

Instructions for Use

Microsart® Validation Standard

For the validation of bacteria (except mycoplasma), fungi, and yeast species

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*
Prod. No. SMB95-2037 *Candida albicans*
Prod. No. SMB95-2038 *Aspergillus brasiliensis*
Prod. No. SMB95-2039 *Aspergillus fumigatus*
Prod. No. SMB95-2040 *Penicillium chrysogenum*
Prod. No. SMB95-2041 *Candida glabrata*
Prod. No. SMB95-2042 *Candida krusei*
Prod. No. SMB95-2043 *Candida tropicalis*

For use in research and quality control

Symbols

LOT

Lot No.

REF

Order No.



Expiry date



Store at



Content



Manufacturer

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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 Advanced Therapy Medicinal Products (ATMPs, e.g. cell autologous transplants).

2. Explanation of the Test

Microsart® Validation Standard is required to be validated with respect to sample matrix and lab precision. In addition, the analytical method used for sterility testing shall show a performance equal or better than the compendial culture procedure. As culturing living bacteria and fungi for validation purposes is highly demanding for most test and cell culture labs, Microsart® ATMP Sterile Release was validated as the method of choice for the sterility testing of cell culture samples including ATMPs. The direct comparison of Microsart® ATMP Sterile Release with the compendial culture method showed an equal performance of both methods.

Microsart® Validation Standards are not infectious and therefore safe to use. They are titrated to 99 Colony-forming Units/ml (CFU/ml), the sensitivity limit for sterility tests according to the Growth Promotion Test and the European Pharmacopoeia (EP) 2.6.1 Sterility guidelines.

The bacteria and fungal 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 particles and plated on agar medium for quantification based on CFU counts.

3. Principle

Each vial contains 99 CFU of inactivated particles. The relevant sample matrix is added directly into the tube. The prepared 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® ATMP Extraction (Sartorius Prod. No. SMB95-2001). The DNA extract can directly be used for PCR. We recommend to use Microsart® ATMP Bacteria (Sartorius Prod. No. SMB95-1008) or Microsart® RESEARCH Bacteria (Sartorius Prod. No. SMB95-1009) for the detection of validation standards (Sartorius Prod. No. SMB95-2005 to -2010) and Microsart® ATMP Fungi (Sartorius Prod. No. SMB95-1012) or Microsart® RESEARCH Fungi (Sartorius Prod. No. SMB95-1013/1014) for the detection of validation standards (Sartorius Prod. No. SMB95-2037 to -2043).

4. Reagents

Each kit contains 6 vials of inactivated bacteria or fungi particles as well as 2 vials containing the same carrier matrix as the bacteria/fungi vials for the preparation of comparable negative controls. All samples are lyophilized for product stability reasons. All particles have been inactivated prior 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 used directly after 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		
<i>Clostridium sporogenes</i>	SMB95-2008		
<i>Bacteroides vulgatus</i>	SMB95-2009		
<i>Staphylococcus aureus</i>	SMB95-2010		
<i>Candida albicans</i>	SMB95-2037	6 × lyophilized	green
<i>Aspergillus brasiliensis</i>	SMB95-2038		
<i>Aspergillus fumigatus</i>	SMB95-2039		
<i>Penicillium chrysogenum</i>	SMB95-2040		
<i>Candida glabrata</i>	SMB95-2041		
<i>Candida krusei</i>	SMB95-2042		
<i>Candida tropicalis</i>	SMB95-2043		
Negative Control		2 × 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)
- Certified 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 cycler
- Isolator/glovebox (for PCR-setup)
- Vortex Mixer
- Heat block with optional shaking function
- Microcentrifuge for 1.5 ml/2 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:

- DNA extraction system DNA-free

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

- DNA PCR detection system

For validation standards (Sartorius Prod. No. SMB95-2005 to -2010), we recommend the Microsart® ATMP Bacteria kit (Sartorius Prod. No. SMB95-1008), or the Microsart® RESEARCH Bacteria kit (Sartorius Prod. No. SMB95-1009)

For validation standards (Sartorius Prod. No. SMB95-2037 to -2043), we recommend the Microsart® ATMP Fungi kit (Sartorius Prod. No. SMB95-1012), or the Microsart® RESEARCH Fungi kit (Sartorius Prod. No. SMB95-1013/1014).

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 5000 x g 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 5000 x g.
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® ATMP Extraction (Sartorius Prod. No. SMB95-2001). Negative Control vials have been prepared just as the bacteria/fungi vials but do not contain any 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 or Microsart® ATMP Fungi 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), 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 GmbH. PCR Clean is a trademark of Minerva Biolabs GmbH.

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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
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® 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	
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 × 500 ml
PCR Clean™ Wipes*		
15-2001	DNA Decontamination Wipes	50 wipes
15-2002	DNA Decontamination Wipes, refill sachets	5 × 50 wipes

* Distributed by Minerva Biolabs

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