

## Microsart® Calibration Reagent

For fungi and yeast species

- Prod. No. SMB95-2044 *Candida albicans*
- Prod. No. SMB95-2045 *Aspergillus brasiliensis*
- Prod. No. SMB95-2046 *Aspergillus fumigatus*
- Prod. No. SMB95-2047 *Penicillium chrysogenum*
- Prod. No. SMB95-2048 *Candida glabrata*
- Prod. No. SMB95-2049 *Candida krusei*
- Prod. No. SMB95-2050 *Candida tropicalis*

For use in research and quality control

Symbols

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Lot No.

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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

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 the extraction, the DNA concentration is quantified photometrically (OD<sub>260/280</sub>) 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 is adjusted with TE80 buffer.

The following species are available:

<i>Candida albicans</i>	ATCC 10231; DSM 1386
<i>Candida glabrata</i>	ATCC 90030; DSM 11226
<i>Candida krusei</i>	ATCC 6258; ATCC 749; DSM 6128; DSM 11956
<i>Candida tropicalis</i>	ATCC 4563; ATCC 7349; ATCC 450; DSM 11953
<i>Aspergillus fumigatus</i>	ATCC 9197; DSM 819
<i>Aspergillus brasiliensis</i>	ATCC 16404; DSM 1988
<i>Penicillium chrysogenum</i>	ATCC 9178; DSM 848

### 3. Principle

Each vial contains  $1 \times 10^6$  copies of the complete fungal 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. Notes on the Procedure

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

## 5. Reagents

Each kit contains 1 vial of fungal DNA ( $1 \times 10^6$  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  $\leq -18$  °C after rehydration.

<b>Component Label Information</b>	<b>Order No.</b>	<b>Quantity</b>	<b>Cap Color</b>
Calibration Reagent	SMB95-2044-	1 × lyophilized	green
Buffer	SMB95-2050	3 × 2.0 ml	white

## 6. 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 (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 fungi-specific DNA detection system is required. We recommend the Microsart® ATMP Fungi kit (Sartorius Prod. No. SMB95-1012), or the Microsart® RESEARCH Fungi kit (Sartorius Prod. No. SMB95-1013/1014).

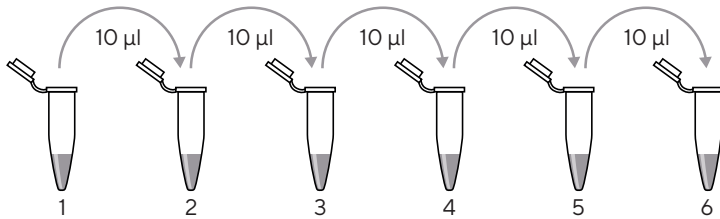


## 7. Test Procedure

### 7.1 Rehydration of the reagents

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

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



### 7.2 Preparation of the dilutions

1. Thaw the resuspended Calibration Reagent if necessary.
2. Label five 1.5 ml reaction tubes consecutively and fill each with 90  $\mu\text{l}$  of the provided Buffer (white cap).
3. Vortex Calibration Reagent for at least 5 sec at medium speed.
4. Add 10  $\mu\text{l}$  of the Calibration Reagent to reaction tube no. 1, close the tube and vortex briefly at medium speed.
5. Add 10  $\mu\text{l}$  of the content of reaction tube no. 1 to reaction tube no. 2.
6. Close the tube and vortex briefly at medium speed.
7. Continue with the following reaction tubes of the dilution series as described above.

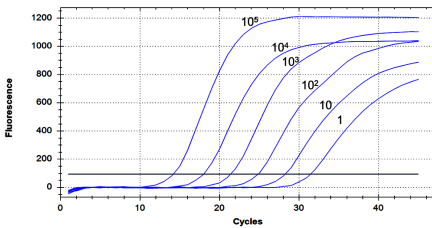
### 7.3 PCR

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:

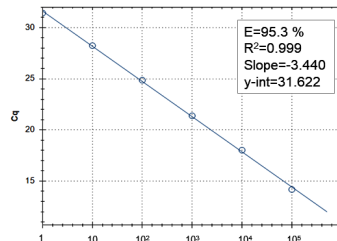
Reaction tube	2 µl sample volume	10 µl sample volume	50 µl sample volume
1.	$2 \times 10^3$ genome copies	$1 \times 10^4$ genome copies	$5 \times 10^4$ genome copies
2.	200 genome copies	1000 genome copies	5000 genome copies
3.	20 genome copies	100 genome copies	500 genome copies
4.	2 genome copies	10 genome copies	50 genome copies
5.	-	1 genome copies	5 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 CFX96 Touch™ instrument.



**Fig. 1:** Amplification curves of a dilution series from  $10^5$  to 1 genome copies/ reaction.



**Fig. 2:** Standard curve generated with CFX96 Touch™ 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, 10<sup>8</sup> genomes / vial, 1 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

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

\*\* Distributed by Minerva Biolabs

Notes

## **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**

CFX96 Touch is a trademark of Bio-Rad Laboratories, Inc. Microsart is a registered trademark of Sartorius Stedim Biotech GmbH. PCR Clean is a trademark of Minerva Biolabs GmbH.

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