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.

Order No.

Expiry date

Storage temperature

Number of reactions

Manufacturer

INDICATION

Food Control™ qPCR is a PCR kit for the 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 agricultural or the food industry via real-time PCR.

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

Table 1.

Pathogen Catalog No.	Description
S. enterica **11-02-01-025**	*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 most often after eating chicken products (including eggs) or (raw) meat. In addition, poor hygiene significantly increases the risk of infection. The Assay detects the *Salmonella enterica* specific sequence of invasion protein (invA) gene.
Y. enterocolica **11-02-02-025**	*Yersinia enterocolitica* is a gram-negative, non-spore-forming bacterium forming 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. The Assay detects the *Yersinia enterocolitica* specific sequence of heat-stable enterotoxin A gene.
Shigella spp. **11-02-03-025**	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 important species are *Shigella flexneri* and *Shigella sonnei.* The target sequence of this assay is a gene specific for both of these species. The Assay detects the *Shigella* genus specific sequence of invasion plasmid antigen (ipaH6) gene.

Campylobacter spp. **11-02-04-025**	*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. The Assay detects the *Campylobacter* genus specific sequence of acyl-[acyl-carrier-protein]--UDP-N-acetylglucosamine O-acyltransferase (lpxA) gene.
C. perfringens **11-02-05-025**	*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. The Assay detects the *Clostridium perfringens* specific sequence of phospholipase C alpha-toxin (plc) gene.
Shiga Toxin 1 ***Shiga Toxin 2*** **11-02-06-025** **11-02-07-025**	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. 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 **11-02-08-025**	*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. The Assay detects the *E. coli O157* specific sequence of wbdR gene.
E. coli O104 **11-02-09-025**	*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. The Assay detects the *E. coli O104* specific sequence of wckD gene.

Listeria spp. **11-02-10-025**	*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. The Assay detects the *Listeria* genus specific DNA sequence of invasion associated protein p60 (iap) gene.
L. monocytogenes **11-02-11-025**	*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. The Assay detects the *Listeria monocytogenes* specific sequence of listeriolysin O (hly) gene.
Salmonella spp. **11-02-12-025**	*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. 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 amplifying the parameters 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 as well as the dual labeling of the probes with fluorescents and quenchers. During PCR the Taq polymerase cleaves and removes annealed probes releasing the previously quenched fluorescent signal.

The user instructions include protocols for analysis of DNA extracted from potentially contaminated food samples (e.g. by using ExtractNow™ Food Control Cat. No. 609-1050 or by using user provided method), after pre-cultivation of the sample in growth medium according to pathogen specific local, regional, national, or international regulations. The entire test needs approximately 90 minutes excluding pre-cultivation and DNA extraction, and, in contrast to culture methods, detection by PCR is considered to be superior in terms of 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, both DNA extraction and qPCR amplification are validated. The internal control amplification is detected at 560 nm (HEX channel), whereas the pathogen-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 Cat. No. 11-02-XX-025	Cap Color
Species qPCR Mix	1Vial, freeze-dried	red
Rehydration Buffer	1 Vial, 1 ml	blue
Positive Control DNA	1 Vial, freeze-dried	green
Internal Control DNA	1 vial, freeze-dried	yellow
PCR grade water	1 vial, 2 ml	white

The lot specific Certificate of Analysis (CoA) can be downloaded from our website (www.minerva-biolabs.com).

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 method, Ethanol > 96 % abs., heat block
- Optional for carry-over prevention: Uracil DNA glycosylase (UNG)
- For more details on pre-culture and sample preparation download the application note from our website (www.minerva-biolabs.com).

SAMPLES

Food samples should be collected according to local standard methods and guidelines. Direct testing of food samples for contamination with pathogenic bacteria is not possible due to an unfavorable food vs. bacterial DNA ratio upon direct extraction. Therefore, a pre-cultivation may be necessary to enrich the contaminating pathogens in the sample. Usually, 25 g of food-material is pre-cultured in a medium suitable for the pathogen under scrutiny before DNA extraction using a suitable method. Direct testing of the cultivation media is not recommended because PCR inhibiting substances may accumulate or be contained 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.

For more details regarding sample preparation and cultivation download the Technical Note from our website, www.minerva-biolabs.com.

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.

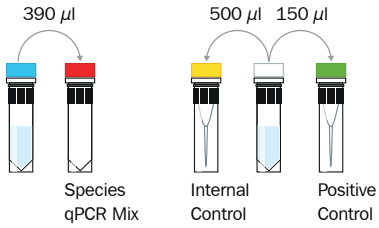
⇨ Set up at least one negative control sample (non-template control, NTC) in each PCR. Use elution buffer for the NTC in case of extracted DNA.

⇨ 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



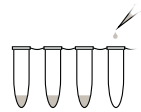
- for 5 min RT
- briefly
- for 5 sec

2. Reaction Mix Preparation !

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

3. Loading the Test Tubes

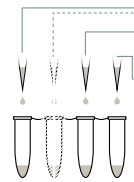
aliquot 15 µl
Reaction Mix



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.

PROCEDURE - STEP BY STEP

1. Reagent 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 Positive Control DNA Internal Control DNA	red cap green cap yellow cap	Spin down all freeze-dried 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 Positive Control DNA Internal Control DNA	red cap green cap yellow cap	Incubate at room temperature for 5 min
6.	Species qPCR Mix Positive Control DNA Internal Control DNA	red cap green cap yellow cap	Vortex briefly and spin down for 5 sec

2. Preparation of PCR reaction mix

The following steps (Reaction mix preparation, adding samples and starting the PCR reaction) should be done in less than 45 minutes to avoid a reduction in the fluorescence signal. Follow these schemes and pipetting sequence to set up the test:

Optional: for verification of the DNA extraction step, Internal Control DNA can be added to the original sample prior extraction (at least 20 µl Internal Control DNA should be added to the sample). In this case, do not add further Internal Control DNA to the PCR mastermix, proceed directly to step 2 and aliquot 15 µl of Species qPCR Mix (red cap) to each PCR reaction tube.

1.	Prepare the required volume of PCR master mix for the number of samples you wish to test. Mix the kit components at room temperature in a 1.5 ml reaction tube for all control and test reactions.			
		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. Adding samples

Add samples according to the following pipetting sequence:

1.	Negative Controls: add 5 µl H_2O or elution buffer from DNA extraction kit
2.	Samples: add 5 µl of extracted DNA from meat or food sample
3.	Positive Control: add 5 µl Positive Control DNA (green cap)
4.	Close the PCR tubes tightly and spin down briefly

4. Start PCR amplification

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

This assay was tested on the following qPCR devices:

qPCR device	Manufacturer
CFX-96™	Bio-Rad Laboratories
ABI Prism® 7500	Applied Biosystems
RotorGene® 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 *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 C_t-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 C_t-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.

- 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.

Trademarks

LightCycler is a registered trademark of a member of the Roche Group. ABI Prism is a registered trademark of Applera Corporation or its subsidiaries in the US and certain other countries. CFX96 Touch, CFX96 Touch Deep Well, CFX Connect, and CFX384 Touch are trademarks of Bio Rad Laboratories. TaqMan is a registered trademark of Roche Molecular Systems, Inc. FAM, and HEX are trademarks of Applied Biosystems LLC. Venor is a registered trademark and Food Control, Meat ID, Vegan Control, ExtractNow, PCR Clean, Lab Clean, WaterShield, ConviFlex, SwabUp, and Cycler Check are trademarks of Minerva Biolabs GmbH, Germany.

Related Products

qPCR Kits for Food Control		
11-02-XX-025	Food Control™ qPCR	25 reactions
11-03-XX-025	Food Contro™ LFA	25 tests
11-04-XX-025	Food Control™ LFA+	25 tests
qPCR Kits for Meat Identification		
12-02-025/-100	Meat ID™ Screen	5/20/40 reactions
12-02-025/-100	Meat ID™ Halal	25/100 tests
qPCR Kits for Vegan Control		
12-05-025/-100	Vegan Control™	25/100 reactions
DNA Extraction kits		
56-1010/1050/1200	Venor®GeM Sample Preparation Kit	10/50/200 extractions
56-2096	Venor®GeM Sample Preparation Kit - IP C16	96 extractions
601-1010/1050/1200	ExtractNow™ DNA Mini Kit	10/50/200 extractions
602-1010/1050/1200	ExtractNow™ Blood DNA Mini kit	10/50/200 extractions
603-1010/1050/1200	ExtractNow™ RNA Mini kit	10/50/200 extractions
604-1010/1050/1200	ExtractNow™ Cleanup kit	10/50/200 extractions
605-1010/1050/1200	ExtractNow™ Plasmid Mini kit	10/50/200 extractions
606-1010/1050/1200	ExtractNow™ Virus DNA/RNA kit	10/50/200 extractions
607-1010/1050	ExtractNow™ Vegan Control	10/50 extractions
608-1010/1050	ExtractNow™ Meat ID	10/50 extractions
609-1010/1050	ExtractNow™ Food Control	
PCR Clean™ (formerly DNA Remover™)		
15-2025/2200	DNA Decontamination Reagent, spray bottle/refill bottles	250 ml/4x 500 ml
15-2201	Wipes	120 wipes in a dispenser box
15-2202	Wipes, refill packs	5 x 120 wipes in a bag
15-2203	Wipes, single wrapped	30 Sachets
Lab Clean™		
15-4100	Molecular Microbiology Lab Cleaner	1 liter
WaterShield™		
15-3015/3020/3050	Water Disinfection Additive for incubators	30 x 5 ml/3 x 50 ml/500 ml
ConviFlex™ DNAmp Mix		
191-025/100/250	PCR Mix with Taq polymerase for conventional and qPCR	25/100/250 reactions
SwabUp™ Lab Monitoring Kits		
181-0010/0050	Sample collection and DNA extraction	10/50 samples
182-0010/0050	Sample collection, DNA extraction and PCR system	10/50 samples
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

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