SARTORIUS

Instructions for Use

Microsart® RESEARCH Bacteria

Bacteria Detection Kit for qPCR
Prod. No. SMB95-1009

Reagents for 25 reactions
For use in research and quality control

Manufactured by:

Minerva Biolabs GmbH | Schkopauer Ring 13 | 12681 Berlin | Germany

Symbols

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

Contents

1. Intended Use

Microsart® RESEARCH Bacteria is used for direct detection of bacterial contaminations in cell cultures and cell culture 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 fluorescent dyes FAM™ and ROX™. The provided protocol is applicable in research and development, for fast and reliable screening of bacteria contamination from cell culture supernatants. The detection procedure can be performed within 3 hours. In contrast to the culture method, samples do not need to contain living bacteria.

3. Test Principle

Bacteria are specifically detected by amplifying a highly conserved rRNA operon, or more specifically, a 16S rRNA coding region of the bacterial genome. The specific amplification is detected at 520 nm (FAM™ channel). The kit includes primers and FAM™-labeled probes, which allow the specific detection of many bacterial species described so far 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 using the internal amplification control, included in the Bacteria RESEARCH Mix. 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. 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 regulatory requirements for S2 labs must be considered.

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 fully understood in order to successfully use 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.

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

8. Assay inhibition may be caused by the sample matrix, but also by elution buffers of DNA extraction kits that are incompatible or that have not been tested with this assay. If DNA extraction is performed, the negative controls should always be set up with the elution buffer used for DNA extraction.

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 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), is recommended.

4.1 Handling and equipment recommendations

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

2. The laminar flow cabinet should be thoroughly decontaminated with PCR Clean™ (Minerva Biolabs, Prod. No. 15-2025) or PCR Clean™ Wipes (Minerva Biolabs, Prod. No. 15-2001) before use.

3. All materials which are introduced in the laminar flow cabinet should be decontaminated thoroughly with PCR Clean™ before beginning the procedure.

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.

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

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 at +2 to +8 °C until use. Lyophilized components must be stored at ≤ -18 °C after rehydration.
Protect the Bacteria RESEARCH Mix from light. The lot specific Certificate of Analysis can be downloaded from the manufacturer's website (www.minerva-biolabs.com).

Kit Component Label Information	Quantity	Cap Color
	25 Reactions Order No. SMB95-1009	
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

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 (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® ATMP Extraction kit, a DNA-free extraction kit for samples containing bacteria and fungi, Sartorius Prod. No. SMB95-2001

Equipment

- qPCR device with filter sets for the detection of the fluorescent dyes FAM™ and ROX™ and suitable for 25 µl PCR reaction volumes
- Minicentrifuge for 1.5 ml reaction tubes and PCR tubes
- Vortex
- Heat block with optional shaking function, when performing DNA extraction
- 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

Cell culture supernatants are ideal samples to be tested with Microsart® RESEARCH Bacteria, directly and without any prior sample preparation or DNA extraction.
However, such cell culture-derived samples contain DNases, which may degrade bacterial DNA even at relatively low temperatures and impair test sensitivity. To avoid such an effect, if the test cannot be performed immediately after sample collection, the specimen should be stabilized by heat inactivation as described in the protocol below. Samples can also be stored at ≤ -18 °C before inactivation. However, we recommend proceeding to the heat inactivation, immediately after thawing.

1. Transfer up to 500 µl of cell culture supernatant or cell culture material into a sterile microcentrifuge tube. The lid should be tightly sealed to prevent opening during heating.
2. Incubate the sample at 95 °C for 10 min.
3. Briefly centrifuge (5 sec) the sample at approx. 13,000 × g to pellet cellular debris.
4. The supernatant can be used for PCR analysis.

If you suspect any PCR inhibitory effects in some of your samples, DNA extraction i.e. with Microsart® ATMP Extraction (Prod. No. SMB95-2001) becomes a mandatory alternative for such samples. Please be aware that samples processed as described above cannot be later used for DNA extraction. For a successful application of Microsart® ATMP Extraction with such samples, skip the protocol described above and proceed directly to DNA extraction with a freshly collected sample.
2 µl of the extract can be used directly as a PCR template.

DNA extracts and heat-inactivated samples can be stored at +2 to +8 °C for 6 days. Longer storage requires a temperature of ≤ -18 °C. Repeated freezing and thawing 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.
To this aim, we recommend Microsart® Calibration Reagents (see Related Products for ordering information). All reagents and samples must be equilibrated to room temperature prior to 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
2.	Bacteria RESEARCH Mix	red cap	Add 600 µl Rehydration Buffer (blue cap)
3.	Positive Control DNA	green cap	Add 300 µl PCR grade Water (white cap)
4.	Bacteria RESEARCH Mix Positive Control DNA	red cap green cap	Incubate 5 min at room temperature. Vortex briefly and spin for 5 sec.

8.2 Preparation of the reaction mix and addition of samples and controls

The preparation of the reaction mix and sample loading should not take longer than 45 minutes to avoid a reduction in the fluorescent signal. The pipetting sequence should be followed strictly and the tubes should be closed after each sample has been loaded.

1. Add 23 µl of rehydrated Bacteria RESEARCH Mix to each PCR tube.
2. Negative controls: add 2 µl of PCR grade Water (white cap) or elution buffer from DNA extraction kit (if DNA extraction was performed, see chapter „Specimen").
3. Sample reaction: add 2 µl of sample.
4. Positive control: add 2 µl of Positive Control DNA (green cap).
5. Close tightly and spin all PCR tubes briefly.

8.3 Start of the qPCR reaction

1. Load the qPCR cycler and check PCR tubes and the cycler lid for tight fit.
2. Program the qPCR cycler or load an appropriate stored temperature profile.

1 cycle	95 °C for 3 min
40 cycles	95 °C for 30 sec
	55 °C for 30 sec
	60 °C for 45 sec (data collection)

   See Appendix for temperature profiles of selected qPCR cyclers.
   Programs for additional cyclers might be available on request.
3. Start the program and data reading.

8.4 Analysis

1. Save the data at the end of the run.
2. Analyze the FAM™ and ROX™ channels and examine the linear representation of the obtained amplification plots.
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. 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 standard curve can be reliably generated with suitable PCR standards (see „Calibration Reagent" in Related Products).

A successfully performed PCR without inhibition is indicated by an increasing fluorescence signal in the internal control (IC) channel (ROX™). Target bacterial DNA and Internal Control DNA are competitors in PCR. Because of the low concentration of Internal Control DNA in the PCR mix, the signal strength in this channel might be reduced with increasing bacteria 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	Bacteria positive
negative (no Ct)	negative**	PCR inhibition*
negative (no Ct)	positive (Ct < 40)**	Bacteria 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, extract the DNA from your sample material and repeat the PCR.

** To be considered valid, the internal control (ROX™) for negative samples (FAM™: no Ct) must show comparable Ct values to those obtained for the negative controls (NTC) (± 2 cycles).

9.2 Detailed analysis and recommended actions

Sample	Result	Interpretation	Action
NTC	negative	Valid PCR	Interpret specimen results
	positive	PCR contamination	Repeat the test
PC	positive	Valid PCR	Interpret specimen results
	negative	Failed PCR	Repeat the test
Specimen	0/2 positive	No contamination	Consider specimen as not contaminated.
	1/2 positive	Possible contamination	Repeat the test. If result is confirmed, consider specimen as contaminated.
	2/2 positive	Contamination	Consider specimen as contaminated.

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 Microsart® RESEARCH Bacteria kit was successfully tested with the following devices:
Rotor-Gene® Q/ Rotor-Gene® (Qiagen), CFX96 Touch™ / CFX96 Touch™ Deep Well (Bio-Rad), ABI Prism® 7500 (Applied Biosystems), Mx3005P™ (Agilent Technologies), AriaMx™ (Agilent Technologies), LightCycler® 480 II (Roche) (available on request).

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 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

3. Analysis:

- Open the menu **Analysis**.
- Select **Quantitation**.
- Check the required filter set (green and orange) 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 performed 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.

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 on 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
		GO TO Step 2, 39 more cycles		

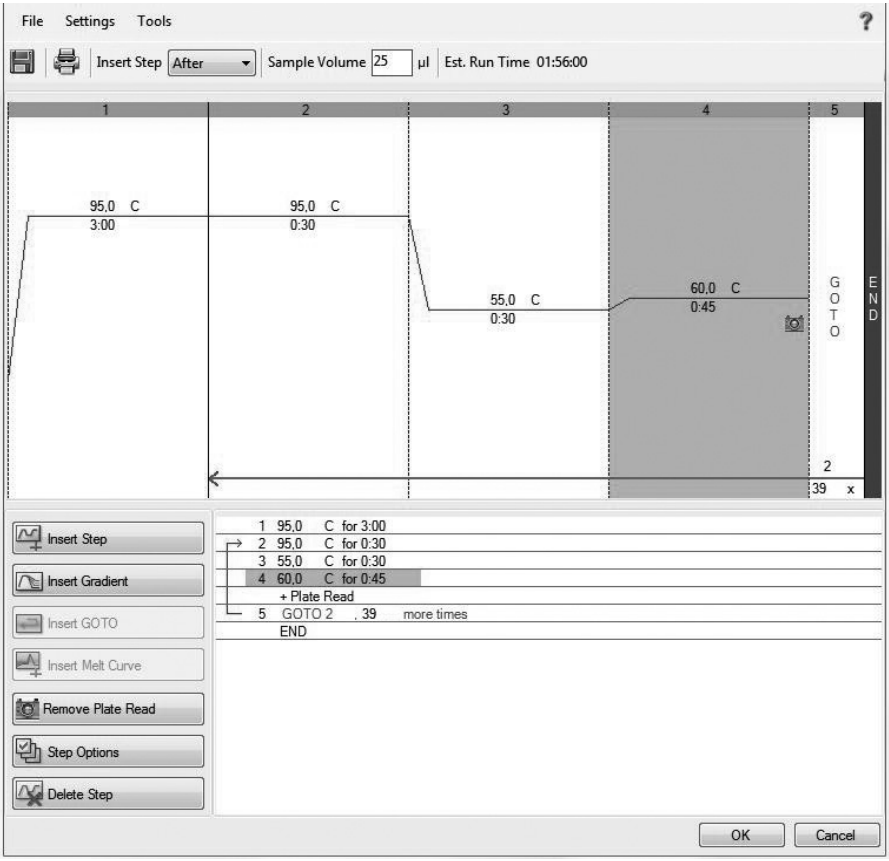
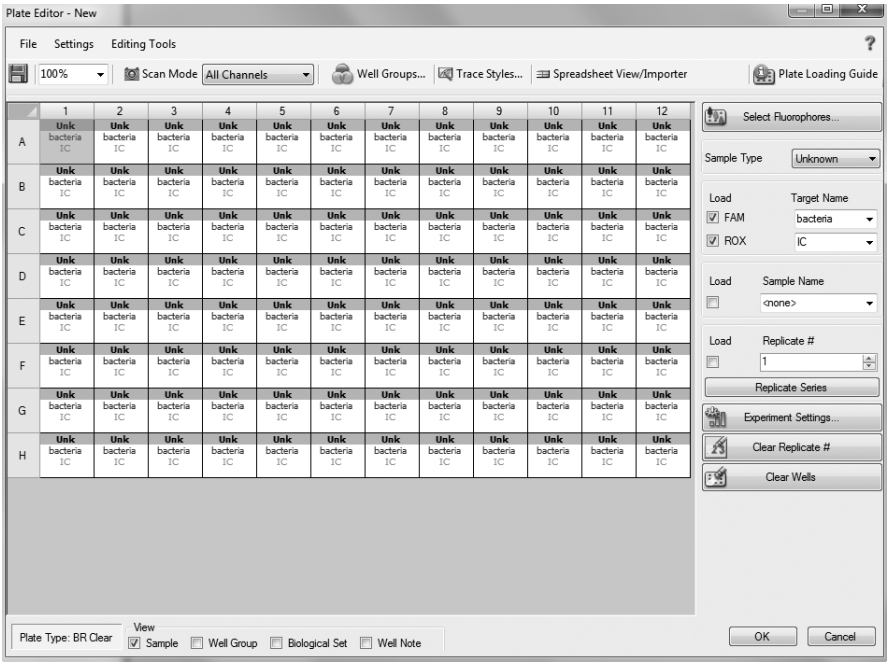


Plate Setup:

- Click File → New → Plate to open the Plate Editor and create a new plate.
- Specify the type of sample with Sample Type.
- Name your samples with 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 the 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.
- View amplification curves of FAM™ channel by selecting the FAM™ checkbox under the amplification plot.

Note: 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.

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

3. 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 cycler by pushing **Start**.

Analysis of raw data:

- In the window **Analysis**, tap on **Analysis Selection / Setup** to analyze the marked positions.
- Ensure that in window **Algorithm Enhancement** all options are activated:

  Amplification-based threshold

  Adaptive baseline

  Moving average

- Click on **Results** and **Amplification Plots** for automatic thresholds.
- To set the thresholds manually: 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
SMB95-1008	Microsart® ATMP Bacteria	100 tests
SMB95-1012	Microsart® ATMP Fungi	100 tests
SMB95-1014/1013	Microsart® RESEARCH Fungi	25/100 tests

Microsart® Calibration Reagent, 10^8 genomes / vial, 1 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, 10^6 genomes / vial, 1 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, 10 CFU / vial, 3 vials each (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, 100 CFU / vial, 3 vials each (Mollicutes)

SMB95-2051	Mycoplasma orale
SMB95-2052	Mycoplasma pneumoniae

Microsart® Validation Standard, 99 CFU / vial, 6 vials each (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® ATMP Extraction (for bacteria and fungi)	50 extractions
SMB95-2003	Microsart® AMP Extraction (for mycoplasma)	50 extractions
SMB95-2002	Microsart® AMP Coating Buffer	20 × 2 ml
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 × 500 ml

PCR Clean™ Wipes**

15-2001	DNA Decontamination Reagent, Wipes	50 wipes
15-2002	DNA Decontamination Reagent, refill sachets	5 × 50 wipes

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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.

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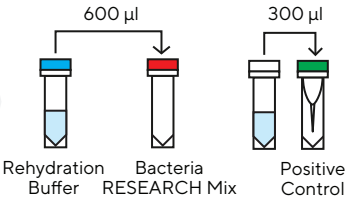
1. Rehydration of Reagents

Bacteria RESEARCH Mix
Positive Control

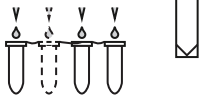


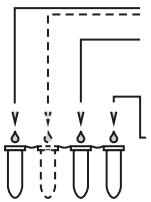
for 5 min RT
briefly
for 5 sec

2. Preparation of PCR Reactions

Loading the test tubes

+ 23 µl
Bacteria RESEARCH Mix
(red cap)

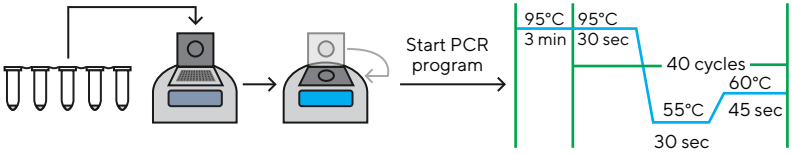




+ 2 µl sample
+ 2 µl Positive Control (green cap)
+ 2 µl elution buffer or PCR grade Water (white cap) (negative control)

Close lid tightly
briefly

3. Start of the qPCR 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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