Kylt® 2x qPCR-Mix

www.kylt.eu

Kylt® 2x qPCR-Mix

A. General

- Kylt® 2x 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 DNA (e.g. viral DNA or bacterial DNA) by Real-Time Polymerase Chain Reaction (Real-Time PCR or qPCR). The qualitative analysis of DNA 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 (Real-Time PCR).
- The Kylt® 2x qPCR-Mix contains all essential components, such as DNA Polymerase, nucleotides and a suitable buffer system. The components are provided in a proprietary buffer system, that enabled the optimal performance of the 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 qPCR-Mix Enzyme Reagent comprises the following reagents:

Reagent	Colour of Lid	100 Reactions Article No 31867	Store at
2x qPCR-Mix	◯ transparent	4 x 280 µl	≤ -18 °C

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

C. Technical Information

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

D. Protocol

Reaction Setup and Amplification (Real-Time PCR)

- Before each use, briefly vortex and spin down the Kylt® 2x 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 DNA 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 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 DNA	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 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.

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	60 sec	
4	Fluorescence Detection	specific channels		

Production:

AniCon Labor GmbH | Muehlenstr. 13 | D-49685 Hoeltinghausen | Germany | www.kylt.eu | info@kylt.eu

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

Management System ISO 9001:2015 TÜVRheinland CERTIFIED www.tuv.com ID 9108634298

Kylt® is a registered trademark.

For *in vitro* use only. Regulatory requirements vary by country, not all of the products described herein may be available in your geographic area.

AC.Kylt.qPCR.02, Rev001, February 2020