

Cyclus® dPCR Tool Box Bacteria Fungi

Primer and Probes Mix
for Digital (Droplet) PCR
Detection



Benefits

- Rapid detection of total bacteria and fungi in cell-based products
- For up to 10 samples
- Time to result: Only a few hours
- Guarantees rapid detection of a broad range of microbes (including all USP 71 relevant ones) in cell-based or short shelf-life products
- Highly specific TaqMan® probes bind to a conserved DNA sequence in the bacterial and fungal genome
- Kit components consist of highly pure reagents
- To be used with the highly efficient and ultra-pure Microsart® ATMP Extraction kit (SMB95-2001)
- Compatible with digital or droplet PCR master mixes (QIAGEN and Bio-Rad)

Product Information

Contaminated cell-based therapeutics pose life-threatening risks for immuno-compromised patients. Microbial release test results prior to treatment are critical to patient safety. Cyclus® dPCR Tool Box Bacteria Fungi enables the detection of bacterial and fungal contamination within a few hours.

To achieve the highest sensitivity and avoid inhibitory effects in dPCR testing, the DNA is extracted prior to PCR.

These ready-to-use primer and TaqMan® probes are compatible with various digital PCR master mix chemistries and digital PCR instruments capable of detecting FAM™ and HEX™ (nanoplate- and droplet-based systems).

Kit Components and Storage

The kit consists of a primer and probes mix for bacteria and fungi detection, Internal Control DNA, Positive Control DNA and PCR grade Water. Color-coded tubes ensure easy handling. For details, see the kit components table below.

Most of the kit components (except of PCR grade Water) are lyophilized and hence highly stable to ensure highest performance and stability. The kit is stored at +2 °C to +8 °C until use. Rehydrated reagents should be stored at –18 °C. The total shelf-life is 18 months. The primer and probes mix should be protected from light.

Test Principle

Cyclus® dPCR Tool Box Bacteria Fungi was optimized for the detection of genomic bacterial and fungal DNA in cell culture derived samples. Bacteria are detected by amplifying a highly conserved region within the 16S rRNA coding region in the bacterial genome or a fragment of the conserved 18S rRNA coding region for fungal detection, respectively.

The kit includes primer and FAM™ labelled probes, which allow the specific detection of > 94% of all sequenced bacteria and clinical relevant fungal contaminants. False negative results due to PCR inhibitors or improper DNA extraction are detected by an inbuilt Internal Control (IC). The amplification of the Internal Control (IC) is detected in the HEX™ channel. The total processing time depends on the number of investigated samples and the used digital PCR system. The QIAGEN QIAcuity® One and Bio-Rad QX200™ digital PCR system were confirmed to be suitable for the described assay.

Key Features

- Broad bacterial and fungal species coverage: e.g. the bacterial assay covers ≥ 94% of all sequenced bacteria (*in silico* analysis), including all USP 71 relevant species, slow growing species or those who go undetected in growth-based methods e.g. *Cutibacterium acnes*. The coverage of specific matrix relevant organisms can be verified upon request.
- Compatible with all common digital PCR devices detecting FAM™ and HEX™. If a dPCR device is available, no new purchase is necessary. The kit has been successfully tested on the most widely used systems, such as the QIAcuity from QIAGEN and the QX System from Bio-Rad.
- Enables the highest sensitivity of any present PCR-based sterile test.
- For a digital PCR in combination with a limit of blank, no clean room environment is required to detect germs in a cell and gene therapy product. However, a DNA-free working environment increases the sensitivity to detect as little as 10 CFU/mL.

Product Certification

This product is meant for use in quality release testing only.

The LOT specific Quality Assurance Certificate can be downloaded from the MySartorius portal <https://my.sartorius.com>.

Kit Components

Label of Tube	Cap Color of Tube	SMB95-6001
Bacteria Primer Probes Mix	red	1 × lyophilized
Fungi Primer Probes Mix	orange	1 × lyophilized
Internal Control DNA	yellow	2 × lyophilized
PCR grade Water	white	3 × 1.5 mL
Positive Control	green	1 × lyophilized

User-Supplied Equipment and Material

- For DNA extraction the Microsart® ATMP Extraction kit from Sartorius is needed
Order No. SMB95-2001
- Microcentrifuge for 1.5 mL reaction tubes
- 1.5 mL reaction tubes (DNase, RNase, DNA, endotoxin free)
- Suitable pipettes for pipetting different volumes (10, 100 and 1000 µL)
- Compatible DNase, RNase, DNA, endotoxin free pipette filter tips

QIAGEN:

- A QIAGEN QIAcuity® dPCR device (FAM™, HEX™) and all related consumables
- 4× QIAcuity® UCP Probe PCR Kit
Cat. No. | ID: 250121

Bio-Rad:

- A Bio-Rad QX ddPCR device with filter sets for the detection of the fluorescence dyes FAM™ and HEX™ and all related consumables for either automated or manual sample preparation
- 2× ddPCR™ Supermix for Residual DNA Quantification
Order No. 1864038

Related Products

Detection Kits for PCR

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
SMB95-6001	Cyclus® dPCR Tool Box Bacteria Fungi	10 samples

Microsart® Calibration Reagent, 10⁸ 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® 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 Kit

SMB95-2001	Microsart® ATMP Extraction (for bacteria and fungi)	50 extractions
SMB95-2003	Microsart® AMP Extraction (for mycoplasma)	50 extractions
SMB95-4000	Microsart® Proteinase K	50 extractions

Cleaning Spray

SMB95-5001	DNA Decontamination Reagent, spray bottle	250 mL
SMB95-5002	DNA Decontamination Reagent, refill canister	5 L

Cleaning Wipes

SMB95-5003	DNA Decontamination Reagent, wipes	50 wipes
SMB95-5004	DNA Decontamination Reagent, refill sachets	5 × 50 wipes

For PCR support and recommendations please contact
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For further information, visit
[sartorius.com](https://www.sartorius.com)