

Microsart® Calibration Reagent

For Mollicutes and other bacteria species

Prod. No. SMB95-2021	Mycoplasma arginini
Prod. No. SMB95-2022	Mycoplasma orale
Prod. No. SMB95-2023	Mycoplasma gallisepticum
Prod. No. SMB95-2024	Mycoplasma pneumoniae
Prod. No. SMB95-2025	Mycoplasma synoviae
Prod. No. SMB95-2026	Mycoplasma fermentans
Prod. No. SMB95-2027	Mycoplasma hyorhinis
Prod. No. SMB95-2028	Acholeplasma laidlawii
Prod. No. SMB95-2029	Spiroplasma citri
Prod. No. SMB95-2030	Bacillus subtilis
Prod. No. SMB95-2031	Pseudomonas aeruginosa
Prod. No. SMB95-2032	Kocuria rhizophila
Prod. No. SMB95-2033	Clostridium sporogenes
Prod. No. SMB95-2034	Bacteroides vulgatus
Prod. No. SMB95-2035	Staphylococcus aureus
Prod. No. SMB95-2036	Mycoplasma salivarium

For use in research and quality control

Symbols

LOT

Lot No.

REF

Order No.



Expiry date



Store at



Content

Contents

1. Intended Use.....	5
2. Explanation of the Product	5
3. Principle	6
4. Notes on the Procedure	6
5. Reagents	7
6. Needed but not included.....	8
7. Test Procedure	9
7.1 Rehydration of the reagents.....	9
7.2 Preparation of the dilutions.....	9
7.3 PCR	10
7.4 Evaluation.....	10
8. Related Products.....	11
Notes.....	13

1. Intended Use

Microsart® Calibration Reagent is titrated genomic DNA and can be used as amplification and sensitivity control for endpoint PCR (gel-based evaluation). If quantitative PCR is required, titrated genomic DNA can be serially diluted and used as PCR templates to create standard curves. The software will generate a standard curve from the known DNA dilutions and use it to determine the DNA concentration of the unknown samples.

2. Explanation of the Product

This product provides isolated genomic DNA of a specific species. An early passage strain of the specific microorganism is inoculated in a suitable culture medium and harvested at the end of the logarithmic growth phase by repeated washing and centrifugation. Following extraction, the DNA concentration is quantified photometrically (OD260/280) and with a double-stranded DNA (dsDNA)-specific ultrasensitive fluorescent dye, calibrated with weight reference standards and controlled by qPCR (compared to exactly quantified calibrator plasmids).

The DNA concentration was adjusted with TE80 buffer.

The following species are available:

<i>Mycoplasma arginini</i>	ATCC 23838; NCTC 10129
<i>Mycoplasma orale</i>	ATCC 23714; NCTC 10112; DSM 25590
<i>Mycoplasma gallisepticum</i>	ATCC 19610; NCTC 10115; DSM 19817
<i>Mycoplasma pneumoniae</i>	ATCC 15531; NCTC 10119; DSM 22911
<i>Mycoplasma synoviae</i>	ATCC 25204; NCTC 10124; DSM 21430
<i>Mycoplasma fermentans</i>	ATCC 19989; NCTC 10117
<i>Mycoplasma hyorhinis</i>	ATCC 17981; NCTC 10130; DSM 25591
<i>Acholeplasma laidlawii</i>	ATCC 23206; NCTC 10116; DSM 23060
<i>Spiroplasma citri</i>	ATCC 27556; NCTC 10164; DSM 21846
<i>Bacillus subtilis</i>	ATCC 6633; NCTC 10400; DSM 347
<i>Pseudomonas aeruginosa</i>	ATCC 9027; DSM 1128
<i>Kocuria rhizophila</i>	ATCC 9341; DSM 348
<i>Clostridium sporogenes</i>	ATCC 19404; NCTC 532; DSM 1664
<i>Bacteroides vulgatus</i>	ATCC 8482; DSM 1447
<i>Staphylococcus aureus</i>	ATCC 6538; NCTC 10788; DSM 799

3. Principle

Each vial contains 1×10^8 copies of the complete bacterial genome. The genomic DNA material can be used directly for PCR and cannot be used for culture methods.

Please note: European Pharmacopoeia does not provide sensitivity limits on DNA level. For process validation, use the corresponding Microsart® Validation Standards (see Related Products).

4. Notes on the Procedure

1. For in vitro use in research and quality control. This kit should be used only by trained staff.
2. This kit does not contain hazardous substances and may be disposed of according to local regulations.
3. This leaflet must be fully understood in order to successfully use the Microsart® Calibration Reagent. 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.
4. Any deviation from the described method can affect the results.
5. For each test setup, at least one negative control should be added. Participation in external quality control programs, such as those offered by Minerva Biolabs GmbH (www.minerva-biolabs.com), is recommended.

5. Reagents

Each kit contains 1 vial of bacterial DNA (1×10^8 genome copies) and 3 vials of buffer for the preparation of dilutions and negative controls. Samples are lyophilized for product stability reasons. The material has been inactivated prior to lyophilization and can be considered as non-infectious. The expiry date of the unopened product 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 rehydration.

Component Label Information	Order No.	Quantity	Cap Color
Calibration Reagent	SMB95-2021-	1 × lyophilized	green
Buffer	SMB95-2036	3 × 2.0 ml	white

6. Needed but not included

Microsart® Calibration Reagent contains all necessary positive and negative material. General industrial supplies and reagents, usually available in PCR laboratories are not included:

Consumables

- Laboratory gloves
- 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)
- 1.5 ml reaction tubes, DNA- and RNA-free

Equipment

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

For PCR analysis, a bacteria- or mycoplasma-specific DNA detection system is required.

- For bacterial DNA, we recommend the Microsart® ATMP Bacteria kit (Sartorius Prod. No. SMB95-1008), Microsart® ATMP Sterile Release kit (Sartorius Prod. No. SMB95-1007), or the Microsart® RESEARCH Bacteria kit (Sartorius Prod. No. SMB95-1009).
- For detection of mycoplasma DNA, we recommend the Microsart® ATMP Mycoplasma (Sartorius Prod. No. SMB95-1003/1004), Microsart® AMP Mycoplasma (Sartorius Prod. No. SMB95-1001/1002), or Microsart® RESEARCH Mycoplasma (Sartorius Prod. No. SMB95-1005/1006).

7. Test Procedure

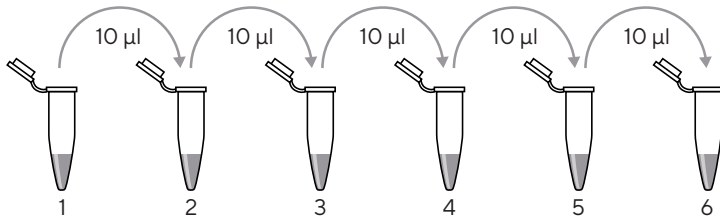
7.1 Rehydration of the reagents

All reagents must be equilibrated to room temperature prior to use.

1. Centrifuge the vial containing the Calibration Reagent (green cap) briefly to collect the lyophilized material at the bottom.
2. Add 100 μl Buffer (white cap) to obtain a concentration of 1×10^6 genomes/ μl .
3. Incubate 5 min at room temperature.
4. Vortex for 10 sec and spin for 5 sec with the “pulse” option or at $5000 \times g$.
5. Aliquot the vial content in DNA-free tubes and freeze at $\leq -18 \text{ }^\circ\text{C}$ or directly proceed to step 7.2.

7.2 Preparation of the dilutions

1. Thaw the resuspended Calibration Reagent if applicable.
2. Label six 1.5 ml reaction tubes as in the figure below and fill each with 90 μl of Buffer (white cap).
3. Vortex Calibration Reagent briefly (at least 5 sec) at medium speed.
4. Add 10 μl of the Calibration Reagent to reaction tube no. 1, close the tube and vortex briefly at medium speed.
5. Add 10 μl of the content of reaction tube no. 1 to reaction tube no. 2.
6. Close the tube and vortex briefly at medium speed.
7. Proceed with the following reaction tubes of the dilution series in the same way.



7.3 PCR

Please follow the instructions given in the manual of the specific PCR kit. The volume used as template for PCR defines the number of genome copies per reaction:

Reaction tube	2 µl sample volume	10 µl sample volume	50 µl sample volume
1.	2x10 ⁵ genome copies	1x10 ⁶ genome copies	5x10 ⁶ genome copies
2.	2x10 ⁴ genome copies	1x10 ⁵ genome copies	5x10 ⁵ genome copies
3.	2x10 ³ genome copies	1x10 ⁴ genome copies	5x10 ⁴ genome copies
4.	200 genome copies	1000 genome copies	5000 genome copies
5.	20 genome copies	100 genome copies	500 genome copies
6.	2 genome copies	10 genome copies	50 genome copies

7.4 Evaluation

In qPCR, the Ct values should decrease linearly with increasing DNA concentrations per reaction, when a suitable PCR assay is used. The software of the qPCR device builds a standard curve and calculates the slope using the DNA concentrations provided by the user and the obtained Ct values. Also, the unknown sample DNA concentrations are automatically determined by interpolation of the Ct values, obtained for these samples, on the standard curve. The following results represent an example of such evaluation and were generated using the Mx3005P™ instrument.

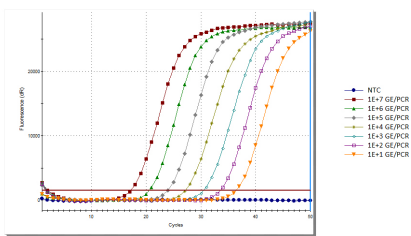


Fig. 1: Amplification curves of a dilution series from 1x10⁷ to 10 genome copies/reaction (GE/PCR).

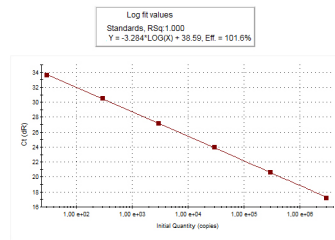


Fig. 2: Standard curve generated with the Mx3005P™ instrument using second derivative maximum method and the data from Fig. 1

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, 10⁶ 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® 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

Microsart® Validation Standard, 99 CFU / vial, 6 vials each (bacteria* and fungi)

SMB95-2005	Bacillus subtilis
SMB95-2006	Pseudomonas aeruginosa
SMB95-2007	Kocuria rhizophila
SMB95-2008	Clostridium sporogenes
SMB95-2009	Bacteroides vulgatus
SMB95-2010	Staphylococcus aureus
SMB95-2037	Candida albicans
SMB95-2038	Aspergillus brasiliensis
SMB95-2039	Aspergillus fumigatus

SMB95-2040	Penicillium chrysogenum
SMB95-2041	Candida glabrata
SMB95-2042	Candida krusei
SMB95-2043	Candida tropicalis

* 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 × 500 ml

PCR Clean™ Wipes**

15-2001	DNA Decontamination Reagent, Wipes	50 wipes
15-2002	DNA Decontamination Reagent, refill sachets	5 × 50 wipes

** Distributed by Minerva Biolabs

Notes

Limited Product Warranty

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Last technical revision:2020-01-10

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Status:
September 2020,
Sartorius Stedim Biotech
GmbH, Goettingen, Germany

Printed in Germany on paper that
has been bleached without any use
of chlorine. | W
Publication No.: SM-6112-e140804
Order No.: 85037-546-77
Ver. 09 | 2020