Kylt® 2x RT-qPCR-Mix

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A. General

- Kylt® 2x RT-qPCR-Mix is a ready-to-use, 2x concentrated Enzyme Reagent, that is to be combined with specific primer- and probe-setups for detection of RNA (e.g. viral RNA) by Real-Time Polymerase Chain Reaction (Real-Time PCR or qPCR) following cDNA synthesis (performed by Reverse Transcription (RT)). The subsequent qualitative analysis of cDNA is based on the amplification of target sequences by the primer pair(s) in the PCR. Amplified target gene fragments are detected via fluorescently labelled probes during the PCR reaction in Real-Time.
- The Kylt® 2x RT-qPCR-Mix contains all essential components, such as Reverse Transcriptase, DNA Polymerase, nucleotides and a suitable reaction buffer. The components are provided in a proprietary system, that enables the optimal performance of the Reverse Transcriptase and amplification as well as highest specificity and sensitivity of the target specific detection in Real-Time.
- This Enzyme Reagent 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® 2x RT-qPCR-Mix Enzyme Reagent comprises the following reagents:

Reagent	Colour of Lid	100 Reactions Article No 31868	Store at
2x RT-qPCR-Mix	◯ transparent	4 x 280 µl	≤ -18 °C

- After receipt, the components are immediately stored at ≤ -18 °C.
- The Kylt® 2x RT-qPCR-Mix is to be used within the indicated shelf life (see label), if stored properly.

C. Technical Information

- A RT-qPCR reaction containing the Kylt® 2x RT-qPCR-Mix can be processed on all commercially available Real-Time PCR thermal cyclers.

D. Protocol

Reaction Setup and Amplification (Real-Time RT-PCR)

- Before each use, briefly vortex and spin down the Kylt® 2x RT-qPCR-Mix.
- To determine the total number of reactions needed, count the number of samples and add two more for the Negative Control and the Positive Control.
- Prepare the Master-Mix without template RNA by combining the reagents as listed in the table below.

Reagent	Volume (µl)		
	per 20 µl Reaction	per 50 µl Reaction	Final concentration
Kylt® 2x RT-qPCR-Mix	10 µl	25 µl	1x
Primer forward	X µl	X µl	300-900 nM
Primer reverse	X µl	X µl	300-900 nM
Probe	X µl	X µl	50-200 nm
Sample RNA	X µl	X µl	10-100 ng
Nuclease free water	To a final volume of 20 µl	To a final volume of 50 µl	-
Total Master-Mix	**20 µl**	**50 µl**	-

- Return the Kylt® 2x RT-qPCR-Mix back to ≤ -18 °C right after application. Avoid the formation of bubbles when pipetting the Master-Mix, samples and controls.
- Program the Real-Time cycler according to the manufacturer's instructions using the following guidelines.

Step No	Description	Temperature	Duration	
1	Reverse Transcription	50 °C	10 min	
2	Activation of Polymerase	95 °C	1 min	
3	Denaturation	95 °C	3 - 10 sec	42 cycles
4	Annealing & Extension	60 °C	30 - 60 sec	
5	Fluorescence Detection	specific channels		

Production:

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Development, manufacturing and distribution of Kylt® *In-Vitro* Diagnostica is certified according to ISO 9001:2015.

Management System ISO 9001:2015
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