Instructions for Use

Microsart® RESEARCH Bacteria

Bacteria Detection Kit for qPCR

Prod. No. SMB95-1009

Reagents for 25 reactions

For use in research

Manufactured by:

Minerva Biolabs GmbH
Koepenicker Strasse 325
12555 Berlin
Germany

Symbols

LOT	**Lot No.**
REF	**Order No.**
	**Expiry date**
	**Store at**
	**Contains reagents for 25 tests**
	**Manufacturer**

Contents

1. Intended Use

Microsart® RESEARCH Bacteria is used for direct detection of bacterial contamination in cell cultures and cell media components in research and development.

2. Explanation of the Test

Microsart® RESEARCH Bacteria utilizes real-time PCR (qPCR). The assay can be performed with any type of real-time PCR cycler able to detect the fluorescence dyes FAM™ and ROX™. The protocol provided is preferred for fast and reliable screening of cell culture supernatants most applicable in research and development. The detection procedure can be performed within three hours. In contrast to the culture method, samples do not need to contain vital bacteria.

3. Test Principle

Bacteria are specifically detected by amplifying a highly conserved rRNA operon, or more specifically, a 16S rRNA coding region in the bacterial genome. The amplification is detected at 520 nm (FAM™ channel). The kit includes primer and FAM™ labeled probes which allow the specific detection of many bacterial species so far described as contaminants of cell cultures and media components. Eukaryotic DNA is not amplified by this primer/probe system.

False negative results due to PCR inhibitors or improper DNA extraction are detected by the internal amplification control which is part of the Bacteria RESEARCH Mix. The amplification of the internal amplification control is detected at 610 nm (ROX™ channel).

4. Notes on the Test Procedure

1. For in vitro use in research. This kit may be disposed of according to local regulations.

2. This kit should be used only by trained persons. You should wear a clean lab coat and use disposable gloves at all times while performing the assay.

3. To avoid DNA cross-contaminations, the complete test must be performed under sterile and DNA-free conditions.

4. Always use a new unopened DNA-free pipette filter tip-box for each assay. Reaction vials should always be closed immediately after every pipetting step.

5. It is recommended to perform the assay in a predecontaminated, UV-treated laminar flow cabinet. Spatial segregation of the sequential steps is recommended.

6. In case of working with living bacteria strains, the local regulatory requirements for S2 labs must be considered.

7. Attention: by aliquoting and freezing your samples you run a high risk of contamination. This should therefore be avoided if possible.

8. This leaflet must be widely understood for a successful use of Microsart® RESEARCH Bacteria. The reagents supplied should not be mixed with reagents from different lots but used as an integral unit. The reagents of the kit should not be used beyond their shelf life.

9. Any deviation from the test method can affect the results.

10. Inhibition may be caused by the sample matrix, but also by sample elution buffer of DNA extraction kits which are not recommended or validated. Please note that by using DNA extraction kits which are not validated you run a high risk of obtaining false-positive or false-negative results.

11. For each test setup, at least one negative control should be included. Positive controls facilitate the evaluation of the test. Typical Ct values for the internal control and positive control are shown on the Certificate of Analysis and can be used as a guideline for quality control.

12. The use of control samples is advised to secure the day-to-day validity of results. The controls should be carried out in the same manner as the samples.

4.1 Handling and Equipment Recommendations

1. The clean bench should be cleaned thoroughly with PCR Clean™ (Minerva Biolabs, Prod. No. 15-2025) or PCR Clean™ Wipes (Minerva Biolabs, Prod. No. 15-2001) before use.

2. All materials which are introduced into the clean bench should be cleaned thoroughly with PCR Clean™ prior the process.

3. Avoid working above open tubes and avoid air turbulences due to rapid movements.

4. Be careful when opening the tubes. Do not touch the inner surface of the lid.

5. Reagents

Each kit contains reagents for 25 reactions. The expiry date of the unopened package is marked on the package label. The kit components are stored until use at +2 to +8 °C and must be stored at ≤ -18 °C after rehydration. Protect the Bacteria RESEARCH Mix from light.

Kit Component Label Information	Quantity 25 Reactions Order No. SMB95-1009	Cap Color
Bacteria RESEARCH Mix	1 × lyophilized	red
Rehydration Buffer	1 × 1.0 ml	blue
Positive Control DNA	1 × lyophilized	green
PCR grade Water	1 × 1.5 ml	white

The lot specific Certificate of Analysis can be downloaded from the manufacturer's website (www.minerva-biolabs.com).

6. Needed but not Included

Microsart® RESEARCH Bacteria contains the reagents for the specific detection of bacteria. General industrial supplies and reagents, usually available in PCR laboratories are not included:

Consumables

- Laboratory gloves
- PCR Clean™ (Minerva Biolabs, Prod. No. 15-2025) and PCR Clean™ wipes (Minerva Biolabs, Prod. No. 15-2001)
- DNA-free PCR reaction tubes (PCR 8-SoftStrips with attached caps from Biozym are recommended: 0.1 ml Low Profile, Prod. No. 710975 and 0.2 ml High Profile, Prod. No. 710970)
- DNA-free pipette filter tips that must be free from bacterial DNA (Biosphere® filter tips from Sarstedt are recommended: 0.5-20 µl, Prod No. 70.1116.210; 2-100 µl, Prod No. 70.760.212; 20-300 µl, Prod. No. 70.765.210; 100-1000 µl. Prod. No. 70.762.211)
- Optional: Microsart® Bacteria Extraction kit, a DNA-free extraction kit, Sartorius Prod. No. SMB95-2001.

Equipment

- qPCR device with filter sets for the detection of the fluorescence dyes FAM™ and ROX™ and suitable for 25 µl PCR reaction volumes
- Minicentrifuge for 2ml reaction tubes and PCR-tubes
- Vortex mixer
- Heat-block
- Pipettes (Sartorius)

mechanical	0.5 – 10 µl Sartorius Prod. No. LH-729020
	10 – 100 µl Sartorius Prod. No. LH-729050
	100 – 1000 µl Sartorius Prod. No. LH-729070
or electrical	0,2 – 10 µl Sartorius Prod. No. 735021
	10 – 300 µl Sartorius Prod. No. 735061
	50 – 1000 µl Sartorius Prod. No. 735081

7. Specimen

Microsart® RESEARCH Bacteria does not require DNA extraction prior use. Samples can be obtained directly from cell culture supernatants.

Samples directly received from cell cultures contain DNases which can degrade bacterial DNA even at lower temperatures. If the test cannot be performed immediately after sampling, samples should be stabilized by heat inactivation at 95 °C for 10 min and stored at ≤ - 18 °C until use.

1. Transfer up to 500 µl of cell culture supernatant or cell culture material with up to 10^6 cells/ml to a sterile micro centrifuge tube. The lid should be tightly sealed to prevent opening during heating.
2. Incubate the sample at 95 °C for 10 minutes.
3. Briefly centrifuge (5 seconds) the sample at approx. 13,000 x g to pellet cellular debris.
4. The supernatant is used for PCR analysis.

If you detect any inhibitory effects, DNA extraction i.e. with Microsart® Bacteria Extraction (Prod No. SMB95-2001) is mandatory. 2 µl of the extract can be used directly as PCR template.

Repeated freezing and thawing of samples should be avoided.

8. Test Procedure

The test should be carried out with negative and positive controls and samples in duplicates. For quantification, set up a dilution series of an appropriate standard. For this, we recommend Microsart® Calibration Reagents (see Related Products for ordering information). All reagents and samples must be equilibrated to +2 to +8 °C prior use.

8.1 Rehydration of the reagents

After reconstitution, the reagents should be stored at ≤ -18 °C. Repeated freezing and thawing should be avoided and the reconstituted Positive Control must be stored in aliquots.

1. Bacteria RESEARCH Mix Positive Control DNA	red cap green cap	Spin all lyophilized components for 5 sec at maximum speed of the microcentrifuge.
2. Bacteria RESEARCH Mix Positive Control DNA	red cap green cap	Add 600 µl Rehydration Buffer (blue cap). Add 300 µl PCR grade water (white cap).
3. Bacteria RESEARCH Mix Positive Control DNA	red cap green cap	Incubate 5 min at room temperature.
4. Bacteria RESEARCH Mix Positive Control DNA	red cap green cap	Vortex briefly

8.2 Loading the test tubes

This process should not take more than 45 minutes to avoid a reduction in the fluorescent signal. The pipetting sequence should be respected and the tubes closed after each sample load.

1. Homogenize the rehydrated Bacteria RESEARCH Mix by vortexing. Spin briefly.
2. Add 23 µl to each PCR tube.
3. Negative control: Add 2 µl PCR grade Water (white cap)
4. Sample reaction: Add 2 µl of sample.
5. Positive control: Add 2 µl Positive Control DNA (green cap).
6. Close and spin all PCR tubes briefly, load the qPCR cycler and start the program.

8.3 Starting the reaction

1. Load the cycler, check each PCR tube and the cycler lid for tight fit.
2. Program the qPCR cycler or check stored temperature profiles.
   See Appendix for temperature profiles of selected qPCR cyclers.
3. Start the program and data reading.

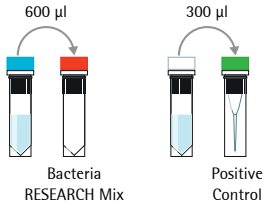
8.4 Analysis

1. Save the data at the end of the run.
2. Show amplification plots for FAM™ and ROX™ in linear mode.
3. Adapt the threshold line to 10 % of the maximum fluorescence level of the positive controls (in case of double determination take the average of the maximum fluorescence levels) (See chapter 11).
4. Read the calculation of the Ct-values for the negative controls, the positive controls and the samples.

9. Short Instructions

1. Rehydration of Reagents

Bacteria RESEARCH Mix and Positive Control

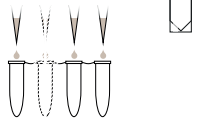


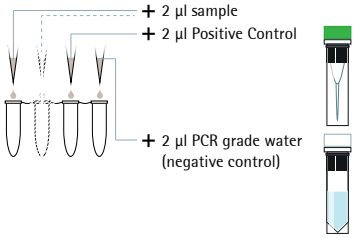
for 5 min RT
briefly
for 5 sec

2. Preparation of PCR Reactions

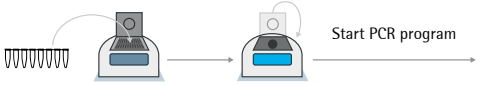
loading the test tubes

+ 23 µl Bacteria RESEARCH Mix

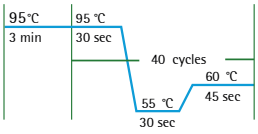




3. Starting the PCR Reaction



Start PCR program



Rehydration Buffer
Bacteria RESEARCH Mix
PCR grade water
Positive Control

incubate
vortex
centrifuge
add

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

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

10. Interpretation of Results

The presence of bacterial DNA in the sample is indicated by an increasing fluorescence signal in the FAM™ channel during PCR. The concentration of the contaminant can be calculated by a software comparing the crossing cycle number of the sample with a standard curve created in the same run.

A successfully performed PCR without inhibition is indicated by an increasing fluorescence signal in the internal control channel (ROX™). Bacterial DNA and Internal Control DNA are competitors in PCR. Because of the very low concentration of Internal Control DNA in the PCR mix, the signal strength in this channel is reduced with increasing bacteria DNA loads in the sample.

10.1 Yes/No Evaluation

Detection of Bacteria FAM™ channel	Internal Control ROX™ channel	Interpretation
positive (Ct < 40)	irrelevant	Bacteria positive
negative (no Ct)	negative **	PCR inhibition *
negative (no Ct)	positive (Ct < 40)	Bacteria negative

* PCR inhibition might be caused by sample matrix. If one out of two replicates is negative for Internal Control (ROX™: No Ct), repeat the PCR. If two out of two replicates are negative for the Internal Control, extract DNA from your sample material and repeat the PCR.
** Internal control of bacteria negative samples (FAM™: no Ct) must show Ct-values in the range of +/- 2 cycles (ROX™) of the no-template control (master mix control).

10.2 Total Analysis and recommended actions

Sample	Expected Outcome	Unexpected Outcome	Action
NTC	negative	NTC positive	Repeat PCR
PC	positive	PC negative	Repeat PCR
Specimen	0/2 positive	Product release	
	1/2 positive	Repeat the whole process New results:	
		0/2 positive	product release
		1/2 positive	Low contamination
		2/2 positive	Contamination
	2/2 positive	Contamination	

In case you want to identify a positive result, please send your PCR product to Minerva Biolabs GmbH. The PCR product will be purified by Minerva Biolabs. Sequencing will be done by an external sequencing service. The interpretation of your sequencing results will be supplied by Minerva Biolabs afterwards.
Attention: in case of a light or multiple contamination, the sequencing analysis might lead to wrong identification.

11. Appendix

The assay of this kit can be performed with any type of real-time PCR cycler able to detect the fluorescence dyes FAM™ and ROX™. The following qPCR cyclers were successfully tested with the Microsart® RESEARCH Bacteria kit:

Mx3005P®, CFX96 Touch™, CFX96 Touch Deep Well™, LightCycler® 480 II, ABI, Rotor-gene, LightCycler I and II

Programming and Data Recording of Different qPCR Devices

LightCycler® 1.0 and 2.0

Program 1: Pre-incubation	
Cycles	1
Analysis Mode	None
Temperature Targets	**Segment 1**
Target Temperature [°C]	95
Incubation time [s]	180
Temperature Transition Rate [°C/s]	20.0
Secondary Target Temperature [°C]	0
Step Size [°C]	0.0
Step Delay [Cycles]	0
Acquisition Mode	None

Important for LC 2.0:
Please check the correct settings for the “seek temperature“ of at least 90° C.

Program 2: Amplification			
Cycles	40		
Analysis Mode	Quantification		
Temperature Targets	**Segment 1**	**Segment 2**	**Segment 3**
Target Temperature [°C]	95	55	60
Incubation time [s]	30	30	45
Temperature Transition Rate [°C/s]	20.0	20.0	20.0
Secondary Target Temperature [°C]	0	0	0
Step Size [°C]	0	0	0
Step Delay [Cycles]	0	0	0
Acquisition Mode	None	None	Single

Program 3: Cooling	
Cycles	1
Analysis Mode	None
Temperature Targets	**Segment 1**
Target Temperature [°C]	40
Incubation time [s]	60
Temperature Transition Rate [°C/s]	20.0
Secondary Target Temperature [°C]	0
Step Size [°C]	0
Step Delay [Cycles]	0
Acquisition Mode	None

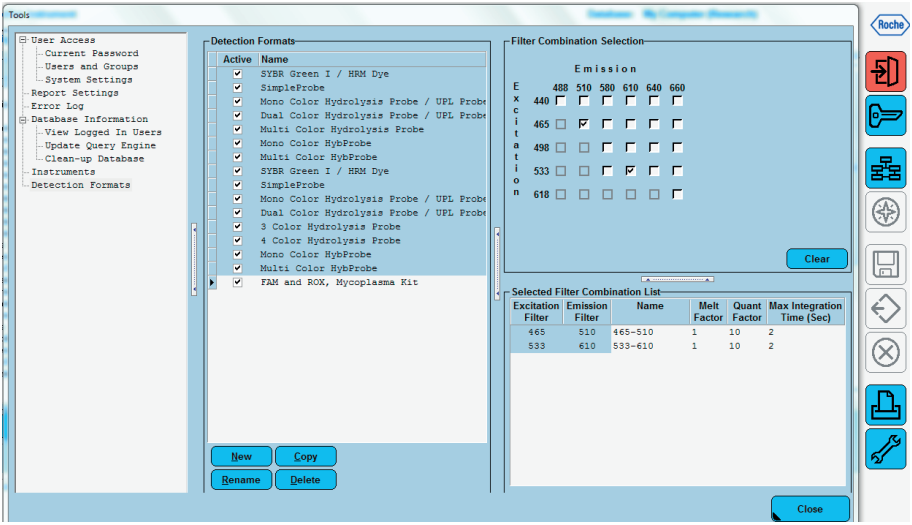
Analysis:
– Select the fluorescence channels Channel 1 (520 nm) and 3 (610 nm)
– Click on Quantification to generate the amplification plots and the specific Ct-values
– The threshold will be generated automatically
- Adapt the threshold line to 10 % of the maximum fluorescence level of the positive control (in case of duplicate determination, take the average of the maximum fluorescence levels)

LightCycler® 480 II

Choosing the correct filter setting:

- To define your filter combination, go to the Tool menu at the lower right-hand corner
- Click on Detection Formats on the left side and create a new detection format by clicking "New"
- Give the new detection format a name, like "Bacteria Kit"
- Select the right filter combination by clicking the checkboxes with an excitation 465 nm/ emission 510 nm (FAM™) and excitation 533 nm/emission 610 nm (ROX™)
- Choose following settings:

Melt Factor	1
Quant Factor	10
Max Integration Time (Sec)	2



Program 1: Pre-incubation	
Cycles	1
Analysis Mode	None
Temperature Targets	**Segment 1**
Target Temperature [°C]	95
Incubation time [s]	180
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	40		
Analysis Mode	Quantification		
Temperature Targets	**Segment 1**	**Segment 2**	**Segment 3**
Target Temperature [°C]	95	55	60
Incubation time [s]	30	30	45
Temperature Transition Rate [°C/s]	4.4	2.2	4.4
Secondary Target Temperature [°C]	0	0	0
Step Size [°C]	0	0	0
Step Delay [Cycles]	0	0	0
Acquisition Mode	None	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
Step Delay [Cycles]	0
Acquisition Mode	None

Data Analysis

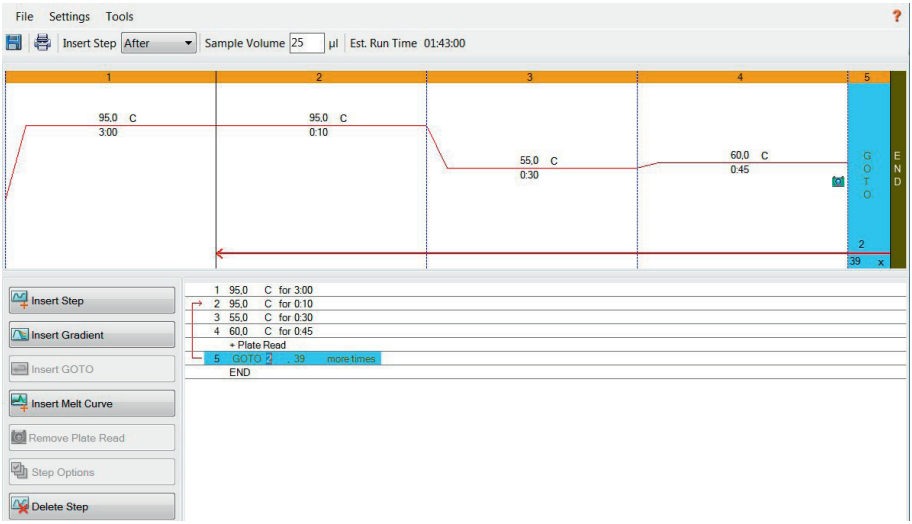
- Adapt the threshold line to 10 % of the maximum fluorescence level of the positive control (in case of duplicate determination, take the average of the maximum fluorescence levels)
- Select the Results tab to view specific Ct values

Bio-Rad CFX96 Touch™ / CFX96 Touch™ Deep Well

Run Setup Protocol Tab:

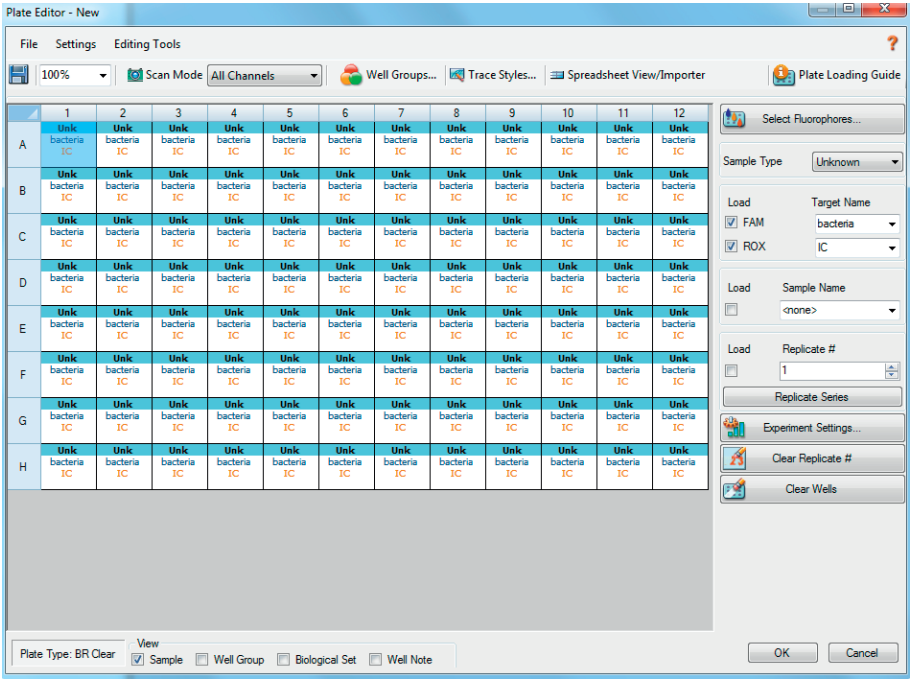
- Click File --> New --> Protocol to open the Protocol Editor and create a new protocol
- Select any step in either the graphical or text display
- Click the temperature or well time to directly edit the value

Segment 1:	1 cycle	3 min	95 °C	
Segment 2:		30 sec	95 °C	
Segment 3:		30 sec	55 °C	
Segment 4:		45 sec	60 °C	data collection
		GOTO Step 2, 39 more cycles		



Run Setup Plate Tab:

- Click File --> New --> Plate to open the Plate Editor and create a new plate
- Specify the type of sample at Sample Type
- Name your samples at Sample Name
- Use the Scan Mode dropdown menu in the Plate Editor Toolbar to designate the data acquisition mode to be used during the run. Select All Channels mode
- Click Select Fluorophores to indicate the fluorophores that will be used in the run. Choose FAM™ for the detection of bacteria amplification and ROX™ for monitoring the amplification of the internal control. Within the plate diagram, select the wells to load
- Choose the fluorophore data you want to display by clicking the fluorophore checkboxes located under amplification chart. Select FAM™ to display data of bacteria detection and ROX™ to display internal control amplification data.



Data Analysis:

- Select Settings in the menu and select Baseline Subtracted Curve Fit as baseline setting and Single Threshold mode as Cq determination
- Remark: Amplification curves for which the baseline is not correctly calculated by the software, can be manually adapted
- By right-click inside the amplification plot choose Baseline Threshold and set baseline cycles manually on basis of your positive control. Set Baseline Begin when fluorescence signal levelled off at a constant level. Set Baseline End before fluorescence signal of positive control increases
- Adapt the threshold line to 10 % of the maximum fluorescence level of the positive control (in case of duplicate determination, take the average of the maximum fluorescence levels)
- Evaluate the Ct-values according to chapter 10

RotorGene® 6000 (5-plex)

For the use of RotorGene® 6000, 0,1ml PCR tubes from Qiagen are recommended (Prod. No. 981106). Those tubes shall imperatively be used with the 72 well rotor from RotorGene® 6000.

1. Check the correct settings for the filter combination:

Target	Bacteria	Internal Control
filter	green	orange
wavelength	470—510 nm	585-610 nm

2. Program the Cycler:

Program 1: Pre-incubation

Setting	Hold
Hold Temperature	95 °C
Hold Time	3 min 0 sec

Program Step 2: Amplification

Setting	Cycling
Cycles	40
Denaturation	95 °C for 30 sec
Annealing	55 °C for 30 sec
Elongation	60 °C for 45 sec —> acquiring to Cycling A (green and orange)
Gain setting	automatic (Auto-Gain)
Slope Correct	activated
Ignore First	deactivated

Analysis:

- Open the menu Analysis
- Select Quantitation
- Check the required filter set (green and orange) according to the following table and start data analysis by double click.
- The following windows will appear:
  Quantitation Analysis - Cycling A (green / orange)
  Quant. Results - Cycling A (green / orange)
  Standard Curve - Cycling A (green / orange)
- In window Quantitation Analysis, select first "Linear Scale" and then "Slope Correct". Threshold setup (not applicable if a standard curve was carried with the samples 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 10 % of the maximum fluorescence level of the positive control (in case of duplicate determination, take the average of the maximum fluorescence levels)
- The Ct-values can be taken from the window Quant. Results. – Samples showing no Ct-value can be considered as negative.

ABI Prism® 7500

1. Check the correct settings for the filter combination:

Target	Bacteria	Internal Control
filter	FAM™	ROX™
wavelength	470-510 nm	585-610 nm
quencher	none	none

Important:

The ROX™ Reference needs to be disabled. Activate both detectors for each well. Measurement of fluorescence during extension.

2. Program the Cycler:

Program Step 1: Pre-incubation

Setting	Hold
Temperature	95 °C
Incubation time	3 min

Program Step 2: Amplification

Cycles	40
Setting	Cycle
Denaturing	95 °C for 30 sec
Annealing	55 °C for 30 sec
Extension	60 °C for 45 sec

Analysis:

- Enter the following basic settings at the right task bar:
  Data: Delta RN vs. Cycle
  Detector: FAM™ and ROX™
  Line Colour: Well colour
- Open a new window for the graph settings by clicking the right mouse button
  Select the following settings 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 "Analyse" within the report window.
- Pull the threshold line into the graph. Adapt the threshold line to 10 % of the maximum fluorescence level of the positive control (in case of duplicate determination take the average of the maximum fluorescence levels)
- Samples showing no Ct-value can be considered as negative

Mx3005P®

- Go to the setup menu, click on “Plate Setup“, check all positions which apply – Click on “Collect Fluorescence Data“ and check FAM™ and ROX™
- Corresponding to the basic settings the “Reference Dye“ function should be deactivated
- Specify the type of sample (no template control or positive control, sample, standard) at “well type“
- Edit the temperature profile at "Thermal Profile Design“:

Segment 1: 1 cycle	3 min	95 °C
Segment 2: 40 cycles	30 sec	95 °C
	30 sec	55 °C
	45 sec	60 °C data collection end

- 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 analyse 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“. The Threshold will be generated automatically
- Adapt the threshold line to 10 % of the maximum fluorescence level of the positive control (in case of duplicate determination take the average of the maximum fluorescence levels)
- Read the Ct-values in “Text Report“
- Evaluate the Ct-values according to chapter 10

Appendix

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 out of the use, the results of use, or the inability to use this product.

Trademarks
LightCycler is a registrated trademark of a member of the Roche Group. ABI Prism is a registrated trademark of Applera Corporation or its subsidiaries in the US and certain other countries. FAM™ and ROX™ are trademarks of Applera Corporation or it's subsidiaries in the US and certain other countries. Mx3005P is a trademark of Agilent Technologies. CFX96 Touch is a trademark of Bio Rad Laboratories, Inc. Microsart is a registered trademark of Sartorius Stedim Biotech. Myocoplasma Off and PCR Clean are trademarks of Minerva Biolabs GmbH, Germany.

Last technical revision: 2018-07-10

12. Related Products

Detection Kits for qPCR

SMB95-1001/1002	Microsart® AMP Mycoplasma	25/100 tests
SMB95-1003/1004	Microsart® ATMP Mycoplasma	25/100 tests
SMB95-1005/1006	Microsart® RESEARCH Mycoplasma	25/100 tests
SMB95-1007	Microsart® ATMP Bacteria Patient	10 patients
SMB95-1008	Microsart® ATMP Bacteria	100 tests

Microsart® Calibration Reagent, 1 vial, 10^8 genomes / vial

SMB95-2021	Mycoplasma arginini
SMB95-2022	Mycoplasma orale
SMB95-2023	Mycoplasma gallisepticum
SMB95-2024	Mycoplasma pneumoniae
SMB95-2025	Mycoplasma synoviae
SMB95-2026	Mycoplasma fermentans
SMB95-2027	Mycoplasma hyorhinis
SMB95-2028	Acholeplasma laidlawii
SMB95-2029	Spiroplasma citri
SMB95-2030	Bacillus subtilis
SMB95-2031	Pseudomonas aeruginosa
SMB95-2032	Kocuria rhizophila
SMB95-2033	Clostridium sporogenes
SMB95-2034	Bacteroides vulgatus
SMB95-2035	Staphylococcus aureus
SMB95-2036	Mycoplasma salivarium

Microsart® Validation Standard, 3 vials each, 10 CFU/vial for Mollicutes (SMB95-2011 - SMB95-2020) and 99 CFU/vial for other bacterial species (SMB95-2005-2010)

SMB95-2011	Mycoplasma arginini
SMB95-2012	Mycoplasma orale
SMB95-2013	Mycoplasma gallisepticum
SMB95-2014	Mycoplasma pneumoniae
SMB95-2015	Mycoplasma synoviae
SMB95-2016	Mycoplasma fermentans
SMB95-2017	Mycoplasma hyorhinis
SMB95-2018	Acholeplasma laidlawii
SMB95-2019	Spiroplasma citri
SMB95-2020	Mycoplasma salivarium
SMB95-2005	Bacillus subtilis
SMB95-2006	Pseudomonas aeruginosa

SMB95-2007	Kocuria rhizophila	
SMB95-2008	Clostridium sporogenes	
SMB95-2009	Bacteroides vulgatus	
SMB95-2010	Staphylococcus aureus	

DNA Extraction Kits

SMB95-2001	Microsart® Bacteria Extraction	50 extractions
SMB95-2003	Microsart® AMP Extraction (only for Mycoplasma qPCR)	50 extractions

PCR Clean™ (formerly DNA Remover™) *

15-2025	DNA Decontamination Reagent, spray bottle	250 ml
15-2200	DNA Decontamination Reagent, refill bottles	4 x 500 ml

PCR Clean™ Wipes*

15-2001	DNA Decontamination Wipes	120 wipes
15-2002	DNA Decontamination Wipes, refill sachets	5 x 120 wipes

Mycoplasma Off™ *

15-1000	Surface Disinfectant Spray, spray bottle	1000 ml
15-5000	Surface Disinfectant Spray, refill bottles	5000 ml

Mycoplasma Off™ Wipes*

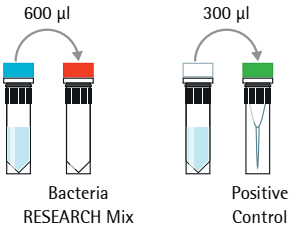
15-1001	Surface Disinfectant Wipes	120 wipes
15-5001	Surface Disinfectant Wipes, refill sachets	5 x 120 wipes

*** Distributed by Minerva Biolabs**

Procedure-Overview

1. Rehydration of Reagents

Bacteria RESEARCH Mix and Positive Control

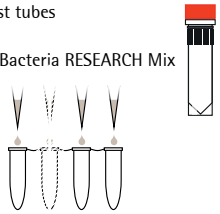


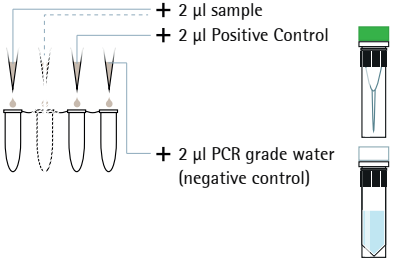
for 5 min RT
briefly
for 5 sec

2. Preparation of PCR Reactions

loading the test tubes

+ 23 µl Bacteria RESEARCH Mix





3. Starting the PCR Reaction

Rehydration Buffer
Bacteria RESEARCH Mix
PCR grade water
Positive Control

incubate
vortex
centrifuge
add

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

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