

**Instructions for Use**

# Microsart<sup>®</sup> ATMP Bacteria

Bacteria Detection Kit for qPCR

Prod. No. SMB95-1008

Reagents for 100 reactions

For use in research and quality control

Manufactured by:



Minerva Biolabs GmbH  
Koepenicker Strasse 325  
12555 Berlin  
Germany

## Symbols

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**LOT**

Lot No.

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**REF**

Order No.

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Expiry date

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Store at

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Contains reagents for  
100 tests

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Manufacturer

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# 1. Intended Use

Microsart® ATMP Bacteria kit is designed for the direct detection of bacteria in cell cultures, cell culture derived biologicals and ATMPs (e.g. autologous transplants), based on real-time PCR (qPCR).

# 2. Explanation of the Test

Microsart® ATMP Bacteria utilizes qPCR as the method of choice for sensitive and robust detection of bacterial contamination. The assay can be performed with any type of real-time PCR cycler able to detect the fluorescence dyes FAM™ and ROX™. The detection procedure can be performed within 2.5 hours (including DNA extraction: 3.5 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 specific 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. The polymerase is part of the Bacteria Mix. 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. The Internal Control DNA can be added directly to the PCR master mix to act as a PCR control or used to monitor the extraction process. The amplification of the internal amplification control is detected at 610 nm (ROX™ channel).

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## 4. Notes on the Test Procedure

1. For *in vitro* use in research and quality control. 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 (see chapter 4.1 for detailed information).
4. In case of working with living bacteria strains, the local regulatory requirements for S2 labs must be considered.
5. Attention: by aliquoting and freezing your samples you run a high risk of contamination. This should therefore be avoided if possible.
6. This leaflet must be widely understood for a successful use of Microsart® ATMP 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.
7. Any deviation from the test method can affect the results.
8. 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.
9. 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.
10. 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.

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## 4.1 Handling and Equipment Recommendations

To avoid false positive results due to improper handling the following actions are recommended:

1. To avoid DNA cross contaminations the complete test must be performed under sterile and DNA-free conditions. Therefore, we recommend the use of an isolator/glovebox with an airlock.
2. The isolator/glovebox should be cleaned thoroughly with PCR Clean™ (Minerva Biolabs, Prod. No. 15-2025) or PCR Clean™ Wipes (Minerva Biolabs, Prod. No. 15-2001) before and during the working process.
3. All materials, which are introduced into the isolator/glovebox should be cleaned thoroughly with PCR Clean™. Don't forget to clean the airlock with PCR Clean™. Pipettes and gloves should be cleaned thoroughly with PCR Clean™ Wipes prior and during the process.
4. Avoid working above open tubes and avoid air turbulences due to rapid movements.
5. Be careful when opening the tubes. Do not touch the inner surface of the lid.

## 5. Reagents

Each kit contains reagents for 100 reactions. The expiry date of the unopened package is marked on the package label. The kit components are stored at +2 to +8°C until use. Lyophilized components must be stored at  $\leq -18$  °C after rehydration. Protect the Bacteria Mix from light.

Kit Component Label Information	100 Reactions Order No. SMB95-1008	Cap Color
Bacteria Mix	4 x lyophilized	red
Rehydration Buffer	4 x 0.5 ml	blue
Positive Control DNA	1 x lyophilized	green
Internal Control DNA	4 x lyophilized	yellow
PCR grade Water	5 x 1.5 ml	white

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



## 6. Needed but not Included

Microsart® ATMP Bacteria contains the reagents, including negative and positive controls and polymerase as a component of the Bacteria Mix, to perform the test. 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)
- Microsart® Bacteria Extraction kit, a DNA-free extraction kit, Sartorius Prod. No. SMB95-2001.

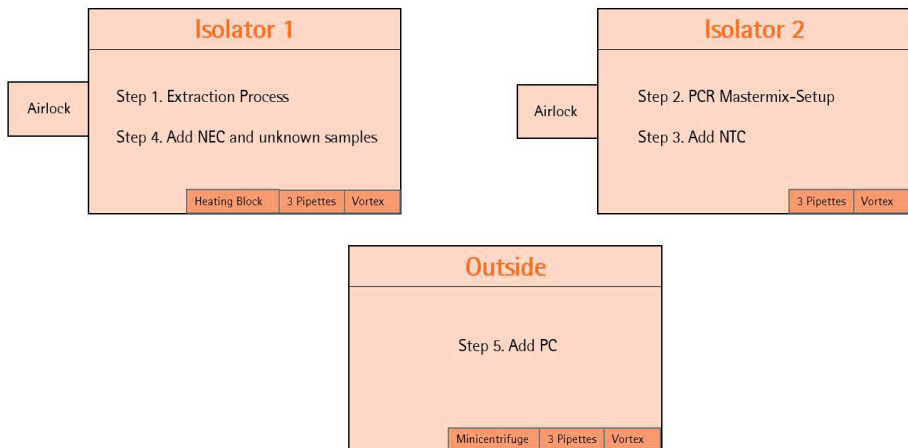
### Equipment

- Isolator/glovebox (further information, supplier and prices are available on request, please contact PCR@sartorius.com)
- 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 reaction tubes and PCR tubes
- Vortex mixer
- Rack for 1.5 ml tubes and for PCR-tube strips
- 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

If you use the Microsart® Bacteria Extraction kit you additionally need the following equipment:

- Isolator/glovebox (for extraction process)
- Vortex mixer
- Set of 3 pipettes (10 µl, 100 µl and 1000 µl; see page 10 for order information)
- Microcentrifuge for 1.5 ml reaction tubes (Centrisart A-14, Sartorius Prod. No. A-14-1EU)
- Heat Block
- Rack for 1.5 ml tubes

Schematical Overview of technical setup and experimental design



It is also possible to connect Isolator 1 and Isolator 2 via an airlock so that you can transfer your PCR-tubes after Step 3 directly from Isolator 2 into Isolator 1. Please note that you need an additional airlock for Isolator 2 in this case.

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## 7. Specimen

Studies showed the strict requirement of DNA extraction for any kind of sample to achieve highest sensitivity. For most test materials a DNA extraction method is available providing templates suitable for PCR. However, most of the DNA extraction kits available on the market are not free of DNA contaminations. Therefore, we recommend the combination of Microsart® ATMP Bacteria with the Microsart® Bacteria Extraction kit (Prod. No. SMB95-2001), a unique state-of-the-art DNA extraction method, which eliminates the risk of DNA contaminations, facilitating the detection of bacteria in cell culture and ATMPs via qPCR.

# 8. Test Procedure

The test should be carried out with negative and positive controls and samples in duplicates. For quantification, a dilution series of an appropriate standard is prepared. For this, Microsart® Calibration Reagents are recommended (see Chapter 12: Related Products for ordering information). All reagents and samples must be equilibrated at +2 to +8 °C prior use.

## 8.1 Recommendation for product release testing

The extraction process (Microsart® Bacteria Extraction) should be carried out with a negative extraction control (NEC) and samples in duplicates. Additionally the PCR test should include a PCR negative control (NTC) and a PCR positive control.

DNA extraction	PCR
2 x Sample	2 x Sample
1 x Negative Extraction Control	1 x Negative Extraction Control
	1 x PCR Positive Control
	1 x PCR Negative Control
$\Sigma$ 3 extractions	$\Sigma$ 5 PCR reactions

## 8.2 Rehydration of the Reagents

After reconstitution, reagents must be stored at  $\leq -18$  °C. In order to avoid repeated freezing and thawing, storage in appropriate aliquots is recommended.

1.	Bacteria Mix	red cap	Centrifuge briefly
	Internal Control DNA	yellow cap	
	Positive Control DNA	green cap	
2.	Bacteria Mix	red cap	Add 390 $\mu$ l Rehydration Buffer (blue cap)
3.	Internal Control DNA	yellow cap	Add 800 $\mu$ l PCR grade Water (white cap)
4.	Positive Control DNA	green cap	Add 300 $\mu$ l PCR grade Water (white cap)
5.	Bacteria Mix	red cap	Incubate 5 min at room temperature
	Internal Control DNA	yellow cap	
	Positive Control DNA	green cap	
6.	Bacteria Mix	red cap	Vortex briefly
	Internal Control DNA	yellow cap	
	Positive Control DNA	green cap	

### 8.3 Preparation of the Reaction Mix

Preparation of the master mix and sample loading 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.

The total volume per reaction is 25  $\mu$ l including 10  $\mu$ l sample. Calculations of reaction setup should include positive (PC) and negative controls (NTC).

If the Internal Control DNA was not added to the sample to monitor the DNA extraction process, follow this protocol:

- 
1. Prepare the master mix at room temperature, by adding 26 $\mu$ l of Internal Control DNA (yellow cap) directly in the Bacteria Mix tube (red cap).
  2. Homogenize the reaction mix by tapping carefully against the tube. Spin briefly.
  3. Add 15  $\mu$ l of the mastermix to 25 PCR tubes. Close PCR tubes. For storage, freeze any mastermix aliquots you do not need for the current assay.  
Attention: in order to avoid contaminations we recommend to freeze the PCR tubes containing the master mix in a closed and clean PCR rack.
- 

#### Attention:

If the Internal Control DNA was added to the sample during DNA extraction, add 15  $\mu$ l of the Bacteria Mix (red cap) directly to each PCR tube. Attention: Don't forget to add 1 $\mu$ l of Internal Control DNA to NTC and PC.

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## 8.4 Loading the Test Tubes

1. Negative controls: add 10 µl Suspension Buffer (violet cap) from the DNA extraction kit or PCR grade Water (white cap). Seal tube before proceeding with the samples.  
Attention: Negative controls should be processed in the isolator/glovebox used for mastermix setup.  
Attention: in order to avoid contaminations make sure to reserve one of the PCR grade water tube (white cap) exclusively to the negative controls.
2. Sample reaction: add 10 µl of sample. Seal tube tightly before proceeding.  
Attention: Samples, including NECs, should be added to the reaction in the isolator/glovebox used for DNA extraction.
3. Positive control: add 10 µl Positive Control DNA (green cap).  
Attention: Positive controls should not be handled in the isolator/glovebox used for mastermix setup or DNA extraction.
4. Close and spin all PCR tubes briefly, load the qPCR cyclers and start the program.

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## 8.5 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.

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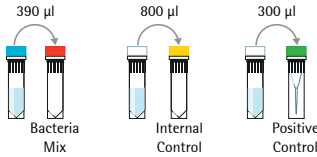
## 8.6 Analysis

1. Save the data at the end of the run.
  2. Analyze the channels for the fluorescence dyes FAM™ and ROX™.
  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. Analyze the calculation of the Ct-values for negative controls, positive controls and samples.
-

# 9. Short Instructions

## 1. Rehydration of Reagents

- ⊕ Bacteria Mix
- ⊕ Positive Control DNA
- ⊕ Internal Control DNA



- ⏱ for 5 min RT
- 🌀 briefly
- ⊕ for 5 sec

## 2. Preparation of PCR Reaction

a) Internal Control added during DNA extraction



+ 15 µl Bacteria Mix (red cap)

don't forget to  
add 1 µl Internal Control  
to NTC and PC

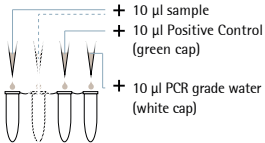
b) Internal Control not added during DNA extraction



Reaction Mix  
+ 15 µl Bacteria Mix  
+ 1 µl Internal Control

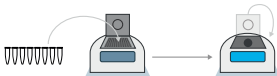


+ 15 µl Reaction Mix

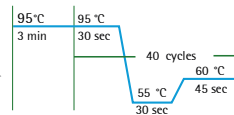


+ 10 µl sample  
+ 10 µl Positive Control  
(green cap)  
+ 10 µl PCR grade water  
(white cap)

## 3. Starting PCR Reaction



Start PCR program



- ⊕ Rehydration Buffer
- ⊕ Bacteria Mix
- ⊕ PCR grade water
- ⊕ Positive Control
- ⊕ Internal Control

- ⏱ incubate
- 🌀 vortex
- ⊕ centrifuge
- +

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



# 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. 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 bacterial 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.

\*\* if used as PCR control, 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). If used as process control, Internal Control of bacteria negative samples (FAM™: no Ct) must show Ct-values in the range of +/- 3 cycles (ROX™) of the no-template control (master mix control).

## 10.2 Total Analysis and recommended actions for product release testing

Sample	Expected Outcome	Unexpected Outcome	Action
NTC	negative	NTC positive	Repeat PCR only
PC	positive	PC negative	Repeat PCR only
NEC	negative	NEC positive	Repeat the whole process incl. DNA extraction and PCR
Specimen	0/2 positive		Product release
		1/2 positive	Repeat the whole process incl. DNA extraction and PCR
			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.

### 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 used for the validation of Microsart® ATMP Bacteria:

CFX96 Touch™ / CFX96 Touch Deep Well™  
Mx3005P®  
ABI Prism® 7500  
RotorGene® 6000

## LightCycler® 1.0 and 2.0

### Program 1: Pre-incubation

Cycles	1
Analysis Mode	None
<b>Temperature Targets</b>	<b>Segment 1</b>
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		
<b>Temperature Targets</b>	<b>Segment 1</b>	<b>Segment 2</b>	<b>Segment 3</b>
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		
<b>Temperature Targets</b>	<b>Segment 1</b>		
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:

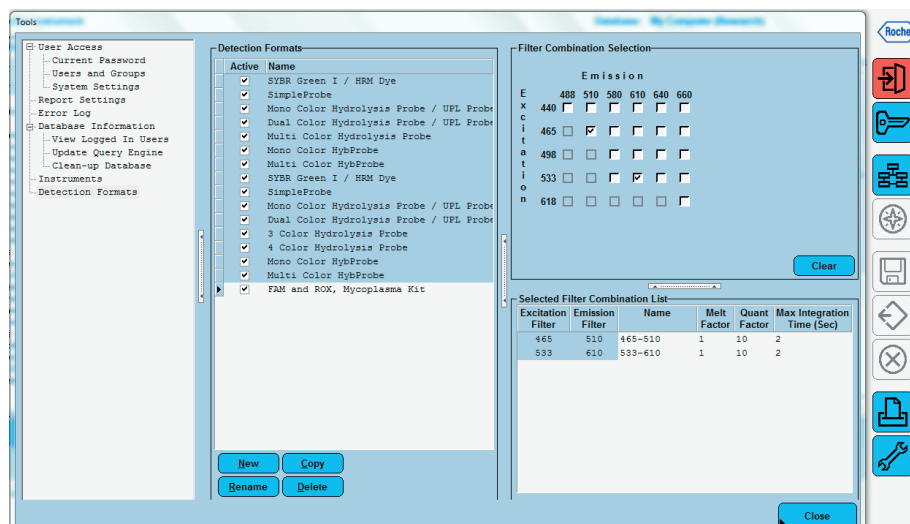
- Select the fluorescence channels Channel 1 (520 nm) and 3 (610 nm)
- Click on Quantification to generate the amplification plots and the specific Ct-values
- The threshold will be generated automatically
- Adapt the threshold line to 10 % of the maximum fluorescence level of the positive control (in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



The screenshot displays the software interface for configuring detection formats. The 'Detection Formats' window is active, showing a list of formats with checkboxes. The 'FAM and ROX, Mycoplasma Kit' format is selected. The 'Filter Combination Selection' window is also open, showing a grid for selecting filter combinations based on excitation and emission wavelengths. The 'Selected Filter Combination List' table is visible below the grid.

Excitation Filter	Emission Filter	Name	Melt Factor	Quant Factor	Max Integration Time (Sec)
465	510	465-510	1	10	2
533	610	533-610	1	10	2

### Program 1: Pre-incubation

Cycles	1
Analysis Mode	None
<b>Temperature Targets</b>	<b>Segment 1</b>
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		
<b>Temperature Targets</b>	<b>Segment 1</b>	<b>Segment 2</b>	<b>Segment 3</b>
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
<b>Temperature Targets</b>	<b>Segment 1</b>
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

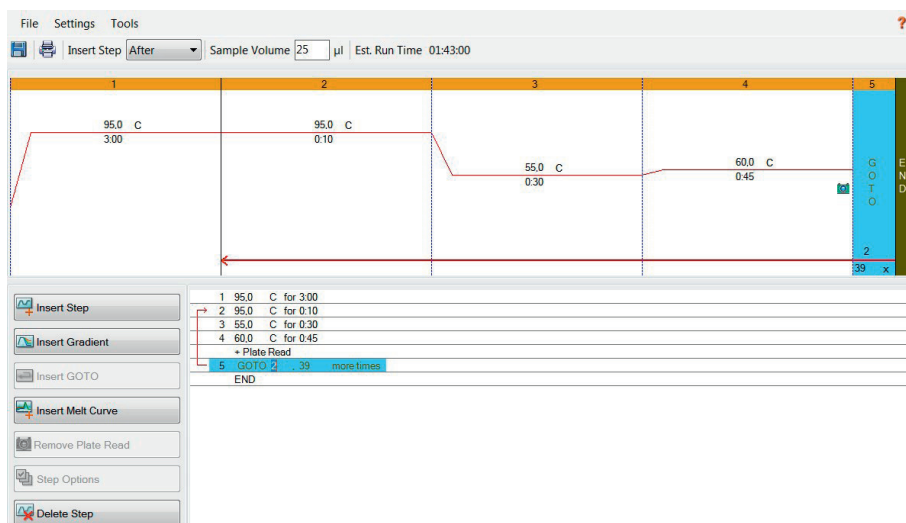
- Adapt the threshold line to 10 % of the maximum fluorescence level of the positive control (in case of duplicate determination, take the average of the maximum fluorescence levels)
- Select the Results tab to view specific Ct values

## 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

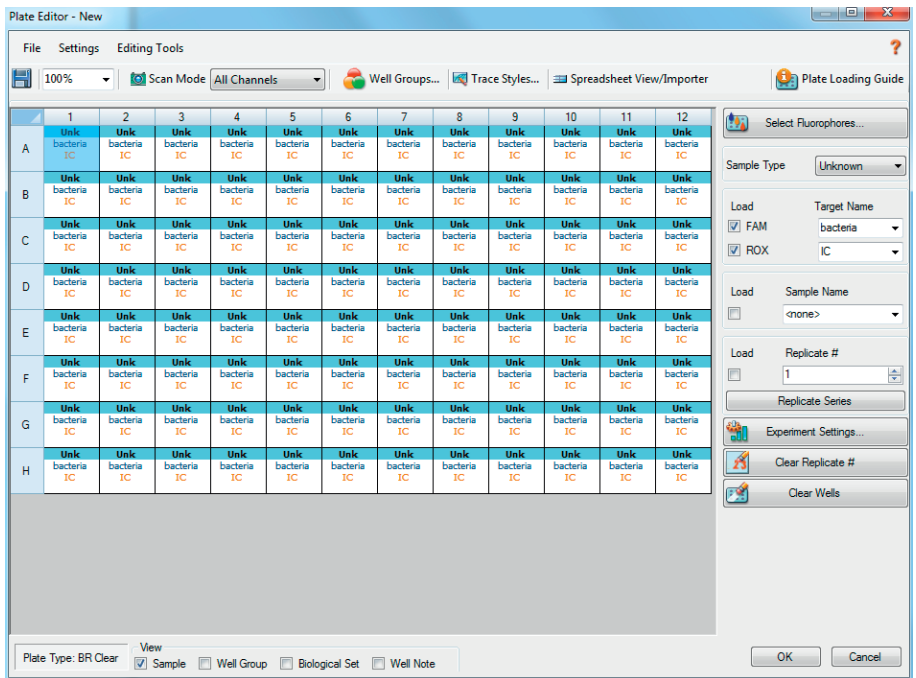
Segment 1:	1 cycle	3 min	95 °C	
Segment 2:		30 sec	95 °C	
Segment 3:		30 sec	55 °C	
Segment 4:		45 sec	60 °C	data collection
			GOTO Step 2, 39 more cycles	





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.



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#### 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 Cyclor:

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

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## 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
SMB95-1009	Microsart® RESEARCH Bacteria	25 tests
SMB95-1007	Microsart® ATMP Bacteria (patient)	10 patients

### Microsart® Calibration Reagent, 1 vial, 10<sup>8</sup> 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	Micrococcus luteus
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	Micrococcus luteus	
SMB95-2008	Clostridium sporogenes	
SMB95-2009	Bacteroides vulgatus	
SMB95-2010	Staphylococcus aureus	
<b>DNA Extraction Kits</b>		
SMB95-2001	Microsart® Bacteria Extraction	50 extractions
SMB95-2003	Microsart® AMP Extraction (only for Mycoplasma qPCR)	50 extractions
<b>PCR Clean™ *</b>		
15-2025	DNA Decontamination Reagent, spray bottle	250 ml
15-2200	DNA Decontamination Reagent, refill bottles	4× 500 ml
<b>PCR Clean™ Wipes*</b>		
15-2001	DNA Decontamination Wipes	120 wipes
15-2002	DNA Decontamination Wipes, refill sachets	5× 120 wipes
<b>Mycoplasma Off™ *</b>		
15-1000	Surface Disinfectant Spray, spray bottle	1000 ml
15-5000	Surface Disinfectant Spray, refill bottles	5000 ml
<b>Mycoplasma Off™ Wipes *</b>		
15-1001	Surface Disinfectant Wipes	120 wipes
15-5001	Surface Disinfectant Wipes, refill sachets	5× 120 wipes

\* Distributed by Minerva Biolabs

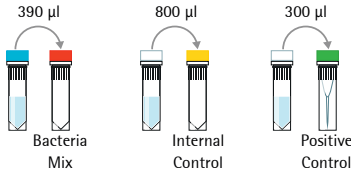


# Short Instructions

Microsart® ATMP Bacteria

## 1. Rehydration of Reagents

- 🧊 Bacteria Mix
- 🧊 Positive Control DNA
- 🧊 Internal Control DNA



- 🕒 for 5 min RT
- 🌀 briefly
- 🌀 for 5 sec

## 2. Preparation of PCR Reaction

a) Internal Control added during DNA extraction



+ 15 µl Bacteria Mix (red cap)

don't forget to add 1 µl Internal Control to NTC and PC

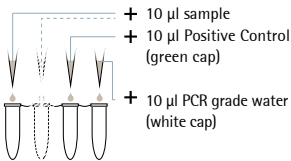
b) Internal Control not added during DNA extraction



Reaction Mix  
+ 15 µl Bacteria Mix  
+ 1 µl Internal Control

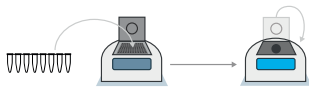


+ 15 µl Reaction Mix

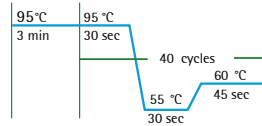


- + 10 µl sample
- + 10 µl Positive Control (green cap)
- + 10 µl PCR grade water (white cap)

## 3. Starting PCR Reaction



Start PCR program



- 🧊 Rehydration Buffer
- 🧊 Bacteria Mix
- 🧊 PCR grade water
- 🧊 Positive Control
- 🧊 Internal Control

- 🕒 incubate
- 🌀 vortex
- 🌀 centrifuge
- + add

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

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