

Microsart® AMP Mycoplasma

Mycoplasma Detection Kit for qPCR
Prod. No. SMB95-1001 | SMB95-1002

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

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1. Intended Use

Microsart® AMP Mycoplasma is used for the direct detection of Mollicutes (*Mycoplasma*, *Acholeplasma*, *Spiroplasma*) in cell cultures, cell culture media components and cell culture derived biologicals according to European Pharmacopoeia (EP) 2.6.7 “Mycoplasmas”.

2. Explanation of the Test

Microsart® AMP Mycoplasma utilizes real-time PCR (qPCR) as the method of choice for sensitive and robust 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 detection procedure can be performed within 3 hours. In contrast to the detection by luminescence-based enzyme assays, fluorescent staining, or culture methods, samples do not need to contain living mycoplasma.

The kit was validated according to EP 2.6.7 “Mycoplasmas” in combination with EP 2.6.21 “Nucleic Acid Amplification Techniques” with respect to detection limit for all listed mycoplasma species, specificity and robustness for cell cultures and autologous cell transplants (e.g. chondrocytes). The kit complies fully with the requirements of EP 2.6.7. The validation report is available on request. However, please note that these validation data are provided for information purposes only. EP 2.6.7 clearly states “Where commercial kits are used ..., documented validation points already covered by the kit manufacturer can replace validation by the user. Nevertheless, the performance of the kit with respect to its intended use has to be demonstrated by the user (e.g. detection limit, robustness, cross-detection of other classes of bacteria)”. Please feel free to contact us if you need further assistance.

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 the specific detection of all mollicute species mentioned in EP 2.6.7 and many more. The polymerase is part of the Mycoplasma Mix.

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).

The kit contains dUTP instead of dTTP, so the option to degrade amplicons from previous analysis by use of uracil-DNA glycosylase (UNG) is available. Thus, the occurrence of false-positive results can be minimized. UNG is not included in the kit.

4. Notes on the Test Procedure

1. For in vitro use in research and quality control. This kit should be used by trained staff, only.
2. All samples should be considered potentially infectious and handled according to local or national regulations. This kit does not contain hazardous substances and may be disposed of according to local regulations.
3. This leaflet must be fully understood in order to successfully use Microsart® AMP 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.
4. Any deviation from the test method can affect the results.
5. Assay inhibition may be caused by the sample matrix but also by sample elution buffers of incompatible or non-validated DNA extraction kits. If DNA extraction is performed, the negative controls should always be prepared with the elution buffer used for DNA extraction.
6. 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.
7. The use of control samples is advised to ensure the day-to-day validity of results. The controls should be handled in the same manner as the samples. It is recommended to run laboratory specific control samples with a high, medial and low level (e.g. $3 \times \text{LOD}_{95}$), or established commercial controls, for example Sartorius Microsart® Validation Standards or Microsart® Calibration Reagents, available for all mycoplasma species listed in EP 2.6.7 (see Related Products).
8. 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 specified on the package label. The kit components are stored at +2 to +8 °C until use and must be stored at ≤ -18 °C after opening and rehydration. Protect the Mycoplasma 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. SMB95-1001 | 100 Reactions Order No. SMB95-1002 | |
| Mycoplasma Mix | 1 × lyophilized | 4 × lyophilized | red |
| Rehydration Buffer | 1 × 1.5 ml | 4 × 1.5 ml | blue |
| Positive Control DNA | 1 × lyophilized | 4 × lyophilized | green |
| Internal Control DNA | 1 × lyophilized | 4 × lyophilized | yellow |
| PCR grade Water | 1 × 1.0 ml | 4 × 1.0 ml | white |

6. Needed but not included

Microsart® AMP Mycoplasma contains all the reagents, including negative and positive controls, and polymerase (as a component of the Mycoplasma Mix) to perform the test. 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 100 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 (Prod. No. SMB95-2003)
- Vivaspin® 6 or 20 (Prod. No. VS0641 / VS2041) and compatible centrifuges
- Microsart® AMP Coating Buffer (Prod. No. SMB95-2002)
- Microsart® Validation Standard available for all EP-listed mycoplasma species (see Related Products)
- Microsart® Calibration Reagent available for all EP-listed mycoplasma species (see Related Products)

7. Specimen for Compliant Testing

7.1 Sample collection and concentration

Please note that it is highly recommended to use preferably native cell culture samples with cells to detect intracellular mycoplasma as well. The kit has been validated using a collection of several representative cell culture samples (e.g. cell supernatants, suspensions etc.) provided by our customers. The assay can be successfully performed with different types of cell culture-derived material. Therefore, the maximum cell number can vary significantly according to the specific characteristics of the sample (e.g. medium, cell type, and a combination of both factors) and suitability should be checked as part of each matrix specific validation.

Standard protocol:

Use 200 µl of the native cell culture or cell culture supernatant directly for DNA extraction (7.3).

Concentration step for sample volumes > 200 µl (alternative protocols):

For samples from 200 µl to 18 ml, an enrichment step is recommended to take advantage of the large sample volume and to increase the sensitivity of the test. Please note that this protocol is suitable for native samples only. For the following test procedures the Microsart® AMP Extraction kit (Prod. No. SMB95-2003) is recommended. This kit was especially designed to complement the Microsart® AMP Mycoplasma qPCR.

Test procedure for sample volumes > 200 µl to 5 ml

1. Add 1 ml Microsart® Coating Buffer to the sample and vortex the mixture briefly. Transfer the mixture into a Vivaspin® 6 unit.

 2. Centrifuge for up to 30 min depending on the maximum speed of the available centrifuge and the viscosity of the sample matrix (see Vivaspin® operation instructions for details). The dead stop volume of the Vivaspin® 6 is 30 µl. The sample is concentrated to a volume < 200 µl.

 3. Estimate the volume of the retentate during transfer into a fresh reaction tube.

 4. Rinse the Vivaspin® 6 unit with the lysis buffer of the DNA extraction kit (Buffer A1 in case of Microsart® AMP Extraction). The wash volume depends on the volume of the retentate. In case of Microsart® AMP Extraction:
wash volume = 400 µl – volume retentate. The wash is added to the retentate.

 5. The complete sample can be used for DNA extraction. The sample (400 µl) contains the starting material (cell culture) and the lysis buffer. Proceed with adding buffer A2 and follow further instructions according to Microsart® AMP Extraction manual (see also 7.3).
-

Test procedure for sample volumes > 5 to 18 ml

1. Add 2 ml Microsart® AMP Coating Buffer to the sample and vortex the mixture briefly. Transfer the mixture into a Vivaspin® 20 unit.
 2. Centrifuge for up to 40 min depending on the maximum speed of the available centrifuge and the viscosity of the sample matrix (see Vivaspin® operation instructions for details). The dead volume of the Vivaspin® 20 is 50 µl. The sample is concentrated to a volume < 200 µl.
 3. Estimate the volume of the retentate during transfer into a fresh reaction tube.
 4. Rinse the Vivaspin® 20 unit with the lysis buffer of the DNA extraction kit (Buffer A1 in case of Microsart® AMP Extraction). The wash volume depends on the volume of the retentate. In case of Microsart® AMP Extraction:
wash volume = 400 µl – volume retentate. The wash is added to the retentate.
 5. The complete sample can be used for DNA extraction. The sample (400 µl) contains the starting material (cell culture) and the lysis buffer. Proceed with adding buffer A2 and follow further instructions according to Microsart® AMP Extraction manual (see also 7.3).
-

7.2 Sample storage for later DNA extraction

Samples directly retrieved from cell culture contain DNases which can degrade mycoplasma DNA even at lower temperatures. If PCR analysis cannot be performed immediately after sampling, it is advised to stabilize the sample material by freezing or heat inactivation:

1. Transfer an appropriate volume of cell culture (e.g. 500 µl) or cell culture supernatant to a sterile reaction tube. 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.
 4. The supernatant (max. 200 µl) can now be used for DNA extraction (see also 7.3).
-

Heat-inactivated samples can be stored at +2 to +8 °C for 6 days. Longer storage times require a temperature of ≤ -18 °C. Repeated freezing and thawing should be avoided.

7.3 DNA extraction

Several studies show that DNA extraction is required to achieve highest sensitivity during testing of any kind of sample. For most types of specimen, a standard DNA extraction method can be applied to obtain suitable PCR templates. However, such DNA extraction method must be applicable to the purification of mycoplasma genomic DNA. Furthermore, the obtained template must be validated in combination with the kit. Therefore, for an optimal performance, we recommend Microsart® AMP Extraction kit (Prod. No. SMB95-2003), which was extensively validated in combination with this testing procedure. The protocol for DNA purification is described in detail in the manual of the DNA extraction kit.

Optional: The EP 2.6.7 requires a test sensitivity of 10 CFU/ml. Therefore, the sample can be spiked with mycoplasma particles by using special reference materials (Microsart® Validation Standard, Mollicutes see Related Products) and processed in parallel.

Recommended: The Internal Control DNA of Microsart® AMP Mycoplasma can be used to monitor the extraction process as follows. Add 5 µl of Internal Control DNA directly to each sample, vortex briefly and proceed to the DNA extraction as described. Please note that only the final elution volume (60 µl DNA extract) is relevant here, whereas the sample volume to be used does not affect the amount of Internal Control DNA. Once added, the Internal Control DNA does not need to be pipetted in the reaction mix any longer (see chapter 8.2). Extracts 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. All reagents and samples must be equilibrated to +2 to +8 °C prior to use.

8.1 Rehydration of the reagents

Reagents should be stored at ≤ -18 °C after reconstitution. We recommend the storage of small aliquots rather than performing repeated cycles (> 4) of freezing and thawing.

| | | | |
|----|--|------------------------------------|--|
| 1. | Mycoplasma Mix Internal Control DNA Positive Control DNA | red cap yellow cap green cap | Spin all lyophilized components for 5 sec at maximum speed |
| 2. | Mycoplasma Mix | red cap | Add 1275 μ l Rehydration Buffer (blue cap) |
| 3. | Internal Control DNA | yellow cap | Add 300 μ l PCR grade Water (white cap) |
| 4. | Positive Control DNA | green cap | Add 300 μ l PCR grade Water (white cap) |
| 5. | Mycoplasma Mix Internal Control DNA Positive Control DNA | red cap yellow cap green cap | Incubate 5 min at room temperature |
| 6. | Mycoplasma Mix Internal Control DNA Positive Control DNA | red cap yellow cap green cap | Vortex briefly and spin for 5 sec |

8.2 Preparation of the reaction mix

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

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

1. Prepare the required amount of reaction mix at room temperature in a 1.5 ml reaction tube for all control and test reactions.

| | Cap Color | For 1 reaction | For 25 reactions |
|----------------------|-----------|----------------|------------------|
| Mycoplasma Mix | red | 49.0 μ l | 1225.0 μ l |
| Internal Control DNA | yellow | 1 μ l | 25 μ l |

2. Mix by gently tapping the tube.
3. Add 50 μ l to each PCR tube.

Attention:

If the Internal Control DNA was added to the sample prior to DNA extraction, add 50 μ l of the Mycoplasma Mix (red cap) directly to each PCR tube.

8.3 Addition of samples and controls

1. Negative controls: add 50 µl elution buffer from the DNA extraction kit (see chapter „Specimen“) or 50 µl PCR grade Water (white cap).

2. Sample: add 50 µl of sample.

3. Positive control: add 50 µl Positive Control DNA (green cap).

Important:

If the Internal Control DNA was added to the samples during DNA extraction, add 1 µl of Internal Control DNA to each negative and positive control.

4. Close tightly and spin all PCR tubes briefly.

8.4 Start of the qPCR reaction

1. Load the qPCR cycler, check the PCR tubes, and the cycler lid for tight fit.

2. Program the qPCR cycler or load an appropriate stored temperature profile. 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.

8.5 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. Set the thresholds following the specific instructions provided in the manual of your cycler or those given in the Appendix, for selected devices. Check fluorescence data individually for typical amplification curves (logarithmic increase of fluorescence) and correct threshold settings manually, if needed.

4. Analyze the calculation of the Ct values for the negative controls, positive controls, and samples.

9. Interpretation of Results

The presence of mollicutes 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 channel. Mollicute 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 mollicute 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). If used as process control, the internal control of negative samples (FAM™ ≥ 40) must show Ct values in the range of +/- 3 cycles (ROX™) of the NTC.

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® AMP Mycoplasma kit:

ABI Prism® 7500
Mx3005P™

According to the device specifications provided by the manufacturer, the following cycler is compatible with the Microsart® AMP Mycoplasma assay:

CFX96 Touch™ deep well

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 |

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 (Pre-Melt): | 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”.

Analysis of raw data:

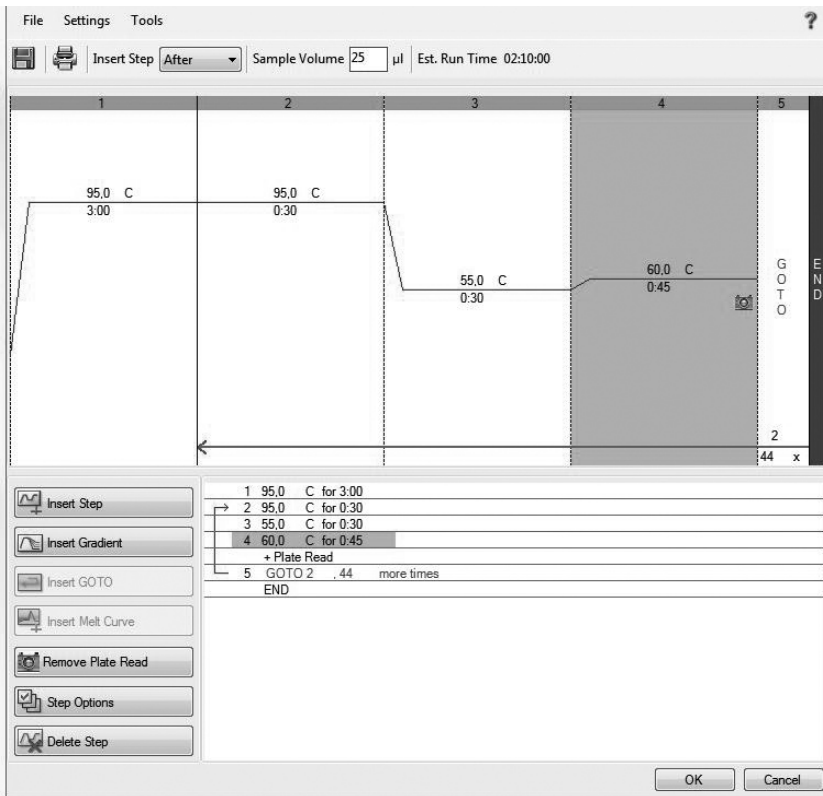
- In the window “Analysis” tap on “Analysis Selection / Setup” to analyze the marked positions.
- Ensure that in window “Algorithm Enhancement” all options are activated:
 - Amplification-based threshold
 - Adaptive baseline
 - Moving average
- Click on “Results” and “Amplification Plots” for automatic thresholds.
- To set the thresholds manually: 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”.

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 |
| GO TO Step 2, 44 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 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.

Plate Editor - New

File Settings Editing Tools

100% Scan Mode All Channels Well Groups... Trace Styles... Spreadsheet View/Importer Plate Loading Guide

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| A | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC |
| B | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC |
| C | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC |
| D | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC |
| E | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC |
| F | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC |
| G | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC |
| H | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC | Unk Mycoplasma IC |

Select Fluorophores...

Sample Type: Unknown

Load Target Name

FAM Mycoplasma

ROX IC

Load Sample Name

<none>

Load Replicate #

1

Replicate Series

Experiment Settings...

Clear Replicate #

Clear Wells

View

Plate Type: BR Clear

Sample Well Group Biological Set Well Note

OK Cancel

Data Analysis:

- Select Settings in the menu and select Baseline Subtracted Curve Fit as baseline setting and Single Threshold mode as C_q 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.

11. Related Products

Detection Kits for qPCR

| | | |
|-----------------|---------------------------------|--------------|
| 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 |
| SMB95-1009 | Microsart® RESEARCH Bacteria | 25 tests |
| SMB95-1012 | Microsart® ATMP Fungi | 100 tests |
| SMB95-1014/1013 | Microsart® RESEARCH Fungi | 25/100 tests |

Vivaspin® and Coating Buffer

| | | |
|------------|---|-----------|
| SMB95-2002 | Microsart® AMP Coating Buffer | 20 × 2 ml |
| VS0641 | Vivaspin® 6 Polyethesulfone 100,000 MWCO | 25 units |
| VS0642 | Vivaspin® 6 Polyethesulfone 100,000 MWCO | 100 units |
| VS2041 | Vivaspin® 20 Polyethesulfone 100,000 MWCO | 12 units |
| VS2042 | Vivaspin® 20 Polyethesulfone 100,000 MWCO | 48 units |

Microsart® Calibration Reagent, 1 vial, 10⁸ 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, 10⁶ 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® ATMP 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 Wipes | 50 wipes |
| 15-2002 | DNA Decontamination Wipes, refill sachets | 5 x 50 wipes |

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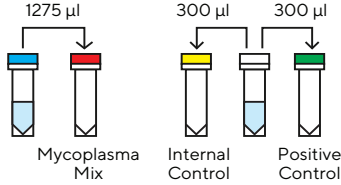
Publication No.: SM-6109-e150102




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Ver. 08 | 2020

1. Rehydration of Reagents

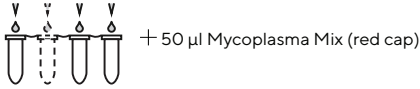
-  Mycoplasma Mix
-  Positive Control DNA
-  Internal Control DNA



-  for 5 min RT
-  briefly
-  for 5 sec

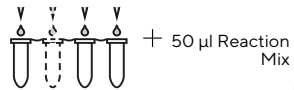
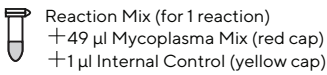
2. Preparation of the Reaction Mix

a) Internal Control added during DNA extraction

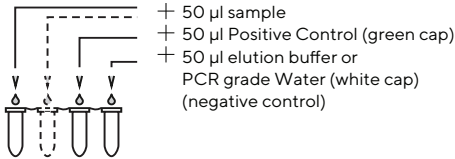



* do not forget to add 1 µl Internal Control to NTC and PC

b) Internal Control not added during DNA extraction

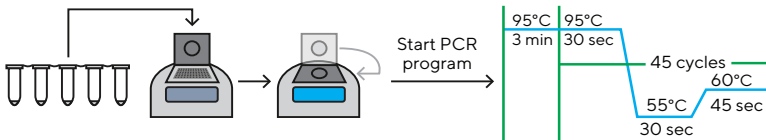







3. Addition of Samples and Controls







Close lid tightly
 briefly

4. Start of the qPCR Reaction



-  Rehydration Buffer
-  Mycoplasma 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@-AMP-Mycoplasma_03_EN

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