SARTORIUS

Instructions for Use

Cyclus® dPCR Tool Box Bacteria Fungi

Bacteria, fungi and yeasts digital (droplet) PCR detection kit
Prod. No. SMB95-6001

Reagents for 10 samples
For use in research and quality control

Manufactured by:

minerva biolabs®

Minerva Biolabs GmbH | Schkopauer Ring 13 | 12681 Berlin | Germany

Symbols

LOT	Lot No.
REF	Order No.
	Expiry date
	Store at
	Contains reagents for 10 samples
	Manufacturer

Contents

1. Intended Use

Cyclus® dPCR Tool Box Bacteria Fungi is designed for digital PCR (dPCR) based detection of bacteria, fungi, and yeasts in cell culture derived biologicals, like Advanced Therapy Medicinal Products (ATMPs) or comparable short shelf-life products.

This product is not intended to be used as a diagnostic tool.

2. Explanation of the Test

This product consists of highly pure primer and probes for total bacteria and fungi detection. Combined with a highly pure sample preparation and dPCR device-specific master mix, it forms an assay for the detection of bacterial or fungal contamination. To achieve highest sensitivity and avoid inhibitory effects in dPCR testing, the DNA is extracted prior to PCR.

For this purpose, the use of Microsart® ATMP Extraction (SMB95-2001), an ultra-pure DNA isolation kit is recommended. To examine a representative fraction of the total sample, at least 10% of the DNA extract should be tested in the dPCR reaction. The subsequent dPCR assay can be performed with a dPCR instrument able to detect the fluorescent dyes FAM™ and HEX™. The QIAGEN QIAcuity® One and BioRad QX200™ digital PCR system were successfully confirmed to be suitable (for the described purpose). The complete detection procedure can be performed within a few hours. In contrast to the culture method, samples do not need to contain viable material as all contaminating cellular material (e.g. viable, dormant, non-culturable or dead) is detected by the PCR method.

3. 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 area of 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 known bacteria and most relevant fungal contaminants. False negative results due to PCR inhibitors or improper DNA extraction are detected by the Internal Control (IC). The amplification of the Internal Control (IC) is detected in the HEX™ channel.

4. Notes on the Test Procedure

1. For in vitro use in research and quality control. This kit may be disposed of according to local regulations.
2. This leaflet must be widely understood for a successful use of Cyclus® dPCR Tool Box Bacteria Fungi. The reagents supplied should not be mixed with reagents from different lots but used as an integral unit. The reagents of the kit should not be used beyond their shelf life.
3. This kit should be used by trained staff, only. A clean lab coat and disposable gloves should be always worn while performing the assay.
4. To avoid DNA cross-contaminations, the complete test must be performed under sterile and DNA-free conditions (see chapter 4.1 for detailed information).
5. In case of work with living microbes, the local regulation for BSL2 laboratories must be followed.
6. This kit is not designed for mycoplasma detection.
7. Any deviation from the protocol can affect the results.
8. For each test setup, an appropriate number of negative extraction controls (NECs) and at least one No Template Control (NTC, PCR negative control) should be included. A positive control (PC) confirms the functionality of the bacterial and fungal assay.
9. Controls should be handled in the same manner as the samples.
10. Assay inhibition may be caused by the sample matrix but also by sample elution buffers of incompatible DNA extraction methods. Do not use reagents from another kit than the Cyclus® dPCR Tool Box Bacteria Fungi and the Microsart® ATMP Extraction kit.

4.1 Handling and equipment recommendations

To avoid false positive results due to improper handling the following actions are recommended:

1. Perform the test under sterile and DNA-free conditions, the use of a laminar flow cabinet is recommended.
2. The laminar flow cabinet and all materials introduced into the laminar flow cabinet should be thoroughly decontaminated with a chlorine-based cleaning agent, e.g. Contec® ProChlor, before and during the work process.
3. Avoid working above open tubes and avoid air turbulences due to rapid movements.
4. Be careful when opening the tubes. Do not touch the inner surface of the lid.

5. Reagents

The kit contains one Bacteria Primer Probe Mix, one Fungi Primer Probe Mix, Internal Control DNA, Positive Control DNA, and PCR grade Water. All components are sufficient for testing up to 10 samples.

The expiry date of the unopened package is marked on the package label. The kit components are stored at +2 – +8 °C until use. Protect the Primer Probe Mixes from light.

Kit Component Label Information	Tubes	Cap Color
Bacteria Primer Probe Mix	1	red cap
Fungi Primer Probe Mix	1	orange cap
Internal Control DNA	2	yellow cap
Positive Control DNA	1	green cap
PCR grade Water	3x 1.5 mL	white cap

The lot specific Quality Assurance Certificate can be downloaded from the MySartorius portal (https://my.sartorius.com).

6. Needed but not included

Cyclus® dPCR Tool Box Bacteria Fungi contains primer and probe mixes to detect bacterial and fungi DNA using a digital PCR instrument. The DNA extraction kit, instrument-specific digital PCR reaction chemistry, and general industrial supplies and reagents, usually available in PCR laboratories are not included.

6.1 DNA extraction kit

The use of the Sartorius Microsart® ATMP Extraction kit (SMB95-2001) for sample preparation and DNA extraction upfront is mandatory. The extraction procedure for the preparation of a dPCR suitable extract is part of the present manual (7.3).

6.2 Consumables

- Laboratory gloves
- Chlorine-based cleaning agents, e.g. Contec® ProChlor
- DNA-free pipette filter tips free from bacterial and fungal DNA (Biosphere® filter tips from Sarstedt are recommended: 20 µL, Prod. No. 70.3020.255; 100 µL, Prod. No. 70.3030.255; 300 µL, Prod. No. 70.3040.255; 1000 µL, Prod. No. 70.3050.255)
- DNA-free PCR reaction tubes (PCR 8-SoftStrips with attached caps from Biozym are recommended: 0.1 mL Low Profile, Prod. No. 710975 and 0.2 mL High Profile, Prod. No. 710970)

6.3 Equipment

- Laminar Flow cabinet
- Heat block with optional shaking function
- Microcentrifuge for 1.5 mL reaction tubes
- Vortex
- dPCR device with filter sets for the detection of the fluorescent dyes FAM™ and HEX™ (QIAGEN – QIAcuity® One, BioRad – QX200™, ect.)
- Minicentrifuge for PCR tubes
- Pipettes (Sartorius)
    - mechanical
        1 – 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. LH-747021
        10 – 300 µL Sartorius Prod. No. LH-747061
        50-1000 µL Sartorius Prod. No. LH-747081
- Rack for 1.5 mL tubes and for PCR-tube strips

6.4 Digital PCR instrument specific components

6.4.1 QIAGEN nanoplate digital PCR

Reaction chemistry

- 4x QIAcuity® UCP Probe PCR Kit (Cat. No. / ID: 250121)

Reaction setup

- QIAcuity® Nanoplate 26k 24-Well (Cat. No. / ID: 250001)
- Nanoplate Tray (supplied with the QIAcuity® instrument - Cat. No. / ID: 250098)
- Plate roller (Cat. No. / ID 911105)

Cycling and data acquisition

- QIAcuity® One, 2plex (FAM™, HEX™) Device (Cat. No. / ID: 911001) or QIAcuity® One, 5plex Device (Cat. No. / ID: 911021)

6.4.2 BioRad droplet digital PCR

Reaction chemistry

- 2x ddPCR™ Supermix for Residual DNA Quantification (#1864038)

Reaction setup automated (droplet generation)

- Automated Droplet Generator (#1864101)
- DG32 Automated Droplet Generator Cartridges (#1864108)
- Automated Droplet Generation Oil for Probes (#1864110)
- Pipette Tips for the AutoDG™ System (#1864120)
- Pipette Tip Waste Bins for the AutoDG™ System (#1864125)

Reaction setup manually (droplet generation)

- QX200™™ Droplet Generator (#1864002)
- DG8™ Cartridge Holder (#1863051)
- Droplet Generation Oil for Probes (#1863005)
- DG8™ Cartridges for QX200™™/QX100™ Droplet Generator (#1864008)
- DG8™ Gaskets for QX200™™/QX100™ Droplet Generator (#1863009)

Plate sealer

- PX1 PCR Plate Sealer (#1814000)
- Sealing Frame (#1814080)
- Plate Support Block (#1814085)
- ddPCR™ Deep Well-Plate (#12001925)
- PCR Plate Heat Seal, foil, pierceable (#1814040)

PCR Cycler

- PTC Tempo Deepwell Thermal Cycler (#12015392)
- C1000 Touch™ Thermal Cycler with 96–Deep Well Reaction Module (#1851197)

Data acquisition

- QX200™ Droplet Reader (#1864003)
- ddPCR™ Droplet Reader Oil (#1863004)

7. Test Procedure

7.1 Recommendation for product release testing

Prior to PCR, DNA extraction is mandatory. Each sample should be extracted in duplicate. Additionally, two negative extraction controls (NEC) should be processed. A product matrix confirmed as sterile and free of microbial DNA should be used for the NEC. Each obtained extract should be transferred into a dPCR reaction for the bacterial and for the fungal detection. Furthermore, each PCR assay should include 1 x PCR negative control (no-template control, NTC) and 1 x PCR positive control (PC). For the NTC, the reaction is supplemented with water provided in the kit. The PC receives additional PC DNA included in the kit.

Samples	DNA extractions	dPCR reactions for Bacteria *		dPCR reactions for Fungi *	
		QIAGEN QIAcuity®	BioRad QX200™	QIAGEN QIAcuity®	BioRad QX200™
10 x samples	2 x per sample = 20	1 x for each extract = 20	2 x for each extract = 40	1 x for each extract = 20	2 x for each extract = 40
2 x NEC	1 x extract per NEC = 2	1 x each NEC = 2	2 x each NEC = 4	1 x each NEC = 2	2 x each NEC = 4
NTC	-	1 x NTC = 1	2 x NTC = 2	1 x NTC = 1	2 x NTC = 2
PC	-	1 x PC = 1	2 x PC = 2	1 x PC = 1	2 x PC = 2
	∑ 22 extractions	∑ 24 dPCR reactions	∑ 48 dPCR reactions	∑ 24 dPCR reactions	∑ 48 dPCR reactions

* To test a representative volume of the extract one dPCR reaction of the QIAGEN QIAcuity® system is required, whereas two reactions of the BioRad QX200™ system are necessary due to the reduced reaction volume. This corresponds to the testing of 20 µL extract per sample. This hardware specific deviation does not compromise the functionality of the Cyclus® dPCR Tool Box Bacteria Fungi kit.

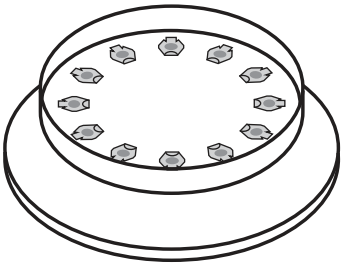
7.2 Sample collection and storage

The Sartorius Microsart® ATMP Extraction kit (SMB95-2001) has been validated using a maximum cell concentration of 10^6 cells/mL.

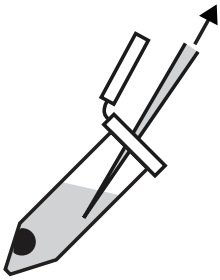
Notably, the assay can be performed with different types of cell culture-derived material. Therefore, the optimal sampling parameters, like volume or cell number, can vary according to the specific characteristics of the sample (e.g. medium, cell type) and may require optimization of the procedure.

If using the Microsart® ATMP Extraction kit, please follow the protocol below:

1.	Transfer 1 mL of cell culture or cell culture supernatant liquid material into a provided DNA-free 1.5 mL processing tube.
2.	Spin down for 15 minutes at a speed of at least 16,200 x g to sediment particles. **Attention:** Make sure to position the tubes in the centrifuge to obtain a pellet on the back side of the tube, as described in the figure below.
3.	Discard the supernatant carefully and completely without touching the pellet. Proceed to DNA extraction. If DNA extraction cannot be performed immediately, freeze samples at ≤ -18 °C. Repeated freezing and thawing should be avoided. **Attention:** Samples can only be inactivated or frozen after this sample collection step.



Make sure to position the tubes with the back side towards the outside of the rotor to obtain a pellet on the back wall of the tube.



Slowly discard all the supernatant without disturbing the pellet.

7.3 DNA extraction process

1.	Add 500 µL Lysis Buffer (transparent cap) to the cell pellet.
2.	Add 20 µL Internal Control DNA to the sample*.
3.	Vortex vigorously until pellet is completely dissolved (≥30 sec.) and pipette up and down if required.
4.	Heat at 80 °C for 20 minutes (optional with shaking).
5.	Spin down at 16,200 x g for 20 minutes. Make sure to position the tubes in the rotor as indicated in the figure in chapter 7.2.
6.	Remove supernatant carefully and completely, following the explanations in chapter 7.2. Make sure not to disturb or aspirate the pellet in the process. **Attention:** Higher risk of inhibition in PCR analysis if residues remain in the tube.
7.	Add 200 µL Suspension Buffer (violet cap) and dissolve the DNA by vortexing.

For best results use the extract directly. Store at +2 to +8 °C during dPCR reaction preparation (<2h). Store ≤-18°C for long term storage. Avoid repeated freezing and thawing.

*The Internal Control DNA allows monitoring of the extraction process.

7.4 Rehydration of the reagents

1.	Bacteria Primer Probe Mix Fungi Primer Probe Mix Internal Control DNA Positive Control DNA	red cap orange cap yellow cap green cap	Centrifuge briefly.
2.	Bacteria Primer Probe Mix Fungi Primer Probe Mix	red cap orange cap	For QIAGEN QIAcuity® use 275 µL of the 4x QIAcuity® UCP Probe PCR kit and incubate 5 min at room temperature, vortex briefly and spin down before adding 275 uL PCR grade Water and vortex briefly and spin down again.
			For BioRad QX200™ use 550 µL of the 2x ddPCR™ Supermix for Residual DNA Quantification.
3.	Internal Control DNA	yellow cap	Add 250 µL PCR grade Water (white cap).
4.	Positive Control DNA	green cap	Add 300 µL PCR grade Water (white cap).
5.	Internal Control DNA Positive Control DNA	yellow cap green cap	Incubate both (IC and PC) 5 min at room temperature vortex briefly and spin down.

Before use, the joining of both tubes of Internal Control after rehydration, briefly vortexing and spin down is recommended.

7.5 Preparation of the reaction mix and reaction setup

Preparation of the master mix and sample loading should not take more than 45 minutes. The pipetting sequence should be respected.

7.5.1 QIAGEN QIAcuity®

1.	Thaw the 4x QIAcuity® UCP Probe PCR Kit (Cat. No. / ID: 250121) vortex briefly, and centrifuge briefly to collect all liquid at the bottom of the tube.
2.	Pipette 275 µL of the 4x QIAcuity® UCP Probe PCR Kit (Cat. No. / ID: 250121) to the lyophilized Bacteria or Fungi Primer Probe Mix.
3.	Incubate 5 min at room temperature, vortex briefly, and centrifuge briefly to collect all liquid at the bottom of the tube.
4.	Pipette 275 µL PCR grade Water to 4x QIAcuity® Mastermix / Bacteria or Fungi Primer Probe Mix, vortex briefly, and centrifuge briefly to collect all liquid at the bottom of the tube. **Note 1:** After adding PCR grade Water, the QIAcuity® Mastermix / Bacteria or Fungi Primer Probe Mix is diluted to a 2x concentration, and must be used in a timely manner and cannot be stored at -20°C for further use **Note 2:** This step might be skipped if 30 µL DNA extract are used as input volume. An example plate layout is shown in 7.5.2. In this case, reverse pipetting is highly recommended. Please consider, that with increasing sample volume matrix effects might also increase.
5.	Aliquot 24x 20 µL of the 2x QIAcuity® master mix / Bacteria or Fungi Primer Probe Mix into 24 PCR tubes. Close PCR tubes.

Assembling test reaction (Bacteria or Fungi)

1.	20 aliquots shall be used to analyse the sample extracts. Add 20 µL of a sample DNA extract to an aliquot. Close lid after liquid transfer is complete.
2.	Use 2 aliquots to analyse the two NEC extracts. Supply each with 20 µL of a NEC DNA extract. Close lid after liquid transfer is complete.
3.	Use 1 aliquot for the NTC. Add 18 µL PCR grade Water and 2 µL IC. Close lid after liquid transfer is complete. **Note:** Rehydrate the IC and complete the reaction in the environment where neither the PC nor other target material is handled. Use dedicated consumables and pipettes.
4.	Use the last aliquot for the PC. Supply it with 16 µL PCR grade Water, 2 µL IC and 2 µL of PC for a PC reaction. Close lid after liquid transfer is complete. **Note:** Rehydrate the PC and complete the reaction in a dedicated environment with dedicated consumables and pipettes.
5.	Briefly vortex all PCR reaction tubes, spin down, and pipette 40 µL of the final dPCR reactions into the QIAcuity® Nanoplate 24x 26k. **Note:** The PC should be handled with the utmost care and loaded last.
6.	Seal the nanoplate as recommended by the manufacturer and place it into the QIAcuity® Cycler.

7.5.2 QIAGEN QIAcuity® proposed plate layout

	1	2	3
A	NTC	Sample 3 Extract 2	Sample 7 Extract 2
B	NEC 1 Extract 1	Sample 4 Extract 1	Sample 8 Extract 1
C	NEC 1 Extract 2	Sample 4 Extract 2	Sample 8 Extract 2
D	Sample 1 Extract 1	Sample 5 Extract 1	Sample 9 Extract 1
E	Sample 1 Extract 2	Sample 5 Extract 2	Sample 9 Extract 2
F	Sample 2 Extract 1	Sample 6 Extract 1	Sample 10 Extract 1
G	Sample 2 Extract 2	Sample 6 Extract 2	Sample 10 Extract 2
H	Sample 3 Extract 1	Sample 7 Extract 1	PC

Control reactions are marked with a dark frame.

7.5.3 QIAGEN QIAcuity® cycling profile

1 x	95 °C	03 min 00 s
40 x	95 °C	00 min 30 s
	55 °C	01 min 15 s

7.5.4 QIAGEN QIAcuity® imaging profile

Channel	Exposure duration	Gain
Green	500 ms	6
Yellow	500 ms	6

7.5.5 BioRad QX200™

1.	Thaw the 2x ddPCR™ Supermix for Residual DNA Quantification (#1864038), vortex briefly, and centrifuge briefly to collect all liquid at the bottom of the tube.
2.	Pipette 550 µL of the 2x ddPCR™ Supermix for Residual DNA Quantification (#1864038) to the lyophilized Bacteria Primer Probe Mix or Fungi Primer Probe Mix.
3.	Incubate 5 min at room temperature, vortex briefly and centrifuge briefly to collect all liquid at the bottom of the tube.
4.	Aliquot 48x 11 µL of the 2x ddPCR™ Supermix / Bacteria Primer Probe Mix into 48 PCR tubes. Close PCR tubes. Discard remaining liquid. **Note:** A 96-well PCR plate can be used instead of the PCR tubes.

Assembling test reaction (Bacteria or Fungi)

Note: To test 10% of the DNA extract, 2x11 µL must be tested for each extract. This will result in two droplet digital™ PCR reactions per extract.

1.	40 aliquots shall be used to analyse the sample extracts. Use two aliquots each with 11 µL for a sample DNA extract. Close lid after liquid transfer is complete.
2.	Use 4 aliquots to analyse the two NEC extracts. Add 11 uL of a NEC DNA extract to each. Close lid after liquid transfer is complete.
3.	Use 2 aliquots for the NTC. Supply each with 10 µL PCR grade Water and 1 µL IC. Close lid after liquid transfer is complete. **Note:** Rehydrate the IC and complete the reaction in the environment where neither the PC nor other target material is handled. Use dedicated consumables and pipettes.
4.	Use the last 2 aliquots for the PC. Supply each with 9 µL PCR grade Water, 1 µL IC and 1 µL of PC. Close lid after liquid transfer is complete. **Note:** Rehydrate the PC and complete the reaction in dedicated environment. Use with dedicated consumables and pipettes.
5.	Briefly vortex all PCR reaction tubes and use for droplet generation as recommended by BioRad.
6.	Execute the PCR cycling program as given in the following.

7.5.6 BioRad QX200™ proposed plate layout

When using the manual droplet generator, treat the samples, negative controls and positive controls separately.

When using an automated droplet generator, an analogue plate layout as shown for the QIAGEN QIAcuity® in 7.5.2 is recommended.

7.5.7 BioRad QX200™ cycling profile

Bacteria

95°C	3 min	
95°C	30 sec	40 cycles
55°C	30 sec	
60°C	45 sec	

Fungi

95°C	3 min	
95°C	30 sec	40 cycles
57°C	30 sec	
60°C	45 sec	

7.5.4 BioRad QX200™ imaging profile

Default setting of the QX200™ Droplet Reader can be used for data readout.

7.6 Data interpretation

Positive Control / Internal Control
Signal lifts in the FAM™ and HEX™ channels confirm the functionality of the Bacteria/ Fungi and IC assays and the reaction chemistry.

No Template Control
The observed signals (concentrations) in the FAM™ channel of the No Template Control (NTC) evaluate the basal contamination originating from the reaction chemistry and the used consumables.

Negative Extraction Control
The observed signals (concentrations) in the FAM™ channel reveal the background contamination present in the process. The determined signals (concentrations) by the NEC represent the Limit of Blank (LoB). For the NEC, using a confirmed sterile and microbial DNA free sample is recommended. The sample should be equivalent to or as similar as possible to the samples being tested.
Note: The Limit of Blank (LoB) depends on the overall process and can decrease with the experience of the operator and the optimisation of the working steps and work environment. During the establishment phase, we recommend generating a relevant number of NEC data within multiple experiments to determine a lab specific LoB.

Positive
We recommend considering the tested sample as positive, when target concentration of the tested sample in the FAM™ channel reaches minimum twice the concentration of the experiment specific LoB average determined by the NECs included in the experiment.

Negative
We recommend considering the tested sample as negative, when target concentration of the tested sample in the FAM™ channel do not reach twice the concentration of the experiment specific LoB average determined by the NECs included in the experiment.

False negative
If a sample was tested negative, confirm the signal (concentration) of the IC in the HEX™ channel. It should be comparable with the signal (concentration) of the NEC. Reaction is inhibited, if the signal (concentration) is significantly reduced or absent. This indicates a false negative result.

Note: Reach out to **PCR@Sartorius.com** if you require assistance with data analyses.

8. 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 Bactera Fungi	10 samples

Microsart® Calibration Reagent, 10^8 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, 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® 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

9. Short Instruction

1. Sample Collection

+ 1 mL
cell culture or cell culture supernatant liquid material

Processing tubes

15 min
≥ 16,200 × g

Discard supernatant

Store at ≤ -18 °C

or Proceed to DNA Extraction

2. DNA Extraction using Microsart® ATMP Extraction

+ 500 µL Lysis Buffer (transparent cap)
+ 20 µL Internal Control DNA

Cell pellet

≥ 30 sec vigorously
20 min, 80 °C
20 min
≥ 16,200 × g

Remove supernatant carefully

+ 200 µL Suspension Buffer (violet cap)

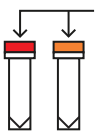
≥ 30 sec vigorously

3. Rehydration of Reagents

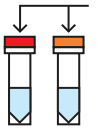
Bacteria Primer Probe Mix,
Fungi Primer Probe Mix,
PC, IC,
4x QIAcuity® UCP Probe PCR Kit or 2x ddPCR™ Supermix

+ briefly

For QIAGEN QIAcuity®

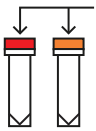


+ 275 mL
4x QIAcuity® UCP Probe PCR Kit
for 5 min RT
+ briefly



+ 275 mL
H_2O
+

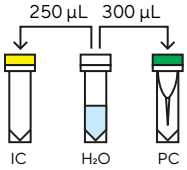
For BioRad QX200™



+ 550 mL
2x ddPCR™ Supermix for Residual DNA Quantification

QIAcuity® Reaction mix or ddPCR™ Reaction mix:

≥ 5 min, RT
briefly
briefly



IC and PC:

5 min, RT
briefly
briefly

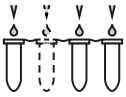
Bacteria Primer Probe Mix
Fungi Primer Probe Mix
Internal Control DNA (IC)
Positive Control DNA (PC)
PCR grade Water (H_2O)

incubate
vortex
centrifuge
add

This procedure overview is not a substitute for the detailed manual.

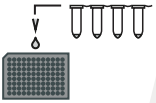
4. Assembling test reaction (Bacteria or Fungi) for QIAGEN QIAcuity®

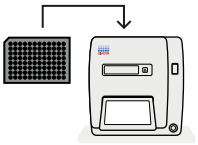
Aliquot 24 x 20 µL QIAcuity® Reaction mix / Bacteria or Fungi Mix



+ 20 x 20 µL sample DNA
+ 2 x 20 µL NEC DNA extract
+ 1 x (2 µL IC + 18 µL H_2O)
+ 1 x (2 µL IC + 2 µL PC + 16 µL H_2O)
briefly

Transfer 40 µL of each final dPCR reaction





Start dPCR program

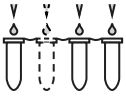
95 °C	95 °C		
3 min	30 sec		
1 cycle		40 cycles	
		55 °C	
		75 sec	

Imaging:

Fluorophore	Exp./Gain
Green	500 ms / 6
Yellow	500 ms / 6

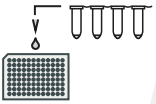
4. Assembling test reaction (Bacteria or Fungi) for BioRad QX200™

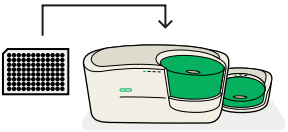
Aliquot 48 x 11 µL ddPCR™ Reaction mix / Bacteria or Fungi Mix



+ 40 x 11 µL sample DNA
+ 4 x 11 µL NEC DNA extract
+ 2 x (1 µL IC + 10 µL H_2O)
+ 2 x (1 µL IC + 1 µL PC + 9 µL H_2O)
briefly

Transfer 40 µl of each final dPCR reaction





Start dPCR program for Bacteria

95 °C	95 °C			
3 min	30 sec			
1 cycle		40 cycles		
			60 °C	
		55 °C	45 sec	
		30 sec		

Imaging: Default setting

Start dPCR program for Fungi

95 °C	95 °C			
3 min	30 sec			
1 cycle		40 cycles		
			60 °C	
		57 °C	45 sec	
		30 sec		

Imaging: Default setting

vortex
add

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