SARTORIUS

Instructions for Use

Microsart® Validation Standard

Prod. No. SMB95-2011	Mycoplasma arginini
Prod. No. SMB95-2012	Mycoplasma orale
Prod. No. SMB95-2013	Mycoplasma gallisepticum
Prod. No. SMB95-2014	Mycoplasma pneumoniae
Prod. No. SMB95-2015	Mycoplasma synoviae
Prod. No. SMB95-2016	Mycoplasma fermentans
Prod. No. SMB95-2017	Mycoplasma hyorhinis
Prod. No. SMB95-2018	Acholeplasma laidlawii
Prod. No. SMB95-2019	Spiroplasma citri
Prod. No. SMB95-2020	Mycoplasma salivarium

10 CFU
For use in research and quality control

Symbols

LOT	Lot No.
REF	Order No.
	Expiry date
	Store at
	Content

Contents

1. Intended Use

Microsart® Validation Standard is used to validate the robustness and sensitivity of NAT-based mycoplasma detection methods in combination with cell cultures, cell culture media components, and cell culture-derived biologicals (e.g. Advanced Therapy Medicinal Products, ATMPs, such as cell autologous transplants), according to the European Pharmacopoeia 2.6.7 (EP 2.6.7 „Mycoplasmas") .

2. Explanation of the Product

In a regulated environment, microbial detection can be extremely time-consuming if it relies on conventional culture methods. Rapid microbial tests such as NAT-based methods (e.g. PCR) are formally valid alternatives to traditional culture methods, only upon a comprehensive assay validation. In this regard, the EP 2.6.7 requires the validation of the test sensitivity and robustness with respect to the sample matrix and lab precision. In addition, the applied analytical method shall show a performance equal or higher than the compendial culture procedure. As culturing living mycoplasma for validation purposes represents a highly demanding when not impracticable task for most laboratories, safe and easy-to-use alternatives are needed.

Microsart® Validation Standards are inactivated and therefore non-infectious mycoplasma preparations. They are titrated to 10 Colony-forming Units/ml (CFU/ml), the sensitivity limit indicated for NAT-based methods like PCR to replace the traditional culture method.

The mycoplasma used for the manufacture of Microsart® Validation Standard are low passage reference strains cultivated in culture broth (Hayflick and Frey medium), as described in EP 2.6.7. The cultures are harvested in the logarithmic growth phase to avoid a high ratio of dead mycoplasma particles, titrated in culture broth and plated on Hayflick and Frey medium for quantification based on CFU.

Each vial contains inactivated mycoplasma particles corresponding to 10 CFU. The relevant sample matrix can be added directly into the vial. The derived sample is expected to be tested positive by a valid NAT-based assay. The inactivated mycoplasma preparation cannot be used for the culture method. For maximal sensitivity, the mycoplasma DNA should be extracted prior to PCR. For this purpose, we developed Microsart® AMP Extraction (Sartorius Prod. No. SMB95-2003). 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. Reagents should not be mixed with reagents from different lots but used as an integral unit. The reagents should 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. Negative controls should be processed with the same sample matrix.
4. For each sample matrix, at least one negative control should be tested. Resulting Ct values can be compared to lot-specific Ct values specified in the respective Certitificate of Analysis.
5. Participation in external quality control programs, such as those offered by Minerva Biolabs GmbH (www.minerva-biolabs.com), is recommended.

4. 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 should be used directly after rehydration. Storage at ≤ -18 °C could interfere with product performance.

Component Label Information	Order No.	Quantity	Cap Color
Mycoplasma Acholeplasma Spiroplasma	SMB95-2011- SMB95-2020	3 × lyophilized	green
Negative Control		2 × lyophilized	white

5. 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

Equipment

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

6. Precautions

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

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 selected sample preparation kit. 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 PCR inhibition and maximize sensitivity.
From a manufacturing point of view, the Negative Control vials contain 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

Microsart® Validation Standard, 100 CFU / vial, 3 vials each (Mollicutes)

SMB95-2051	Mycoplasma orale
SMB95-2052	Mycoplasma pneumoniae

Microsart® Calibration Reagent, 1 vial, 10^8 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
SMB95-2036	Mycoplasma salivarium

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

Vivaspin®

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

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

** Distributed by Minerva Biolabs

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.

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

Phone +49 551 308 0
Fax +49 551 308 3289
www.sartorius.com

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