# Food Control™ qPCR

Kits for the detection of foodborne pathogens via qPCR

**INSTRUCTIONS FOR USE**

**FOR USE IN RESEARCH AND QUALITY CONTROL**

## Symbols


**Lot No.**


**Cat. No.**

**Expiry date**

**Storage temperature**

**Number of reactions**

**Manufacturer**

## INDICATION

Food Control™ qPCR is a kit for fast and reliable detection of foodborne pathogens via real-time PCR. Isolated total DNA from a potentially contaminated food sample serves as starting material, typically after a pre-cultivation of the sample in growth medium.

Food Control™ qPCR kit allows for easy determination of contamination degree in the agricultural or in the food industry via real-time PCR.

The detection assay is available for different pathogens as described in **Table 1.**, with detection sensitivity limits of down to 10 genome copies per PCR (see „Assay Characteristics“ for further information).

**Table 1.**

| Pathogen<br>Catalog No. | Description |
|---|---|
| ***S. enterica***<br>**360-1025** | *Salmonella enterica* is a gram-negative, non-spore-forming bacterium often found in contaminated food and beverages. There are two forms of illnesses caused by *Salmonella*; the typhoid and the more common enteric salmonellosis that is known for abdominal pain, diarrhea, vomiting and, often, high fever. An infection with the enteric *Salmonella* species occurs mostly after eating chicken products (including eggs) or (raw) meat. In addition, poor hygiene significantly increases the risk of infection.<br>The assay detects the *Salmonella enterica*-specific sequence of invasion protein (invA) gene. |
| ***Y. enterocolica***<br>**360-2025** | *Yersinia enterocolitica* is a gram-negative, non-spore-forming bacterium with flagella at a growth temperature below 30°C. The most frequent food-associated infections with *Yersinia enterocolitica* occur after eating raw pork. The infection induces enteritis with diarrhea and abdominal pain.<br>The assay detects the *Yersinia enterocolitica*-specific sequence of heat-stable enterotoxin A gene. |
| ***Shigella spp.***<br>**360-3025** | Shigellae are gram-negative, immotile and non-spore-forming bacteria. Contaminated drinking water and food are the infection sources in humans. In Germany, the most prominent species are *Shigella flexneri* and *Shigella sonnei.* The target sequence of this assay is a gene specific for both of these species.<br>The assay detects the *Shigella* genus-specific sequence of invasion plasmid antigen (ipaH6) gene. |

| | |
|---|---|
| ***Campylobacter spp.***<br>**360-4025** | *Campylobacter* is a gram-negative, rod-shaped bacterium and belongs to the most important pathogens inducing food-borne infections in humans. The most relevant infectious species are *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter lari*. A contamination may occur in food like poultry and poultry related products (excluding eggs), unpasteurized milk, drinking water or pets.<br>The assay detects the *Campylobacter* genus-specific sequence of acyl-[acyl-carrier-protein]--UDP-N-acetylglucosamine O-acyltransferase (lpxA) gene. |
| ***C. perfringens***<br>**360-5025** | *Clostridium perfringens* is a gram-positive, obligate anaerobe, spore-forming bacterium and spores occurring in the environment, in soil, water and dust. The bacterium is associated with a multitude of human diseases. Its frequent occurrence as a food poisoning agent especially in meat, oysters and other sea food contributes to its significance.<br>The assay detects the *Clostridium perfringens*-specific sequence of phospholipase C alpha-toxin (plc) gene. |
| ***Shiga Toxin 1***<br>***Shiga Toxin 2***<br>**360-6025**<br>**360-7025** | Shiga toxins Stx1 and Stx2 are produced by *Shigella dysenteriae* as well as enterohemorrhagic *Escherichia coli* (EHEC). The syndromes associated with shiga toxin include dysentery, hemorrhagic colitis, and hemolytic uremic syndrome.<br>The assay detects the *Shiga Toxin 1*-specific sequence of Shiga Toxin 1 (stx1) gene and the *Shiga Toxin 2*-specific sequence of Shiga Toxin 2 (stx2) gene. |
| ***E. coli O157***<br>**360-8025** | *Escherichia coli* O157 is an enterohemorrhagic serotype of the bacterium *Escherichia coli* and a cause of illness, typically through consumption of contaminated food. Infection may lead to hemorrhagic diarrhea, and to kidney failure. Transmission is via the fecal-oral route, and most illnesses have occurred after ingestion of contaminated raw green-leaf vegetables and undercooked meat.<br>The assay detects the *E. coli O157*-specific sequence of wbdR gene. |
| ***E. coli O104***<br>**360-9025** | *Escherichia coli* O104 is an enterohemorrhagic serotype of the bacterium *Escherichia coli* and a cause of illness, typically through consumption of contaminated food. Infection may lead to hemorrhagic diarrhea, and to kidney failure. Transmission is via the fecal-oral route, and most illnesses have occurred after ingestion of contaminated raw green-leaf vegetables and undercooked meat.<br>The assay detects the *E. coli O104*-specific sequence of wckD gene. |

| | |
|---|---|
| ***Listeria spp.*** **361-1025** | *Listeria* is a genus of gram-positive, facultatively anaerobic, non-spore-forming bacteria that contains 10 species. The major human pathogen in the *Listeria* genus is *L. monocytogenes*. *Listeria ivanovii* is a pathogen of mammals, specifically ruminants, and has rarely caused listeriosis in humans.<br>The assay detects the *Listeria* genus-specific DNA sequence of invasion associated protein p60 (iap) gene. |
| ***L. monocytogenes*** **361-2025** | *Listeria monocytogenes* is an aerobic, gram-positive, non-spore-forming bacterium. It is ubiquitous in the environment and able to contaminate different types of food. A contamination may occur in food like poultry and poultry products, fresh milk and cheese made of raw milk, fish and "ready-to-eat" salad. Along with induced diarrheal diseases, *Listeria monocytogenes* is also one of the most common pathogens in perinatal infections.<br>The assay detects the *Listeria monocytogenes*-specific sequence of listeriolysin O (hly) gene. |
| ***Salmonella spp.*** **361-3025** | *Salmonella* is a genus of gram-negative, non-spore-forming bacteria of the Enterobacteriaceae family. There are only two species of *Salmonella*, *Salmonella bongori* and *Salmonella enterica*. *Salmonella bongori* causes a gastrointestinal disease called salmonellosis, characterized by cramping and diarrhea in cold-blooded animals, unlike other members of the genus, and is most frequently associated with reptiles.<br>The assay detects the *Salmonella* genus-specific DNA sequence in the spacer-region between 16S and 23S RNA genes. |

## TEST PRINCIPLE

Food pathogenic species are detected by amplification of the species-specific targets as described in **Table 1**, whereas eukaryotic or other bacterial DNA is not amplified by the Food Control™ qPCR assays. The assay is based on the TaqMan® principle, which relies on the 5' → 3' exonuclease activity of Taq polymerase and on the dual labeling of the probes with fluorophores and quenchers. During qPCR, the Taq polymerase cleaves and removes annealed probes releasing the previously quenched fluorescent signal.

The Instructions for Use include protocols for the analysis of DNA extracted from potentially contaminated food samples (e.g. by using ExtractNow™ Food Control Cat. No. 609-1050 or an appropriate user-based method), after pre-cultivation of the sample in growth medium according to pathogen-specific local, regional, national, or international regulations.

The entire test is completed in approximately 90 minutes, excluding pre-cultivation and DNA extraction. Compared to culture methods, qPCR-based detection has the advantage of having higher sensitivity.

False negative results caused by PCR inhibition and/or DNA extraction issues will be reliably identified by means of the Internal Control DNA. The Internal Control DNA is either added directly to the PCR master mix to function as a PCR amplification control, or is alternatively added to the original sample prior to DNA extraction. By adding the Internal Control DNA directly to the sample prior to DNA extraction, both DNA extraction and qPCR amplification are monitored. The amplification of the Internal Control DNA is detected at 560 nm (HEX™ channel), whereas the mycoplasma-specific amplification is detected at 520 nm (FAM™ channel).

The kit contains dUTP instead of dTTP to facilitate the degradation of amplicon carry-over by use of uracil-DNA glycosylase (UNG). Thus, the probability of false-positive results is minimized. Please note that UNG is not included in the Food Control™ qPCR kit.

## REAGENTS

Each kit contains reagents for 25 reactions. The expiry date of the unopened package is marked on the package label. The kit components must be stored at +2 to +8 °C until use.
The rehydrated components must be stored at ≤ -18 °C.

| Component | 25 reactions<br>Cat. No. 36x-x025 | Cap Color |
|---|---|---|
| Species qPCR Mix<br>(species-specific) | 1 vial, lyophilized | red |
| Rehydration Buffer | 1 vial, 1 ml | blue |
| Positive Control DNA | 1 vial, lyophilized | green |
| Internal Control DNA | 1 vial, lyophilized | yellow |
| PCR grade Water | 1 vial, 2 ml | white |

The lot-specific quality control certificate (Certificate of Analysis) can be downloaded from our website (www.minerva-biolabs.com / www.minervabiolabs.us).

## USER-SUPPLIED CONSUMABLES AND EQUIPMENT

The Food Control™ qPCR kit contains reagents for the specific detection of pathogens as indicated in **Table 1**. Additional consumables and equipment are supplied by the user:

- qPCR device with filter sets for detecting the fluorescence dyes FAM™ and HEX™
- PCR reaction tubes and caps for the specific qPCR device
- DNase-free 1.5 ml reaction tubes
- Microcentrifuge for 1.5 ml and 0.2 ml reaction tubes
- Pipettes with corresponding filter-tips (10, 100, and 1000 µl)

  Required for extraction:

- DNA extraction kit (e.g. ExtractNow™ Food Control kit, order No. 609-1050), or user-based method; Ethanol > 96 % abs.; Heat block
- Optional for carry-over prevention: Uracil DNA glycosylase (UNG)

## SAMPLES

Food samples should be collected according to local guidelines and recommended standard methods. Direct testing of food for contamination with pathogenic bacteria is hindered by the unfavorable food to bacterial DNA ratio. A pre-cultivation may help circumventing this issue and obtaining an enrichment of the contaminating pathogens in the sample. Usually, 25 g of food material are pre-cultured in a medium suitable for the pathogen of interest before DNA extraction. Direct testing of the culture media is also not recommended due to the potential presence or accumulation of PCR inhibiting substances in the media. Extracted DNA may be stored at +2 to +8 °C for up to 6 days or at ≤ –18 °C for long term storage.

## RECOMMENDATIONS

Optional: If further investigation is desired, we recommend using additional positive controls. These DNA samples can be purchased separately from Minerva Biolabs.

Food samples should be collected according to local standard methods and guidelines. This product is for *in vitro* use only. It can be used in research and industry for the detection of bacterial species in meat and other foods. Do not use for clinical or diagnostic application or for testing of patient samples.

## PRECAUTIONS

Food Control™ qPCR kit should be used by trained laboratory staff only. All samples should be considered potentially infectious and handled with all due care and attention. Always wear a suitable lab coat and disposable gloves. This kit does not contain hazardous substances. Waste is disposable according to local regulations.

## IMPORTANT NOTES

⇨ These instructions must be understood to successfully use the Food Control™ qPCR kit. The reagents supplied should not be mixed with reagents from different batches but used as an integral unit. The reagents of the kit must not be used beyond their shelf life.

⇨ Follow the exact protocol. Any deviations may affect the test method and results.

⇨ PCR inhibition is likely to be caused by the sample matrix, or, in case of extracted DNA, by the elution buffer. Thus, we recommend our ExtractNow™ Food Control kit for sample preparation. Any other DNA extraction kit needs to be qualified and validated.

⇨ It is important to include control samples on a regular basis to monitor the reliability of your results. Positive and negative controls are essential in case of troubleshooting.

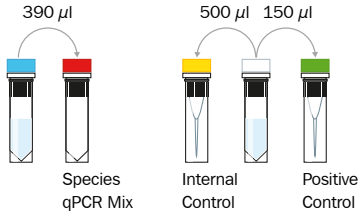
⇨ Set up at least one negative control sample (no template control, NTC) in each PCR. Use elution buffer for the NTC in case of extracted DNA.

⇨ The control samples must be processed in the same manner as the test samples. You may want to include other laboratory specific control samples such as high, median and low DNA levels.

## PROCEDURE – OVERVIEW

### 1. Reagent Preparation

- Species qPCR Mix
- Positive Control
- Internal Control




- for 5 min RT
- briefly
- for 5 sec

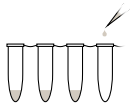
### 2. Reaction Mix Preparation !

**1 reaction**

15 µl Species qPCR Mix (red cap)
1 µl Internal Control (yellow cap)

### 3. Loading the Test Tubes

aliquot 15 µl
Reaction Mix



### 4. Adding Samples

+ 5 µl DNA extract
+ 5 µl Positive Control (green cap)
+ 5 µl Elution Buffer (Negative Control)

briefly

### 5. PCR Amplification


* see manual for details

! If Internal Control was already added during DNA extraction, skip step 2 and proceed directly to step 3 and aliquot 15 µl Species qPCR Mix (red cap).

- Rehydration Buffer
- Species qPCR Mix
- PCR grade water
- Positive Control
- Internal Control

- incubate
- vortex
- centrifuge
- add

storage 2-8 °C
after rehydration ≤ -18 °C

This procedure overview is not a substitute for the detailed manual.

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## PROCEDURE - STEP BY STEP

### 1. Reagents preparation

The test should be carried out with negative and positive controls and samples in duplicates. All reagents and samples must be equilibrated to +2 to +8 °C prior use. After reconstitution, the reagents should be stored at ≤ -18 °C. Repeated freezing and thawing should be avoided and reconstituted controls (Internal Control and Positive Control) should be stored in aliquots.

| | | | |
|---|---|---|---|
| 1. | Species qPCR Mix<br>Positive Control DNA<br>Internal Control DNA | red cap<br>green cap<br>yellow cap | Spin down all lyophilized components at max speed for 5 sec. |
| 2. | Species qPCR Mix | red cap | Add 390 µl Rehydration Buffer (blue cap) |
| 3. | Positive Control DNA | green cap | Add 150 µl PCR grade Water (white cap) |
| 4. | Internal Control DNA | yellow cap | Add 500 µl PCR grade Water (white cap) |
| 5. | Species qPCR Mix<br>Positive Control DNA<br>Internal Control DNA | red cap<br>green cap<br>yellow cap | Incubate at room temperature for 5 min |
| 6. | Species qPCR Mix<br>Positive Control DNA<br>Internal Control DNA | red cap<br>green cap<br>yellow cap | Vortex briefly and spin down for 5 sec |

### 2. PCR reaction mix preparation

The following steps (2. PCR reaction mix preparation, 3. Addition of samples and controls, and 4. PCR amplification) should be performed within 45 minutes to avoid a significant reduction in the fluorescence signal. We recommend following strictly this protocol and pipetting sequence:

Optional: To monitor the DNA extraction performance, the Internal Control DNA can be added to the original sample before beginning the extraction (at least 20 µl Internal Control DNA should be added to the sample). In this case, do not add the Internal Control DNA to the PCR reaction mix and proceed directly to step 2.2. by aliquoting 15 µl of Species qPCR Mix (red cap) to each PCR reaction tube.

| | | | | |
|---|---|---|---|---|
| 1. | Calculate the required volume of master mix for all the control and test reactions and prepare it as indicated below. Pipet and mix the components at room temperature in a 1.5 ml reaction tube. | | | |
| | | **Cap Color** | **for 1 reaction** | **for 25 reactions** |
| | Species qPCR Mix | red | 15 µl | 375 µl |
| | Internal Control DNA | yellow | 1 µl | 25 µl |
| 2. | Aliquot 15 µl of PCR master mix to each PCR tube, discard remaining material. | | | |

### 3. Addition of samples and controls

Add samples according to the following pipetting sequence:

| | |
|---|---|
| 1. | Negative Controls: add 5 µl of PCR grade Water or elution buffer from the DNA extraction kit. |
| 2. | Samples: add 5 µl of extracted DNA from meat or food samples. |
| 3. | Positive Control: add 5 µl of Positive Control DNA (green cap). |
| 4. | Close the PCR tubes tightly and spin down briefly |

### 4. PCR amplification

| | |
|---|---|
| 1. | Place the PCR tubes in the qPCR cycler and close the lid. |
| 2. | Program the qPCR cycler (see appendix I for detailed cycler programs of selected qPCR cyclers.<br>Programs for additional cyclers are available upon request). |
| 3. | Start the program. |

This assay has been successfully performed on the following qPCR devices:

| qPCR device | Manufacturer |
|---|---|
| CFX-96™ | Bio-Rad Laboratories |
| ABI Prism® 7500 | Applied Biosystems |
| Rotor-Gene® 6000 | Corbett Research |
| Mx3005P™ | Agilent Technologies |

## DATA INTERPRETATION

This method is qualitative due to the pre-culture step. The exact procedure for obtaining $C_t$-values including baseline calculation/normalization depends on the particular qPCR device and cycler control software. Please see the documentation of your device for further details. We recommend the assessment of the amplification curve progression of all samples including control samples.

A positive PCR is indicated by $C_t < 40$. PCR reactions with $C_t \geq 40$ are considered negative. In addition, a successful PCR is displayed by an increasing fluorescence signal in either the FAM™ or the HEX™ channel (given the Internal Control was added), or both. The presence of a contaminating pathogenic species is indicated by an increasing fluorescence signal in the FAM™ channel. The pathogenic bacterial DNA and Internal Control function as competitors in the PCR. Thus, the more DNA of the pathogen under investigation is in the sample, the higher the signal in the FAM™ channel and the lower the Internal Control signal in the HEX™ channel. The following table will help with the interpretation of PCR results:

| Detection of Pathogen FAM™ channel | Internal Control HEX™ channel | Interpretation |
|---|---|---|
| positive | irrelevant | Pathogen positive |
| negative | negative | PCR inhibition |
| negative | positive | Pathogen negative |

## ASSAY CHARACTERISTICS

### 1. Sensitivity

The detection limit was determined using dilution series of isolated foodborne pathogen DNA (as indicated in Table 1). Apart from *S.enterica*, all assays showed a sensitivity down to 10 genome copies per PCR. Sensitivity limit of *S.enterica* detection is 100 genome copies per PCR.

### 2. Specificity

The specificity of this assay was verified using DNA of the following selected relevant food contaminating species:

*Campylobacter coli* (DSM No.: 4689), *Campylobacter jejuni* (DSM No.: 4688), *Campylobacter lari* (DSM No.: 11375), *Clostridium perfringens* (DSM No.: 756), *Escherichia coli* (DSM No.: 498, 8579, 10806, 10809), *Salmonella enterica* (DSM No.: 17420, 17058), *Shigella flexneri* (DSM No.: 4782), *Shigella sonnei* (DSM No.: 5570), *Staphylococcus aureus* (DSM No.: 11822, 17091, 18586, 18587, 18588, 18589, 19040, 19045) and *Yersinia enterocolitica* (DSM No.: 11502 , 11503). *Listeria welshimeri* (DSM 20650), *Listeria monocytogenes* (DSM No.: 20600). No cross-reactivity was observed.

### 3. Temperature Profile of Different Bacteria Species

**Table 2.** Adjust your temperature profile according to your species of interest.

| Bacteria Species | Annealing Temperature °C |
|---|---|
| *S. enterica* | 53 |
| *Y. enterocolica* | 55 |
| *Shigella spp.* | 55 |
| *Campylobacter spp.* | 60 |
| *C. perfringens* | 55 |
| *Shiga Toxin 1*<br>*Shiga Toxin 2* | 60 |
| *E. coli O157* | 60 |
| *E. coli O104* | 60 |
| *Listeria spp.* | 53 |
| *L. monocytogenes* | 53 |
| *Salmonella spp.* | 60 |

## APPENDIX I

These protocols were created on the basis of in-house testing and customer reports. Minerva Biolabs does not warrant or assume responsibility for the performance of these protocols.

### Programming the LightCycler® 2.0

Program 1: Pre-incubation

| | |
|---|---|
| Cycles | 1 |
| Analysis Mode | None |
| ***Temperature Targets*** | ***Segment 1*** |
| Target Temperature [°C] | 95 |
| Incubation time [min] | 5:00 |
| Temperature Transition Rate [°C/s] | 20.0 |
| Secondary Target Temperature [°C] | 0 |
| Step Size [°C] | 0.0 |
| Step Delay [Cycles] | 0 |
| Acquisition Mode | None |

Program 2: Amplification

| | | |
|---|---|---|
| Cycles | 45 | |
| Analysis Mode | Quantification | |
| ***Temperature Targets*** | ***Segment 1*** | ***Segment 2*** |
| Target Temperature [°C] | 95 | ×× **(s. Table 2)** |
| Incubation time [s] | 30 | 45 |
| Temperature Transition Rate [°C/s] | 20.0 | 20.0 |
| Secondary Target Temperature [°C] | 0 | 0 |
| Step Size [°C] | 0.0 | 0.0 |
| Step Delay [Cycles] | 0 | 0 |
| Acquisition Mode | None | Single |

Program 3: Cooling

| | |
|---|---|
| Cycles | 1 |
| Analysis Mode | None |
| ***Temperature Targets*** | ***Segment 1*** |
| Target Temperature [°C] | 40 |
| Incubation time [s] | 30 |
| Temperature Transition Rate [°C/s] | 20.0 |
| Secondary Target Temperature [°C] | 0 |
| Step Size [°C] | 0.0 |
| Step Delay [Cycles] | 0 |
| Acquisition Mode | None |

Result Reading:

- Select the fluorescence channels 1 and 2
- Click on *Quantification* to generate amplification plots and $C_t$-values
- The threshold will be generated automatically.
- Samples showing no significant increase in the amplification plot can be considered as negative.

**Programming of RotorGene® 6000 (5-plex)**

Program Step 1: Pre-incubation

| | |
|---|---|
| Setting | Hold |
| Hold Temperature | 95 °C |
| Hold Time | 5 min 0 sec |

Program Step 2: Amplification

| | |
|---|---|
| Setting | Cycling |
| Cycles | 45 |
| Denaturation | 95 °C for 30 sec |
| Annealing | ×× °C for 45 sec |
| | → **acquiring to Cycling A (green and yellow) (s. Table 2)** |
| Gain setting | automatic (auto Gain) |
| Slope Correct | activated |
| Ignore First | deactivated |

**Please check the correct settings for the filter combination:**

| | |
|---|---|
| **green filter (510):** | **Pathogen-Species** |
| **yellow filter (555):** | **Internal Control** |

Result Reading:

- Open the menu *Analysis*
- Select *Quantitation*
- Check the required filter set (green and yellow) according to the following table and start data analysis by double click.
- The following windows will appear:

  *Quantitation Analysis - Cycling A* (green or yellow)

  *Quant. Results - Cycling A* (green or yellow)

  *Standard Curve - Cycling A* (green or yellow)
- In window *Quantitation Analysis*, select first linear scale and then slope correct
- Threshold setup (not applicable if a standard curve was included in parallel and auto threshold was selected)

  In window *CT Calculation* set the threshold value to 0-1

  Pull the threshold line into the graph. Adapt the threshold line to the initial linear section of the positive control reaction.
- The Ct-values can be taken from the window *Quant. Results.*

**Programming of ABI Prism® 7500**

Detector Settings:

| | |
|---|---|
| Target Probe: | Reporter - FAM™ / Quencher - none |
| Internal Control Probe: | Reporter - HEX™ / Quencher - none |

The “ROX Reference” function needs to be disabled, as no ROX dye is included in the mix. Activate both detectors for each well.
Measure fluorescence during annealing.

Program Step 1: Pre-incubation

| | |
|---|---|
| Setting | Hold |
| Temperature | 95 °C |
| Incubation time | 5:00 min |

Program Step 2: Amplification

| | |
|---|---|
| Cycles | 45 |
| Setting | Cycle |
| Denaturing | 95 °C for 30 sec |
| Annealing | ×× °C for 45 sec & data reading **(s. Table 2)** |

Result Reading:

- Enter the following basic settings at the right task bar:

  | | |
  |---|---|
  | Data: | Delta RN vs. Cycle |
  | Detector: | FAM™ and HEX™ |
  | Line Color: | Well Color |

- Open a new window with for the Graph settings by clicking the right mouse button
  Select the following setting and confirm with ok:

  | | |
  |---|---|
  | Real Time Settings: | Linear |
  | Y-Axis Post Run Settings: | Linear and Auto Scale |
  | X-Axis Post Run Settings: | Auto Scale |
  | Display Options: | 2 |

- Initiate the calculation of the Ct-values and the graph generation by clicking on Analyze within the report window.
- Pull the threshold line into the graph. Adapt the threshold line to the initial linear section of the positive control reaction.
- Samples showing no Ct-value can be considered as negative.

**Programming the Mx3005P®**

- Go to the setup menu, click on *„Plate Setup“*, check all positions which apply
- Click on *„Collect Fluorescence Data“* and check FAM and HEX
- Corresponding to the basic settings the *„Reference Dye“* function should be deactivated
- Specify the type of sample (negative or positive control, sample, standard) at *„well type“*
- Edit the temperature profile at *„Thermal Profile Design“:*

| | |
|---|---|
| Segment 1: | 95 °C for 5 min |
| Segment 2: | |
| Denaturing | 95 °C for 30 sec |
| Annealing | ×× °C for 45 sec & data collection end **(s. Table 2)** |
| 45 cycles | |

- at menu *„Run Status“* select *„Run“* and start the cycler by pushing *„Start“*

**Analysis of raw data:**

- In the window *„Analysis“* tab on *„Analysis Selection / Setup“* to analyze the marked positions
- Ensure that in window *„algorithm enhancement“* all options are activated:
  Amplification-based threshold
  Adaptive baseline
  Moving average
- Click on *„Results“* and *„Amplification Plots“* for an automatic threshold
- Read the $C_t$ values at *„Text Report“*

**Programming the LC 480**

Program 1: Pre-incubation

| | |
|---|---|
| Cycles | 1 |
| Analysis Mode | None |
| ***Temperature Targets*** | ***Segment 1*** |
| Target Temperature [°C] | 95 |
| Incubation time [min] | 5:00 |
| Temperature Transition Rate [°C/s] | 4.4 |
| Secondary Target Temperature [°C] | 0 |
| Step Size [°C] | 0.0 |
| Step Delay [Cycles] | 0 |
| Acquisition Mode | None |

Program 2: Amplification

| | | |
|---|---|---|
| Cycles | 45 | |
| Analysis Mode | Quantification | |
| ***Temperature Targets*** | ***Segment 1*** | ***Segment 2*** |
| Target Temperature [°C] | 95 | ×× **(s. Table 2)** |
| Incubation time [s] | 30 | 45 |
| Temperature Transition Rate [°C/s] | 4.4 | 2.2 |
| Secondary Target Temperature [°C] | 0 | 0 |
| Step Size [°C] | 0.0 | 0.0 |
| Step Delay [Cycles] | 0 | 0 |
| Acquisition Mode | None | Single |

Program 3: Cooling

| | |
|---|---|
| Cycles | 1 |
| Analysis Mode | None |
| ***Temperature Targets*** | ***Segment 1*** |
| Target Temperature [°C] | 40 |
| Incubation time [s] | 30 |
| Temperature Transition Rate [°C/s] | 2.2 |
| Secondary Target Temperature [°C] | 0 |
| Step Size [°C] | 0.0 |
| Step Delay [Cycles] | 0 |
| Acquisition Mode | None |

**Before starting the LC480, make sure that the filter setting is correct:**

| LightCycler 480 | Pathogen | Internal Control |
|---|---|---|
| Instrument I | 533 nm | 568 nm |
| Instrument II | 510 nm | 580 nm |

**Programming the CFX 96 Touch, CFX96 Touch Deep Well, CFX Connect, and CFX384 Touch (Bio-Rad)**

**Performing Runs**

*Run Setup - Protocol Tab*

- Click **Create New** to open the Protocol Editor to create a new protocol.
- Select any step in either the graphical or text display. The selected step becomes highlighted in blue. Click the temperature or incubation time to directly edit the value.

| | Step 1 | Step 2 | Step 3 | Step 4 |
|---|---|---|---|---|
| Temperature | 95.0 °C | 95.0 °C | ×× °C **(s. Table 2)** | GO TO STEP 2 |
| Incubation time | 05:00 min | 00:30 sec | 00:45 sec | × 45 |

*Run Setup - Plate Tab*

- Click **Create New** to open the Plate Editor to create a new plate.
- Use the **Scan Mode** dropdown menu in the Plate Editor toolbar to designate the data acquisition mode to be used during the run. Important!!! Select the **All Channels** mode.
- Click the **Select Fluorophores** button to indicate the fluorophores that will be used in the run.
- Select the wells to be loaded within the plate diagram.

*Run Setup – Start Run Tab*

- View the selected Protocol file, Plate file, and data acquisition Scan Mode setting in the **Run Information** pane.
- Select one or more blocks and edit run parameters if necessary in Start Run on Selected Block(s) pane.
- Click the Start Run button to begin the run.

**Data Analysis**

*Quantification Tab*

The amplification chart data in this tab display the relative fluorescence (RFU) collected from each well at every cycle of the run.

- Choose the fluorophore data you want to display by clicking the fluorophore checkboxes located under the amplification chart.

*Data Analysis Settings*

- The Software uses two modes for quantification cycle determination. Select **Settings** from the menu bar and select **Baseline Subtracted Curve Fit** as baseline setting and **Single Threshold Mode** as Cq Determination Mode.
- In the **Single Threshold Mode**, click and drag the threshold line to manually position the line. Adapt the threshold line to the initial linear section of the positive control reaction.
- Samples showing no $C_t$-value can be considered as negative.

## APPENDIX II

*Limited Product Warranty*

This warranty limits our liability for replacement of this product. No warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided. Minerva Biolabs shall have no liability for any direct, indirect, consequential, or incidental damages arising from of the use, the results of use, or the inability to use this product.

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## Related Products

| | | |
|---|---|---|
| **qPCR Kits for Food Contamination Testing** | | |
| 36X-X025 | Food Control™ qPCR | 25 reactions |
| **qPCR Kits for Meat Identification** | | |
| 370-1025/-1100 | Meat ID™ Halal | 25/100 reactions |
| **qPCR Kits for Vegan Control** | | |
| 370-2025/-2100 | Vegan Control™ | 25/100 reactions |
| **qPCR Kits for Water Contamination Testing** | | |
| 33-2025/-2100/-2250 | AquaScreen® Legionella species | 25/100/250 reactions |
| 34-2025/-2100/-2250 | AquaScreen® Legionella pneumophila | 25/100/250 reactions |
| 34-6025/-6100/-6250 | AquaScreen® Pseudomonas aeruginosa | 25/100/250 reactions |
| 34-7025/-7100/-7250 | AquaScreen® Escherichia coli | 25/100/250 reactions |
| **DNA Extraction kits** | | |
| 609-1010/1050 | ExtractNow™ Food Control | 10/50 extractions |
| 607-1010/1050 | ExtractNow™ Vegan Control | 10/50 extractions |
| 608-1010/1050 | ExtractNow™ Meat ID | 10/50 extractions |
| 601-1010/1050 | ExtractNow™ DNA Mini Kit | 10/50 extractions |
| 602-1010/1050 | ExtractNow™ Blood DNA Mini kit | 10/50extractions |
| 603-1010/1050 | ExtractNow™ RNA Mini kit | 10/50 extractions |
| 604-1010/1050 | ExtractNow™ Cleanup kit | 10/50extractions |
| 605-1010/1050 | ExtractNow™ Plasmid Mini kit | 10/50 extractions |
| 606-1010/1050 | ExtractNow™ Virus DNA/RNA kit | 10/50 extractions |
| 56-1010/1050/1200 | Venor®GeM Sample Preparation Kit | 10/50/200 extractions |
| **PCR Cycler Validation** | | |
| 57-2102 | PCR Cycler Check™ Advance | 6 strips, 8 vials each |
| 57-2103 | PCR Cycler Check™ OneStep | 100 reactions |
| 57-2202 | qPCR Cycler Check™ | 100 reactions |
| **Lab Monitoring Kits** | | |
| 181-0010/-0050 | SwabUp™ Lab Monitoring, For sample collection and DNA extraction | 10/50 samples |
| **PCR Mix** | | |
| 191-0025/-0100/-0250 | ConviFlex™ DNAmp Mix, PCR Mix with Taq polymerase for conventional and qPCR | 25/100/250 reactions |
| 192-0025/-0100/-0250 | ConviFlex™ RT-Taq Mix, RT-PCR Mix with Taq polymerase and retrotrascriptase for conventional and RT-qPCR | 25/100/250 reactions |
| **PCR Clean™** | | |
| 15-2025/-2200 | Decontamination Reagent, spray bottle/refill canister | 250 ml/4×500 ml |
| 15-2500 | Decontamination Reagent, refill canister | 5 l |
| 15-2001 | Decontamination Reagent, Wipes in dispenser box | 50 wipes |
| 15-2002 | Decontamination Reagent, Wipes, refill pack | 5 × 50 wipes |
| **LabClean™** | | |
| 15-4100 | Molecular biology lab cleaner | 1 l |
| **WaterShield™** | | |
| 15-3015/3020/3050 | Water Disinfection Additive for incubators | 30 × 5 ml/3 × 50 ml/500 ml |

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