

## Microsart® 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 | Schkopauer Ring 13 | 12681 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 reactions

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Manufacturer

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# Contents

1. Intended Use . . . . .	5
2. Explanation of the Test . . . . .	5
3. Test Principle . . . . .	5
4. Notes on the Test Procedure . . . . .	6
4.1 Handling and Equipment Recommendations . . . . .	7
5. Reagents . . . . .	8
6. Needed but not included . . . . .	9
7. Specimen . . . . .	11
8. Test Procedure . . . . .	11
8.1 Recommendation for Product Release Testing . . . . .	11
8.2 Rehydration of the Reagents . . . . .	12
8.3 Preparation of the Reaction Mix . . . . .	13
8.4 Addition of Samples and Controls . . . . .	14
8.5 Start of the qPCR Reaction . . . . .	14
8.6 Analysis . . . . .	14
9. Interpretation of Results . . . . .	15
9.1 Yes/No Evaluation . . . . .	15
9.2 Total Analysis and recommended Actions for Product Release Testing . . . . .	16
10. Appendix . . . . .	17
QuantStudio™ . . . . .	17
Bio-Rad CFX96 Touch™ / CFX96 Touch™ deep well . . . . .	19
Rotor-Gene® 6000 (5-plex) . . . . .	22
ABI Prism® 7500 . . . . .	24
Mx3005P™ . . . . .	26
11. Related Products . . . . .	27
Short Instructions . . . . .	inside back cover



# 1. Intended Use

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

# 2. Explanation of the Test

Microsart® ATMP Bacteria uses qPCR as method of choice to enable sensitive and robust detection of bacterial contaminations. The assay can be performed with any type of real-time PCR cycler able to detect the fluorescent dyes FAM™ and ROX™.

The detection procedure can be carried out in approximately 2.5 hours (3.5 hours including DNA extraction). In contrast to the culture method, this approach is compatible also with samples that do not contain living bacteria, and detects live as well as dormant or non-culturable bacteria.

# 3. Test Principle

Bacteria are specifically detected by amplification of a highly conserved region within the bacterial genome: the 16S rRNA gene. The specific amplification of this gene is detected at 520 nm (FAM™ channel).

The kit contains all the reagents necessary for a FAM™-labeled fluorogenic 5′ nuclease assay. The Bacteria Mix includes primers, FAM™-labeled probes, and polymerase. 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 (or Internal Control). The Internal Control DNA can be added directly to the PCR master mix and function as a PCR inhibition control or to the samples before DNA extraction to monitor the extraction process. The amplification of the internal control is detected at 610 nm (ROX™ channel).

## 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 by trained staff, only. A clean lab coat and disposable gloves should be worn 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 work with living bacteria strains, the local regulation for S2 laboratories must be followed.
5. Aliquoting and freezing the samples can increase the risk of sample contaminations. Therefore, this should be avoided whenever 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. Assay inhibition may be caused by the sample matrix, but also by sample elution buffers of incompatible or non-validated DNA extraction kits. Using DNA extraction kits that are not validated frequently increases the rate of false-positive or false-negative results.
9. We recommend running control reactions in each test to assess inter-assay variability. Controls should be always handled in the same manner as the samples.
10. For each test setup, at least one negative extraction control and one PCR 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.
11. Participation in external quality control programs, such as those offered by Minerva Biolabs GmbH ([www.minerva-biolabs.com](http://www.minerva-biolabs.com)), is recommended.

## 4.1 Handling and Equipment Recommendations

To avoid false positive results, we recommend following the guidelines below:

1. To perform the test under sterile and DNA-free conditions, we recommend the use of an isolator/glovebox with an airlock.
2. The isolator/glovebox should be thoroughly decontaminated with PCR Clean™ (Minerva Biolabs, Prod. No. 15-2025) or PCR Clean™ Wipes (Minerva Biolabs, Prod. No. 15-2001) before and during the work process.
3. All materials introduced into the isolator/glovebox should be thoroughly decontaminated with PCR Clean™ before beginning the procedure.
4. Avoid working above open tubes and avoid air turbulences due to rapid movements.
5. Be careful while 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 ≤ -18 °C after rehydration. Protect the Bacteria Mix from light.

<b>Kit Component Label Information</b>	<b>100 Reactions Order No. SMB95-1008</b>	<b>Cap Color</b>
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 all 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 (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® ATMP Extraction kit, a DNA-free extraction kit for samples containing bacteria and fungi, Sartorius Prod. No. SMB95-2001

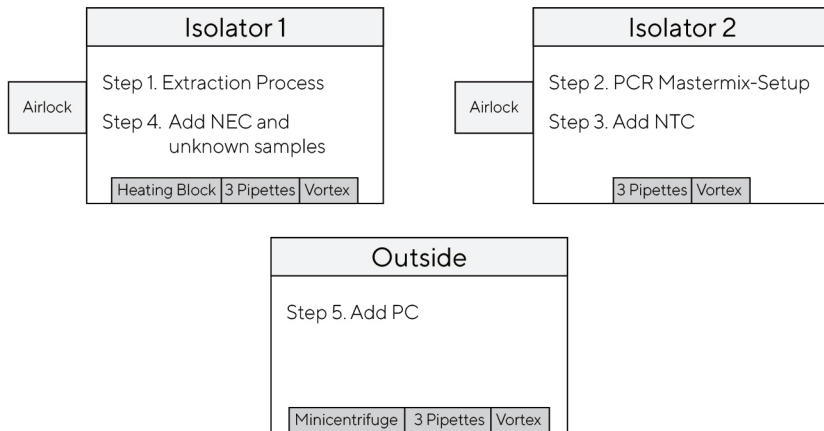
### Equipment

- 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/strips
- Vortex
- 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® ATMP Extraction kit, you also need the following equipment:

- Isolator/glovebox (for extraction process)
- Vortex
- Set of 3 pipettes (10 µl, 100 µl and 1000 µl; see previous page for ordering information)
- Microcentrifuge for 1.5 ml reaction tubes (Centrisart A-14, Sartorius Prod. No. A-14-1EU)
- Heat block with optional shaking function, when performing DNA extraction
- Rack for 1.5 ml tubes

Schematic 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 the 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.

# 7. Specimen

Studies showed that DNA extraction prior to PCR is required to achieve highest sensitivity for any kind of sample. While a DNA extraction method providing templates suitable for PCR is available for most types of test samples, several commercially available DNA extraction kits are not DNA- or contaminations-free. For this reason, we recommend combining Microsart® ATMP Bacteria with Microsart® ATMP Extraction kit (Prod. No. SMB95-2001): a unique, state-of-the-art DNA extraction method, which eliminates the risk of DNA contaminations and facilitates the detection of bacteria in cell cultures 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 should be prepared. To this aim, we recommend using Microsart® Calibration Reagents as standard material (see Related Products).

## 8.1 Recommendation for Product Release Testing

The extraction process (Microsart® ATMP 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 (PC).

DNA extraction	PCR
2 x Sample	2 x Sample (or extracts)
1 x NEC	1 x NEC
	1 x PC
	1 x NTC
Σ 3 extractions	Σ 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.

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

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### 8.3 Preparation of the Reaction Mix

In order to avoid fluorescence decrease, we recommend preparing the master mix and loading the samples in  $\leq 45$  minutes.

The total volume/reaction is 25  $\mu\text{l}$ , including 10  $\mu\text{l}$  of sample. When calculating the total reaction volume, positive (PC) and negative controls (NTC) should be taken into account.

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

- 
1. Prepare the master mix at room temperature, by adding 26  $\mu\text{l}$  of Internal Control DNA (yellow cap) directly in the Bacteria Mix tube (red cap).
- 
2. Vortex and spin briefly.
- 
3. Add 15  $\mu\text{l}$  of the master mix to each PCR tube and close the lid.  
Freeze aliquots of any remaining master mix that you might not need for the current assay.  
**Attention:** In order to avoid contaminations, we recommend freezing the master mix aliquots in PCR tubes and in a closed and clean rack for PCR.
- 

**Attention:** If the Internal Control DNA was added to the sample during DNA extraction, add 15  $\mu\text{l}$  of the Bacteria Mix (red cap) directly to each PCR tube. In this case, do not forget to add 1  $\mu\text{l}$  of Internal Control DNA to NTC and PC.

## 8.4 Addition of Samples and Controls

Follow the pipetting sequence and close the tubes after each sample has been loaded.

1. Negative controls: add 10 µl Suspension Buffer (violet cap) from the DNA extraction kit or PCR grade Water (white cap). Seal tube tightly before proceeding.  
**Note:** These negative controls should be processed in the isolator/glovebox used for master mix setup.  
**Important:** In order to avoid false positives, do not use the same PCR grade Water tube for NTC and rehydration of the Positive Control DNA.  
**Important:** If the Internal Control DNA was added to the samples during extraction, add 1 µl of Internal Control DNA (yellow cap) also to each negative and positive (step 3.) control.
2. Sample(s): add 10 µl of sample. Seal tube tightly before proceeding.  
**Note:** Samples and NECs, should be added in the isolator/glovebox used for DNA extraction.
3. Positive controls: add 10 µl Positive Control DNA (green cap). Seal tube tightly.  
**Note:** Positive controls **should not** be handled in the isolator/glovebox used for master mix setup or DNA extraction.
4. Spin all PCR tubes briefly.

## 8.5 Start of the qPCR Reaction

1. Load the cycler, check the PCR tubes 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.

## 8.6 Analysis

1. Save the data at the end of the run.
2. Analyze FAM™ and ROX™ fluorescent signals.
3. FAM™: adapt the threshold line to 10 % of the maximum fluorescence level of the positive controls. ROX™: adapt the threshold line to 10 % of the maximum fluorescence level of the NTCs. With replicates, take the average of the maximum fluorescence levels. See chapter 10.
4. Analyze the Ct values calculated for controls and samples.

## 9. Interpretation of Results

The presence of bacterial DNA in the sample is indicated by an increasing fluorescence signal in the FAM™ channel during PCR. Based on the obtained Ct values, the unknown concentration of the contaminant can be interpolated along 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 (IC) channel. Target bacterial DNA and Internal Control DNA are competitors in the PCR. Because of the 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.

### 9.1 Yes/No Evaluation

Detection of bacteria FAM™ channel	Internal Control (IC) ROX™ channel	Interpretation	
positive (Ct < 40)	irrelevant	Bacterial DNA positive	
negative (no Ct)	negative**	if IC used as PCR control	PCR inhibition*
		if IC used as process control	Extract or/and PCR inhibition
negative (no Ct)	positive (Ct < 40)	Bacterial DNA negative	

\*PCR inhibition might be caused by the sample matrix.

If one out of two replicates is negative in the Internal Control channel (ROX™: no Ct), repeat the PCR. If two out of two replicates are negative in the Internal Control channel, repeat the DNA extraction and the PCR.

\*\*If used as PCR control, the internal control (ROX™) for negative samples (FAM™: no Ct) must show comparable Ct values to those obtained for the negative controls (NTC) ( $\pm 2$  cycles). If used as a process control, the internal control (ROX™) for negative samples (FAM™: no Ct) must show comparable Ct values to those obtained for the NTCs ( $\pm 3$  cycles).

## 9.2 Total Analysis and recommended Actions for Product Release Testing

Sample	Result	Interpretation	Action
NTC	negative	Valid PCR	Interpret specimen results
	positive	PCR contamination	Repeat PCR only
PC	positive	Valid PCR	Interpret specimen results
	negative	Failed PCR	Repeat PCR only
NEC	negative	Valid PCR	Interpret specimen results
	positive	Contamination during extraction or PCR	Repeat the whole process* incl. DNA extraction and PCR.
	0/2 positive	No contamination	Release
Specimen	1/2 positive	Possible contamination	Repeat the whole process* incl. DNA extraction and PCR. If result is confirmed, no release.
	2/2 positive	Contamination	No release

\* Repeat PCR only, if NTC is also positive.

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 performed by an external sequencing service. The interpretation of your sequencing results will be supplied by Minerva Biolabs afterwards.

### Attention:

In case of light or multiple contamination, the sequencing analysis might lead to wrong identification.



# 10. Appendix

The protocol can be performed with any type of real-time PCR cycler able to detect the fluorescent dyes FAM™ and ROX™.

The following qPCR cyclers were used for the validation of Microsart® ATMP Bacteria: Mx3005P™, CFX96 Touch™, CFX96 Touch Deep Well™, ABI Prism® 7500, Rotor-Gene® Q/ Rotor-Gene®.

In addition, Microsart® ATMP Bacteria kit was successfully tested with QuantStudio™ and LightCycler® 480 II (protocol available on request).

## QuantStudio™

- Click File -> New Experiment -> Experiment Setup, to open the setup menu.
- Select "Properties" from the menu bar to open the "Experiment Properties" tab. In this tab, assign an experiment name ("Name") and make sure that "Chemistry" is set to "TaqMan® Reagents" and that the "Run mode" option is set to "Standard"
- Select "Method" in the menu bar to open the "Experiment Method" tab.
- Adjust the reaction volume and the cover temperature by setting "Volume" to 25 µl and "Cover" to 105 °C.
- Program a "Hold Stage" of 3 min at 95 °C.
- In "PCR Stage", perform "Step1" (denaturation step) at 95 °C for 30 sec, "Step2" (annealing step) at 55 °C for 30 sec, and "Step3" (elongation step) at 60 °C for 45 sec. Enable the data readout during the elongation step by clicking on the camera symbol. Set the number of cycles to 40. See also figure below for an overview of these settings.
- Select "Plate" in the menu bar to open the "Assign Targets and Samples" tab.  
**Important:** Set the "Passive Reference" to "None"!!!!
- Click on the "Advanced Setup" tab to define your targets and samples.
- In the "Targets" section: for the assay target, choose FAM™ as a reporter and NFQ-MGB as a quencher. For the internal control target, choose ROX™ as a reporter and NFQ-MGB as a quencher.
- Enter your samples names in the "Samples" section.
- Select the wells containing your samples (from the graphical overview of the plate), enable the targets, and assign the appropriate sample name by clicking on the respective check boxes.
- Select "Run" from the menu bar and click on START RUN to start the PCR run.

Experiment Method Action Save

Volume	Cover
25 $\mu$ L	105.0 $^{\circ}$ C

**Hold Stage**

1.6  $^{\circ}$ C/s, 95.0  $^{\circ}$ C, 03:00

Step1

**PCR Stage**

1.6  $^{\circ}$ C/s, 95.0  $^{\circ}$ C, 00:30

1.6  $^{\circ}$ C/s, 55.0  $^{\circ}$ C, 00:30

1.6  $^{\circ}$ C/s, 60.0  $^{\circ}$ C, 00:45

Step1 Step2 Step3

40 x

**Legends:** Data Collection On Data Collection Off Pause On Pause Off Advanced Settings VeriFlex

Previous Next

## Data Analysis

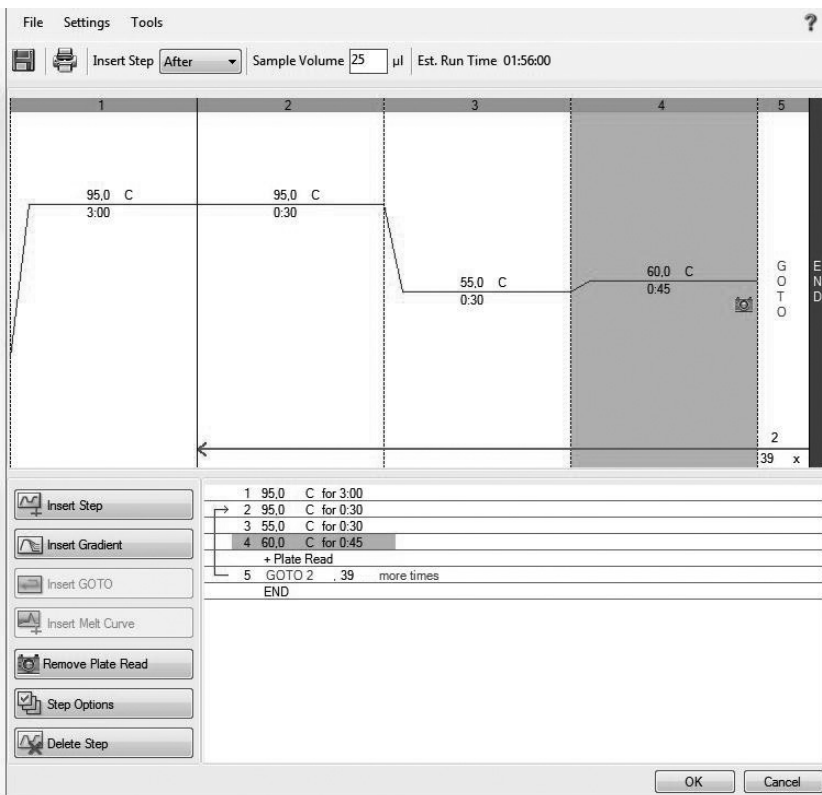
- To enable thresholds setting, please follow the specific instructions provided in the manual of your cyclor.
- FAM™: adapt the threshold line to 10 % of the maximum fluorescence level of the positive control. ROX™: adapt the threshold line to 10 % of the maximum fluorescence level of the NTCs. With replicates, 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:

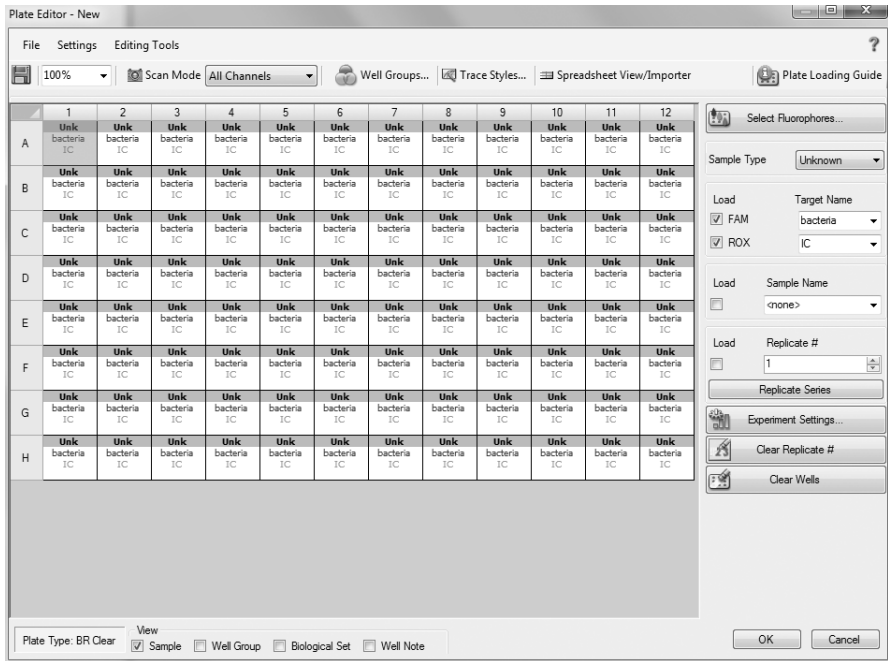
- Click File -> New -> Protocol to open the Protocol Editor and create a new protocol.
- Select any step in either the graphical or text display.
- Click the temperature or well time to directly edit the value.

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 C<sub>q</sub> determination.
- Remark: Amplification curves for which the baseline is not correctly calculated by the software can be manually adapted.
- To enable thresholds setting, please follow the specific instructions provided in the manual of your cycler.
- FAM™: adapt the threshold line to 10 % of the maximum fluorescence level of the positive controls. ROX™: adapt the threshold line to 10 % of the maximum fluorescence level of the NTCs. With replicates, take the average of the maximum fluorescence levels.
- Evaluate the Ct values according to chapter 9.

## Rotor-Gene® 6000 (5-plex)

For the use of Rotor-Gene® 6000, 0.1 ml PCR tubes from Qiagen are recommended (Prod. No. 981106). Those tubes shall imperatively be used with the 72 well rotor from Rotor-Gene® 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 Cyclcr:

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. FAM™: adapt the threshold line to 10 % of the maximum fluorescence level of the positive control. ROX™: adapt the threshold line to 10 % of the maximum fluorescence level of the NTCs. With replicates, 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. FAM™: adapt the threshold line to 10 % of the maximum fluorescence level of the positive control. ROX™: adapt the threshold line to 10 % of the maximum fluorescence level of the NTCs. With replicates, 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 that 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 cyclor 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”. The threshold will be generated automatically.
- FAM™: adapt the threshold line to 10 % of the maximum fluorescence level of the positive controls. ROX™: adapt the threshold line to 10 % of the maximum fluorescence level of the NTCs.
  - With replicates, take the average of the maximum fluorescence levels.
- Read the Ct values in “Text Report”.
- Evaluate the Ct values according to chapter 9.

# 11. 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 Sterile Release	10 samples
SMB95-1009	Microsart® RESEARCH Bacteria	25 tests
SMB95-1012	Microsart® ATMP Fungi	100 tests
SMB95-1014/1013	Microsart® RESEARCH Fungi	25/100 tests

## Microsart® Calibration Reagent, 1 vial, 10<sup>8</sup> genomes / vial (bacteria, including Mollicutes)

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® Calibration Reagent, 1 vial, 10<sup>6</sup> genomes / vial (fungi)

SMB95-2044	Candida albicans
SMB95-2045	Aspergillus brasiliensis
SMB95-2046	Aspergillus fumigatus
SMB95-2047	Penicillium chrysogenum
SMB95-2048	Candida glabrata
SMB95-2049	Candida krusei
SMB95-2050	Candida tropicalis

## Microsart® Validation Standard, 3 vials each, 10 CFU / vial (Mollicutes)

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

**Microsart® Validation Standard, 6 vials each, 99 CFU / vial (bacteria\* and fungi)**

SMB95-2005	Bacillus subtilis
SMB95-2006	Pseudomonas aeruginosa
SMB95-2007	Kocuria rhizophila
SMB95-2008	Clostridium sporogenes
SMB95-2009	Bacteroides vulgatus
SMB95-2010	Staphylococcus aureus
SMB95-2037	Candida albicans
SMB95-2038	Aspergillus brasiliensis
SMB95-2039	Aspergillus fumigatus
SMB95-2040	Penicillium chrysogenum
SMB95-2041	Candida glabrata
SMB95-2042	Candida krusei
SMB95-2043	Candida tropicalis

\* except for Mollicutes

**DNA Extraction Kit**

SMB95-2001	Microsart® ATPM Extraction (for bacteria and fungi)	50 extractions
SMB95-2003	Microsart® AMP Extraction (for mycoplasma)	50 extractions
56-0002	Proteinase K**	50 extractions

**PCR Clean™ \*\***

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 Reagent, Wipes	50 wipes
15-2002	DNA Decontamination Reagent, refill sachets	5 x 50 wipes

\*\* Distributed by Minerva Biolabs

## **Limited Product Warranty**

This warranty limits our liability for replacement of this product.

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Sartorius Stedim Biotech GmbH  
August-Spindler-Str. 11  
37079 Goettingen, Germany

Phone +49 551 308 0  
Fax +49 551 308 3289

 [www.sartorius.com](http://www.sartorius.com)

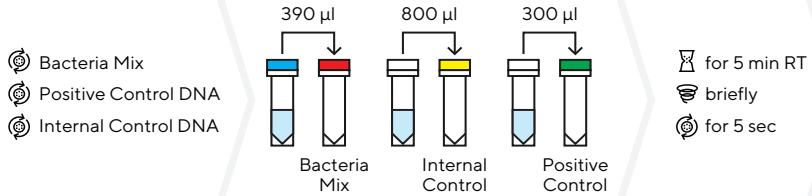
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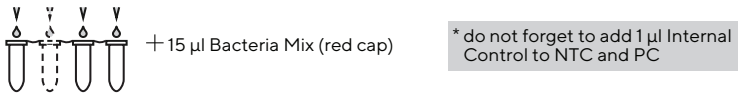
Printed in Germany on paper that  
has been bleached without any use  
of chlorine. | W  
Material No.: 1000054394  
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## 1. Rehydration of Reagents

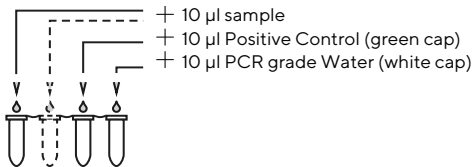


## 2. Preparation of PCR Reaction

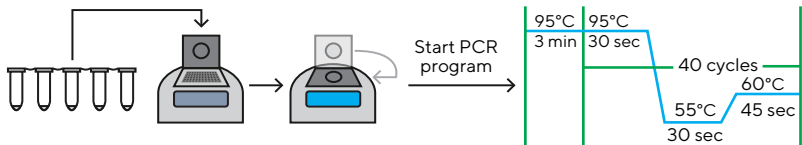
a) Internal Control added during DNA extraction



b) Internal Control not added during DNA extraction



## 3. Starting PCR Reaction



- Rehydration Buffer
- Bacteria 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.

ST\_SI\_Microsart®-ATMP-Bacteria\_O4\_EN

Sartorius Stedim Biotech GmbH  
August-Spindler-Str.11  
37079 Goettingen, Germany

Phone +49 551 308 0  
Fax +49 551 308 3289

 [www.sartorius.com](http://www.sartorius.com)

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