4everyone Functionality Kit 100ul

User Guide

Check of real time PCR instrument functions

For research *in vitro* use only

SKU #2400-1001 **4everyone Functionality Kit 100ul**

Includes low profile 100 µl PCR tubes, clear

The 4everyone Functionality Kit contains reagents and PCR tubes for 16 wells of a thermoblock to test the function of a real time PCR thermocycler.

Kit includes buffer and master mix which are necessary for running a real time PCR together with PCR tubes. The PCR tubes are containing specific primers and probes for a 2-channel real time PCR together with target DNA in different concentrations, all aliquoted in a dried format.

4everyone Functionality Kits are extremely temperature stable and ship at room temperature. Storage at 2-8 °C upon arrival is recommended.

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Section 1

4everyone Functionality

This test checks the measurement capabilities of any 2-channel real time PCR thermoycler in a 200 µL or 100 µl tube format.

The 4everyone Functionality Kit is optimized but not limited to two-channel real time PCR thermocyclers using FAM channel (495/520 nm) and VIC/HEX channel (530/550 nm) to verify the correct function of both the hardware and software of the thermocycler.

Whenever there are unspecific results from a PCR test and it is unknown if the failure originates from a hardware error or from false sample handling, you can check the correct function of your thermocycler instrument by just running a functionality test. It is especially useful after moving the instrument or after a calibration was done. With the Functionality Kit, you can easily verify that your sample DNA is measured correct and the software read-out gives out the results within the correct concentration ranges, too.

Section 2

Introduction to the 4everyone Detection Kit Technology

Today, the use of PCR is accepted as the standard method for detecting nucleic acids from numerous microorganisms in a diversity of food and beverages, both functional species as well as spoilers. Real time PCR is one of the most powerful, specific and reliable methods for the quantitative detection and identification of microorganisms at an early process stage to prevent spoilage and to maintain overall product quality.

The 4everyone Detection Kit system is based on DNA amplification and detection of microorganisms by real time PCR. The specific PCR reagents, primers and probes, come in a ready-made dried format in the PCR tubes for unrivalled ease of use and temperature stability.

All PCR tests use the FAM channel (495/520 nm) for detection of the target microorganisms and the VIC/HEX channel (530/550 nm) for an internal control reaction. This allows 4everyone Detection Kits to prevent false negatives due to sample inhibition, allowing you to be truly confident about negative results.

A typical workflow includes the following four steps:

Section 3

Kit Components

The 4everyone Functionality Kit contains sufficient reagents for 16 reactions.

Kit material for DNA isolation and analysis	Amount	Storage
Rehydration buffer B (blue cap)	1 x 275 µL	2-8 °C
2 x Master mix (white cap with blue sealing ring)	1 x 264 µL	
PCR tubes (strips of 8) with mix of primers and probes	2	
Cap strips (strips of 8) for covering the PCR reaction tubes	2	

Table 1: Materials supplied

Section 4

Shelf Life and Storage

Once received, the kit must be stored at 2-8 °C. Reagents stored at this temperature can be used until the expiration date indicated on the package label.

Section 5

Materials Required but Not Supplied

Equipment	Supplies
Real time PCR thermocycler for 0.1 or 0.2 mL tubes with detection channels for FAM (520 nm emission) and VIC/HEX (550 nm emission)	Pipette tips with filters
Centrifuge for 8-tube strips 0.1 or 0.2 mL or adaptor for benchtop microcentrifuge	Gloves, powder free
Reaction tube mixer (Vortexer) (optional)	
Microliter pipettes for DNA extraction 100-1,000 µL variable volume	

Table 2: Additional materials required

Section 6

Detailed Instructions

Warning! Read the manual and the Safety Data Sheets before starting the analysis. Safety Data Sheets are available in the download area from www.pika-weihenstephan.com. All handling steps should be performed under sterile conditions. Wear appropriate protective clothing and powder free gloves. The use of filter tips is recommended.

DNA Analysis by real time PCR

Each of the PCR tubes in a Functionality Kit contains all components for a two channel PCR reaction in a dried format, including the target and internal control DNAs. The only exception is master mix which needs to be added as a reconstitution solution.

The target DNA is contained in different amounts per tube which will result in detection of different concentrations, while the internal control is inculded in equal amounts in all PCR tubes. PCR will give negative and positive results in channel 1 (FAM), showing three different concentrations in the positive results. All tubes should give equal results for the internal control reaction which is measured in channel 2 (VIC/HEX). The results are read out automatically using the automated read-out of results from the Open qPCR software.

The expected results are shown in the following table:

	A1	A2	A3	A4	A5	A6	A7	A8	
A	(high)	valid negative (NTC)	medium	low	low	medium	negative (NTC)	high	H

	B1	B2	B3	B4	B5	B6	B7	B8	
A	high	negative (NTC)	medium	low	low	medium	negative (NTC)	high	H

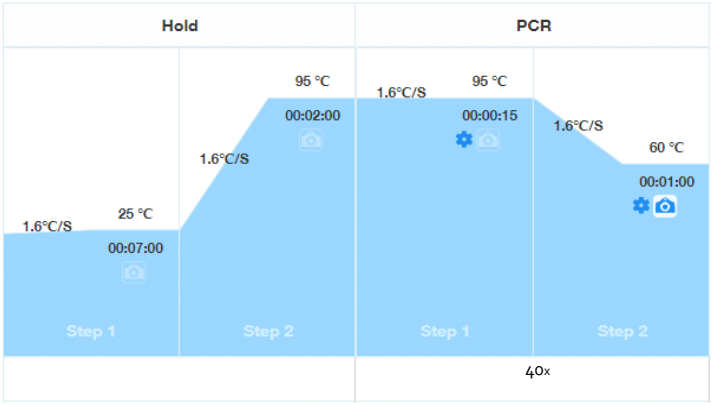
Table 3: Results per tube from 4everyone Functionality Kit

Preparation and Distribution of the Reconstitution Solution for PCR

1. Prepare the reconstitution solution by pipetting **264 µL of Rehydration buffer B (blue cap)** into the delivered vial 2 x Master Mix (white cap with blue sealing ring)
2. After adding the Rehydration buffer, re-close the reaction tube with the white cap which now contains the reconstitution solution. Mix briefly by vortexing or inverting the tube a couple times. Follow up with a quick spin down to collect the liquid at the bottom of the tube
3. Pipet **30 µL** of the reconstitution solution **into each of the 16 PCR tubes**
4. Close all PCR tubes with the provided cap strips
5. Spin down the PCR tubes shortly (10-15 seconds) to collect the liquid at the bottom (max. 2,000 rpm), and check for trapped air bubbles
6. If trapped bubbles are present, repeat step 5.
7. Transfer all PCR tubes into the thermocycler and follow instructions according to the instrument's software

Instrument and Software Setup

Set up a PCR test with the following temterature and time protocol.



Detectors: FAM (520 nm emission)
VIC/HEX (550 nm emission)

Quencher: TAMRA or BHQ

Data collection

Sample voume 30 µL

All times given are in hours:minutes:seconds

Fig. 1 : Temperature scheme of thermocycler

Data Analysis

1. Follow user manual of thermocycler instrument
2. Evaluate the thermocycler results
    - Verify the curves
    - Expected values for the different included DNA concentrations Ct values are given in table 4
        - FAM channel detects two üarallels of all target DNAs in 3 different concen–trations plus the negative control
        - VIC/HEX channel detects internal positive control reaction in all PCR tubes, expected value is Ct 30-33 for all tubes, independent from FAM value

Concentration	high	medium	low	negative
Expected Ct value in FAM channel	10.00-23.99	24.00-29.99	30.00-38.00	> 38.00

Table 4: Expected FAM channel results for different PCR tubes - manual evaluation

Use of Chai Open qPCR thermocycler

1. Select "RUN A TEST KIT"
2. Select Kit manufacturer "PIKA"
3. Select "Single spoiler detection"
4. Select "Omit Positive Control"
5. Assign wells for experiment with different DNA concentrations as shown below
6. Run test

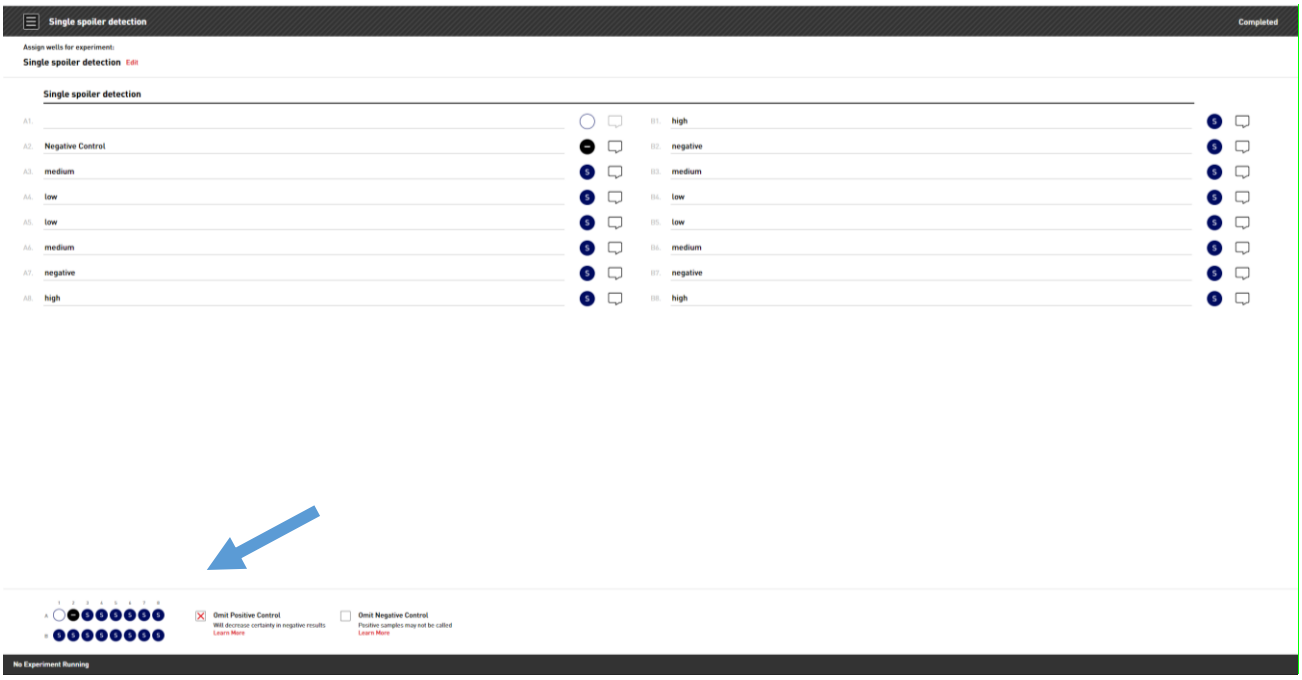


Fig. 2: Assigned wells for Open qPCR experiment with omitted Positive Control

Data Analysis with Open qPCR

All data can be analyzed directly at the end of the PCR run or at a later time by opening the stored experiment. Follow instructions of the corresponding Open qPCR software manual for opening experiments and setting the data analysis parameters.

Once the run is completed, the software displays the full data interpretion in a table format, an example is given in table 5. Full data including amplification curves can be seen in 'Full Results' view

Single spoiler detection

Well	Sample	Result	C_q	Quantity
A2	**Negative Control**	Valid		
A3	**medium**	Positive	28.97	Medium
A4	**low**	Positive	31.92	Low
A5	**low**	Positive	31.79	Low
A6	**medium**	Positive	29.17	Medium
A7	**negative**	Negative		Not Detectable
A8	**high**	Positive	19.04	High
B1	**high**	Positive	19.03	High
B2	**negative**	Negative		Not Detectable
B3	**medium**	Positive	28.95	Medium
B4	**low**	Positive	32.18	Low
B5	**low**	Positive	32.00	Low
B6	**medium**	Positive	29.24	Medium
B7	**negative**	Negative		Not Detectable
B8	**high**	Positive	19.09	High

Table 5: Results from Chai Dual Channel Open qPCR™ thermocycler

Section 7

Precautions and Recommendations for Best Results

- ✓ This test must be performed by trained persons
- ✓ All potentially infectious material should be autoclaved before disposal
- ✓ The quality of results depends on strict compliance with Good Laboratory Practices (for example, the EN ISO 7218 standard), especially concerning PCR:
    - The laboratory equipment (pipets, tubes, etc.) must not circulate from one workstation to another
    - It is essential to use positive and negative controls for each series of amplification reactions
    - Do not use reagents after their expiration date
    - Periodically verify the accuracy and precision of pipets and the correct functioning of the instruments
- ✓ Change gloves often, especially if you suspect they are contaminated
    - Clean work spaces periodically with at least 5% bleach or other DNA decontaminating agents such as DNA AWAY
    - Use powder-free gloves and avoid fingerprints and writing on tube caps as this can interfere with data acquisition
- ✓ It is strongly advised to follow the general requirements described in the standard EN ISO 22174:2005 (Microbiology of food and animal feeding stuffs Polymerase chain reaction (PCR) for the detection of food pathogens General requirements and definitions)

Section 8 Appendix

Trademarks and Property Rights

Trademarks:
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Use of product:
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