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

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 for validating robustness and sensitivity of NAT-based mycoplasma detection methods in combination with cell cultures, cell culture media components and cell culture derived biologicals according to European Pharmacopoeia (EP) 2.6.7 Mycoplasmas.

2. Explanation of the Product

European Pharmacopoeia 2.6.7 Mycoplasmas requires validation of sensitivity and robustness with respect to sample matrix and lab precision. In addition, the method shall show a performance equal or better to the classical cultivation procedure. As most test and cell culture labs are frighten to cultivate mycoplasma, such comparison is not easy to accomplish. Microsart® Validation Standards are not infectious and therefore safe in use. They are titrated to 10 CFU/ml, the sensitivity limit 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 described in EP 2.6.7. The cultures are harvested in the early logarithmic phase of the growth to avoid an atypical high ratio of dead mycoplasma particles, titrated immediately in culture broth and plated on Hayflick and Frey medium for quantification based on colony forming units (CFU).

3. Principle

Each vial contains 10 culture forming units (CFU) of inactivated mycoplasma particles. The relevant sample matrix is added directly into the tube. The arising sample should be tested positive. The inactivated sample material cannot be used for the cultivation method. The suspension should be DNA extracted for highest sensitivity. The DNA extract can directly be used for PCR. EP 2.6.7 does not provide sensitivity limits on DNA level. Microsart® Validation Standard was therefore not titrated for genome copies (GC).

4. Reagents

Each kit contains 3 vials of mycoplasma particles as well as 2 vials containing the same carrier matrix as the mycoplasma vials for the preparation of comparable negative controls. All samples are lyophilized for product stability reasons. All particles have been inactivated prior lyophilisation. 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.

Kit Component Label Information	Quantity	Cap Color
Mycoplasma Acholeplasma Spiroplasma	3 x lyophilized	green
Negative Control	2 x lyophilized	white

5. Needed, but not Included in the Kit

Microsart® Validation Standard contains the reagents to perform the test. General industrial supplies and reagents, usually available in PCR laboratories are not included:

- PCR device
- PCR reaction tubes
- 1.5 ml reaction tubes, DNA- and RNA-free
- Micro centrifuge for 1.5 ml tubes
- Pipettes with corresponding filter tips (10, 100 and 1000 µl)
- Mycoplasma PCR Detection Kit, e.g. Microsart® AMP Mycoplasma (Order No. SMB95-1001/1002) or Microsart® ATMP Mycoplasma (Order No. SMB95-1003/1004)

6. 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.

7. Procedure

1.	Centrifuge the tubes briefly to collect the lyophilized material at the bottom of the tube
2.	Add 1 ml of your sample material to each vial.
3.	Incubate 5 min at room temperature.
4.	Vortex for 10 sec. and spin for 5 sec.
5.	Use amount of sample according to the needs of the kit used for sample preparation

All reagents and samples must be equilibrated to room temperature prior use.
It is highly recommended to perform suitable DNA extraction of the samples prior PCR application to reduce risk for inhibition and to maximize sensitivity. The Negative Control vials have been prepared just as the Mycoplasma vials but do not contain any mycoplasma particles. The Negative Controls should be rehydrated with the same sample matrix and processed in parallel with a suitable number of replicates to verify the test results as correct positive.

8. Notes on the Procedure

1. This leaflet must be widely understood for a successful use of the Microsart® Validation Standard. The reagents supplied should not be mixed with reagents from different lots and used as an integral unit. The reagents of the kit 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 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 offered by Minerva Biolabs GmbH (www.minerva-biolabs.com) on an biannual base, is recommended.

Appendix

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. Sartorius Stedim Biotech GmbH 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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9. 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® Calibration Reagent, $1x10^8$ genomes

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

DNA Extraction Kit

SMB95-2003	Microsart® AMP Extraction	50 extractions

Vivaspin and Coating Buffer

SMB95-2002	Microsart® AMP Coating Buffer	20x 2 ml
VS 0641	Vivaspin 6 Polyethesulfone 100,000 MWCO	25 units
VS 0642	Vivaspin 6 Polyethesulfone 100,000 MWCO	100 units
VS 2041	Vivaspin 20 Polyethesulfone 100,000 MWCO	12 units
VS 2042	Vivaspin 20 Polyethesulfone 100,000 MWCO	48 units

UNG Carry over prevention*

54-1001	Uracil-DNA Clycosylase (UNG), heat-labile	100 u, 1 u/µl

DNA Remover™ *

15-2025	DNA Decontamination Reagent, spray bottle	250 ml
15-2200	DNA Decontamination Reagent, refill bottles	4x 500 ml

Mycoplasma Off® *

15-1000	Surface Disinfectant Spray, spray bottle	1000 ml
15-5000	Surface Disinfectant Spray, refill bottles	5x 1000 ml

Mycoplasma Off® Wipes*

15-1001	Surface Disinfectant Wipes	120 wipes
15-5001	Surface Disinfectant Wipes, refill sachets	5 x 120 wipes

UNG Carry over prevention*

54-1001	Uracil-DNA Clycosylase (UNG), heat-labile	100 u, 1 u/µl

*** Distributed by Minerva Biolabs**

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