

### Microsart® Validation Standard

Prod. No. SMB95-2051    *Mycoplasma orale*  
Prod. No. SMB95-2052    *Mycoplasma pneumoniae*

100 CFU  
For use in research and quality control

## Symbols

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**LOT**

Lot No.

---

**REF**

Order No.

---



Expiry date

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Store at

---



Content

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# 1. Intended Use

Microsart® Validation Standard 100 CFU is used to validate the robustness and sensitivity of NAT-based detection methods in combination with cell cultures, cell culture media components and cell culture derived biologicals like Advanced Therapy Medicinal Products (ATMPs, e.g. cell autologous transplants).

# 2. Explanation of the Product

In a regulated environment, microbial detection can be extremely time-consuming if it relies on conventional culture methods. For rapid microbial tests such as NAT-based methods (e.g. PCR) to replace the traditional culture method, a comprehensive assay validation is required. Particularly, the test sensitivity and robustness must be evaluated as functions of the sample matrix and lab precision.

In addition, the applied analytical method shall show a performance equal or better than the compendial culture procedure. As culturing living mycoplasma for validation purposes represents a highly demanding task for most laboratories, safe and easy-to-use alternatives are needed.

Microsart® Validation Standards 100 CFU are inactivated and therefore non-infectious mycoplasma preparations. They are titrated to 100 Colony-forming Units/ml (CFU/ml), the sensitivity limit for sterility tests according to the United States Pharmacopoeia (USP) <71>. Furthermore, these standards provide an additional test concentration to the Microsart® Validation Standards 10 CFU (Sartorius Prod. No. SMB95-2011 - SMB95-2020) for further validation procedures of PCR-based detection tests.

Each vial contains 100 CFU of inactivated mycoplasma particles. The relevant sample matrix is added directly into the tube. The derived sample is expected to test positive. The inactivated sample material cannot be used for the culture method. For maximal sensitivity, the DNA should be extracted with a DNA-free kit. For this purpose we developed Microsart® AMP Extraction (Sartorius Prod. No. SMB95-2003). The DNA extract can directly be used for PCR. We recommend using Microsart® AMP Mycoplasma (SMB95-1001/ SMB95-1002), Microsart® ATMP Mycoplasma (Sartorius Prod. No. SMB95-1003/ SMB95-1004), or Microsart® RESEARCH Mycoplasma (Sartorius Prod. No. SMB95-1005/1006).

### 3. Notes on the Test Procedure

1. This leaflet must be fully understood in order to successfully use Microsart® Validation Standard. The reagents supplied should not be mixed with reagents from different lots but used as an integral unit. The reagents must not be used beyond their shelf life.
2. Any deviation from the described method can affect the results.
3. Inhibition of PCR may be caused by the sample matrix added to the reagents. Negative controls should always be completed with the same sample matrix.
4. For each test setup, at least one negative control should be added that includes the sample preparation. Typical Ct-values for the analysis of this preparation using the Microsart® AMP Mycoplasma kit are shown on the Certificate of Analysis.
5. 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. Precautions

For *in vitro* use in research and quality control. This kit should be used only by trained persons. All samples should be considered potentially infectious and handled at the local or national regulations. This kit does not contain hazardous substances and may be disposed of according to local regulations.

### 5. Reagents

Each product contains 3 vials of mycoplasma particles as well as 2 vials containing the same carrier matrix as the mycoplasma vials for the preparation of corresponding negative controls. All components are lyophilized for maximal product stability. All particles have been inactivated prior to lyophilization. The expiry date of the unopened package is specified on the package label. The kit components are stored until use at +2 to +8 °C and must be stored after opening and rehydration at ≤ -18 °C.

| Component Label Information | Order No.  | Quantity        | Cap Color |
|-----------------------------|------------|-----------------|-----------|
| Mycoplasma orale            | SMB95-2051 | 3 × lyophilized | green     |
| Mycoplasma pneumoniae       | SMB95-2052 |                 |           |
| Negative Control            |            | 2 × lyophilized | white     |

## 6. Needed but not included

Microsart® Validation Standard contains the positive and negative preparations 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)

### Equipment

- PCR cyclers
- Microcentrifuge for 1.5 ml reaction tubes (Centrisart A-14, Prod. No. A-14-1EU)
- 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

For DNA extraction and PCR analysis, the following kits are required additionally:

- Mycoplasma DNA extraction system. We recommend the Microsart® AMP Extraction (Prod. No. SMB95-2003).
- Mycoplasma DNA PCR detection system. We recommend the Microsart® AMP Mycoplasma (Prod. No. SMB95-1001/1002), Microsart ATMP Mycoplasma (Prod. No. SMB95-1003/1004) or Microsart® RESEARCH Mycoplasma (Prod. No. SMB95-1005/1006).

## 7. Test Procedure

1. Centrifuge the tube(s) briefly to collect the lyophilized material at the bottom.
2. Add 1 ml of the sample matrix of interest to each vial.
3. Incubate for 5 min at room temperature.
4. Vortex for 10 sec and spin down for 5 sec.
5. Use the volume of sample required by the kit selected and used for sample preparation. After DNA extraction, proceed to PCR.

All reagents and samples must be equilibrated to room temperature before use. It is highly recommended to perform suitable DNA extraction of the samples prior to PCR in order to reduce the risk of reaction inhibition and maximize sensitivity. From a manufacturing point of view, the Negative Control vials contains exactly the same components (carrier matrix) as the Mycoplasma vials except for the mycoplasma particles. For a valid interpretation of the test results, the Negative Controls should be rehydrated with the sample matrix of interest and processed in parallel to the samples, in a suitable number of replicates.



## 8. 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    |
| SMB95-1009      | Microsart® RESEARCH Bacteria    | 25 tests     |
| SMB95-1012      | Microsart® ATMP Fungi           | 100 tests    |
| SMB95-1014/1013 | Microsart® RESEARCH Fungi       | 25/100 tests |

### 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, 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 |
| SMB95-2002 | Microsart® AMP Coating Buffer                       | 20 × 2 ml      |
| 56-0002    | Proteinase K**                                      | 50 extractions |

## **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.

## **Trademarks**

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Last technical revision: 2020-08-12



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Status:  
November 2020,  
Sartorius Stedim Biotech  
GmbH, Goettingen, Germany

Printed in Germany on paper that  
has been bleached without any use  
of chlorine. | W  
DIR No.: 2661225  
Ver. 11 | 2020