SARTORIUS

Instructions for Use

Microsart® Research Mycoplasma

Mycoplasma Detection Kit for qPCR
Prod. No. SMB95-1005 | SMB95-1006

Reagents for 25 | 100 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 or 100 reactions
	Manufacturer

Contents

1. Intended Use

Microsart® Research Mycoplasma is used for direct detection of Mollicutes (*Mycoplasma, Acholeplasma, Spiroplasma*) contamination in cell cultures and cell culture media components in research and development.

2. Explanation of the Test

Microsart® Research Mycoplasma utilizes real-time PCR (qPCR) as the method of choice for fast detection of mycoplasma contaminations. 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 enables fast and reliable mycoplasma screening of cell culture supernatants and is therefore best suited for use in research and development.
The detection procedure can be performed within 3 hours. In contrast to the detection with luminescence-based enzyme assays, fluorescent staining or culture methods, the samples for this screening do not need to contain live mycoplasma.

3. Test Principle

Mycoplasma are specifically detected by amplifying a highly conserved rRNA operon, or more precisely, a 16S rRNA coding region in the mycoplasma genome. The mycoplasma-specific amplification is detected at 520 nm (FAM™ channel). The kit includes primers and FAM™-labeled probes, which allow specific detection of all mollicute species so far described as contaminants of cell cultures and media components. Eukaryotic DNA does not represent a relevant cause of false positive results as it is not amplified by this primer/probe system.

False negative results due to PCR inhibitors or improper DNA extraction are detected by the internal amplification control, which is already included in the Mycoplasma Master Mix. The amplification of the internal amplification control is detected at 610 nm (ROX™ channel).

The kit contains dUTP instead of dTTP, in order to degrade amplicons from previous analysis by using uracil-DNA glycosylase (UNG). This minimizes the occurrence of false-positive results. UNG is not included in the kit.

4. Notes on the Test Procedure

1. For *in vitro* use in research. This kit should be used by trained staff, only.

2. This leaflet must be fully understood for a successful use of Microsart® Research Mycoplasma kit. 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.

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

4. 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.

5. 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.

6. The use of control samples is advised to secure the day-to-day validity of results. It is recommended to run laboratory-specific control samples with a high, medium, and low DNA level (e.g. 100 copies/µl), or established commercial controls, for example Sartorius Microsart® Calibration Reagents (see Related Products).

7. We recommend the participation in external quality control programs, such as those offered by Minerva Biolabs.

5. Reagents

Each kit contains reagents for 25 or 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 upon arrival, at low humidity conditions. Lyophilized components must be stored at ≤-18 °C after rehydration. Protect the Mycoplasma Master Mix from light.
The lot specific Certifices of Analysis can be downloaded from the MySartorius portal (https://my.sartorius.com).

Kit Component Label Information	Quantity		Cap Color
	25 Reactions Order No. SMB95-1005	100 Reactions Order No. SMB95-1006	
Mycoplasma Mix	1 × lyophilized	4 × lyophilized	red
Rehydration Buffer	1 × 1.0 ml	3 × 1.0 ml	blue
Positive Control DNA	1 × lyophilized	1 × lyophilized	green
PCR grade Water	1 × 1.0 ml	1 × 1.0 ml	white

6. Needed but not included

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

- qPCR device with filter sets for the detection of the fluorescent dyes FAM™ and ROX™ and suitable for 25 µl PCR reaction volumes
- Vortex
- PCR reaction tubes for the specific qPCR device
- 1.5 ml reaction tubes, DNA- and RNA-free
- Microcentrifuge for 1.5 ml PCR reaction tubes
- Pipettes with corresponding filter tips to prepare and dispense the reaction mix (10, 100 and 1000 µl)

  Optional:
- Microsart® AMP Extraction kit (Prod. No. SMB95-2003)

7. Specimen

The assay can be successfully performed with different types of cell culture-derived material. Cell culture and cell culture supernatants are ideal samples to be tested with this assay, directly and without any prior sample preparation.
Cell cultures should be tested preferably when they reach 80 - 90 % confluence. PCR inhibiting substances may accumulate in the medium over time and might be significantly present in older/overconfluent cultures. Importantly, the maximum cell number that does not interfere with the test can vary significantly, according to specific characteristics of the sample (e.g. medium, cell type, and a combination of both factors). Therefore, results interpretation should always be performed on a case-by-case basis and take these matrix-specific differences into account. Penicillin and streptomycin in culture media do not inhibit mycoplasma or affect test sensitivity.

Typically, positively tested samples like contaminated spent media or supernatants contain average titers of 10^6 mycoplasma particles/ml and maximum titers of 10^8 mycoplasma particles/ml. In these conditions, sufficient DNA is available for PCR, even for cell membrane-associated species or in the rare eventuality of an intracellular contamination. With known or suspected sample-related sensitivity issues, we recommend testing native, cells-containing cell culture samples.

Standard protocol

Use 2 µl of the native cell culture or cell culture supernatant directly for PCR analysis (see 8.2).

Storage of samples for later qPCR analysis

Samples collected from cell cultures contain DNases, which can degrade mycoplasma DNA even at low temperatures. Therefore, if the PCR cannot be performed immediately after sample collection, it is advised to stabilize the sample material 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 500 µl of cell culture supernatant or cell culture. The lid should be sealed tightly to prevent opening during heating.
2. Boil or incubate the sample at 95 °C for 10 min.
3. Centrifuge the sample briefly (5 sec) at approx. 13,000 × g to pellet cellular debris.
   Note: Please use the heat-inactivated samples only after a centrifugation step. This step is critical to avoid potential interferences of the matrix on the test sensitivity.
4. The supernatant can now be used for qPCR analysis.

DNA extraction prior to PCR becomes mandatory for samples with higher matrix complexity such as cell pellets, cryostocks, samples from long-term cultures, fetal calf serum (FCS), vaccines and paraffin-embedded samples. To this aim, we recommend using Microsart® AMP Extraction (Prod. No. SMB95-2003) or an alternative procedure. Please read carefully the specific instructions given with the isolation protocol before starting the test. After the extraction, 2 µl of the extract can be used directly as 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, 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). 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 should be stored in aliquots.

1.	Mycoplasma Mix Positive Control DNA	red cap green cap	Spin all lyophilized components for 5 sec at maximum speed.
2.	Mycoplasma 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.	Mycoplasma 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 Mycoplasma Mix to each PCR tube.
2.	Negative controls: add 2 µl of PCR grade Water (white cap) or elution buffer from DNA extraction kit (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
45 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 the initial linear section of the positive control reaction. ROX™: adapt the threshold line to the initial linear section of the NTCs.

4. Analyze the Ct values calculated for controls and samples.

9. Interpretation of Results

The presence of mycoplasma DNA in the sample is indicated by an increasing fluorescence signal in the FAM™ channel during PCR. A successfully performed PCR without inhibition is indicated by an increasing fluorescence signal in the internal control (ROX™) channel. Mycoplasma 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 is reduced with increasing mycoplasma DNA load in the sample.

Detection of Mollicutes FAM™ channel	Internal Control ROX™ channel	Interpretation
positive (Ct < 40)	irrelevant	Mollicutes positive
negative (Ct ≥ 40)*	negative**	PCR inhibition
negative (Ct ≥ 40)*	positive (Ct < 40)**	Mollicutes negative

* Samples showing Ct values ≥ 40 need to be evaluated individually. First, check their amplification curves for significant fluorescence increase in comparison to the background noise of the negative control. In case of substantial amplification, the curve should display the typical shape associated with a logarithmic "amplification" function. In general, we recommend repeating the testing of samples with such Ct values. In fact, even if a Ct ≥ 40 is not necessarily indicating amplification of mycoplasma DNA, it can reflect sample matrix effects caused by incorrect sample preparations or setup errors.

** If used as PCR control, the internal control of negative samples (FAM™ ≥ 40) must show Ct values in the range of ± 2 cycles (ROX™) of the negative control (master mix control, NTC).

10. Appendix

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

The Microsart® Research Mycoplasma kit was successfully tested with the following devices:
LightCycler® 1.0 and 2.0 (Roche), Rotor-Gene® (Qiagen), ABI Prism® 7500 (Applied Biosystems), Mx3005P™ (Agilent Technologies), CFX96 Touch™ / CFX96 Touch™ Deep Well (Bio-Rad), AriaMx™ (Agilent Technologies).

LightCycler® 1.0 and 2.0

Program 1: Pre-incubation

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

Important for LC 2.0:
Please check the correct settings for Seek Temperature of at least 90 °C.

Program 2: Amplification

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

Program 3: Cooling

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

Analysis:

- Select the fluorescence channels Channel 1 (520 nm) and Channel 3 (610 nm).
- Click on Quantification to generate the amplification plots and the specific Ct values.
- The threshold will be generated automatically.

Rotor-Gene® 6000 (5-plex)

1. Check the correct settings for the filter combination:

Target	Mollicutes	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	45
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 the initial linear section of the positive control reaction. ROX™: adapt the threshold line to the initial linear section of the NTCs.
- 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	Mollicutes	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	3:00 min

Program Step 2: Amplification

Cycles	45
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 the initial linear section of the positive control reaction. ROX™: adapt the threshold line to the initial linear section of the NTCs.

- 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:	45 cycles	30 sec	95 °C	
		30 sec	55 °C	
		45 sec	60 °C	data collection

Analysis mode: non adaptive baseline (baseline correction)

- at menu **Run Status** select **Run** and start the cycler by pushing **Start**.

<u>Analysis of raw data:</u>

- 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: for FAM™, adapt the threshold line to the initial linear section of the positive controls; for ROX™, adapt the threshold line to the initial linear section of the NTCs.
- Read the Ct values in **Text Report**.

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
		GO TO Step 2, 44 more cycles		

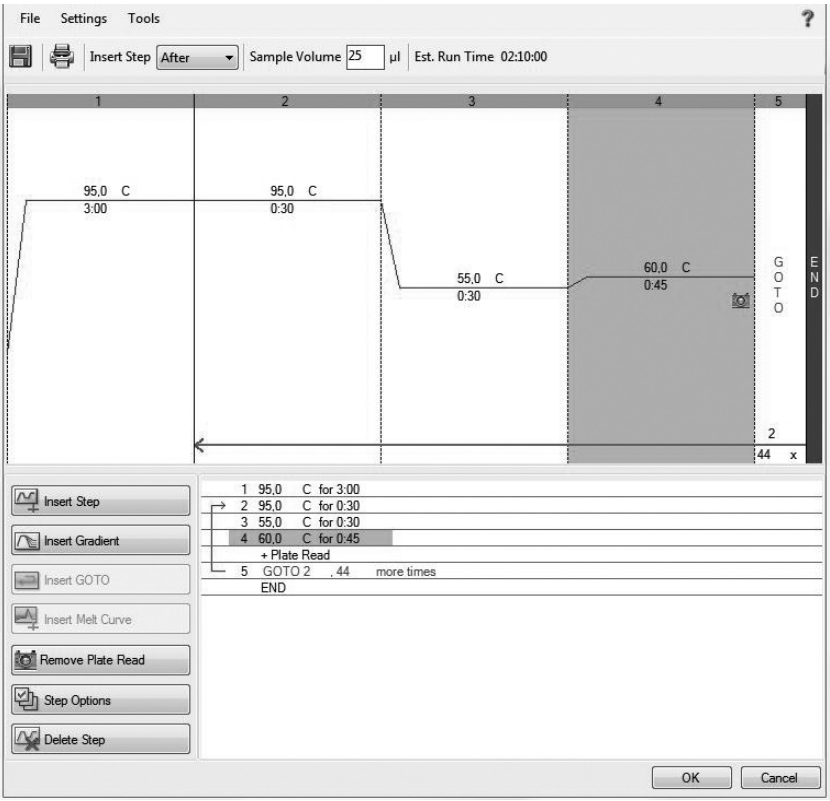
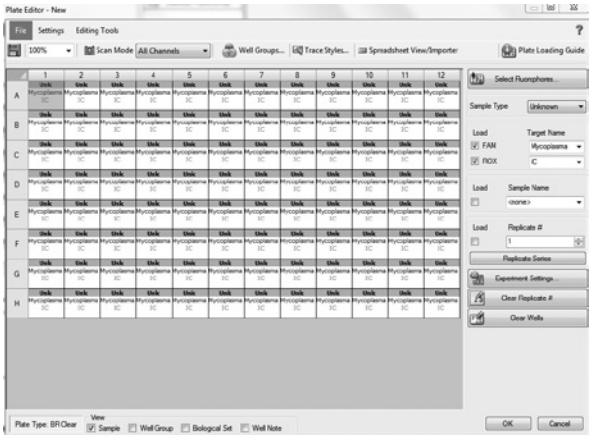


Plate Setup:

- 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 mycoplasma 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 mycoplasma 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.
- To enable thresholds setting, please follow the specific instructions provided in the manual of your cycler.
- FAM™: adapt the threshold line to the initial linear section of the positive controls. ROX™: adapt the threshold line to the initial linear section of the NTCs.
- Evaluate the Ct values according to chapter 9.

AriaMx™

- Click on New Experiment and Quantitative PCR - Fluorescence Probe.
- Go to the Plate Setup menu and check all positions which apply.
- Choose the fluorophore data you want to display by clicking the fluorophore checkboxes located under Well Types drop down menu. Select FAM™ to display data of mycoplasma detection and ROX™ to display internal control amplification data.
- Corresponding to the basic settings the Reference Dye function should be deactivated.
- Use the Well Types drop down menu to specify the type of sample.
- Name the samples.
- Edit the temperature profile by changing to Thermal profile tab.

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

- Start the run by clicking Run Experiment in the Thermal Profile tab in the top right-hand corner.

Data Analysis:

- In the area Analysis click on Analysis Criteria and mark the wells you want to analyze.
- By clicking the checkbox at the top left-hand of the plate screen you can select all wells.
- By changing to Graphical Display tab you can see the amplification plot.
- Choose ΔR (baseline corrective raw fluorescence) under Fluorescence Term and turn the Smoothing option on. The threshold will be generated automatically.
- Please ensure that the automatically calculated threshold line is in the initial linear section of the reference sample (for FAM™: positive controls; for ROX™: NTCs). If not, change to Log view by clicking the triangle under Smoothing options to see advanced options for the amplification plot. Choose the Log option for the graph type and adapt the threshold line manually to the middle of the linear section of the reference sample.
- Read specific Ct values at Result table.

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
SMB95-1007	Microsart® ATMP Sterile Release	10 samples
SMB95-1008	Microsart® ATMP Bacteria	100 tests
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, 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

SMB95-2001	Microsart® ATMP Extraction (for bacteria and fungi)	50 extractions
SMB95-2003	Microsart® AMP Extraction (for mycoplasma)	50 extractions
SMB95-4000	Microsart® Proteinase K	50 extractions

Cleaning Spray

SMB95-5001	DNA Decontamination Reagent, spray bottle	250 ml
SMB95-5002	DNA Decontamination Reagent, refill canister	5 l

Cleaning Wipes

SMB95-5003	DNA Decontamination Reagent, wipes	50 wipes
SMB95-5004	DNA Decontamination Reagent, refill sachets	5 × 50 wipes

Limited Product Warranty

This warranty limits our liability for replacement of this product.
No warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided. Minerva Biolabs shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

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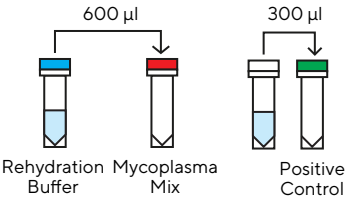
1. Rehydration of Reagents

Mycoplasma Mix
Positive Control

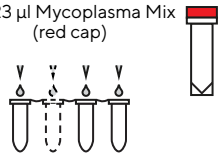


for 5 min RT
briefly
for 5 sec

2. Preparation of PCR Reactions

Loading the test tubes

+ 23 µl Mycoplasma Mix (red cap)

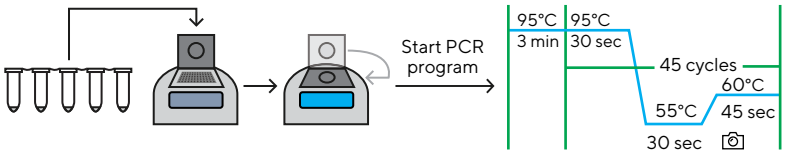


+ 2 µl sample
+ 2 µl Positive Control (green cap)
+ 2 µl elution buffer or PCR grade Water (white cap) (negative control)

Close lid tightly

briefly

3. Start of the qPCR Reaction



Rehydration Buffer
Mycoplasma Mix
PCR grade Water
Positive Control

incubate
vortex
centrifuge
add

data reading

storage +2 to +8 °C upon arrival
after rehydration ≤ -18 °C

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

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