ tissues and organs (e.g. lung, cecal tonsils) ▪ swab samples (e.g. cloacal, choanal, tracheal) ▪ isolates derived from cultural processes
Target HEX channel (550 nm)	beta-actin
Target FAM channel (520 nm)	Influenza A - H9
Pooling	Up to 5 samples
Temperature profile	Kylt® Profile I

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

The RNA target sequences are reverse transcribed (Reverse Transcription (RT)) and amplified in parallel with 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. 31070	25 Reactions Article no. 31071	Storage temperature
2x RT-qPCR-Mix	Transparent	4× 280 µl	1× 280 µl	<= -18°C
Detection-Mix	Yellow	4× Lyoph. a final 150 µl	1× Lyoph. a final 150 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.
- Components from different batches may not be mixed or interchanged.
- The components are to be used within the indicated shelf life (see box label).
- The **Detection-Mix** needs to be stored and handled protected from light.

5. REAGENT PREPARATION

- Before first use, rehydrate the lyophilized **Detection-Mix**: add 150 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

- 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).
- 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.
- The **Internal Control** is based on detection of **beta-actin**, which is ubiquitous in the cells of the host that the sample is derived from. The beta-actin target gene is co-amplified with every single reaction and allows for evaluation of sufficient sampling, sample storage and shipment, sample preparation and the Real-Time PCR run itself.
- It is recommended to run one or more of a **RNA Isolation Control (RIC)** per set of RNA preparation. The RIC is a "mock sample" composed of the plain sterile buffer used for the preparation of the raw samples. It is processed as a normal sample and allows the detection of potential contaminations of the reagents used (in addition to the Negative Control reaction) as well as for the detection of potential carryover contamination between individual samples, e.g. during the RNA preparation process.

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 16 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 RT-qPCR-Mix	10 µl	70 µl
Detection-Mix	6 µl	42 µl
Total Volume Master-Mix	**16 µl**	**112 µl (dispense 16 µl per reaction)**
RNA (Negative Control / sample RNA / RIC(s) / Positive Control)	4µl	
Total Volume per Reaction	**20 µl**	

- Keep exposure of the 2x RT-qPCR-Mix, Detection-Mix and prepared Master-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** (including **RIC(s)**, if processed) 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 Kylt Profile I.
- 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 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:
- If one or more of a RNA Isolation Control (RIC(s)) is processed, its curves must be negative.

Control Reactions	Channel			
	HEX		FAM	
	Interal Control (beta-actin)		Pathogen-Specific Channel	
Negative Control	negative	Ct > 35	negative	Ct > 42
Positive Control	**positive**	**Ct ≤ 35**	**positive**	**Ct > 15 and ≤ 35**

11. TEST EVALUATION - SAMPLES

Target	Channel	Signal		
Interal Control (beta-actin)	HEX	**positive**	positive / negative	negative
Influenza A - H9	FAM	negative	**positive**	negative
The sample is ***Influenza A - H9***		negative	**positive**	inhibited

- The **Interal Control (beta-actin)** is positive: Ct-value ≤ 35.
- The **pathogen-specific channel** is positive: Ct-value ≤ 42.
- Please note that the vaccine Newflend® ND H9 (Ceva) is detected as a positive result with this PCR kit.
- **Recommendation:** In the case of an inhibited sample the test may be repeated with a dilution of the RNA preparation at e.g. 1:4. The Negative Control is used as the diluting agent. Preferably, the entire RNA 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
Rev007	November 2019	Positive Control includes beta-Actin (Signal in HEX) from Batch no. 20H9:01 on
Rev008	March 2025	New Layout/Design, Added note about detection of the Newflend® ND H9 (Ceva) vaccine (11. Test Evaluation - Samples), changed TÜV logo

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Development, production and distribution of KYLT® In-Vitro diagnostics is certified to ISO 9001:2015.

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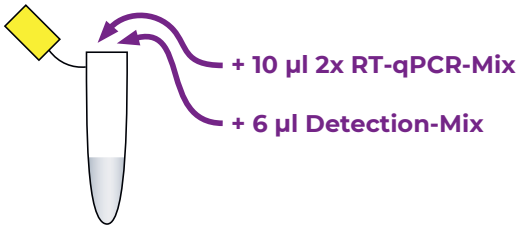
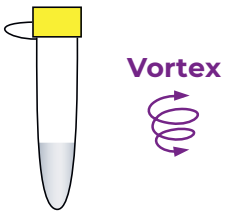
PROTOCOL AT A GLANCE
REAL-TIME PCR SETUP

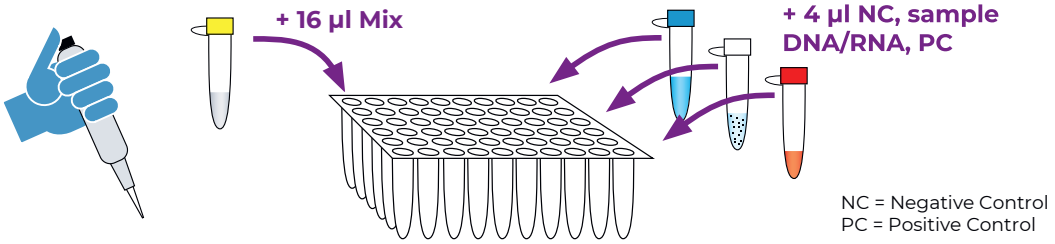
1 Prepare a Master-Mix



2 Pulse-vortex and spin down

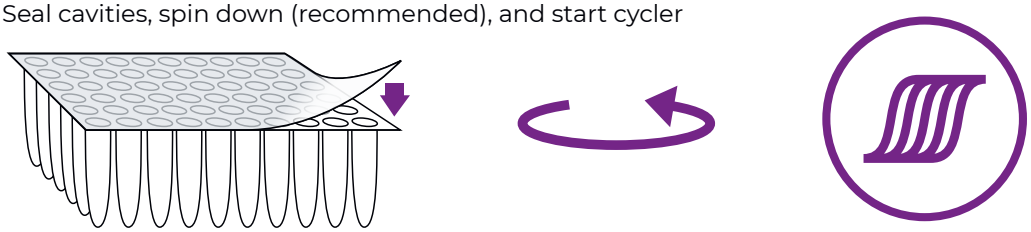
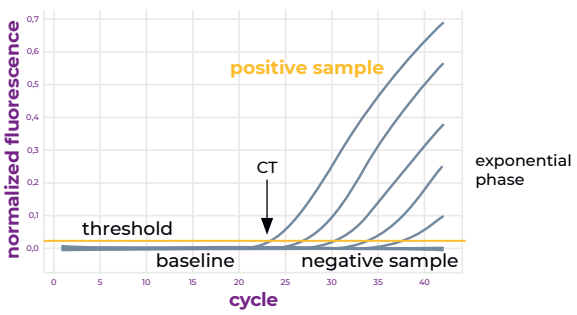


3 Dispense Master-Mix and add 4 µl NC, sample DNA/RNA, PC



NC = Negative Control
PC = Positive Control

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



5 Analysis