

Instructions for Use

Microsart® Validation Standard

For the validation of bacteria species (excluding mycoplasma)

- Prod. No. SMB95-2005 *Bacillus subtilis*
- Prod. No. SMB95-2006 *Pseudomonas aeruginosa*
- Prod. No. SMB95-2007 *Kocuria rhizophila*
- Prod. No. SMB95-2008 *Clostridium sporogenes*
- Prod. No. SMB95-2009 *Bacteroides vulgatus*
- Prod. No. SMB95-2010 *Staphylococcus aureus*

For use in research and quality control

Manufactured by:



Minerva Biolabs GmbH
Koepenicker Strasse 325
12555 Berlin
Germany

Symbols

LOT

Lot No.

REF

Order No.



Expiry date



Store at



Content



Manufacturer

Contents

1. Intended Use	5
2. Explanation of the Test	5
3. Principle	6
4. Reagents	7
5. Needed but not Included	8
6. Precautions.	10
7. Procedure.	11
8. Notes on the Procedure.	12
9. Related Products.	14



1. Intended Use

Microsart® Validation Standard is used for validating robustness and sensitivity of NAT-based detection methods in combination with cell cultures, cell culture media components and cell culture derived biologicals like cell autologous transplants and ATMPs.

2. Explanation of the Test

Microsart® Validation Standard requires to be validated with respect to sample matrix and lab precision. In addition, the method analysis for sterility testing shall show performance equal or better to the classical cultivation procedure. As most test and cell culture labs are frightened to cultivate bacteria, Microsart® ATMP Bacteria was validated as a method of choice for the sterility testing of cell culture samples including ATMPs. The direct comparison of Microsart® ATMP Bacteria with the classical culture method showed an equal performance between both methods.

Microsart® Validation Standards are not infectious and therefore safe in use. They are titrated to 99 CFU/ml, the sensitivity limit for sterility tests according to European Pharmacopoeia (EP) 2.6.1 Sterility. The bacteria strains used for the manufacture of Microsart® Validation Standard are low passage reference strains cultivated in suitable culture broth. The cultures are harvested in the early logarithmic phase of the growth to avoid an atypical high ratio of dead bacteria and plated on Agar medium for quantification based on Colony-forming Units (CFU).

3. Principle

Each vial contains 99 Colony-forming Units (CFU) of inactivated bacterial 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 culture method. For highest sensitivity, the DNA should be extracted with a DNA-free kit. For this purpose we developed Microsart® Bacteria Extraction (SMB95-2001). The DNA extract can directly be used for PCR (we recommend to use Microsart® ATMP Bacteria SMB95-1008 or Microsart® RESEARCH Bacteria SMB95-1009).

4. Reagents

Each kit contains 6 vials of inactivated bacteria particles as well as 2 vials containing the same carrier matrix as the bacteria 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 at ≤ -18 °C after opening and rehydration.

Kit Component Label Information	Cat. No.	Quantity	Cap Color
<i>Bacillus subtilis</i>	SMB95-2005		
<i>Pseudomonas aeruginosa</i>	SMB95-2006		
<i>Kocuria rhizophila</i>	SMB95-2007	6 x lyophilized	green
<i>Clostridium sporogenes</i>	SMB95-2008		
<i>Bacteroides vulgatus</i>	SMB95-2009		
<i>Staphylococcus aureus</i>	SMB95-2010		
Negative Control		2 x lyophilized	white

5. Needed but not Included

Microsart® Validation Standard contains the positive and negative samples 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 pipette filter tips that must be free from bacterial DNA (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 cycler
- Isolator/glovebox (for PCR-setup)
- Vortex Mixer
- Heat block
- Microcentrifuge for 1.5 ml reaction tubes (Centrisart A-14, Prod. No. A-14-1EU)
- Pipettes
 - 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. 73508

- Rack for 1.5 ml tubes

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

- Bacterial DNA extraction system DNA-free.

Attention: Most DNA-extraction kits on the market are not DNA-free. For this reason, we recommend the Microsart® Bacteria Extraction kit (Sartorius Prod. No. SMB95-2001) intended for further Bacterial DNA amplification through qPCR.

- Bacterial DNA PCR detection system. We recommend the Microsart® ATMP Bacteria kit (Sartorius Prod. No. SMB95-1008), or the Microsart® RE-SEARCH Bacteria kit (Sartorius Prod. No. SMB951009)

6. Precautions

For in vitro use in research and quality control. This kit should be used only by trained persons. This kit does not contain hazardous substances and may be disposed of according to local regulations.

7. Procedure

1. Centrifuge the tubes 5 sec. with the "pulse" option or at 5000g to collect the lyophilized material at the bottom of the tube
2. Add 1 ml of your sample matrix to each vial
3. Incubate 5 min at room temperature
4. Vortex for 10 sec. and spin 5 sec. with the "pulse" option or at 5000g.
5. For DNA extraction use the recommended volume according to user instructions for DNA isolation

All 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. We recommend the DNA-free kit Microsart® Bacteria Extraction (SMB95-2001). Negative Control vials have been prepared just as the bacteria vials but do not contain any bacteria particles. The Negative Controls should be rehydrated with the same sample matrix and processed in parallel with a suitable number of replicates to validate the interpretation of 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 supplied material should not be mixed with material 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® ATMP Bacteria 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 a 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.

Trademarks

Microsart is a registered trademark of Sartorius Stedim Biotech. Mycoplasma Off and PCR Clean are trademarks of Minerva Biolabs GmbH, Germany.

Last technical revision: 2018-06-18

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
SMB95-1007	Microsart® ATMP Bacteria patient	10 patients
SMB95-1008	Microsart® ATMP Bacteria	100 tests
SMB95-1009	Microsart® RESEARCH Bacteria	25 tests

Microsart® Calibration Reagent, 1 vial, 10⁸ genomes/vial

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® Validation Standard, 3 vials each, 10 CFU/vial for Mollicutes (SMB95-2011 - SMB95-2020) and 99 CFU/vial for other bacterial species (SMB95-2005 - SMB95-2010)

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

DNA Extraction Kit

SMB95-2001	Microsart® Bacteria Extraction	50 extractions
SMB95-2003	Microsart® AMP Extraction (only for Mycoplasma qPCR)	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 Glycosylase (UNG), heat-labile	100 u, 1 u/μl
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PCR Clean™ *

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

PCR Clean™ Wipes*

15-2001	DNA Decontamination Wipes	120 wipes
15-2002	DNA Decontamination Wipes, refill sachets	5× 120 wipes

Mycoplasma Off™ Wipes *

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

Mycoplasma Off™ Wipes *

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

* Distributed by Minerva Biolabs

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