

## Instructions for Use

# Microsart® Calibration Reagent

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

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**LOT**

Lot No.

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**REF**

Order No.

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Expiry date

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Store at

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Content

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# 1. Intended Use

Titred genomic DNA can be used as amplification and sensitivity control of end point PCR (gel-based evaluation). For quantitative PCR, titred genomic DNA can be used to create standard curves by performing dilution series of the material as template for PCR. The software of various devices will be able to calculate from qPCR data corresponding concentrations and will generate a standard curve, which can be used to determine unknown DNA concentrations.

## 2. Explanation of the Product

This product provides isolated genomic DNA of a specific species. To cultivate the microorganisms a culture medium was inoculated with an early passage strain and harvested at the end of the logarithmic growth phase by repeated washing and centrifugation. Following the extraction, the DNA concentration was quantified photometrically (OD260/280) and with an ultrasensitive fluorescent nucleic acid stain for quantifying double-stranded DNA (dsDNA) calibrated to weight reference standards and controlled by qPCR (compared against exactly quantified calibrator plasmids). The DNA concentration was adjusted with regular TE80 buffer.

The following species are used

<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

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## 3. Principle

Each vial contains  $1 \times 10^8$  copies of the complete bacterial genome. The material cannot be used for cultivation methods. The genome standard can directly be used for PCR.

Please note: European Pharmacopoeia does not provide sensitivity limits on DNA level. For process validation use Microsart® Validation Standards.

## 4. Reagents

Each kit contains 1 vial of bacterial DNA ( $1 \times 10^8$  genome copies) and 3 vials of buffer for the preparation of dilutions and negative controls. All samples are lyophilized for product stability reasons. The material has been inactivated prior lyophilisation and can be considered as non-infectious. The expiry date of the unopened product is specified on the package label. The kits components are stored until use at +2 to +8 °C and must be stored at  $\leq -18$  °C after rehydration.

Kit Component Label Information	Quantity	Cap Color
Calibration Reagent	1 × lyophilized	green
Buffer	3	white



## 5. Needed but not Included

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

### Consumables

- Laboratory gloves
- 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

- Microcentrifuge for 1.5 ml reaction tubes (Centrisart A-14, Prod. No. A-14-1EU)
- Vortex
- 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 PCR analysis, a bacteria or mycoplasma DNA PCR detection system is required.

- For Bacterial DNA we recommend the Microsart® ATMP Bacteria kit (Sartorius Prod. No. SMB95-1008), 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)

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## 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

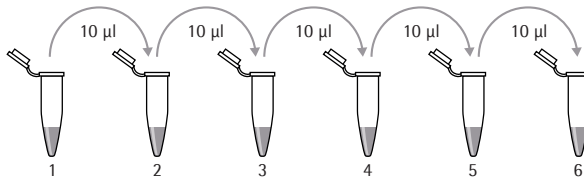
## 7.1 Rehydration of the reagents

All reagents must be equilibrated to room temperature prior use.

1. Centrifuge the tube briefly to collect the lyophilized material at the bottom of the tube.
2. Add 100  $\mu$ l Buffer (white cap) to the vial containing the Calibration Reagent (green cap) to obtain a concentration of  $1 \times 10^6$  genomes/  $\mu$ l.
3. Incubate 5 min at room temperature.
4. Vortex for 10 sec. and spin for 5 sec. with the "pulse" option or at 5000 g.
5. Aliquots the vial content in DNA-free tubes and freeze  $\leq -18$  °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 consecutively and fill each with 90  $\mu$ l of Buffer (white cap).
3. Vortex Calibration Reagent briefly (1 to 2 seconds) at medium speed.
4. Add 10  $\mu$ l of the Calibration Reagent to reaction tube no. 1, close the tube and vortex briefly at medium speed.
5. Add 10  $\mu$ l 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 PCR kit manual. 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 <sup>5</sup> genome copies	1x10 <sup>6</sup> genome copies	5x10 <sup>6</sup> genome copies
2.	2x10 <sup>4</sup> genome copies	1x10 <sup>5</sup> genome copies	5x10 <sup>5</sup> genome copies
3.	2x10 <sup>3</sup> genome copies	1x10 <sup>4</sup> genome copies	5x10 <sup>4</sup> 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 ascending DNA concentration per reaction, when a suitable PCR assay is used. The software of the qPCR device calculates a standard curve and slope using the DNA concentrations stated by the user and the appendant Ct-values. Also, the Ct-values of samples with unknown DNA concentrations are automatically compared to the standard curve, and concentrations are assigned. The following figures were generated using the Mx 3005p instrument.

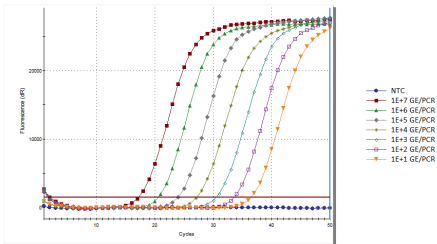


Fig. 1: Amplification curves of a dilution series from 1x10<sup>7</sup> to 10 genome copies/reaction.

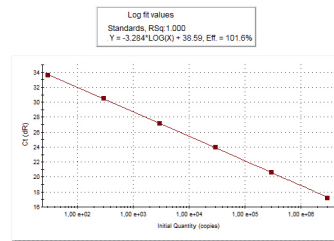


Fig. 2: Standard curve generated with the Mx 3005p instrument using second derivative maximum method and the data from Fig. 1

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## 8. Notes on the Procedure

1. This leaflet must be widely understood for a successful use of 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.
2. Any deviation from the described method can affect the results.
3. For each test setup, at least one negative control should be added.
4. Participation in external quality control programs, such as offered by Minerva Biolabs GmbH ([www.minerva-biolabs.com](http://www.minerva-biolabs.com)) on an biannual base, is recommended.

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## 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. Mycoplasma Off and PCR Clean are a trademark of Minerva Biolabs.

Last technical revision: 2018-07-10

## 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® Validation Standard

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
SMB95-2005	Bacillus subtilis
SMB95-2006	Pseudomonas aeruginosa
SMB95-2007	Kocuria rhizophila
SMB95-2008	Clostridium sporogenes
SMB95-2009	Bacteroides vulgatus
SMB95-2010	Staphylococcus aureus

### 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
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

**UNG Carry over prevention\***

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

**PCR Clean™ (formerly DNA Remover™) \***

15-2025 DNA Decontamination Reagent, spray bottle 250 ml

15-2200 DNA Decontamination Reagent, refill bottles 4x 500 ml

**PCR Clean™ Wipes\***

15-2001 DNA Decontamination Wipes 120 wipes

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

**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 Glycosylase (UNG), heat-labile 100 u, 1 u/μl

\* Distributed by Minerva Biolabs









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