



# 4everyone Functionality Kit

## USER GUIDE

### *Check of real time PCR instrument functions*

For research *in vitro* use only

**SKU #2400-2001**      **4everyone Functionality Kit 200ul**  
Includes skirted 200 µl PCR tubes, frosted

**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 Kit

This test checks the measurement capabilities of any 2-channel real time PCR thermocycler 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 our website <https://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 included 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 as follows:

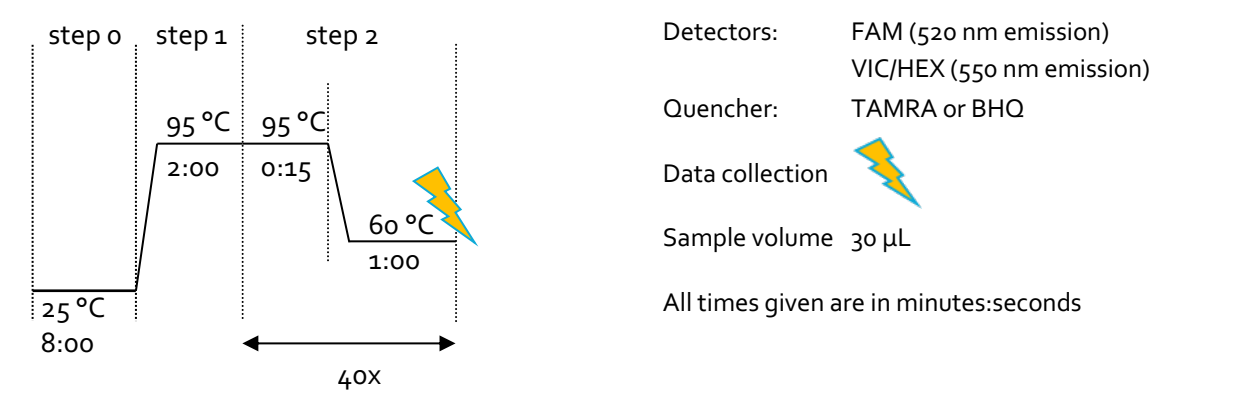


Fig. 2: Temperature scheme of thermocycler

1. Only when using Chai Dual Channel Open qPCR™ real time PCR thermocycler:
- i. Select "RUN A TEST KIT"

ii. Select Kit manufacturer "PIKA"

iii. Select "Single spoiler detection"

iv. Select "Omit Positive Control" and assign wells for experiment as shown below

v. Run test

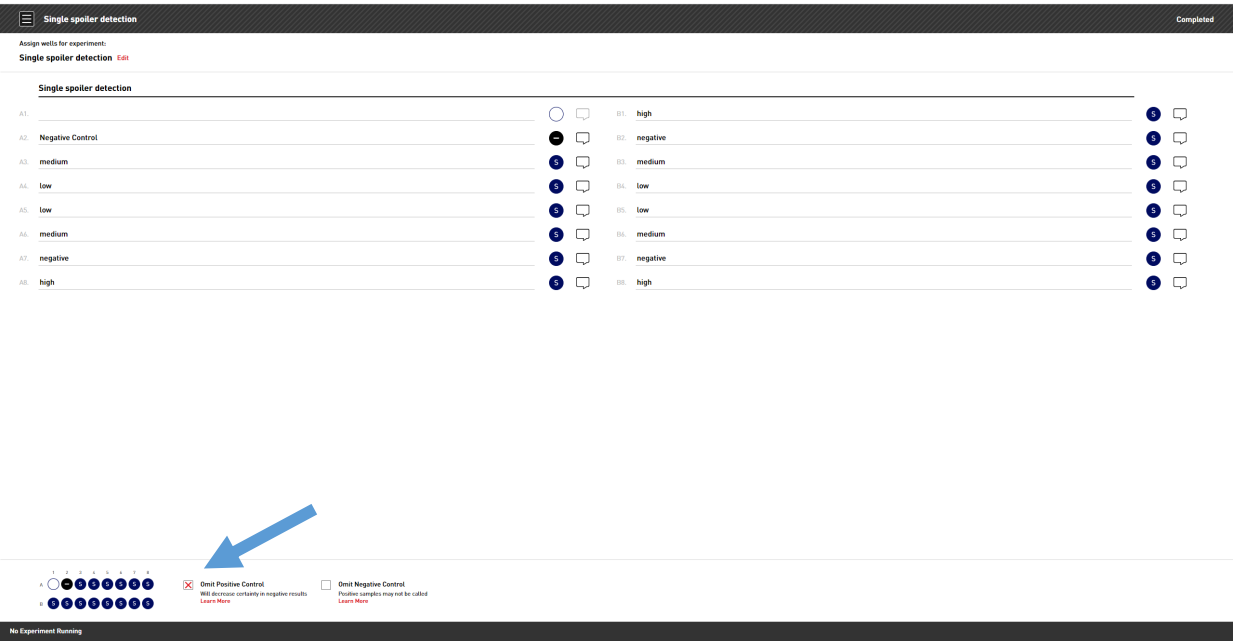


Fig. 2: Assigned wells for experiment with omitted Positive Control

## Data Analysis

1. Real time PCR thermocyclers with microliter plate block format
  - Follow user manual of thermocycler instrument
  - Evaluate the thermocycler results as follows:
    - Verify the curves
    - Evaluation of the measured Cq/Ct values is shown in table 5
      - FAM channel detects target DNA in different concentrations
      - VIC/HEX channel detects internal positive control reaction

Cq/ Ct value (FAM channel)	Internal control reaction (HEX channel)	Result from analysis
<b>10.0-24.00</b>	<b>+</b>	Positive control: <b>high</b> DNA amount detected
<b>24.01-30.00</b>	<b>+</b>	Positive control: <b>medium</b> DNA amount detected
<b>30.01-38.00</b>	<b>+</b>	Positive control: <b>low</b> DNA amount detected
<b>-</b>	<b>+</b>	Negative control: no curve in FAM channel, only Cq/Ct value in HEX channel (30.00 ± 2)

Table 4: Results for manual evaluation of PCR

2. Only for Chai Dual Channel Open qPCR™ real time PCR thermocycler
  - 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 in the corresponding Open qPCR software manual for opening experiments and setting the data analysis parameters
  - Interpreting Results
    - i. Once the run is complete, the software displays the full data interpretation in a table format, an example is given in table 5.
    - ii. Full data including amplification curves can be seen in 'Full Results' view

#### Single spoiler detection

Well	Sample	Result	C <sub>q</sub>	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)

# Trademarks and Property Rights

### Trademarks:

FastOrange and PIKA Weihenstephan are registered trademarks or trademarks of PIKA Weihenstephan, Pfaffenhofen, Germany, in Germany and other countries.

### Use of product:

4everyone Detection Kit is to be used for *in vitro* research purposes only.

### Property Rights:

For any commercial use of the kit or parts of it, licensing from PIKA Weihenstephan GmbH is required. The use of our products may touch property rights of third parties. The purchase of this product does not implement any rights for the performance of PCR or its use for diagnostic purposes. We point out that licensed accessories (thermocycler instrument) have to be used for any PCR application of our kits. PIKA Weihenstephan GmbH does assume no responsibility for the lawful use of this kit; this responsibility lies expressly and solely at the user. The process of polymerase chain reaction is covered by several patents. For commercial use, licensing by either of the companies Roche and/or Applied Biosystems is needed.

"No license under these patents to use the PCR process is conveyed expressively or by implication to the purchaser by the purchase of this product. Further information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at Roche Molecular Systems, Inc., 1145 Atlantic Avenue, Alameda, California 94501."



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