SARTORIUS

Instructions for Use

Cyclus® 10 CFU

SMB95-3011	Cyclus® 10 CFU *Mycoplasma arginini*
SMB95-3012	Cyclus® 10 CFU *Mycoplasma orale*
SMB95-3013	Cyclus® 10 CFU *Mycoplasma gallisepticum*
SMB95-3014	Cyclus® 10 CFU *Mycoplasma pneumoniae*
SMB95-3015	Cyclus® 10 CFU *Mycoplasma synoviae*
SMB95-3016	Cyclus® 10 CFU *Mycoplasma fermentans*
SMB95-3017	Cyclus® 10 CFU *Mycoplasma hyorhinis*
SMB95-3018	Cyclus® 10 CFU *Acholeplasma laidlawii*
SMB95-3019	Cyclus® 10 CFU *Spiroplasma citri*
SMB95-3020	Cyclus® 10 CFU *Mycoplasma salivarium*

Validation Standard and External Positive Control
For use in research and quality control

Symbols

LOT	Lot No.
REF	Order No.
	Expiry date
	Store at
	Content
	Manufacturer

Contents

1. Intended Use

Cyclus® 10 CFU are intended for the method validation and routine control of nucleic acid amplification technology (NAT)-based mycoplasma detection methods in accordance with European Pharmacopoeia (EP) 2.6.7 (Edition 12.2), as well as current NAT concepts described in US Pharmacopoeia (USP) <77> and Japanese Pharmacopoeia (JP) 18 G3.

They are used for product-specific method validation, including Limit of Detection (LOD) determination, and as low-concentration controls in routine testing when applied as External Positive Control (EPC) described in EP 2.6.7 or Extraction Inhibition Control (EIC) described in USP <77> within the complete NAT workflow.

The standards are suitable exclusively for molecular NAT methods and are not intended for culture-based mycoplasma assays.

2. Test Principle

Cyclus® 10 CFU contain purified, non-infectious inactivated mycoplasma particles at a defined concentration of 10 colony forming units (CFU) per vial.
The material is spiked into the product-specific matrix prior to nucleic acid extraction and processed through the complete NAT workflow. As the nucleic acids are contained within inactivated mycoplasma particles, extraction is required before amplification.

By undergoing the full extraction and amplification procedure, the standards enable evaluation of overall workflow performance, including extraction efficiency and amplification sensitivity, using PCR.

3. Reagents

The Cyclus® 10 CFU are manufactured accordingly with ≤10 CFU equivalent and have a GC/CFU ratio of <10. Testing using dPCR is part of QC testing. Lot-specific information is provided in the accompanying Quality Assurance Certificate.

Component	Prod. No.	Quantity	Cap color
Mycoplasma arginini	SMB95-3011	3 vials ≤10 CFU (lyophilized)	green
Mycoplasma orale	SMB95-3012		
Mycoplasma gallisepticum	SMB95-3013		
Mycoplasma pneumoniae	SMB95-3014		
Mycoplasma synoviae	SMB95-3015		
Mycoplasma fermentans	SMB95-3016		
Mycoplasma hyorhinis	SMB95-3017		
Acholeplasma laidlawii	SMB95-3018		
Spiroplasma citri	SMB95-3019		
Mycoplasma salivarium	SMB95-3020		
PCR grade Water	Included in all products	1 vial	white

Storage and Stability (unopened product):

- Store at +2 °C to +8 °C.
- The expiry date of the unopened product is indicated on the package label.

After Reconstitution / Rehydration:
Each vial is intended for single use only.
Rehydrated material must not be stored or reused.

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

4. Needed but not included

Cyclus® 10 CFU require only basic laboratory consumables for reconstitution and handling.

All additional materials depend on the NAT-based detection method used downstream and must be provided by the user.

Consumables

- RNase-free, DNase-free low-bind tubes (1.5 mL or 5 mL)
- RNase-free, DNase-free pipette filter tips (100 – 1000 µL)
- Personal protective equipment (laboratory gloves, protective masks)
- Sartorius Cleaning Spray (SMB95-5001/SMB95-5002) or Cleaning Wipes (SMB95-5003/SMB95-5004) for contamination control
- PCR reaction tubes, strips or plates suitable for the chosen amplification platform

Laboratory equipment

- Pipettes (Suitable pipettes and corresponding filter tips are available from Sartorius)
- Vortex mixer
- Microcentrifuge for 1.5 mL tubes
- Suitable PCR cycler
- Tube racks and general laboratory equipment applicable to the chosen detection method

Recommended extraction and detection systems

For optimal performance and to ensure recovery of both DNA and RNA, the following systems are recommended:

- Cyclus® Bead Extraction (SMB95-6000)
- Cyclus® RT-qPCR Mycoplasma (SMB95-6002)

These assays are optimized for sensitive and reliable NAT-based mycoplasma detection and are fully compatible with the Cyclus® 10 CFU.

5. Sample

Cyclus® 10 CFU are intended for spiking into product-specific sample matrices requiring mycoplasma testing. The standards themselves do not require a specific matrix composition. Instead, each user must evaluate whether their own product-specific matrix is compatible with the chosen nucleic acid extraction and NAT-based amplification method.

Applicable sample types

The standards can be used with all matrices typically evaluated in NAT-based mycoplasma testing, including:

- Cell culture supernatants
- Suspension and adherent cell cultures
- Viral vector preparations (e.g., AAV, LV, adenovirus)
- Plasmid-, DNA- or mRNA-based products
- Upstream and downstream processing intermediates
- Formulated drug substances and drug products
- Cryopreservation media and supplements
- Buffer systems, process intermediates and stabilizers

Depending on product type and regulatory expectations, testing may be required for both cellfree and cell-containing samples.

Matrix considerations

Many matrices contain DNases and/or RNases, which may degrade nucleic acids. To prevent loss of genomic material, follow the handling instructions provided in this manual:

- Protocol 1: For nuclease-free matrices
- Protocol 2: For matrices with potential nuclease activity

Highly viscous or hydrophobic matrices may impair dissolution or rehydration of the standard. In such cases, ensure thorough mixing and evaluate whether additional steps are required to achieve complete homogenization.

Use in method validation

The standards support matrix-specific validation of NAT-based mycoplasma detection methods. The specific validation design (e.g. number of product batches, organism selection, representative matrices) must be defined by the user based on regulatory requirements and internal quality guidelines.
Matrices used for validation should represent the final product or the relevant manufacturing stage, as defined in the validation plan.
This document does not define a complete validation protocol. Such procedures must comply with applicable regulatory requirements, including EP 2.6.7, USP <77>, JP 18 G3, and internal risk-based assessments.

Product matrix suitability test

The "Method Suitability Test" (USP <77>) or the "Test for Inhibitory Substances" (EP 2.6.7) are required to demonstrate that the user's product-specific matrix does not interfere with nucleic acid extraction or PCR amplification.

For this purpose, Cyclus® 10 CFU may be spiked into the matrix and processed through the complete NAT workflow. Successful detection confirms that the matrix supports efficient extraction and amplification without inhibitory effects.

If inhibition is observed, the matrix composition or sample preparation procedure must be adjusted before routine testing or method validation is performed.

6. Precautions

Cyclus® 10 CFU are intended for in vitro use only and must be handled by experienced laboratory personnel following good laboratory practice.

The product contains purified, non-infectious inactivated mycoplasma particles and does not pose a biological infection risk. Nevertheless, all samples and spiked matrices should be treated as potentially infectious.

It is extremely important to always wear appropriate personal protective equipment, including a lab coat, disposable gloves and a protective mask, to prevent contamination of the sample and exposure to biological aerosols.

PCR carry-over contamination may lead to false-positive results. Use dedicated pipettes, RNase-/DNase-free consumables, and contamination control reagents such as Sartorius Cleaning Spray (SMB95-5001/SMB95-5002) or Cleaning Wipes (SMB95-5003/SMB95-5004).

Avoid repeated freeze-thaw cycles and do not use the product beyond its expiry date.

The standards are not suitable for culture-based mycoplasma assays, as they contain no viable organisms.

The standards are not suitable for sample concentration by centrifugation.

Dispose of unused materials and consumables according to local laboratory waste regulations.

7. Additional Notes

1. Matrix handling and nuclease considerations

- If the matrix is nuclease-free, rehydrate the standard directly in the matrix (Protocol 1).
- In matrices containing nucleases, the nucleic acids must be protected from degradation. If the matrix contains DNases and/or RNases (e.g. fetal calf serum, cell lysates), rehydrate the standard in lysis buffer before adding it to the matrix (Protocol 2).
- Once the standard has been mixed with matrix (or with lysis buffer + matrix), stability cannot be guaranteed; immediate processing is recommended.

2. Use as controls in NAT-based mycoplasma testing

- Extraction inhibition control (EIC)
  When spiked into the matrix before nucleic acid extraction, Cyclus® 10 CFU can be used to verify extraction efficiency and matrix compatibility within NAT workflows.
- External Positive Control (EPC)
  When used according to the validated NAT workflow, Cyclus® 10 CFU may serve as a low-concentration (EPC).
  The standard added prior to nucleic acid extraction. The EPC verifies overall assay performance close to the detection limit and confirms that the complete workflow functions as intended.

3. General laboratory handling

- Use aerosol-resistant filter tips and change tips between pipetting steps to prevent carry-over contamination.
- Reagents must not be mixed with components from other lots and must not be used beyond their expiry date.
- Any deviation from the instructions in this document may affect functionality of the standard and must be validated by the user.
- These instructions must be fully understood and carefully followed for successful application of this product.

4. Application of PCR grade Water

- PCR grade Water is DNase- and RNase-free and suitable for use in PCR-related workflows where nuclease-free conditions are required. It may be used for rehydration of the standards or for the preparation and dilution of internal controls (IC).
- It can also be used for preliminary experiments prior to matrix-specific validation. In such experiments, the performance of the standard dissolved in PCR grade Water can be compared with the standard spiked into the product matrix to assess potential inhibitory effects of the matrix.
- Rehydration in PCR grade Water may additionally be useful for familiarization with the handling, rehydration, and analytical performance of the standards before performing matrix-specific experiments.

8. Test Procedure

Protocol 1 – Nuclease-free matrices

1.	Centrifuge the vial briefly for 5 sec at maximum speed.
2.	Add 1 mL of the product-specific matrix to the vial.
3.	Incubate for at least 5 min. **Note:** Solubility depends on the matrix. For challenging matrices, extend incubation (e.g. 15 min) to support rehydration.
4.	Mix thoroughly by pulse-vortexing (3 × 10 sec). **Note:** Avoid foam formation. If necessary, mix by gentle pipetting without drawing air.
5.	Centrifuge briefly to collect any liquid from the lid.
6.	Proceed with analysis according to the instructions of the selected NAT-based mycoplasma detection method.

Protocol 2 – Nuclease-containing matrices

1.	Centrifuge the vial briefly for 5 sec at maximum speed.
2.	Add 1 mL of lysis buffer (Cyclus® Bead Extraction SMB95-6000). Additional lysis buffer available as **Cyclus® Bead Extraction Lysis Buffer** (SMB95-6003) if needed. **Note:** When using extraction systems from other manufacturers, the volumes may need to be adjusted accordingly.
3.	Incubate for at least 5 min.
4.	Mix thoroughly by pipetting at least 10 times, avoiding air intake.
5.	Add the entire 1 mL rehydrated standard to 1 mL of the product-specific matrix to generate 4 technical replicates.
6.	Mix thoroughly again by pipetting at least 10 times.
7.	Centrifuge briefly to collect any liquid from the lid.
8.	Aliquot 500 µL per extraction (corresponding to 250 µL sample).
9.	Add 20 µL Proteinase K (from the Cyclus® Bead Extraction (SMB95-6000)) per extraction.
10.	Perform extraction according to the instructions of the Cyclus® Bead Extraction (SMB95-6000)

Note on Internal Control (IC):
For workflows using an internal control as a process control, add the IC to the lysis buffer before rehydrating the standard. For the Cyclus® RT-qPCR Mycoplasma (SMB95-6002), add 6.4 µL IC to 1 mL lysis buffer (based on a 100 µL IC stock).

9. Related Products

Detection Kits for qPCR or dPCR

SMB95-6001	Cyclus® dPCR Tool Box Bacteria Fungi	10 samples
SMB95-6002	Cyclus® RT-qPCR Mycoplasma	25 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

Cyclus® 100 GC , 3 vials

SMB95-3001	Cyclus® 100 GC *Mycoplasma arginini*
SMB95-3002	Cyclus® 100 GC *Mycoplasma orale*
SMB95-3003	Cyclus® 100 GC *Mycoplasma gallisepticum*
SMB95-3004	Cyclus® 100 GC *Mycoplasma pneumoniae*
SMB95-3005	Cyclus® 100 GC *Mycoplasma synoviae*
SMB95-3006	Cyclus® 100 GC *Mycoplasma fermentans*
SMB95-3007	Cyclus® 100 GC *Mycoplasma hyorhinis*
SMB95-3008	Cyclus® 100 GC *Acholeplasma laidlawii*
SMB95-3009	Cyclus® 100 GC *Spiroplasma citri*
SMB95-3010	Cyclus® 100 GC *Mycoplasma salivarium*

Cyclus® 10 CFU, 3 vials

SMB95-3011	Cyclus® 10 CFU *Mycoplasma arginini*
SMB95-3012	Cyclus® 10 CFU *Mycoplasma orale*
SMB95-3013	Cyclus® 10 CFU *Mycoplasma gallisepticum*
SMB95-3014	Cyclus® 10 CFU *Mycoplasma pneumoniae*
SMB95-3015	Cyclus® 10 CFU *Mycoplasma synoviae*
SMB95-3016	Cyclus® 10 CFU *Mycoplasma fermentans*
SMB95-3017	Cyclus® 10 CFU *Mycoplasma hyorhinis*
SMB95-3018	Cyclus® 10 CFU *Acholeplasma laidlawii*
SMB95-3019	Cyclus® 10 CFU *Spiroplasma citri*
SMB95-3020	Cyclus® 10 CFU *Mycoplasma salivarium*

Microsart® Calibration Reagent, 10^8 genomes / vial, 1 vial (bacteria)

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, 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*

DNA Extraction Kit

SMB95-6000	Cyclus® Bead Extraction (for mollicutes)	100 extractions
SMB95-6003	Cyclus® Bead Extraction Lysis Buffer	27.5 mL
SMB95-2001	Microsart® ATMP Extraction (for bacteria and fungi)	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

Limited Product Warranty

This warranty limits our liability for replacement of this product.

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Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Str. 20
37079 Goettingen, Germany

Phone +49 551 308 0
Fax +49 551 308 3289
www.sartorius.com

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