SARTORIUS

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

99 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 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

Microsart® Validation Standard is a quantified, non-infectious microbiological preparation of 99 Colony-forming Units (CFU), available for several bacterial and fungal species.

In a regulated environment such as in ATMPs manufacturing, product sterility testing relies on compendial culture methods for microbial detection. As in-house culturing of bacteria and fungi is not always feasible, rapid alternative methods are often applied and validated for quality control testing.
Microsart® Validation Standard is a unique tool for comprehensive validation of alternative NAT-based methods, including for the determination of robustness, sensitivity, and specificity on specific sample matrices. For meaningful comparisons, each Microsart® Validation Standard contains < 100 CFU, the sensitivity limit for sterility tests according to the Growth Promotion Test and the European Pharmacopoeia (EP) 2.6.1 Sterility guidelines.

Each vial contains inactivated, lyophilized microorganisms particles corresponding to 99 CFU. The relevant sample matrix can be added directly into the vial to yield a final concentration of 99 CFU/ml. The derived sample is then expected to be tested positive by a valid NAT-based assay. The inactivated bacterial or fungal preparation cannot be used for the culture method.

For maximal sensitivity, the DNA should be extracted with a DNA-free kit prior to PCR. We developed several kits for efficient extraction and sensitive detection, as reported in the chapter „Needed but not included" (Table 1).

The bacteria and fungal strains used to manufacture Microsart® Validation Standard are low passage reference strains cultivated in suitable culture broth. The cultures are harvested in the early logarithmic growth phase to avoid a high ratio of dead particles and plated on agar medium for quantification based on CFU counts. They are titrated to 99 CFU/ml, inactivated, and lyophilized.

Microsart® Validation Standards have been extensively tested in combination with Microsart® ATMP Sterile Release, Microsart® ATMP Extraction, Microsart® ATMP Bacteria, Microsart® ATMP Fungi (see Related Products).

3. Notes on the 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 Certificate 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 6 vials of bacteria or fungi particles as well as 2 vials containing the same carrier matrix as the bacteria/fungi 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
Bacillus subtilis	SMB95-2005		
Pseudomonas aeruginosa	SMB95-2006		
Kocuria rhizophila	SMB95-2007		
Clostridium sporogenes	SMB95-2008		
Bacteroides vulgatus	SMB95-2009		
Staphylococcus aureus	SMB95-2010		
Candida albicans	SMB95-2037	6 × lyophilized	green
Aspergillus brasiliensis	SMB95-2038		
Aspergillus fumigatus	SMB95-2039		
Penicillium chrysogenum	SMB95-2040		
Candida glabrata	SMB95-2041		
Candida krusei	SMB95-2042		
Candida tropicalis	SMB95-2043		
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)
- 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)
- Microcentrifuge for 1.5 ml reaction tubes (Centrisart A-14, Prod. No. A-14-1EU)
- Vortex
- Rack for 1.5 ml tubes
- Heat block with optional shaking function
- 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 electronical
        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:

- 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 (see table below) intended for further DNA amplification through qPCR.

- DNA PCR detection system

Please see the table below for our recommended detection systems for bacterial and fungal validation standards.

Table 1. Related products recommendations and intended use.

Recommended kit	Sartorius Prod. No.	Extraction	Detection	
			For SMB95-2005 to -2010 (Bacteria)	For SMB95-2037 to -2043 (Fungi)
Microsart® ATMP Extraction	SMB95-2001	×		
Microsart® ATMP Bacteria	SMB95-1008		×	
Microsart® ATMP Fungi	SMB95-1012			×
Microsart® RESEARCH Bacteria	SMB95-1009		×	
Microsart® RESEARCH Fungi	SMB95-1013 SMB95-1014			×
Microsart® ATMP Sterile Release	SMB95-1007	×	×	×

6. Precautions

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

7. Test Procedure

1.	Centrifuge the tube(s) for 5 sec with the „pulse“ option or at 5000 × g 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 with the „pulse“ option or at 5000 × g.
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. We recommend the DNA-free kit Microsart® ATMP Extraction (Sartorius Prod. No. SMB95-2001). From a manufacturing point of view, the Negative Control vials contain exactly the same components (carrier matrix) as the bacteria/fungi vials except for the microorganisms 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® Calibration Reagent, 10^6 genomes / vial, 1 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® 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
SMB95-2030	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, 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, 100 CFU / vial, 3 vials each (Mollicutes)

SMB95-2051	Mycoplasma orale	
SMB95-2052	Mycoplasma pneumoniae	

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

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. 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.

Last technical revision: 2019-11-15

Notes

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

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

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.: 2543683-000-00
Ver. 11 | 2020